EXCHANGE STUDIES OF P^{32} AND P^{31} IN PLANTS

DISSERTATION

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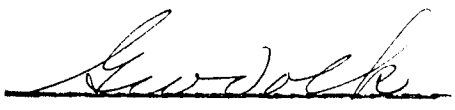

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INTRODUCTION

The dilution of a radioactive isotope with a stable isotope of the same element is one of the fundamental techniques used in various phases of work. This technique is based on the assumption that all properties of different isotopes of an element are the same except the mass and the radioactivity, so that the different isotopes become distributed homogeneously in the solution with the ordinary isotope acting as a carrier. Plants absorb them in the same proportion as in the culture medium, however, it has been reported that some exchange reaction between the different isotopes may take place. Hevesy (5) has demonstrated that radiolead was rapidly taken up by the roots of the horse bean plant (Vicia faba). When the roots were subsequently immersed in a solution containing a high concentration of normal lead, most of the radiolead diffused into the external solution. This phenomenon may be explained as a result of the exchange reaction between radiolead and normal lead. At equilibrium, the radiolead previously accumulated in the roots has been exchanged with the normal lead in the solution. Overstreet and his associates (2, 8, 14), working with radioactive potassium, have observed a similar exchange phenomenon between the roots of barley and the culture medium. McAulliffe et al (12), by using radiophosphorus P^{32}, were able to study the exchange between phosphate on the surfaces of soil minerals with

phosphate in the solution. They concluded that a rapid exchange between phosphate in the solution and phosphate on the surface takes place. Olsen (13), taking the advantage of this exchange property of the isotopes has measured the amount of phosphate on the surfaces of hydroxylapatite and phosphate rock with radioactive phosphorus P^{32}. Whether the same exchange reaction occurs within the plant and its possible significance in plant studies using radioisotopes as tracers have not been reported. It is for these purposes that the present investigation was conducted. The isotopes used were radioactive phosphorus, P^{32} and the stable form P^{31}.

METHODS OF EXPERIMENTATION

Cultural Method

All experiments in this study were carried out in the greenhuose at the Ohio Agricultural Experiment Station during 1953 and 1954. The plants used were corn, tomato and soybean. The corn plants were grown in gravel cultures consisting of a 3-gallon sloping bottom pot with a 5-gallon bottle serving as a reservoir for the mineral nutrient solution. The tomato plants were grown in 3-gallon glazed, flat bottom pots in which the lower part was filled with quartz gravel and the upper half with sand. The use of sand kept the small, light seeds of tomato in place when they were being irrigated. The soybean plants were grown in pure sand cultures. Fourteen liters of the mineral nutrient solution were kept in the reservoir for tomato and soybean plants, and fifteen and two tenth liters for corn. The cultures were irrigated seven times a day, once every two hours from 8 a. m. until 6 p. m. with the seventh irrigation being made at midnight. The time for irrigations was controlled automatically by a time clock. The irrigation system was so arranged that, when, irrigating, the mineral nutrient solution in the reservoirs was forced up into the pots by low air pressure from a small Crowell-type air pump. When the pump stopped, the solution drained back into the reservoir by allowing the air in the solution bottle to be forced back through the pump and the by-pass valves.

Rain water was used instead of the mineral nutrient solution in the reservoir at first and then replaced by the latter one week

to ten days after the seedlings appeared. The composition of the mineral nutrient solutions for the different species of plants is given in Table I. The solutions for corn and soybean were those which have been in use at the Ohio Agricultural Experiment Station, while for tomato, Arnon and Hoagland's solution was used (1). These mineral nutrient solutions were found very satisfactory in the present studies. Beside the major nutrient elements, the micrometabolic elements were also used in the solution. They were boron, manganese, zinc, copper, molybdenum, vanadium, chromium, nickel, cobalt and tungsten. A concentration of 0.25 ppm was used in the nutrient solutions for boron and manganese, 0.025 ppm for zinc, 0.01 ppm for copper and 0.005 ppm for all others. Iron was added in the form of finely ground magnetite directly to the gravel cultures for Experiments I and III, and for Experiments II, IV and V, an iron-containing chelating compound "Sequestrene NaFe" at a concentration of 3 ppm of iron was used. This is a technical compound of sodium ferric ethylenediamine tetra-acetate monohydrate, a product of the Alrose Chemical Company. The mineral nutrient solution was first introduced at half unit strength in all experiments except Experiment II, and thereafter renewed every other week with full unit strength until the plants reached the stage of growth at which the different treatments were made. In Experiment II, a full unit strength solution was used at the beginning and a half unit phosphorus-free solution was added to the original one two weeks later. The solution remained

Table I. Composition of the Mineral Nutrient Solutions Used in the Experiments

Element	Chemical form	Experiment I (Corn)	II (Corn)	III (Corn)	IV (Tomato)	V (Soybean)
		ppm	ppm	ppm	ppm	ppm
Sodium	Sulfate	29	29	29	0	0
Potassium	Chloride	38	38	38	0	58
	Nitrate	69	69	69	390	0
	Phosphate	13	26	13	0	58
	Total	120	133	120	390	116
Calcium	Chloride	0	0	0	0	51
	Nitrate	100	100	100	120	129
	Total	100	100	100	120	180
Magnesium	Sulfate	20	20	20	48	55
Nitrogen (NH_4-N)	Sulfate	5	5	5	0	10
	Phosphate	0	0	0	28	0
	Total	5	5	5	28	10
Nitrogen (NO_3-N)	Ca-salt	70	70	70	84	90
	K-salt	25	25	25	141	0
	Total	95	95	95	225	90
Phosphorus (PO_4-P)	NH_4-salt	0	0	0	62	0
	K-salt	10	20	10	0	45
	Total	10	20	10	62	45
Sulfur (SO_4-S)	NH_4-salt	6	6	6	0	12
	Mg-salt	26	26	26	64	75
	Na-salt	20	20	20	0	0
	Total	52	52	52	64	87
Chlorine (Cl-Cl)	Ca-salt	0	0	0	0	90
	K-salt	35	35	35	0	53
	Total	35	35	35	0	143

* The figures underlined indicate the total concentration of the element in the mineral nutrient solution. Those from the salts of the micrometallic elements are not included.

unchanged until the plants were harvested.

Description Of The Experiments

In this study, a total of five greenhouse experiments were conducted, three for corn and one each for tomato and soybean. Since the dates of planting and harvesting as well as the amounts of P^{32} and P^{31} used were not the same, it is necessary to describe each experiment separately.

Experiment I This experiment was conducted in the summer of 1953. Four seeds of W64, an Ohio double cross corn hybrid, were planted in each pot on April 24. The seedlings emerged on April 30. They were thinned on May 11 to two per pot and the mineral nutrient solution was introduced. On June 5, the plants in each pot were thinned to one and on July 7, when the corn entered the tasseling stage, the tassel was cut off and a new mineral nutrient solution containing all elements but phosphorus was introduced in each culture. Different levels of P^{32} and P^{31} were added to the solution to give the different treatments. Each treatment was replicated three times. The amount of P^{32} added, the concentration of P^{31} in the solution and their unit ratio are tabulated as shown on the following page.

Treatment	Total P^{32} added mc*	Conc. of P^{31} in solution ppm	Unit+ ratio of P^{32} : P^{31}
A	0	10	0 : 1
B	1	0	1 : 0
C	1	10	1 : 1
D	1	200	1 : 20

* Assayed on July 2, 1953 at the Oak Ridge Laboratory and the quantity of P^{32} was not converted to that at the time the isotope was used.

\+ One mc. of P^{32} in 15.2-liter solution and a concentration of 10 ppm of P^{31} are considered as one unit of P^{32} and P^{31} respectively.

Ten days after the treatments were made, the plants were harvested and dissected into upper and lower leaves, stem, ear and roots. All samples were oven-dried at 70 °C for three days.

Experiment II Four corn seeds of W64 were planted in each culture on July 28, 1954 right after the first irrigation of the washed gravel. The seedlings appeared on August 1. On August 17, they were thinned to two in each pot. The mineral nutrient solution was introduced on August 18. Previous to this, the cultures were irrigated with rain water. Since this experiment was designed to serve as a reference for exchange studies between P^{31} and P^{32}, the different treatments were made in the very beginning before any accumulation of P^{31} in the tissues could occur. Initially a whole unit of the mineral nutrient solution containing P^{31} was introduced with additional P^{31} and different amounts of P^{32} for various treatments. Two weeks after this, a half unit strength solution excluding phosphorus was added to the original solution in order to meet the nutrient requirement of rapidly growing plants. The amounts of P^{32}

and P^{31} added to the solution for different treatments were:

Treatment	Total P^{32} added mc*	Conc. of P^{31} in solution ppm	Unit+ ratio of P^{32} : P^{31}
A	0.156	20	1 : 1
B	0.156	40	1 : 2
C	0.156	100	1 : 5
D	0.312	40	2 : 2
E	0.780	100	5 : 5

* Assayed on August 16, 1954 at the Oak Ridge Laboratory and the quantity of P^{32} was not converted to that at the time the isotope was used.

\+ 0.156 mc. of P^{32} in 15.2-liter solution and a concentration of 20 ppm of P^{31} are considered as one unit of P^{32} and P^{31} respectively.

On September 15, four weeks after the introduction of different treatments, the plants were harvested and dissected into leaves, stems and roots. The tissues from the two plants in each pot were combined for analysis.

Experiment III This experiment was a repetition of the one conducted in 1953. The plants were seeded on April 26, 1954 with four corn seeds of W64 in each pot. Five days later, the seedlings appeared and on May 7, a one half unit strength of the mineral nutrient solution was introduced. A week later, the plants were thinned to two and on May 17, to one in each pot. On June 10, the treatments as shown on next page were given.

Treatment	Total P^{32} added mc*	Conc. of P^{31} in solution ppm	Unit+ ratio of P^{32} ; P^{31}
A	0.27	0	1 : 0
B	0.27	10	1 : 1
C	0.27	50	1 : 5
D	0.27	100	1 : 10
E	0.54	20	2 : 2
F	1.35	50	5 : 5

* Assayed on June 7, 1954 at the Oak Ridge Laboratory. The quantity of P^{32} was not converted to that at the time the isotope was used.

\+ 0.27 mc. of P^{32} in 15.2-liter solution and a concentration of 10 ppm of P^{31} are considered as one unit of P^{32} and P^{31} respectively.

The tassels appeared on June 15. However, no pollen had been formed when the plants were harvested on June 21. The plants were dissected into tassel, upper and lower leaves, stems and roots. Ears had started to form but were combined with sheath and stem for analysis.

Experiment IV Tomato plants were used in this experiment. Fifteen to twenty tomato seeds of Rutgers variety were planted in each pot on May 20, 1954. The seedlings were visible in five days. The plants were thinned to five on June 15, and to two in each pot on June 21. These two plants were allowed to grow until mature but only one of them was used for analysis. The plants grew vigorously throughout the experiment. Flowers appeared on July 15 and one week later, fruits started to form. On August 5, the following treatments were made:

Treatment	Total P^{32} added mc*	Conc. of P^{31} in solution ppm	Unit+ ratio of P^{32} : P^{31}
A	0.256	0	1 : 0
B	0.256	62	1 : 1
C	0.256	124	1 : 2
D	0.256	248	1 : 4
E	0.512	124	2 : 2
F	1.024	248	4 : 4

* Assayed on July 22, 1954 at the Oak Ridge Laboratory. The quantity of P^{32} was not converted to that at the time the isotope was used.

\+ 0.256 mc. of P^{32} in 14-liter solution and a concentration of 62 ppm of P^{31} are considered as one unit of P^{32} and P^{31} respectively.

The plants were harvested on August 20, 1954 and dissected into leaf blades, stem (including branch) and fruits. Roots were not analyzed.

Experiment V Eight soybean seeds of Lincoln variety were planted in each pot on June 16. The plants were thinned to five in each pot on June 27. The plants grew vigorously and started flowering on July 21 and by August 5, pods had formed. Various treatments were made on August 18 as follows:

Treatment	Total P^{32} added mc*	Conc. of P^{31} in solution ppm	Unit+ Ratio of P^{32} : P^{31}
A	0.156	0	1 : 0
B	0.156	45	1 : 1
C	0.156	90	1 : 2
D	0.156	225	1 : 5
E	0.312	90	2 : 2
F	0.780	225	5 : 5

* Assayed on August 16, 1954 at the Oak Ridge Laboratory.

\+ 0.156 mc. of P^{32} in 14-liter solution and a concentration of 45 ppm of P^{31} are considered as one unit of P^{32} and P^{31} respectively.

The plants were harvested on August 30. They were then dissected into blades, stems and pods (with seeds). Roots were not sampled.

Preparation Of Samples And Methods Of Analysis

After the plants were harvested and dissected, the samples were kept in a 70 ^{o}C oven for three days, then ground and passed through a 30-mesh screen in a laboratory Wiley mill. Each sample was thoroughly mixed before being analyzed.

One gram of plant tissue was weighed out in 100- or 150-ml. tall form beakers and digested with 3:1 nitric-perchloric acid mixture. After complete digestion, the solution was cooled and diluted to 100 ml. with distilled water. One ml. aliquot was pipetted out into the planchet for the measurement of the radioactivity of P^{32} and one or more milliliters for P^{31} depending upon the phosphorus content of the tissue. In most cases, one ml. was suitable for the determination. Duplicate samples were used for analysis.

For the measurement of the radioactivity of P^{32}, the solution in the planchet was first evaporated on a steam plate to dryness. The planchet was then put on the second shelf under the Geiger tube of a counter manufactured by Potter Instrument Company. The radioactivity was measured and recorded as counts per minute. This was corrected for background counts, coincidence and tube sensitivity factors. Since the radioactivity of all samples from different tissues could not be measured at the same time, the counts were all corrected to a reference date, the date of harvest, by using the

decay factor of P^{32} which has a half-life of fourteen and three tenth days. Since no absolute value in quantity of P^{32} was needed in this study, the yield factor was not used.

The determination of P^{31} was carried out by placing 1 ml. of the solution from the digest of the plant tissue into a 50-ml. volumetric flask. A volume of about 40 ml. of distilled water, 2 ml. of 2.5 % ammonium molybdate in 8.5 N H_2SO_4 and 1 ml. of 1-amino-2-naphthol-4-sulfonic acid solution were added and then diluted to 50 ml. with distilled water. After mixing, the blue color developed and the contents were allowed to stand for fifteen minutes. The percentage of transmission was measured by using a red filter of 650 mμ in a Fisher electrophotometer. The amount of P^{31} in the plant tissue was calculated from a standard curve prepared by using various known amounts of P^{31}. The 1-amino-2-naphthol-4-sulfonic acid solution was prepared every three weeks by dissolving 0.5 gram of the acid in 250 ml. distilled water containing 5 grams of sodium metasulfite.

The ratio of P^{32}:P^{31} in plant tissues was obtained by dividing the number of counts per minute of P^{32} by the amount of P^{31} in micrograms (abbreviated as r) in a given amount of the plant sample.

RESULTS AND DISCUSSION

EXPERIMENT I: CORN (1953)

$P^{32}:P^{31}$ Ratio in the Culture Solution

In order to determine if the absorption of the mixture of different isotopes of the same atomic number by the plant follows the assumption that they are absorbed in the same proportions, the actual concentration of P^{32} and P^{31} in each of the mineral nutrient solutions supplied to each treatment was determined. The samples were taken from each bottle after the P^{32} and P^{31} were thoroughly mixed with the mineral nutrient solution before irrigation. For comparison, samples of the solution at the end of the experiment were also collected. The results are given in Table II.

When the ratios of $P^{32}:P^{31}$ in the solutions at the beginning and at the end of the treatment are compared, it is found that the ratios at the end of the treatment were lower than at the beginning. The difference is more significant in Treatment C than D. In the former, the ratios at the end of the treatment were about twenty-five percent lower than at the beginning, while in the latter, there was no significant difference. Since the radioactivity of both samples had been converted into that at the time the plants were harvested, theoretically the ratios of $P^{32}:P^{31}$ should be the same. The fact that they were not the same indicates that some preferential absorption or adsorption of different isotopes may have occurred. However, it has been one of the fundamental assumptions in tracer work that plants exhibit no preferential selection between the

Table II. Concentrations of P^{32} and P^{31} and Their Ratio in the Mineral Nutrient Solutions

Treatment	Unit* ratio	Replicate	Solution at beginning+			Solution at end			$P^{32}:P^{31}$ on gravel
			P^{32}	P^{31}	$P^{32}:P^{31}$	P^{32}	P^{31}	$P^{32}:P^{31}$	
	$P^{32}:P^{31}$	Pot no.	10^2ct/m/ml	ppm	10^2ct/m/rπ	10^2ct/m/ml	ppm	10^2ct/m/r	10^2ct/m/r
A	0 : 1	1	-----	10.30	-----	-----	1.15	-----	-----
		9	-----	10.30	-----	-----	1.70	-----	-----
		19	-----	11.30	-----	-----	1.95	-----	-----
B	1 : 0	3	84.28	-----	-----	3.85	0.18	21.39	0.41
		11	84.15	-----	-----	2.99	0.14	21.36	0.24
		21	86.05	-----	-----	1.88	0.22	8.55	0.46
C	1 : 1	5	116.42	10.30	11.30	10.77	1.55	6.95	0.34
		13	119.40	10.30	11.59	16.16	1.70	9.51	0.44
		23	122.29	10.40	11.76	15.13	1.55	9.76	0.38
D	1 : 20	7	120.44	217.00	0.56	79.09	154.00	0.51	0.17
		17	116.08	217.00	0.53	87.13	164.00	0.53	0.19
		25	115.21	209.00	0.55	81.57	159.00	0.51	0.15

* 1 unit of P^{32} = 1 mc. (assayed on July 2, 1953).
1 unit of P^{31} = 10 ppm.

\+ For comparison, the radioactivity of the solution samples at the beginning of the treatment was converted to that at the time the plants were harvested.

π r = microgram.

radioactive and stable isotopes and the results of this investigation,which will be shown later, verify this assumption. It might be a possibility that gravel would adsorb P^{32} and P^{31} in a higher ratio than in the solution. But the data in the last column of Table II showed the opposite effect. The extremely low ratios of P^{32}:P^{31} in the gravel are believed a result of the considerable high content of P^{31} which had been fixed by magnetite in gravel and other mechanisms of phosphorus fixation previous to the treatment, and was brought into the hydrochloric acid extract when analyzed. Therefore it is impossible to conclude that the differential adsorption of P^{32} and P^{31} had occurred on the gravel surface. Since the plants had been growing in the optimum mineral nutrient solution before the P^{32} and the different levels of P^{31} were applied, gravel may have retained some phosphorus in the solution around the surface. After the treatments were made, the irrigation might result in a dilution of the P^{32}:P^{31} ratio by the phosphorus which had remained in the gravel. This is more likely the cause, however, it is still doubtful whether the quantity of phosphorus thus retained was large enough to exert much influence on the P^{32}:P^{31} ratio in 15.2 liters of the mineral nutrient solution. According to the results obtained by Dr. J. D. Sayre and the author* in a study of daily absorption of phosphorus, potassium and sodium by the corn plant in

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* Unpublished data.

gravel cultures with an optimum mineral nutrient solution of the same composition as used here, it was found that corn plants absorbed most of the phosphorus present in the solution in the first three or four days after the solution was renewed. Therefore, it is probable that a few days after the different treatments were made, the concentration of total phosphorus in the solution was greatly reduced. This may have caused an increase in the amount of P^{32} adsorption on the walls of the container as happened in Treatment B where the amount of P^{32} introduced was the same as those introduced to the mineral nutrient solutions in Treatments C and D. But in the solution samples taken right after mixing, it was found that the radioactivity of P^{32} in the solutions of Treatment B was only 72 % as much as in Treatments C and D (see Table II). As a result of such increased adsorption of P^{32}, the ratio of $P^{32}:P^{31}$ in the solution was thus lowered. In the case of Treatment D, a large amount of P^{31} still remained in the solution at the harvest time and therefore prevented P^{32} from the increased adsorption on the wall of the reservoir, the connecting tubes in the irrigation system and perhaps also on the surface of gravel particles. This may be illustrated by the data shown in Table II. The difference of $P^{32}:P^{31}$ ratio of the mineral nutrient solutions at the beginning and the end of the treatment in C was very great while in Treatment D, the difference was negligible. This phenomenon is also shown in other experiments of this study. It is believed that the reduced $P^{32}:P^{31}$ ratio at the end of the treatment was probably due to the

increased adsorption of P^{32}. The further discussion will be made later.

P^{32}:P^{31} Ratio in Various Corn Tissues

Table III shows the P^{32}:P^{31} ratios in different tissues of the corn plant. It is apparent that the ratio was much higher in the leaves than in any of the other tissues. Also, the ratio in the upper leaves was higher than in the lower leaves, although there was not much difference in the P^{31} content of both tissues (Table IV). The ratios of P^{32}:P^{31} were nearly the same in the other parts of the plant except for the roots which had a higher ratio than either stem or ear. It seems that, the tissues with the lower metabolic activities had lower ratios which tended to reach a somewhat constant value. It is also noticed that the ratios of P^{32}:P^{31} were higher in Treatment B than in Treatment C, especially in the upper leaves and the roots. However, as shown in Table V, both the total radioactivity and the radioactivity per unit dry weight of the plant tissues grown in Treatment B were even lower than in Treatment C. The higher ratios in Treatment B might be explained by the fact that the plants of both treatments absorbed radioactive P^{32} in somewhat quantity the same as they were in the solution. Since there was only P^{32} in the mineral nutrient solution of Treatment B, therefore, plants in this treatment had a lower P^{31} in the tissues. As a result, the P^{32}:P^{31} ratios were naturally higher than in the plant tissues of Treatment C. The higher ratios of P^{32}:P^{31} in the upper leaves

Table III. P^{32}:P^{31} Ratio* in Various Corn Tissues in Experiment I.

Treatment	Unit+ ratio	Replicate	Upper leaves	Lower leaves	Stem	Ear	Roots	Plant as a whole	Solu. at beginning
B	1 : 0	# 3	5.25	3.69	2.69	2.21	4.49	2.81	-----
		11	4.74	3.25	1.96	2.04	5.05	2.61	-----
		21	4.45	2.84	2.52	2.23	4.82	2.91	-----
C	1 : 1	5	4.11	3.50	1.91	2.26	2.42	2.57	11.30
		13	3.90	2.97	2.01	2.17	2.73	2.49	11.59
		23	4.42	2.89	2.57	2.53	2.28	2.76	11.76
D	1 : 20	7	0.47	0.41	0.25	0.21	0.38	0.32	0.56
		17	0.48	0.37	0.24	0.23	0.41	0.34	0.53
		25	0.55	0.40	0.32	0.34	0.56	0.43	0.55

* All ratios except the unit ratio are in 10^2 c/m/r.
\+ 1 unit of P^{32} = 1 mc.; 1 unit of P^{31} = 10 ppm in the mineral nutrient solution.

Table IV. Average Contents of P^{32} and P^{31} in Various Corn Tissues in Experiment I

Plant tissue	Treatment							
	A		B		C		D	
	P^{32}	P^{31}	P^{32}	P^{31}	P^{32}	P^{31}	P^{32}	P^{31}
	10^2c/m/gm	r*/gm	10^2c/m/gm	r/gm	10^2c/m/gm	r/gm	10^2c/m/gm	r/gm
Leaves, upper	-----	2840	9765	2000	13104	3170	4769	9550
lower	-----	2590	6772	2070	9799	3130	3959	9970
Stem	-----	1100	1449	650	2462	1120	657	2480
Ear	-----	2590	4514	2090	6136	2650	921	3550
Roots	-----	1310	3889	810	3942	1610	2741	6280

* r = microgram; the weight of corn tissues is on oven-dry basis.

Table V. P^{32} Content of the Corn Plants in Treatments B and C

Treatment	Replicate	Dry wt.* of plant	Radioactivity of P^{32} in whole tissue	Radioactivity of P^{32} per gm. tissue	Radioactivity of P^{32} per ml. solu.	$\frac{P^{32}/gm\ tissue}{P^{32}/ml\ solu.}$
	Pot no.	gm	10^4c/m	10^4c/m	10^2c/m	
B	3	142.3	5876	41.29	84.28	48.99
	11	160.3	6639	41.42	84.15	49.22
	21	126.1	4961	39.34	86.05	45.72
	Average	142.9	5825	40.76	84.83	48.05
C	5	122.7	6655	54.24	116.42	46.58
	13	128.5	6686	52.03	119.40	43.58
	23	117.7	6620	56.24	122.29	45.99
	Average	123.0	6654	54.17	119.37	45.38
B/C	Average	-----	----	0.75	0.71	1.06

* On oven-dry basis (at 70°C).

and roots may be assumed to be related to the mechanism of the exchange reaction between these two isotopes in plants. The proposed mechanism will be discussed under the heading "General Discussion".

As for Treatment D, the ratios of $P^{32}:P^{31}$ in the corn tissues were much lower than those in the tissues of the other treatments. Since the supply of phosphorus was in great excess in the solution, only a small part was absorbed by the plant. The total uptake of P^{32} was therefore greatly reduced because of the large dilution of P^{32} by P^{31}. As a result, the ratios of $P^{32}:P^{31}$ in the plant tissues were lowered. When the values of $P^{32}:P^{31}$ ratios in the tissues of Treatments C and D are compared, it is found that, as shown in Table III, the ratios in the latter were about one eighth of those in the former instead of one twentieth. This can be easily understood since the mixture of P^{32} and P^{31} absorbed were diluted differently by the P^{31} accumulated before the treatments were made in the tissues of different treatments. The P^{31} previously accumulated is considered a part of the total P^{31} on which the calculation of the ratio of $P^{32}:P^{31}$ is based.

$P^{32}:P^{31}$ Ratio of the Isotope Mixture Accumulated in Various Corn Tissues During the Treatment Period

If the average percentage of P^{31} in various tissues of the corn plants in Treatment B is used as the percentage of P^{31} accumulated in these tissues before the treatments, we are able to calculate the amount of stable P^{31} absorbed during the treatment

period. The results are shown in Table VI. It is found that during the ten-day treatment period, the increases of P^{31} content in the plants of Treatment C and Treatment D were approximately 80 and 351 milligrams; while the amounts of P^{32} absorbed in terms of radioactivity were 6654×10^4 and 1825×10^4 counts per minute respectively. The ratio of P^{32}:P^{31} in 10^2 counts per minute per microgram was 8.23 in the former and 0.52 in the latter. These values are very close to those in the nutrient solutions at the end of the experiment. It appears, therefore, that plants do absorb the mixture of different isotopes in the same proportion that they are present in culture medium. However, it is revealed in Table VI that although the P^{32}:P^{31} ratio of the isotope mixture absorbed was the same as that in the culture solution, the ratios of the concentrations of P^{32} and P^{31} translocated to or accumulated in various tissues were apparently different. They were much higher in leaves and ear than in the stem and roots.

Summary of Experiment I

Several interesting facts have been observed from the data obtained in this experiment. They are summarized as follows:

1. The mineral nutrient solutions had a lower P^{32}:P^{31} ratio at the end than at the beginning of the treatment; this was probably due to the increased adsorption of P^{32} as P^{31} concentration decreased.

2. The proportion of P^{32} to P^{31} absorbed by the plant during

Table VI. Average Amounts and the Ratio of P^{32} and P^{31} Accumulated in the Replicated Corn Tissues During the Treatment Period in Experiment I

Tissue	Treatment	P^{31} at harvest	P^{31}* before treatment	P^{31}+ increased during treatment	Total P^{31} accumulated during treatment	Total P^{32} accumulated	$P^{32}:P^{31}$
		%	%	%	r‡	10^2c/m	10^2c/m/r
Leaves, Upper	C	0.317	0.200	0.117	10647	117858	11.07
	D	0.955	0.200	0.755	59645	37290	0.63
	C/D	-----	-----	-----	-----	-----	17.57
Lower	C	0.313	0.207	0.106	9328	87740	9.41
	D	0.997	0.207	0.790	71890	36548	0.51
	C/D	-----	-----	-----	-----	-----	18.45
Stem	C	0.112	0.064	0.048	18480	98829	5.35
	D	0.248	0.064	0.184	57224	20427	0.36
	C/D	-----	-----	-----	-----	-----	14.86
Ear	C	0.265	0.209	0.056	25256	275918	10.92
	D	0.355	0.209	0.146	59422	36958	0.62
	C/D	-----	-----	-----	-----	-----	17.61
Roots	C	0.161	0.081	0.080	17120	85554	5.00
	D	0.628	0.081	0.547	102836	51264	0.50
	C/D	-----	-----	-----	-----	-----	10.00
Whole plant	C	0.208	0.147	0.061	80831†	665377	8.23
	D	0.472	0.147	0.325	351017†	182487	0.52
	C/D	-----	-----	-----	-----	-----	15.83
Solution at end	C	-----	-----	-----	-----	-----	8.74
	D	-----	-----	-----	-----	-----	0.52
	C/D	-----	-----	-----	-----	-----	16.81

* Average percentage of the triplicate tissues in Treatment B.
\+ Differences between the average percentages of the plant tissues in Treatment B and Treatment C or D.
† Sum of average P^{31} content of all plant tissues in Treatment C or D.
‡ r = microgram.

the treatment period was the same as that in the mineral nutrient solution. But in different plant tissues, the amounts of P^{32} and P^{31} accumulated during that period did not give the same proportion.

3. Plant tissues which had accumulated a higher concentration of P^{31}, accumulated more P^{32} and this resulted in a higher ratio of P^{32}:P^{31} in the isotope mixture accumulated during the treatment period.

4. In this particular experiment, leaves and roots had a much higher final ratio of P^{32}:P^{31}.

EXPERIMENT II: CORN (1954)

In order to clarify the relationship between P^{32} and P^{31} in the plant, several additional experiments were designed in 1954. The first stage of the growth of corn was considered in this experiment. It seems that the previous accumulation of P^{31} in different tissues had an effect on the ratio of $P^{32}:P^{31}$. In this experiment, this factor was eliminated by introducing P^{32} at the very beginning with different levels of P^{31} so that the results may serve as a reference for other experiments.

$P^{32}:P^{31}$ Ratio in Various Corn Tissues

The results of the experiment are shown in Table VII. It is apparent that, despite the different contents of P^{31}, the ratios of $P^{32}:P^{31}$ in different tissues were the same except the roots in which the ratios were a little lower although not much. This fact indicates that if there is no previous accumulation of P^{31} in the plant, the propertion of P^{32} to P^{31} in the mixture translocated to different tissues will be the same no matter how much the concentration of P^{31} varies in different tissues. In other words, the previously accumulated P^{31} will have something to do with the final $P^{32}:P^{31}$ ratios, i.e. there is a certain reaction between the previously accumulated P^{31} and the prevailing P^{32} in the P^{31} and P^{32} mixture. This relationship will be discussed later. Of course, in the practical conditions of any experiment, at least some phosphorus from the seed enters in the plant. The amount is so small

Table VII. $P^{32}:P^{31}$ Ratio in Various Corn Tissues in Experiment II.

Treatment	Unit* ratio of $P^{32}:P^{31}$	Replicate	Leaves	Stem	Roots	Whole plant	Solution+
		Pot no.	10c/m/r¶	10c/m/r	10c/m/r	10c/m/r	10c/m/r
A	1 : 1	1	2.87	3.02	2.66	2.89	3.70
		6	3.10	2.87	2.53	2.94	3.58
		11	3.09	2.95	2.70	2.97	3.35
B	1 : 2	2	1.78	1.72	1.64	1.74	1.92
		7	1.64	1.69	1.66	1.67	1.84
		12	1.69	1.73	1.61	1.70	1.72
C	1 : 5	3	0.81	0.76	0.76	0.78	0.80
		8	0.72	0.80	0.71	0.75	0.75
		13	0.71	0.75	0.71	0.72	0.75
D	2 : 2	4	3.42	3.35	3.07	3.33	3.75
		9	3.37	3.32	3.07	3.30	3.66
		14	3.36	3.36	3.17	3.33	3.62
E	5 : 5	5	3.75	3.85	3.66	3.76	3.95
		10	3.70	3.68	3.57	3.62	3.81
		15	3.88	3.67	3.45	3.69	3.77

* 1 unit of P^{32} = 0.156 mc. (assayed on August 16, 1954).
1 unit of P^{31} = 20 ppm in the mineral nutrient solution.

\+ at the beginning of the treatment.

¶ r = microgram.

when compared with the amount absorbed by the plant during its growth in the mineral nutrient solution that it may be neglected.

It is important to point out that the leaves and roots did not contain P^{32} and P^{31} in as high a ratio as found in Experiment I. The similar results were found in other experiments also.

Effect of Different P^{32}:P^{31} Ratios in the Mineral Nutrient Solutions on the Ratios in Corn Tissues

Among the five treatments of this experiment, Treatment A, B and C had different P^{31} concentrations but the same amount of P^{32}. In Treatments B and D, and also Treatments C and E, the reverse was true. Before we enter into the discussion, we should point out that, according to the results obtained from Experiment I, the P^{32}:P^{31} ratio in the mineral nutrient solution at the beginning of the treatment did not serve well as an index of the P^{32}:P^{31} ratio in the plant, especially in the solutions with a low P^{31} concentration. It was the P^{32}:P^{31} ratio at the end of the treatment, which corresponded more closely to the ratio of the isotopes accumulated in the plant as a whole during the treatment period. In this and the following experiments, no solution samples were collected at the end of the treatment period because of the wide variations of the extremely low P^{31} concentrations in the triplicate solutions. For the following discussion, the P^{32}:P^{31} ratio in the plant as a whole is selected to represent the actual ratio in the nutrient solution rather than the ratio in the solution at the beginning of

the treatment. It is reasonable and quite safe to do this because in this experiment, no previous accumulation of P^{31} occurred in the plant.

If the $P^{32}:P^{31}$ ratios in each plant tissue and in the mineral nutrient solution represented by that in the whole plant in Treatment C are used as the index of 100%, and the relative percentages of $P^{32}:P^{31}$ ratios in the plant tissues of Treatments A and B are calculated and plotted against that in the mineral nutrient solution, Figure 1 is obtained. It is evident that although the concentration of P^{31} in the nutrient solutions varied widely, the ratios of $P^{32}:P^{31}$ in various plant tissues were directly proportional to the actual ratio in the nutrient solution. The slight deviation of the relative percentages in the figure probably resulted from the experimental error. The relations between the ratios in plant tissues and in the mineral nutrient solution of Treatments B and D, and Treatments C and E are shown in Figures 2 and 3 respectively. These two figures also show clearly that the $P^{32}:P^{31}$ ratio in the plant tissues follows the ratio directly in the culture medium.

The relative percentages of the $P^{32}:P^{31}$ ratios in the mineral nutrient solutions at the beginning of the treatment are also plotted in the figures. In Figure 1, The solution in Treatment A had a much higher relative percentage than it should have, while in Figures 2 and 3, no such phenomenon is observed in the solutions of Treatments Band D, and also in those of Treatments C and E, where the solutions

Figure 1. Effect of Different Ratios of P^{32};P^{31} in the Mineral Nutrient Solutions on the P^{32};P^{31} Ratio in the Corn Tissues in Treatments A, B and C

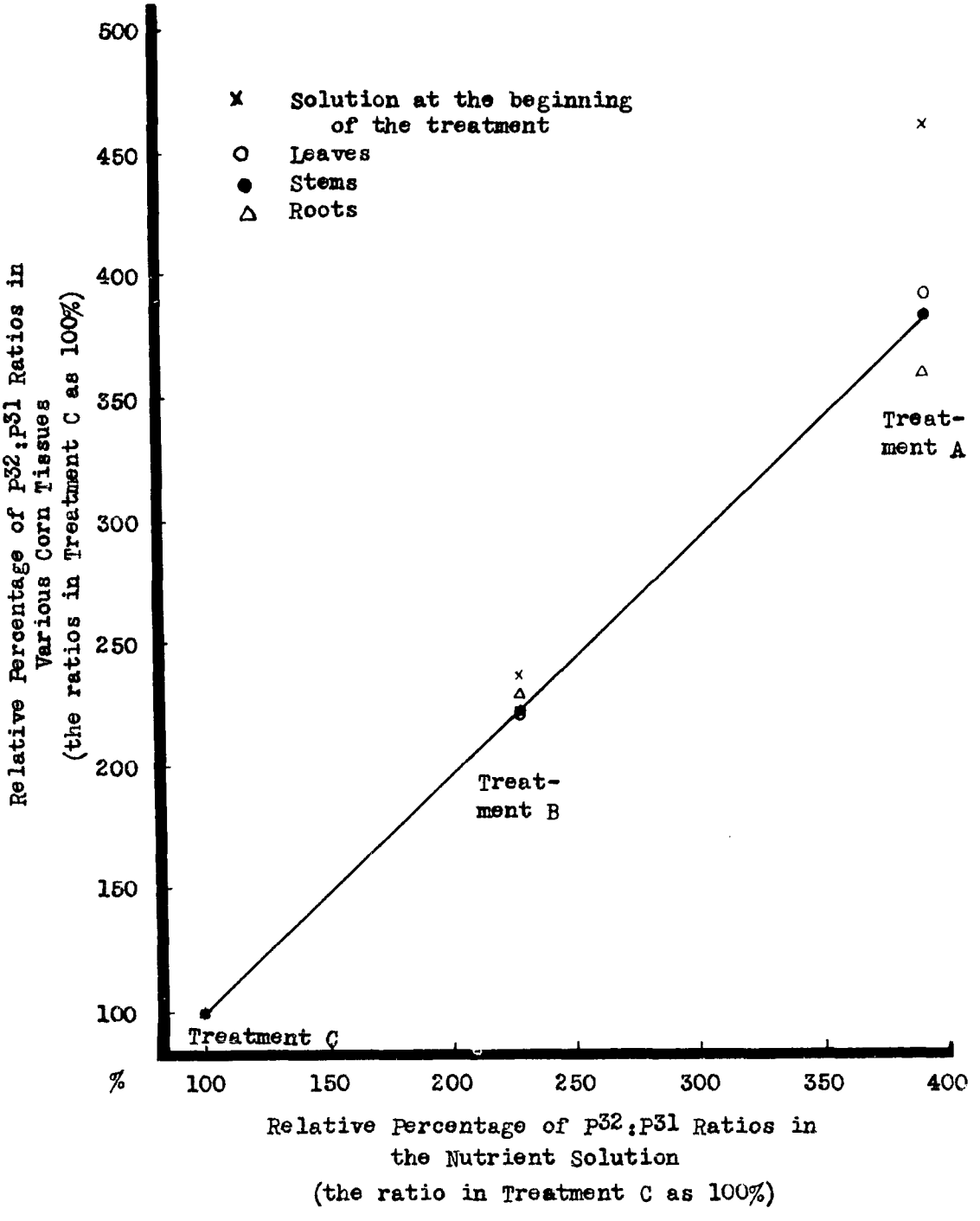


Figure 2. Effect of Different Ratios of $P^{32}:P^{31}$ in the Mineral Nutrient Solutions on the $P^{32}:P^{31}$ Ratio in the Corn Tissues in Treatments B and D

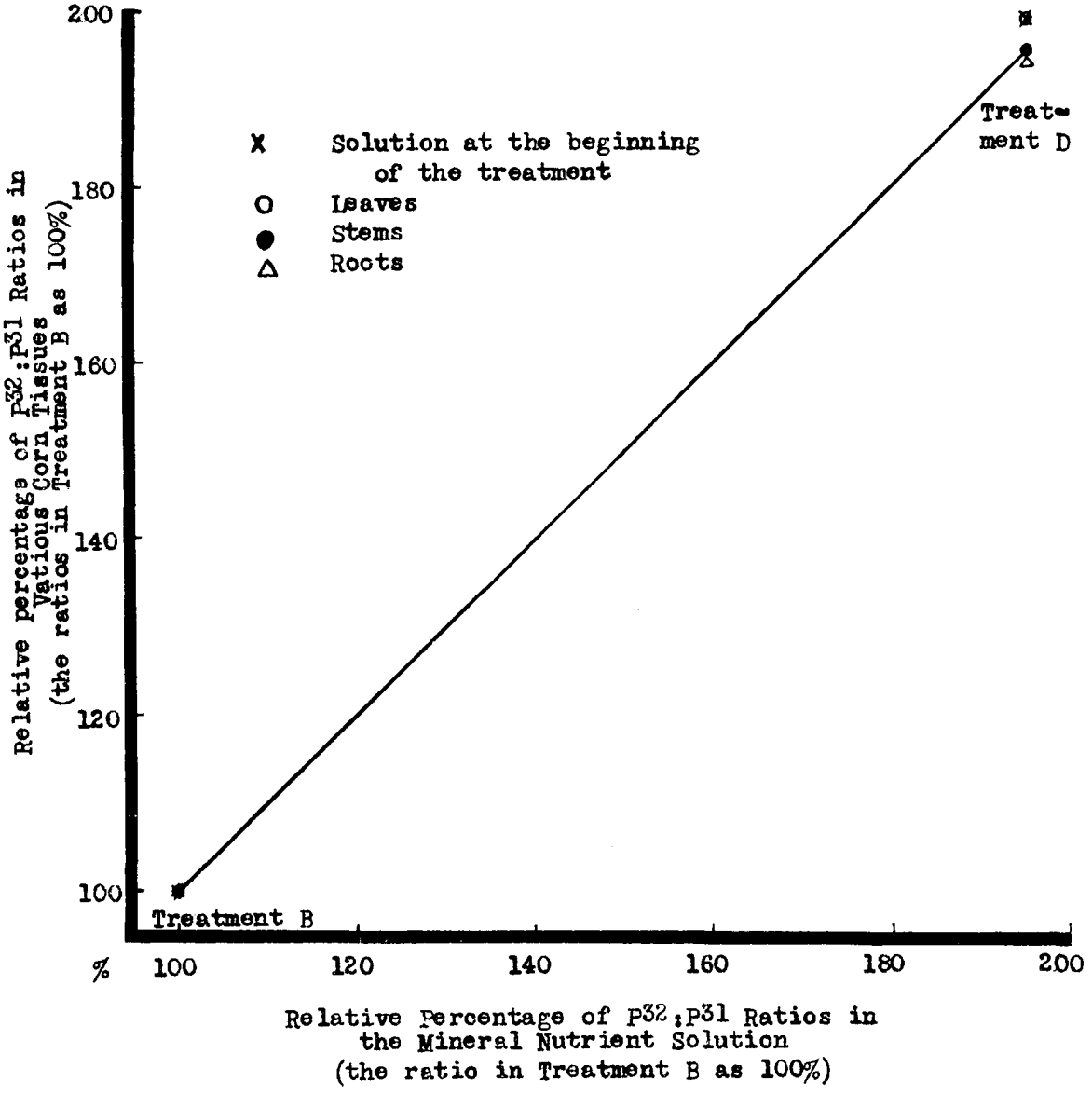
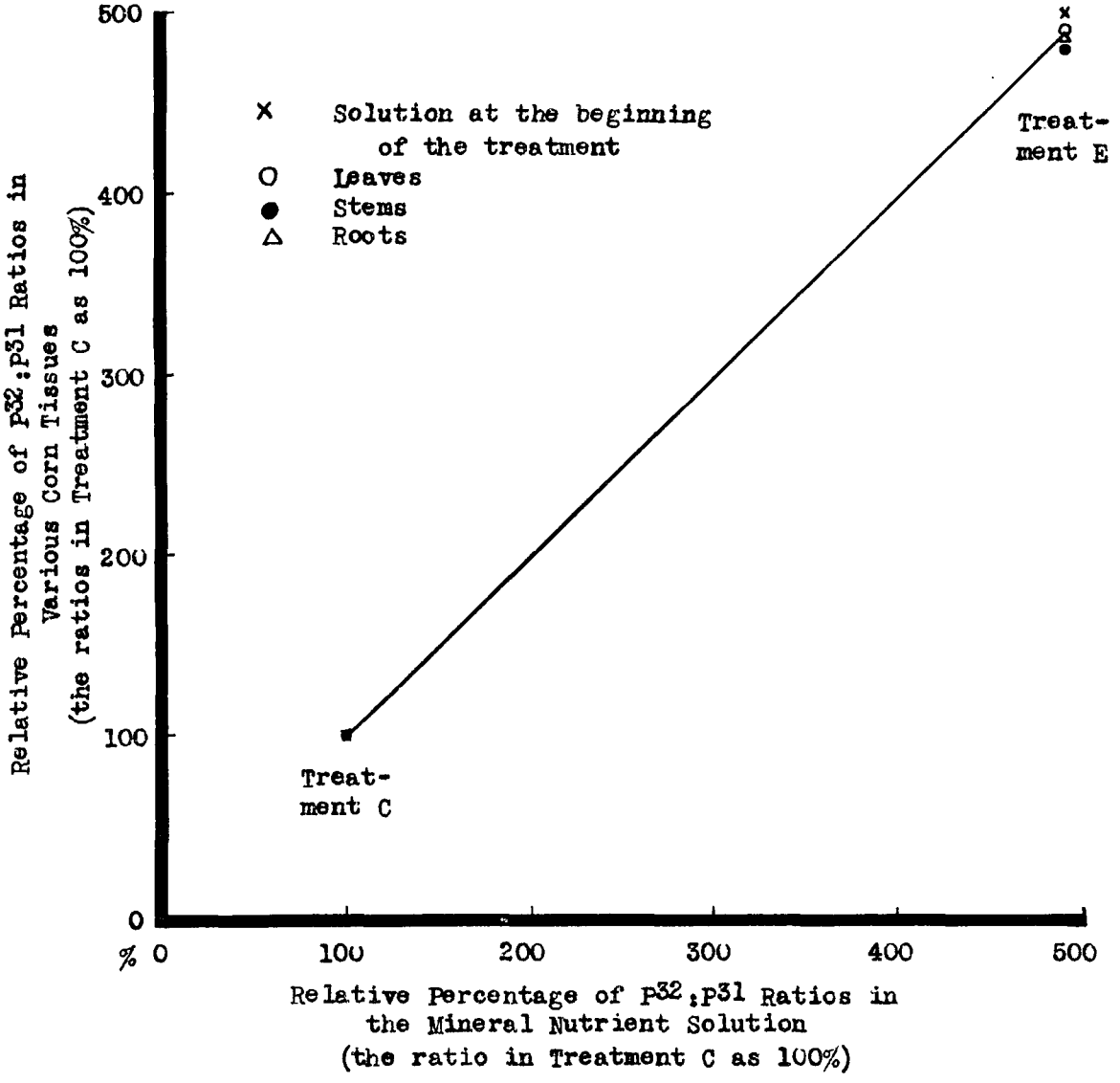


Figure 3. Effect of Different Ratios of P^{32}:P^{31} in the Mineral Nutrient Solutions on the P^{32}:P^{31} Ratio in the Corn Tissues in Treatments C and E

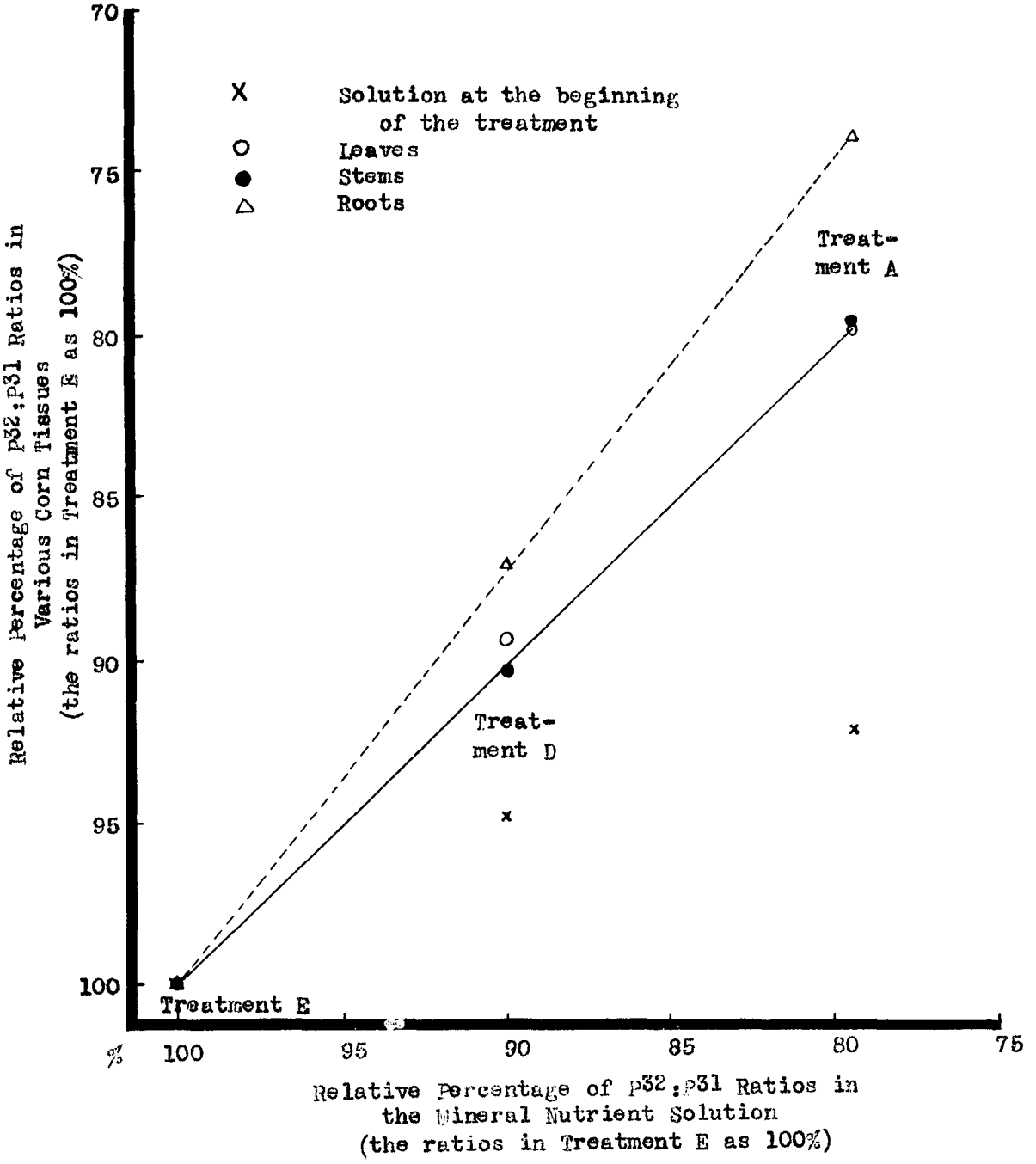


had the same concentrations of P^{31}. The reduced ratio of $P^{32}:P^{31}$ in the mineral nutrient solution at the end of the treatment, represented by the ratio in the plant as a whole here, can not be considered as a result of the experimental error but a result of the adsorption of the relative larger proportion of P^{32} in relation to P^{31} when the concentration of the latter becomes very low. The relation between the adsorption of P^{32} and the concentration of P^{31} in the solution is not necessary inversely proportional although no attempt was made to clarify this point. The adsorption of a larger proportion of P^{32} under low P^{31} concentration will be discussed in detail later.

Effect of Same $P^{32}:P^{31}$ Ratio But Different Concentrations of P^{32} and P^{31} in the Mineral Nutrient Solution on the Ratio in Corn Tissues

By using the similar procedure as described in the previous section, the relationship of Treatments A, D and E is shown in Figure 4. It shows that the ratios of $P^{32}:P^{31}$ in both leaf and stem tissues seem to be directly proportional to that of the whole plant or of the mineral nutrient solution at the end of the experiment. Since these treatments had the same $P^{32}:P^{31}$ ratio but different concentrations of both P^{32} and P^{31} in the mineral nutrient solution, the much higher relative percentages of the $P^{32}:P^{31}$ ratio in the solution at the beginning of the experiment in Treatments D and A mean that the lowering of $P^{32}:P^{31}$ ratio at the end of the experiment is probably due to the adsorption of a larger portion of P^{32} in

Figure 4. Effect of Same Ratio of P^{32}:P31 But Different Concentrations of P^{32} And P31 in the Mineral Nutrient Solutions on the P^{32}:P31 Ratio in the Corn Tissues in Treatments A, D and E



relation to P^{31} when the P^{31} concentration decreases as a result of plant absorption.

It is also noted that the relative percentages of P^{32}:P^{31} ratio of Treatments E, D and A do not have a linear relationship in the figure. In Treatment A where there was a lowest P^{31} concentration, the relative percentage of the ratio in the solution at the beginning of the treatment is much higher. In other words, the lowering of P^{32}:P^{31} ratio at the end of the treatment was much greater than that in Treatment D when that of Treatment E is used as an index. This might be used as an illustration to show the possibility that a larger portion of P^{32} would adsorb on the wall of the container and in the irrigation system when the P^{31} concentration is lowered.

There is a marked lowering of the relative percentage of P^{32}:P^{31} ratios in root tissues in Figure 4. However, it is found that this lowering has a linear relationship to the ratio of the mineral nutrient solution at the end of the treatment. Therefore, from the relations observed, it may be concluded that little effect on P^{32}:P^{31} ratio in the plant tissues is caused by the different concentrations of P^{32} and P^{31} in the mineral nutrient solution if their ratio is kept constant.

Summary of Experiment II

No previous accumulation of P^{31} occurred in the plants in this experiment except a small amount which was originally present in the seed. As soon as the corn seedlings emerged from the gravel,

different levels of P^{32} and P^{31} were introduced to the mineral nutrient solutions. The results are summarized as follows:

1. All corn tissues in the same treatment had almost the same ratio of P^{32}:P^{31}, although the ratio was a little lower in the root tissues.

2. The P^{32}:P^{31} ratios in corn tissues were affected proportionally by the ratios in the mineral nutrient solutions.

3. In the treatments where the ratios of P^{32}:P^{31} were the same, the concentrations of P^{32} and P^{31} in the mineral nutrient solutions had little or no effect on the ratio of P^{32}:P^{31} in the plant tissues.

4. When the concentrations of P^{31} were different in the mineral nutrient solutions , the lower the concentration of P^{31} remained in the solution, the more was the loss of P^{32} probably due to the adsorption and thus resulted a lower P^{32}:P^{31} ratio. No difference in loss of P^{32} in term of the P^{32}:P^{31} ratio was observed in the solutions with a same concentration of P^{31} but different amounts of P^{32}.

EXPERIMENT III: CORN (1954)

P^{32}:P^{31} Ratio in Various Corn Tissues

This experiment was similar to the one conducted in the summer of 1953. The corn plants were grown to the same stage but the number of treatments and the levels of both P^{32} and P^{31} were different. The data in Table VIII show that the root tissues had the highest ratio of P^{32}:P^{31} while the lower leaves had the lowest. There was not much difference among the other tissues. Although there were differences in the P^{32}:P^{31} ratio in different plant tissues, they were not very great.

In Treatments A and B, the same amount of P^{32} was introduced to the mineral nutrient solution. The only difference was that in the former no P^{31} was applied while the latter had a concentration of 10 ppm of P^{31} in the nutrient solution. The final ratios of P^{32}:P^{31} in the plant tissues were higher in Treatment A than Treatment B. This phenomenon has been observed and discussed in Experiment I, however, in this experiment, the difference was much greater.

As in Experiment II, the treatments are also used to study the effect of the different P^{32}:P^{31} ratios and that of the same ratio but with different concentrations of P^{32} and P^{31} in the solutions on the ratios in the plant tissues. As a result of the dilution of the ratio by P^{31} previously accumulated in this experiment, the P^{32}:P^{31} ratios in various corn tissues were not the same as in the mineral nutrient solutions of various treatments. However, the data

Table VIII. $P^{32}:P^{31}$ Ratio in Various Corn Tissues in Experiment III

Treatment	Unit* ratio $P^{32}:P^{31}$	Replicate	Upper leaves	Lower leaves	Tassel	Stem	Roots	Whole plant	Solution at beginning
		Pot no.	10^2c/m/r+	10^2c/m/r	10^2c/m/r	10^2c/m/r	10^2c/m/r	10^2c/m/r	10^2c/m/r
A	1 : 0	1	0.83	0.63	0.92	0.86	0.90	0.85	----
		7	0.91	0.74	0.99	0.99	0.92	0.92	----
		13	0.83	0.71	0.82	0.82	0.92	0.84	----
B	1 : 1	2	0.57	0.55	0.52	0.57	0.69	0.58	2.79
		8	0.58	0.51	0.63	0.68	0.69	0.64	2.72
		14	0.61	0.53	0.62	0.63	0.68	0.62	2.69
C	1 : 5	3	0.31	0.29	0.28	0.34	0.35	0.33	0.64
		9	0.27	0.27	0.26	0.31	0.40	0.31	0.63
		15	0.32	0.26	0.27	0.33	0.40	0.33	0.63
D	1 : 10	4	0.15	0.13	0.14	0.16	0.23	0.18	0.32
		10	0.19	0.14	0.14	0.16	0.21	0.18	0.31
		16	0.14	0.12	0.13	0.20	0.25	0.19	0.30
E	2 : 2	5	0.94	0.80	0.89	0.95	1.03	0.94	2.96
		11	0.87	0.77	0.76	0.87	1.03	0.87	3.07
		17	0.85	0.75	0.80	0.86	1.01	0.86	2.87
F	5 : 5	6	1.29	1.29	1.16	1.41	1.84	1.44	3.16
		12	1.34	1.24	1.16	1.38	1.78	1.41	2.80
		18	1.47	1.18	1.37	1.45	1.61	1.47	3.10

* 1 unit of P^{32} = 0.27 mc. (assayed on June 7, 1954).
1 unit of P^{31} = 10 ppm.

\+ r = microgram.

show some close relationships. Figure 5 illustrates a linear relationship occurred between the P^{32}:P^{31} ratios in the tissues and in the solutions of Treatments B, C and D where the same amount of P^{32} but different concentration of P^{31} were first introduced.

P^{32}:P^{31} Ratio of the Isotope Mixture Accumulated in Various Corn Tissues During the Treatment Period

Since the plants in Treatment A did not receive any P^{31} during the treatment period, the P^{31} content of various corn tissues may be regarded as the content before the different treatments were introduced. The increase of P^{31} during the treatment period in various plant tissues may, therefore, be calculated by subtracting the percentage content of P^{31} of the plant tissues in different treatments by that of the corresponding tissues in Treatment A. The results are presented in Table IX. The data do not show a uniform increase in different tissues. Generally speaking, roots and stem seemed to accumulate more phosphorus during the treatment period than the other tissues. The upper leaves accumulated much less than the lower ones. In different treatments, these differences did not have a proportional relationship. If we divide the amount of P^{32} by the amount of P^{31} accumulated during the treatment period, we obtain the results shown in Table X. The ratios of P^{32}:P^{31} accumulated in various tissues were quite different. However, in terms of the plant as a whole, the P^{32}:P^{31} ratio appeared to be the same as that in the mineral nutrient solution. This again confirms the assumption that plants absorb the isotopes in a same ratio as in the culture medium.

Figure 5. Relation Between the $P^{32}:P^{31}$ Ratios in the Corn Tissues and in the Mineral Nutrient Solution

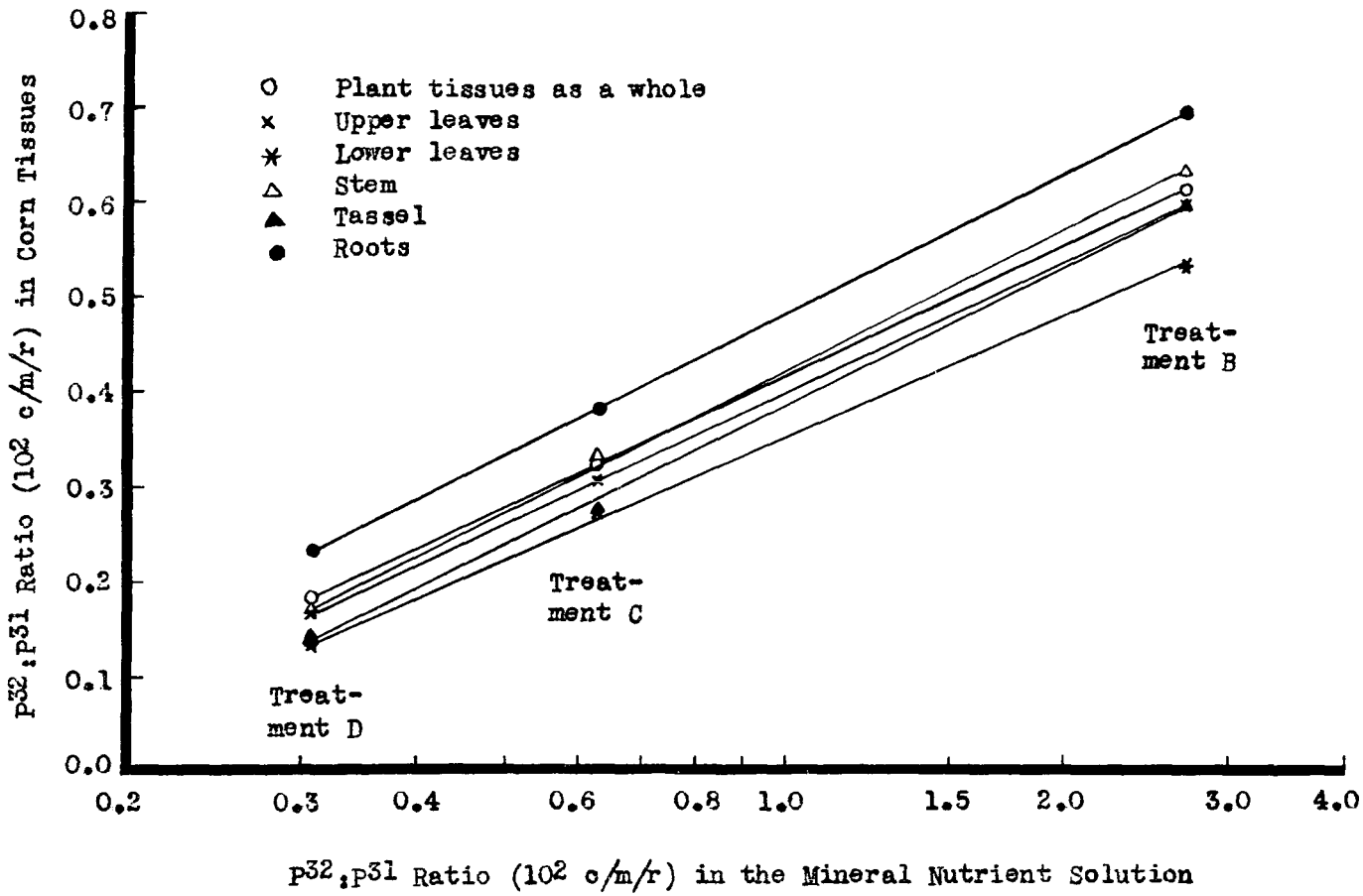


Table IX. Increase* of P^{31} in Corn Tissues During the Treatment Period in Experiment III

Treatment	Unit* ratio of P^{32} : P^{31}	Upper leaves	Lower leaves	Tassel	Stem	Roots	Plant as a whole
		%	%	%	%	%	%
A	1 : 0	-----	-----	-----	-----	-----	-----
B	1 : 1	0.027	0.040	0.041	0.103	0.080	0.073
C	1 : 5	0.157	0.215	0.130	0.335	0.288	0.265
D	1 : 10	0.122	0.184	0.091	0.203	0.546	0.239
E	2 : 2	0.067	0.093	0.074	0.178	0.111	0.127
F	5 : 5	0.120	0.172	0.106	0.252	0.235	0.202

* Expressed as percentage of the dry weight of plant tissue or the difference between the percentage contents of P^{31} in tissues in various treatments and Treatment A.

* 1 unit of P^{32} = 0.27 mc. (assayed on June 7, 1954); 1 unit of P^{31} = 10 ppm.

Table X. Average Ratio of P^{32}:P^{31} of the Isotope Mixture Accumulated in Various Corn Tissues During the Treatment Period in Experiment III

Treatment	Unit* ratio	Upper leaves	Lower leaves	Tassel	Stem	Roots	Plant as a whole	Solution at beginning
	P^{32}:P^{31}	10^2c/m/r+	10^2c/m/r	10^2c/m/r	10^2c/m/r	10^2c/m/r	10^2c/m/r	10^2c/m/r
A	1 : 0	----	----	----	----	----	----	----
B	1 : 1	7.02	3.70	5.88	1.55	2.30	2.40	2.73
C	1 : 5	0.86	0.59	1.06	0.49	0.63	0.59	0.63
D	1 : 10	0.55	0.30	0.71	0.32	0.31	0.34	0.31
E	2 : 2	4.80	2.77	4.86	1.75	2.71	2.41	2.97
F	5 : 5	4.74	2.95	5.53	2.38	3.13	3.00	3.02

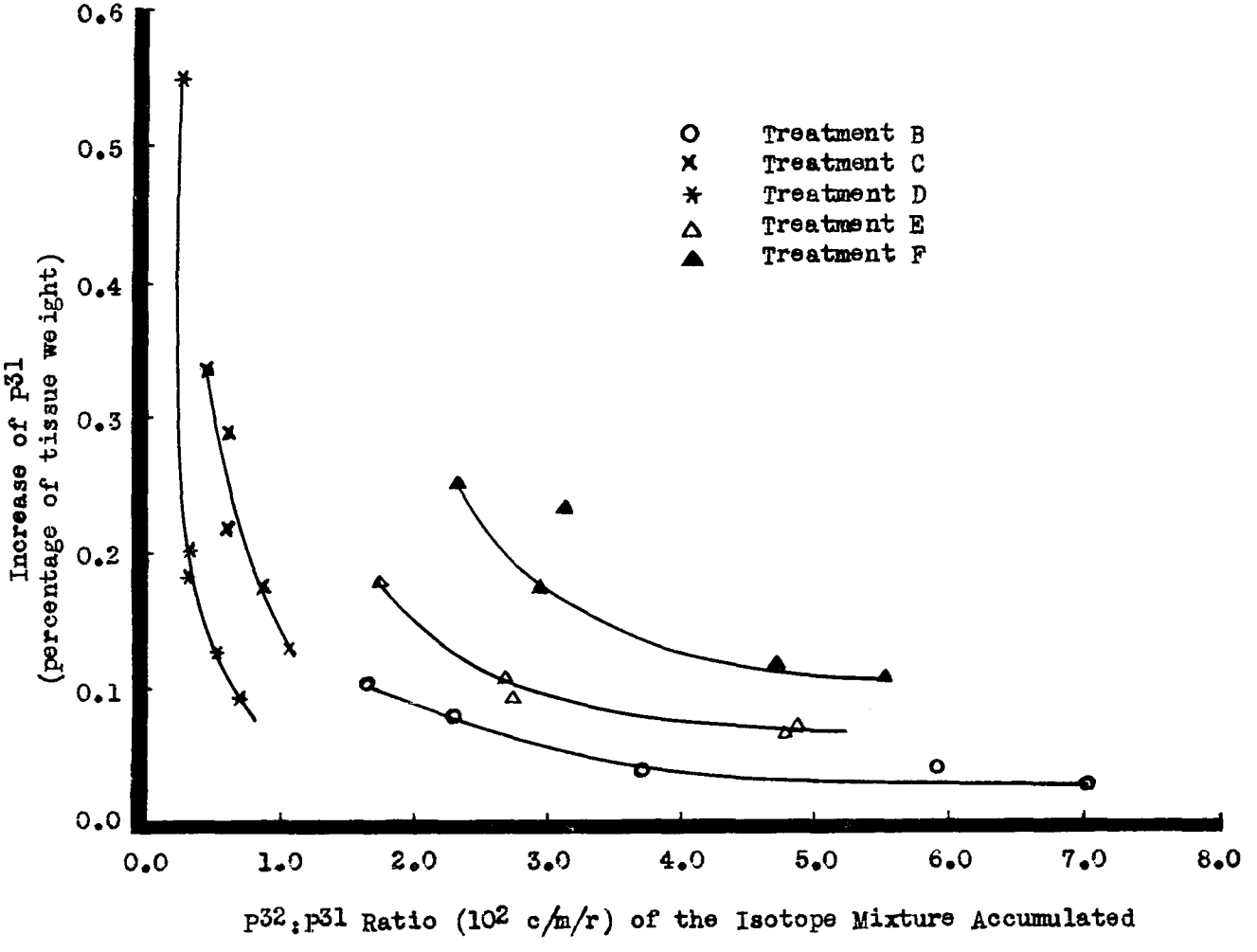
* See Table IX.

+ r = microgram.

If we examine the data presented in Tables IX and X closely, it is evident that some relationship exists between the increase of P^{31} and the $P^{32}:P^{31}$ ratio of the isotope mixture accumulated in various tissues during the treatment period as shown in Figure 6. The less the increase of P^{31}, the higher will be the $P^{32}:P^{31}$ ratio in the absorbed isotope mixture accumulated there. The relationship is not linear and this is apparently due to the effect of the P^{31} previously accumulated.

It has been stated that the isotope mixture of P^{32} and P^{31} accumulates in different tissues with different $P^{32}:P^{31}$ ratios which are influenced by the amounts of P^{31} previously accumulated. The higher the concentration of P^{31} previously accumulated, the higher will be the ratio of $P^{32}:P^{31}$ in the isotope mixture which will accumulate in that plant tissue. Eventrally, the $P^{32}:P^{31}$ ratio in all plant tissues assumes a more or less constant value. However, according to the results shown in Tables IX and X and Figure 6, another statement may be made. The lower the concentration of P^{31} accumulated during the treatment period in a certain tissue, the larger will be the amount of P^{32} which will accumulate in that tissue. It is known that the capacities of various tissues for phosphorus accumulation are different and limited, therefore, plant tissues with the same amounts of previously accumulated P^{31} but with different capacities will not necessarily accumulate the same amounts of P^{32} and P^{31} mixture during the treatment period. The

Figure 6. Relation Between the Increase of P^{31} and the $P^{32}:P^{31}$ Ratio of the Isotope Mixture Accumulated in the Corn Tissues During the Treatment Period



amount of the isotope mixture accumulated may depend on the different degrees of deficiency with respect to the capacity for P^{31} accumulation. Therefore, the more the isotope mixture or the higher the concentration of P^{31} accumulated during the treatment period in a certain plant tissue, the less will be the extra amount of P^{32} which is required by the previously accumulated P^{31} for the exchange in that tissue in order for a relatively constant $P^{32}:P^{31}$ ratio to result throughout the plant. However, the amount of P^{31} previously accumulated also influences the amounts of P^{31} accumulated during the treatment period. In other words, the amount of P^{31} accumulated before the treatment and the amount of P^{31} during the treatment are both factors which determine the ratio of $P^{32}:P^{31}$ of the isotope mixture accumulated in different tissues.

Summary of Experiment III

The results of this experiment may be summarized as follows:

1. No extremely high $P^{32}:P^{31}$ ratio was found in the leaves as in Experiment I.

2. There were some differences in the ratio of $P^{32}:P^{31}$ among various corn tissues, but the differences were not great.

3. The final ratios of $P^{32}:P^{31}$ in plant tissues formed a linear relationship with that in the mineral nutrient solution.

4. When there is previous accumulation of P^{31} in the plant tissues, the ratio of $P^{32}:P^{31}$ of the isotope mixture accumulated during the treatment period varies depending on both the amounts of P^{31} previously accumulated and accumulated during the treatment.

EXPERIMENT IV: TOMATO (1954)

P^{32}:P^{31} Ratio in Various Tomato Tissues

Tomato plants were used in this experiment. The ratios of P^{32}:P^{31} in various tomato tissues excluding roots are shown in Table XI. There was some difference in the ratios among those tissues. However, the difference was even smaller than the variations among the replicates in the same treatment. The variations may be very wide as seen in Treatment A, in which the plant tissues from Pot no. 1 had P^{32}:P^{31} ratios about fifty percent higher than those in Pot no. 7. In Pot no. 3, the ratios of P^{32}:P^{31} in the tissues were twenty to thirty percent lower than those in Pot no. 9. In other treatments, considerable variations were also observed although the percentage differences were not as great. Since the ratio of the isotopes after introduction to the mineral nutrient solutions in the same treatment did not vary much at first, the large variations must have occurred later on. Perhaps different amounts of P^{32} adsorption took place in the nutrient solutions of the same treatment, especially the treatments with low phosphorus concentrations. The different amounts of P^{31} absorption by the different plants in the same treatment could also produce the same effect in the replicate solutions. These might have resulted the increase or decrease of the P^{32}:P^{31} ratios in the mineral nutrient solutions. The increase or decrease of P^{32}:P^{31} ratio, in turn, affects the amounts and the ratio of P^{32} and P^{31} in the isotope mixture absorbed by the plant. The P^{32}:P^{31}

Table XI. $P^{32}:P^{31}$ Ratio in Various Tomato Tissues in Experiment IV

Treatment	Unit* ratio of $P^{32}:P^{31}$	Replicate	Leaves	Stem	Fruit	Top as a whole	Solution
		Pot no.	10c/m/r+	10c/m/r	10c/m/r	10c/m/r	10c/m/r
A	1 : 0	1	1.35	1.42	1.47	1.41	----
		7	0.97	0.79	0.98	0.88	----
		13	1.15	0.82	1.24	1.02	----
B	1 : 1	2	0.86	0.73	0.80	0.79	2.23
		8	0.86	0.68	1.09	0.79	2.04
		14	0.89	0.85	0.91	0.87	1.98
C	1 : 2	3	0.44	0.43	0.44	0.43	1.17
		9	0.60	0.50	0.60	0.55	1.15
		15	0.54	0.48	0.47	0.51	1.15
D	1 : 4	4	0.29	0.26	0.29	0.27	0.58
		10	0.38	0.24	0.24	0.29	0.63
		16	0.36	0.20	0.30	0.27	0.54
E	2 : 2	5	0.97	0.81	1.09	0.89	2.21
		11	1.13	0.88	0.95	0.99	2.29
		17	0.94	0.75	0.89	0.83	2.09
F	4 : 4	6	1.35	1.03	1.07	1.16	2.16
		12	1.07	1.05	1.09	1.06	2.21
		18	1.10	0.81	0.95	0.95	2.09

* 1 unit of P^{32} = 0.256 mc. (assayed on July 22, 1954).
1 unit of P^{31} = a concentration of 62 ppm.

\+ r = microgram.

ratios in various plant tissues are therefore influenced.

Since the difference of P^{32}:P^{31} ratios in various tissues were much less variable than those among the replicates, it seems, according to the results obtained (Table XI), that all plant tissues had a tendency to reach a somewhat equal value of P^{32}:P^{31} ratio as has been shown in Experiments II and III. However, when we examine the data, the ratio in the stem was a little lower than that in the leaves and fruits. It may still be true as assumed in Experiment I, that the tissues with high metabolic activities may have a higher P^{32}:P^{31} ratio.

P^{32}:P^{31} Ratio of the Isotope Mixture Accumulated in Various Tomato Tissues During the Treatment Period

The increase of P^{31} concentration and the average ratio of P^{32}:P^{31} in the isotope mixture accumulated in different tomato tissues during the treatment period have also been calculated. The results are shown in Tables XII and XIII. Leaves accumulated more P^{31} than any other tissues. The fruits only had an increase of one third as much as the stem. When we examine the data presented in Table XIII, it is very interesting to note that in each treatment, the P^{32}:P^{31} ratio in the isotope mixture accumulated in the fruits during the treatment period was three times that in the stem. The ratio in the isotope mixture accumulated in stem tissues was also higher than that in leaves where the P^{31} increase was greater. It confirms that, as discussed in Experiment III, when there is previous accumulation

Table XII. Increase* of P^{31} in Tomato Tissues During the Treatment Period in Experiment IV

Treatment	Unit*ratio of P^{32}:P^{31}	Leaves	Stem	Fruits	Top as a whole
		%	%	%	%
A	1 : 0	-----	-----	-----	-----
B	1 : 1	0.277	0.235	0.083	0.231
C	1 : 2	0.312	0.273	0.083	0.258
D	1 : 4	0.298	0.257	0.093	0.248
E	2 : 2	0.292	0.210	0.083	0.222
F	4 : 4	0.387	0.285	0.070	0.287

* Expressed as percentage of the dry weight of plant tissue or the difference between the percentage contents of P^{31} in tissues in various treatments and in Treatment A.
* 1 unit of P^{32} = 0.256 mc. (assayed on July 22, 1954).
1 unit of P^{31} = a concentration of 62 ppm.

Table XIII. Average Ratio of P^{32}:P^{31} of the Isotope Mixture Accumulated in Various Tomato Tissues During the Treatment Period in Experiment IV

Treatment	Unit*ratio of P^{32}:P^{31}	Leaves	Stem	Fruits	Top as a whole	Solution at beginning
		10c/m/r+	10c/m/r	10c/m/r	10c/m/r	10c/m/r
A	1 : 0	----	----	----	----	----
B	1 : 1	2.30	2.37	7.58	2.57	2.08
C	1 : 2	1.30	1.33	4.07	1.45	1.15
D	1 : 4	0.87	0.69	2.04	0.84	0.58
E	2 : 2	2.59	2.74	7.98	2.92	2.20
F	4 : 4	2.53	2.67	9.81	2.88	2.15

* 1 unit of P^{32} = 0.256 mc. (assayed on July 22, 1954).
1 unit of P^{31} = a concentration of 62 ppm.
+ r = microgram.

of P^{31}, the less the P^{31} concentration increased in the plant tissue during the treatment period, the higher will be the P^{32}:P^{31} ratio in the isotope mixture that will accumulate in that tissue.

Summary of Experiment IV

The results of this experiment may be summarized as follows:

1. A wide variation of P^{32}:P^{31} ratios in the plant tissues of the replicates of the same treatment may occur. It may be due to the increase or decrease of P^{32}:P^{31} ratio in the mineral nutrient solutions, affected by the amounts of P^{31} absorption and P^{32} adsorption.

2. Stem tissues had a lower P^{32}:P^{31} ratio than leaves and fruits. It seems that the tissues with a high metabolic activities will have a higher ratio. However, all tissues have a tendency to reach a more or less equal value.

3. During the treatment period, the less the amount of P^{31} increased in a tissue, the higher the P^{32}:P^{31} ratio was found in the isotope mixture which accumulated in that tissue during the treatment period.

EXPERIMENT V: SOYBEAN (1954)

P^{32}:P^{31} Ratio in Various Soybean Tissues

Soybean plants in sand cultures were used in this experiment. The results are shown in Table XIV. It is noticed that, like Experiment IV, there were wide variations in P^{32}:P^{31} ratios among the plants in the replicate pots especially in Treatment A. The variation in P^{32}:P^{31} ratio in all tissues of the plant was about the same when compared with the corresponding tissues from the plants in other pots of the same treatment. This result may serve as an evidence for what has been discussed in Experiment IV that the difference in P^{32}:P^{31} ratio among the plants in the replicate pots was a result of the change of the P^{32}:P^{31} ratio in the corresponding mineral nutrient solution. This change may vary to a different degree when affected by the different rates of P^{31} absorption and P^{32} adsorption.

There was a great difference in P^{32}:P^{31} ratios between various plant tissues in Treatments A and B, in which none and a concentration of 45 ppm of P^{31} were added to the phosphorus-free mineral nutrient solutions respectively. The explanation for the difference has been suggested. In this experiment, however, the ratios of P^{32}:P^{31} in Treatment A were much lower than in Treatment B, a fact just opposite to that found in other experiments. This can be explained by the fact that the concentration of P^{32} in the solutions of Treatment A was much lower than that of Treatment B (see Appendices). This difference is undoubtedly due to the adsorpeion of P^{32}

Table XIV. P^{32}:P^{31} Ratio in Various Soybean Tissues in Experiment V

Treatment	Unit* ratio of P^{32}:P^{31}	Replicate	Leaves	Stem	Pods	Top as a whole	Solution
		Pot no.	10c/m/r+	10c/m/r	10c/m/r	10c/m/r	10c/m/r
A	1 : 0	1	0.31	0.29	0.44	0.35	----
		7	0.34	0.37	0.54	0.41	----
		13	0.52	0.79	0.80	0.69	----
B	1 : 1	2	0.79	0.71	0.94	0.81	3.77
		8	0.74	0.74	1.02	0.82	3.73
		14	0.72	0.77	1.04	0.84	3.81
C	1 : 2	3	0.61	0.62	0.76	0.65	1.93
		9	0.49	0.46	0.55	0.49	1.90
		15	0.63	0.65	0.75	0.67	1.87
D	1 : 5	4	0.29	0.26	0.31	0.29	0.78
		10	0.34	0.37	0.38	0.36	0.78
		16	0.32	0.32	0.40	0.34	0.83
E	2 : 2	5	1.33	1.13	1.37	1.28	4.11
		11	1.17	1.03	1.41	1.19	3.67
		17	1.36	1.43	1.61	1.44	3.96
F	5 : 5	6	1.56	1.56	1.62	1.57	4.05
		12	1.75	1.71	1.84	1.76	4.10
		18	1.85	1.99	2.15	1.88	4.05

* 1 unit of P^{32} = 0.156 mc. (assayed on August 16, 1954).
1 unit of P^{31} = a concentration of 45 ppm.

\+ r = microgram.

in Treatment A.

Among the various tissues, the pods had a higher ratio of P^{32}:P^{31} than leaves and stem, while the latter two had about the same ratio. This also indicates that a higher ratio of P^{32}:P^{31} may exist in the tissues with high metabolic activities.

P^{32}:P^{31} Ratio of the Isotope Mixture Accumulated in Various Soybean Tissues During the Treatment Period

During the treatment period, the amounts of P^{32} and P^{31} absorbed by the plants, and their amounts and ratio accumulated in different tissues excluding the roots are shown in Table XV. It is apparent that the leaf tissue accumulated much more P^{31} than any other tissues in the above ground portion of the soybean plants. The stem accumulated much less than the leaves and the pods the least. In the case of P^{32}, about the same amount of the isotope was found in pods as in the stem, however, the amount was far less than that accumulated in the leaves. As far as the ratio of P^{32}:P^{31} is concerned, the leaves, despite having the largest amounts of both P^{32} and P^{31} accumulation, had the lowest P^{32}:P^{31} ratio, while the pods had the highest. From the data of Table XV, it is clearly shown that the ratio of P^{32}:P^{31} of the isotope mixture accumulated in each plant tissue of the same treatment is inversely related to the increase in the P^{31} concentration. This has been discussed both in Experiments III and IV.

Table XV. Average Amounts and the Ratio of P^{32} and P^{31} Accumulated in the Replicated Soybean Tissues During the Treatment Period in Experiment V

Treatment	Unit*ratio of P^{32}:P^{31}	Isotope+	Leaves	Stem	Pods	Top as a whole	Solution
A	1 : 0	P^{32}	3380	3160	3773	3440	87.5
		P^{31}	----	----	----	----	----
		P^{32}:P^{31}	----	----	----	----	----
B	1 : 1	P^{32}	10190	6023	6930	7527	161.4
		P^{31}	5220	1730	650	2250	42.8
		P^{32}:P^{31}	1.95	3.48	10.66	3.35	3.77
C	1 : 2	P^{32}	10440	5233	5140	6660	169.3
		P^{31}	9530	2600	1190	4020	89.2
		P^{32}:P^{31}	1.10	2.01	4.32	1.66	1.90
D	1 : 5	P^{32}	8270	3347	3157	4653	166.0
		P^{31}	17550	4070	2320	7160	207.7
		P^{32}:P^{31}	0.47	0.82	1.36	0.65	0.80
E	2 : 2	P^{32}	23940	12623	11650	13850	343.7
		P^{31}	10120	4070	1690	4990	87.8
		P^{32}:P^{31}	2.37	3.10	6.89	2.78	3.91
F	5 : 5	P^{32}	50940	17530	16633	26540	842.5
		P^{31}	21080	3730	2650	8200	207.3
		P^{32}:P^{31}	2.42	4.70	6.28	3.24	4.07

* 1 unit of P^{32} = 0.156 mc.; 1 unit of P^{31} = 45 ppm.

\+ P^{32} in 10 counts per minute per gram oven-dry plant tissue or in 10 counts per milliliter solution.

P^{31} in microgram (r) per gram plant tissue or in microgram per milliliter solution.

P^{32}:P^{31} in 10 counts per minute per microgram.

Summary of Experiment V

From the data of this experiment, similar results as found in Experiments III and IV have been obtained. They are briefly stated as follows:

1. A higher ratio of P^{32}:P^{31} was observed in the pods. The leaf and stem tissues had nearly the same P^{32}:P^{31} ratio although their P^{31} contents were different.

2. During the treatment period, the ratios of P^{32}:P^{31} of the isotope mixture accumulated in different plant tissues were different. There was an inverse relationship between the P^{32}:P^{31} ratio and the amount of P^{31} accumulated.

GENERAL DISCUSSION

The most interesting result of this study is that no matter how different the percentage of P^{31} accumulated in different plant tissues before or after treatment, the ultimate ratios of P^{32}:P^{31} were almost the same. This indicates that some close relationship existed between the isotopes P^{32} and P^{31} in plant tissues. Phosphorus, as is known, is absorbed by the plant and incorporated into various organic compounds which form the basic constituents of the plant cells. Whenever the supply of phosphorus is abundant, a luxury consumption may result. Under the experimental conditions of this study, the phosphorus supply was amply sufficient, and a part of the phosphorus absorbed may have existed in the plants in the unassimilated or inorganic form, and most probably in an ionic state. The results of this investigation show that when the plants with the previous accumulation of P^{31} were treated with P^{32} or a mixture of P^{32} and P^{31}, P^{32} and the ratio of P^{32}:P^{31} of the mixture absorbed by the plants was not only diluted by this inorganic form P^{31}, but also diluted by the P^{31} which had been assimilated into the tissues as various organic compounds. These types of dilution especially the latter probably can only be accomplished by direct exchange reaction between P^{32} and P^{31}. It seems that the P^{31} fixed in the plant tissues can exchange continuously and freely with P^{32} newly absorbed until an equilibrium is reached between them.

That an exchange reaction occurs among the atoms or ions of the

same element is known. Even an insoluble inorganic compound, when put into water, will have at least a small number of molecules which come into the solution in the form of ions. An equilibrium between these few ions and the undissolved molecules is then established. The equilibrium is believed to be dynamic in nature. Although the concentration of the ions in the solution remains constant, the ions in the solution may again become a part of the insoluble compound while an equal number of ions comes out to the solution at the same time. Such phenomenon may be considered as an exchange among the same kind of ions. But whether this is only a surface reaction or whether the exchange reaction may take place among all molecules of the insoluble compound is not known. Furthermore, whether such exchange can only occur in the inorganic electrolytes or whether it may also take place in the very complex organic compounds is also a question. With some information now available, the author tries to discuss them in the following paragraphs.

Since an exchange reaction exists among the atoms of the same elements, it naturally must exist among the different isotopes with the same atomic number, for all properties of different isotopes of an element are assumed to be the same except the mass and, for unstable isotopes, the radiation. Such exchange has been demonstrated by many investigators in various phases of their work. However, the exchange is subject to some limitations. Douglas et al (3) found that no exchange greater than the experimental error occurred between carbon disulfide and hydrogen sulfide containing radioactive S^{35} in

benzene solution after ninety-five hours at 120°; but Tudge and Thode (17) claimed that the exchange might take place in carbon disulfide solution through a reaction by the formation of the intermediate compound, $H2CS_3$. Hevesy (6) reviewed the work done by various investigators and indicated that no exchange interaction occurred between the sulfur atoms of sulfide or sulfite and sulfate; and between the phosphorus atoms of different forms of inorganic phosphates. From these results, we may conclude that the exchange between the isotopes may be affected and inhibited to some extent by several factors, such as the chemical form of the isotopes, the solvent or medium they are in, and even the temperature as in the examples given by Hevesy in his book (6). This may help us to explain the $P^{32}:P^{31}$ ratio in the hydrochloric acid extract of the gravel in Experiment I. Its extremely low ratio may be due to the failure of P^{32} in the mineral nutrient solution to exchange with the P^{31} previously fixed in the gravel in certain non-exchangeable inorganic forms through various mechanisms.

Exchange between the isotopes in organic compounds does not seem to take place. Lukovnikov et al (10) heated the dioxane solutions of equivalent amounts of $(CH_3C_6H_4)_3PO_4$ and $H_3P^{32}O_4$, $(C_4H_9)_3PO_4$ and $Na_2HP^{32}O_4$, $(iso\text{-}C_4H_9)_3PO_4$ and $Na_2HP^{32}PO_4$, $(C_6H_{13})_3PO_4$ and $Na_2HP^{32}O_4$, and $(C_2H_5)_3PO_4$ and $Na_2HP^{32}O_4$ in sealed tubes for twenty to thirty hours at 100-300 °C, and concluded that no exchange occurred. Gourley (4) in his study, mixed the acid soluble phosphate esters with tri-sodium phosphate and P^{32}. After being adjusted to

pH 8, the mixtures were shaken for three hours on a Warburg apparatus at a rate of about 70 oscillations per minute with the temperature maintained at 37.0 ± 0.02 °C. He concluded that there was no simple exchange between inorganic phosphate and the phosphate groups of either glucose-1-phosphate, adenylic acid, 2,3-diphosphoglyceric acid or adenosine triphosphate occurred. Hevesy (6) has pointed out the similar results obtained by other workers with respect to the exchange of the labeled phosphate with the solution of hexose monophosphate, glycerophosphate, lecithin, casein, or nucleic acid. He also discussed the formation of labeled phosphatides in the organism and attributed their formation to the enzymic process which is coupled with an energh-producing system. The results obtained by Chaikoff et al were also discussed which showed the exchange was not due to the collision or any simple "physical" exchange between the labeled phosphate and non-labeled phosphatide molecules, and moreover, not due to the reversibility of their degradation. The above information leads one to the conclusion that exchange between the isotopes is not likely to occur in the organic forms. However, the results of this study clearly show that an exchange reaction between P^{32} absorbed and P^{31} in various organic forms in the plant must have taken place. The $P^{32}:P^{31}$ ratios in different tissues which had had different P^{31} contents were so uniform that we can not expect their ultimate equal ratios resulted from the degradation and re-synthesis of all complex phosphorus-containing organic compounds in the plant during the two week treatment period. If our reasoning is right,

the almost equal ratios of P^{32}:P^{31} in various plant tissues may be a result of the exchange between P^{32} in inorganic form and P^{31} both in inorganic and in various complex organic forms. The exchange reaction is very likely an intermolecular and interionic one. The extent of such exchange depends solely on the total P^{32} and P^{31} present in the plant not on the amount of P^{31} in inorganic or organic form.

If it is the isotopic exchange which resulted in the same ratio of P^{32}:P^{31} in various plant tissues of this study, then the exchange process may be postulated as follows: As soon as the roots absorbed the mixture of P^{32} and P^{31} from the mineral nutrient solution, two processes took place simultanuously. One was the accumulation of a portion of the isotope mixture in the roots and the other was the exchange of P^{32} in the mixture with P^{31} previously accumulated. Therefore, as far as the ratio of P^{32} and P^{31} which accumulated in root tissues at that moment is concerned, the roots had a higher ratio of P^{32}:P^{31} than originally present in the solution. The isotope mixture with a relatively low ratio of P^{32}:P^{31} was carried upward by the transpiration stream or other mechanisms to the different parts of the plant. In other words, the P^{32}:P^{31} ratio in the transpiration stream already differed from that present in the culture solution. As the translocation of phosphorus continued, a portion of the isotope mixture accumulated and a portion of the P^{32}in the isotope mixture being translocated continued to exchange with the P^{31} already present in the different tissues. Therefore the ratio of

$P^{32}:P^{31}$ in the mixture which was being translocated was gradually lowered. At that time, the $P^{32}:P^{31}$ ratios in various plant tissues may not be the same. Since phosphorus is an easily redistributed element in the plant, the isotope mixture moved in and moved out of the tissues continuously. The direction of its movement was not only from the roots to aerial organs but also from the aerial organs to the roots; and then, the isotope mixture was translocated upward again. The continuous accumulation of the isotope mixture and exchange between P^{32} and P^{31} during the process of translocation resulted in a constantly changing equilibrium between the $P^{32}:P^{31}$ ratios in the plant tissues and the isotope mixture being translocated. When the ratio of $P^{32}:P^{31}$ in a certain tissue was lower than that in the circulated mixture, an extra portion of P^{32} was received by that tissue. When it was higher, an extra portion of P^{32} was given to the isotope mixture in circulation. Finally when the ratios in all tissues were in equilibrium with the $P^{32}:P^{31}$ ratio in the mixture translocated, an equal value was attained. Since the plant absorbs the mineral elements continuously as long as they are present in the culture medium, a slight difference of the $P^{32}:P^{31}$ ratios in various plant tissues might always exist. The time required for equilibrium to be reached among the ratios of $P^{32}:P^{31}$ in different plant tissues is short. In this study, the plants were harvested one to two hours after the last irrigation. It indicates a new balance of P^{32} and P^{31} through exchange in the plant can be accomplished within one to two hours. However, if the time is too short, a complete balance may not

be reached. Then, the different ratios of P^{32}:P^{31} in different plant tissues may be observed. Unfortunately, how long after the last irrigation the plant samples were taken in Experiment I was not recorded. Otherwise, some information might be available to explain the much higher results of P^{32}:P^{31} ratios in leaves and roots.

The decrease of P^{32}:P^{31} ratio in the mineral nutrient solution at the end of the treatment as compared with that at the beginning is probably due to the increasing adsorption of P^{32} on the walls of the container and the connections in the cultural system and some other causes, especially when the concentration of the carrier becomes lower and lower. The adsorptionof P^{32} on the wall of the container has been noticed and studied by Ishibashi et al (7) and Martin et al (11), but no information is available on the amount of such adsorption in the presence of different levels of P^{31}. According to the basic assumptions in the use of radioisotopes in tracer work, the adsorption of phosphorus on the wall of the container involves both P^{32} and P^{31}. Since the properties of them are assumed to be exactly the same except mass and radiation, their ratio in the mixture adsorbed must be also the same under any condition. In other words, there should not be any differential adsorption. However, we have carefully calculated the data and observed that the plant did not have the preferential absorption between the two. The ratio of P^{32}:P^{31} in the isotope mixture absorbed by the plant followed closely the ratio in the mineral nutrient solution at the end of the treatment. Since the radioactivity of P^{32} in the solution at the beginning

has been converted to that at the end of the treatment, the much lower $P^{32}:P^{31}$ ratio in the solution at the end of the treatment and in the plant as well must be due to the loss of P^{32} in the low P^{31} solution during the treatment period. It was noticed that in all experiments conducted in 1954, the different treatments with the same ratio of $P^{32}:P^{31}$ but different amounts of P^{32} and P^{31} had different $P^{32}:P^{31}$ ratios in the solution even at the beginning of the treatment period before irrigation (see Table XVI). Since the solution samples were taken right after the P^{32} and P^{31} were added to and mixed with the solution, the possibility of the differential adsorption of P^{32} and P^{31} is great. Table XVI shows that the $P^{32}:P^{31}$ ratio increased with the increase of P^{31} concentration in the solution. In the solutions with lower concentrations of P^{31}, the loss of P^{32} in proportion to P^{31} is larger. Therefore, the reduced ratio of $P^{32}:P^{31}$ at the end of the treatment period may be a result of the differential adsorption of P^{32} and P^{31}, when the concentration of the latter became very low as a result of plant absorption.

The proposed mechanism for the reduced $P^{32}:P^{31}$ ratio at the end of the treatment period does not seem to be in agreement with what we generally believe. However, it has been found that isotope fractionation can occur. Thode et al (16) found that in nature, sulfates whether present as gypsum deposits or in solution were enriched in the heavier isotopes, while the hydrogen sulfide of sulfuretted well waters was usually low in these isotopes even though present in the same solution with the sulfates. Szabo et al (15) claimed that in

Table XVI. Influence of the P^{31} Concentration on the $P^{32}:P^{31}$ Ratio in the Mineral Nutrient Solution at the Beginning of the Treatment Period in Experiments II, III, IV and V

Experiment	Treatment	Unit* ratio of $P^{32}:P^{31}$	Concentration in solution		$P^{32}:P^{31}$ ¶ observed
			p32+ (μc/l)	p31 (ppm)	
II	A	1 : 1	9.87	20	2.73
	D	2 : 2	19.74	40	2.93
	E	5 : 5	49.34	100	3.02
III	B	1 : 1	15.22	10	2.08
	E	2 : 2	30.43	20	2.20
	F	5 : 5	76.32	50	2.15
IV	B	1 : 1	9.28	62	3.77
	E	2 : 2	18.56	124	3.91
	F	4 : 4	37.12	248	4.07
V	B	1 : 1	10.71	45	3.54
	E	2 : 2	21.43	90	3.64
	F	5 : 5	53.57	225	3,85

* See Tables VII, VIII, XI and XIV.

\+ The amount has been converted to the date when the treatments were made.

¶ The ratio is in $10c/m/r$ except in Experiment III where the ratio is in $10^2c/m/r$.

the conversion of sulfate to hydrogen sulfide, perhaps by action of sulfur bacteria, the isotopes of sulfur will be fractionated, favoring S^{34} and S^{36} in the sulfate ion and S^{32} in the sulfide ion. The explanation for the marked different isotopic abundances of sulfur in sulfate and hydrogen sulfide present together in the ground water samples were suggested by Thode et al (16) as the result of the differences in chemical properties of the sulfur isotopes and the chemical and physical processes that occur in nature or in the laboratory. Tudge and Thode (17) pointed out that the isotopes of other light elements do differ in their chemical properties and that the energy, entropy and free energy of isotopic substances depend on the vibrational frequencies of the molecules, which in turn depend on the masses of the atoms in the molecules so that there is a theoretical basis for the differences in the properties of the isotopic substances. Lindsay et al (9) made a study on the relative rate of decarboxylation of two isotopic species of malonic acid, one containing only C^{12} carbon atoms and the other containing a C^{13} carbon atom in a carboxyl position. They found a 3.3% faster rate of reaction for the molecule containing only C^{12} carbon atoms and a 2% greater probability of rupture of a C^{12}-C^{12} bond than a C^{12}-C^{13} bond in the molecule containing a C^{13} carbon. The results of the above-mentioned investigations reveal a fact that there are greater differences in the physical and chemical properties among the isotopes of the same element than we have hitherto realized. It is therefore,

probable that a differential adsorption of P^{32} and P^{31} may occur under the condition of low P^{31} concentrations. Of course, the surface exchange of P^{32} with P^{31} fixed as insoluble phosphorus compounds in the gravel or sand before the treatments may also contribute to the reduced ratio of P^{32}:P^{31} in the solution at the end of the treatment period.

If the reduced ratio of P^{32}:P^{31} in the nutrient solution at the end of the treatment period is due to the increasing adsorption of P^{32} when the P^{31} concentration becomes lower, the results of this study may have some practical importance. The use of radioactive isotopes in soil fertility and plant studies must be practised with some precautions, because the radioisotopes, P^{32} as an example, may adsorb to a greater extent on the surface of soil particles when the amount of the carrier is gradually decreased as a result of plant absorption. In this case, the ratio of P^{32}:P^{31} found in the plant does not represent the ratio originally present in the labelled fertilizer so that the quantitative estimation from P^{32} will give an inaccurate result. However, if an excess or a large amount of carrier is applied, since enough quantity is left to prevent the radioactive isotope from becoming increasingly adsorbed, the errors may become very small. The latter case has been shown in the treatments with high P^{31} concentration in this study. In these treatments, little difference between the ratios of P^{32}:P^{31} at the beginning and at the end of the treatment was found. However, the probable

effect of isotopic exchange in the soil between the radioelement added and the ordinary isotope originally present in the soil may also be very important in these studies. The ratio of the radioisotope to the carrier and to various forms of the ordinary isotope in the soil, both at the beginning and at the end of the experiment, should also be taken into consideration.

Some other information from the results of this study may also be helpful in tracer work. This is the observed exchange phenomenon of different isotopes in the plant. If there is previous accumulation of the ordinary isotope, the analytical results of the radioactive isotope from a certain single plant tissue fails to indicate how much ordinary isotope is absorbed after the radioisotope is introduced. However, when the whole plant is analyzed or if there is no previous accumulation of the carrier, the data of the radioactivity of the unstable isotope can then be satisfactorily used.

CONCLUSION AND SUMMARY

The exchange phenomenon of P^{32} and P^{31} in the plant was studied with five experiments including three crops of corn and one crop each of tomato and soybean which were grown in gravel, gravel-sand and sand cultures respectively. The results are concluded and summarized as follows:

1. An isotopic exchange between P^{32} and P^{31} was found in the plant tissues of all experiments in this investigation. The P^{32}:P^{31} ratios in various plant tissues were approximately the same no matter how different the P^{31} contents in different tissues were and whether or not there was previous accumulation of P^{31}. P^{32} not only exchanged with P^{31} in inorganic or unassimilated form but also in various complex organic forms. It is suggested that the isotopic exchange of P^{32} and P^{31} in plants is intermolecular and interionic in nature.

2. Although there was exchange between P^{32} and P^{31} in the plant, the total P^{32} and P^{31} absorbed during the treatment period were in the same ratio as the P^{32} and P^{31} in the mineral nutrient solution. In other words, the amount of the stable phosphorus P^{31} previously accumulated in the plant may have an effect on the amounts of P^{32} and P^{31} absorption but has no effect on their ratio.

3. When there was previous accumulation of P^{31} in the plant, the portions of the isotope mixture absorbed during the treatment

period accumulated in different tissues in widely different ratios, depending upon both the amounts of P^{31} previously accumulated and P^{31} accumulated during that period. A positive relationship was observed with the former and with the latter there was a negative relationship.

4. It is proposed that when there is previous accumulation of P^{31}, the final almost same ratios of P^{32}:P^{31} in different tissues are brought about by the continuous exchange between P^{31} previously accumulated and P^{32} in the isotope mixture being translocated upward and downward during the treatment period.

5. The ratio of P^{32}:P^{31} in the mineral nutrient solution at the end of the treatment period was found to be lower than at the beginning. The ratio in overall plant tissues was thus affected. It is suggested that the lowering of P^{32}:P^{31} ratio was mainly due to the adsorption of a larger portion of P^{32} in relation to P^{31} on the wall of the containers and connections in the cultural system, especially when the concentration of P^{31} in the solution became very low as a result of plant absorption. When there was P^{31} fixed in the gravel or in the sand previous to the P^{32}-P^{31} treatment, at least the surface exchange of P^{32} with P^{31} fixed in insoluble form may also contribute to the reduced P^{32}:P^{31} ratio in the solution.

6. Since the exchange between P^{32} and P^{31} occurs, several factors, such as the isotopic exchange in the culture medium, the concentration of the carrier, previous accumulation of the carrier in the plant, and possibly others, must be taken into consideration

in evaluating quantitatively the absorption of phosphorus in soil fertility and plant nutritional studies. The measurement of P^{32} in any single plant tissue cannot be relied on as a true indication of P^{31} absorption when there has been previous accumulation of P^{31} in the plant.

APPENDICES

Appendix Table I. Dry Weight* of Various Corn Tissues in Experiment I

Treatment	Unit* ratio	Replicate	Upper leaves	Lower leaves	Stem	Ear	Roots	Total
	P^{32}:P^{31}	Pot no.	gm	gm	gm	gm	gm	gm
A	0 : 1	1	10.6	9.2	38.4	68.4	23.1	149.7
		9	7.3	8.5	26.8	63.6	14.5	120.7
		19	7.0	10.3	32.6	50.8	23.2	123.9
B	1 : 0	3	8.4	9.7	36.6	69.9	17.7	142.3
		11	9.9	13.1	40.5	72.3	24.5	160.3
		21	10.0	10.4	38.5	39.5	27.7	126.1
C	1 : 1	5	7.1	9.5	32.4	56.5	17.2	122.7
		13	9.9	8.9	37.0	46.7	26.0	128.5
		23	10.4	8.1	46.0	32.0	21.2	117.7
D	1 : 20	7	9.3	9.8	34.4	49.7	17.4	120.6
		17	7.2	7.9	29.6	35.3	21.6	101.6
		25	7.2	9.7	29.3	37.0	17.3	100.5

* On dry basis (oven-dry at 70 °C).

+ 1 unit of P^{32} = 1 mc. (assayed on July 2, 1953 but not converted to that at the time the isotope was used) in 15.2-liter mineral nutrient solution. 1 unit of P^{31} = a concentration of 10 ppm.

Appendix Table II. Radioactivity*of P^{32} in the Treatment Solution and Various Corn Tissues in Experiment I

Treatment	Unit+ ratio	Replicate	Upper leaves	Lower leaves	Stem	Ear	Roots	Whole plant	Treatment solution
	$P^{32}:P^{31}$	Pot no.	10^2c/m/g	10^2c/m/g	10^2c/m/g	10^2c/m/g	10^2c/m/g	10^2c/m/g	c/m/ml
B	1 : 0	3	11447	7685	780	4600	3774	4129	8428
		11	9853	7093	1902	4114	4039	4142	8415
		21	7694	5537	1665	4828	3853	3934	8605
C	1 : 1	5	14918	11715	1412	6224	2958	5424	11642
		13	12104	8921	2679	5864	3707	5203	11940
		23	12290	8761	3296	6319	5160	5624	12229
D	1 : 20	7	4222	3440	651	790	2805	1521	12044
		17	4411	3278	651	790	2613	1587	11608
		25	5673	5159	670	1182	2805	2018	11521

* All radioactivity was converted to that when the plants were harvested.

\+ See Appendix Table I.

Appendix Table III. Percentage Content of P^{31} in the Treatment Solution and Various Corn Tissues in Experiment I

Treatment	Unit* ratio	Replicate	Upper leaves	Lower leaves	Stem	Ear	Roots	Whole plant	Treatment solution
	$P^{32}:P^{31}$	Pot no.	%	%	%	%	%	%	ppm
A	0 : 1	1	0.290	0.265	0.068	0.248	0.126	0.187	10.30
		9	0.313	0.268	0.138	0.276	0.132	0.230	10.30
		19	0.248	0.243	0.123	0.254	0.136	0.196	11.30
B	1 : 0	3	0.218	0.208	0.029	0.208	0.084	0.147	0.00
		11	0.208	0.218	0.097	0.202	0.080	0.159	0.00
		21	0.173	0.195	0.066	0.216	0.080	0.135	0.00
C	1 : 1	5	0.363	0.335	0.074	0.276	0.122	0.211	10.30
		13	0.310	0.300	0.133	0.270	0.136	0.209	10.30
		23	0.278	0.303	0.128	0.250	0.226	0.204	10.40
D	1 : 20	7	0.905	0.830	0.263	0.375	0.735	0.473	217.00
		17	0.925	0.880	0.270	0.340	0.645	0.468	217.00
		25	1.035	1.280	0.210	0.350	0.505	0.475	209.00

* See Appendix Table I.

Appendix Table IV. Dry Weight* of Various Corn Tissues in Experiment II

Treatment	Unit+ ratio	Replicate	Leaves	Stem	Roots	Total
	$P^{32}:P^{31}$	Pot no.	gm	gm	gm	gm
A	1 : 1	1	17.3	10.6	6.3	34.2
		6	23.0	14.8	6.5	44.3
		11	21.7	16.5	7.3	45.5
B	1 : 2	2	21.2	12.8	6.2	40.2
		7	20.7	15.1	6.5	42.3
		12	16.3	11.2	4.6	32.1
C	1 : 5	3	19.3	10.6	7.3	37.2
		8	18.0	11.6	6.3	35.9
		13	16.8	10.9	6.0	33.7
D	2 : 2	4	20.8	16.0	7.7	44.5
		9	20.4	13.6	6.4	40.4
		14	20.7	14.6	6.1	41.4
E	5 : 5	5	13.6	7.0	4.4	25.0
		10	15.8	12.8	6.3	34.9
		15	17.1	11.6	5.4	34.1

* On oven-dry basis.

\+ 1 unit of P^{32} = 0.156 mc. (assayed on August 16, 1954 but not converted to that at the time the isotope was used) in 15.2-liter mineral nutrient solution.
1 unit of P^{31} = a concentration of 20 ppm.

Appendix Table V. Radioactivity* of P^{32} in the Treatment Solution and Various Corn Tissues in Experiment II

Treatment	Unit+ ratio	Replicate	Leaves	Stem	Roots	Whole plant	Treatment solution
	$P^{32}:P^{31}$	Pot no.	10^2c/m/g	10^2c/m/g	10^2c/m/g	10^2c/m/g	c/m/ml
A	1 : 1	1	1146	1646	997	1274	739
		6	1101	1220	811	1098	663
		11	973	1152	1052	1051	670
B	1 : 2	2	1119	1342	1009	1173	729
		7	838	1207	988	993	688
		12	979	1479	997	1156	653
C	1 : 5	3	387	503	634	469	714
		8	415	613	677	525	686
		13	317	491	659	434	683
D	2 : 2	4	1573	1957	1567	1710	1405
		9	1363	1909	1506	1569	1371
		14	1781	2671	1899	2112	1318
E	5 : 5	5	1967	3000	3549	2535	3534
		10	1811	2433	3967	2428	3466
		15	2271	3342	4537	2994	3407

* All radioactivity was converted to that when the plants were harvested.

\+ See Appendix Table IV.

Appendix Table VI. Percentage Content of P^{31} in the Treatment Solution and Various Corn Tissues in Experiment II

Treatment	Unit+ ratio	Replicate	Leaves	Stem	Roots	Whole plant	Treatment solution
	$p^{32}:p^{31}$	Pot no.	%	%	%	%	ppm
A	1 : 1	1	0.400	0.545	0.375	0.440	20.5
		6	0.355	0.425	0.320	0.373	18.5
		11	0.315	0.390	0.390	0.354	20.0
B	1 : 2	2	0.630	0.780	0.615	0.675	38.0
		7	0.510	0.715	0.595	0.596	37.5
		12	0.580	0.855	0.620	0.682	38.0
C	1 : 5	3	0.480	0.665	0.835	0.602	89.0
		8	0.580	0.765	0.950	0.705	91.0
		13	0.445	0.655	0.930	0.599	91.0
D	2 : 2	4	0.460	0.585	0.510	0.514	37.5
		9	0.405	0.575	0.490	0.476	37.5
		14	0.530	0.795	0.600	0.634	37.5
E	5 : 5	5	0.525	0.780	0.970	0.675	89.5
		10	0.490	0.680	1.110	0.672	91.0
		15	0.585	0.910	1.315	0.811	90.5

+ See Appendix Table IV.

Appendix Table VII. Dry Weight* of Various Corn Tissues in Experiment III

Treatment	Unit+ ratio	Replicate	Upper leaves	Lower leaves	Tassel	Sheath & Stem	Roots	Total
	$P^{32}:P^{31}$	Pot no.	gm	gm	gm	gm	gm	gm
A	1 : 0	1	23.9	13.9	6.3	50.6	20.8	115.5
		7	17.8	12.0	9.0	39.5	16.4	94.7
		13	19.4	13.8	8.3	58.3	22.1	121.9
B	1 : 1	2	17.2	12.8	7.0	45.6	18.1	100.7
		8	26.2	11.8	10.4	58.7	25.6	132.7
		14	17.9	14.1	7.1	41.4	16.4	96.9
C	1 : 5	3	14.6	11.0	10.8	52.1	19.5	108.0
		9	16.5	10.5	10.5	38.8	17.2	93.5
		15	19.2	9.8	9.4	51.5	13.8	103.7
D	1 : 10	4	24.8	15.3	8.2	63.8	26.6	138.7
		10	19.5	18.0	9.5	47.7	20.5	115.2
		16	15.2	14.8	7.5	42.9	15.4	95.8
E	2 : 2	5	16.9	14.0	8.0	38.0	20.3	97.2
		11	16.5	15.2	5.5	40.1	15.0	92.3
		17	16.5	13.1	6.3	33.3	15.0	84.2
F	5 : 5	6	16.8	15.6	6.8	44.0	18.4	101.6
		12	12.9	13.0	5.8	32.3	15.1	79.1
		18	22.3	12.4	9.4	49.2	19.3	112.6

* On oven-dry basis.

\+ 1 unit of P^{32} = 0.27 mc. (assayed on June 7, 1954 but not converted to that at the time the isotope was used) in 15.2-liter mineral nutrient solution. 1 unit of P^{31} = a concentration of 10 ppm.

Appendix Table VIII. Radioactivity* of P^{32} in the Treatment Solution and Various Corn Tissues in Experiment III

Treatment	Unit+ ratio	Replicate	Upper leaves	Lower leaves	Tassel	Stem	Roots	Whole plant	Treatment solution
	$P^{32}:P^{31}$	Pot no.	10^2c/m/g	10^2c/m/g	10^2c/m/g	10^2c/m/g	10^2c/m/g	10^2c/m/g	c/m/ml
A	1 : 0	1	2353	1478	3072	1411	1952	1802	2564
		7	2782	1738	3598	1591	1813	2063	2411
		13	2433	1762	3263	1535	1422	1880	2051
B	1 : 1	2	1884	1463	2094	1602	1757	1717	2848
		8	1712	1174	2445	1546	1785	1662	2718
		14	2090	1622	2694	1964	1980	1994	2720
C	1 : 5	3	1311	1189	1438	1648	1794	1561	2765
		9	1380	1369	1422	1625	1999	1599	2740
		15	1345	1217	1274	1681	1683	1538	2681
D	1 : 10	4	676	585	623	598	1590	802	2809
		10	733	470	631	553	1525	750	2769
		16	607	619	675	801	1971	920	2658
E	2 : 2	5	3116	2555	3821	3182	3179	3132	5449
		11	3003	2393	3154	2708	3012	2785	5406
		17	3520	2771	3823	3442	2835	3273	5279
F	5 : 5	6	5004	4662	4639	4661	7474	5226	14044
		12	5582	4979	5627	5766	7084	5848	14008
		18	6492	5558	7318	7572	7502	7103	13642

* All radioactivity was converted to that when the plants were harvested.

\+ See Appendix Table VII.

Appendix Table IX. Percentage Content of P^{31} in the Treatment Solution and Various Corn Tissues in Experiment III

Treatment	Unit+ ratio	Replicate	Upper leaves	Lower leaves	Tassel	Stem	Roots	Whole plant	Treatment solution
	$P^{32}:P^{31}$	Pot no.	%	%	%	%	%	%	ppm
A	1 : 0	1	0.285	0.235	0.335	0.165	0.190	0.212	0.0
		7	0.307	0.235	0.365	0.160	0.197	0.223	0.1
		13	0.292	0.247	0.400	0.187	0.177	0.223	0.1
B	1 : 1	2	0.332	0.297	0.400	0.282	0.255	0.296	10.2
		8	0.295	0.232	0.390	0.227	0.257	0.259	10.0
		14	0.340	0.307	0.435	0.312	0.292	0.322	10.1
C	1 : 5	3	0.425	0.410	0.510	0.490	0.507	0.478	43.4
		9	0.505	0.500	0.540	0.520	0.497	0.513	43.4
		15	0.427	0.462	0.440	0.507	0.425	0.471	42.6
D	1 : 10	4	0.437	0.442	0.430	0.367	0.692	0.454	89.0
		10	0.392	0.330	0.440	0.347	0.720	0.426	89.0
		16	0.422	0.497	0.505	0.407	0.790	0.493	88.0
E	2 : 2	5	0.330	0.320	0.430	0.335	0.310	0.335	18.4
		11	0.345	0.310	0.415	0.310	0.292	0.320	17.6
		17	0.412	0.367	0.480	0.402	0.280	0.383	18.4
F	5 : 5	6	0.387	0.362	0.400	0.330	0.407	0.363	44.5
		12	0.417	0.402	0.485	0.417	0.397	0.416	50.0
		18	0.442	0.470	0.535	0.522	0.465	0.485	44.0

+ See Appendix Table VII.

Appendix Table X. Dry Weight* of Various Tomato Tissues in Experiment Iv

Treatment	Unit+ratio	Replicate	Leaves	Stem	Fruit	Top total
	$P^{32}:P^{31}$	Pot no.	gm	gm	gm	gm
A	1 : 0	1	41.8	52.7	16.1	110.6
		7	40.2	57.2	14.0	111.4
		13	43.0	59.5	25.6	128.1
B	1 : 1	2	54.8	82.8	19.6	157.2
		8	37.8	50.5	8.9	97.2
		14	44.1	65.3	11.3	120.7
C	1 : 2	3	49.8	67.4	16.4	133.6
		9	47.1	73.9	24.0	145.0
		15	44.4	57.2	17.2	118.8
D	1 : 4	4	50.5	68.4	10.7	129.6
		10	61.9	86.7	31.9	180.5
		16	47.6	65.2	11.8	124.6
E	2 : 2	5	55.4	81.0	7.9	144.3
		11	78.4	99.7	28.9	207.0
		17	48.4	71.2	16.7	136.3
F	4 : 4	6	49.8	68.2	8.3	126.3
		12	46.9	70.8	24.0	141.7
		18	36.6	51.0	25.6	113.2

* On oven-dry basis.
\+ 1 unit of P^{32} = 0.256 mc. (assayed on July 22, 1954 but not converted to that at the time the isotope was used) in 14-liter mineral nutrient solution.
1 unit of P^{31} = a concentration of 62 ppm.

Appendix Table XI. Radioactivity* of P^{32} in the Treatment Solution and Various Tomato Tissues in Experiment IV

Treatment	Unit+ ratio	Replicate	Leaves	Stem	Fruit	Whole top	Treatment solution
	$P^{32}:P^{31}$	Pot no.	10^2c/m/g	10^2c/m/g	10^2c/m/g	10^2c/m/g	c/m/ml
A	1 : 0	1	616	753	940	728	1008
		7	404	349	550	394	1155
		13	562	436	722	535	1266
B	1 : 1	2	578	496	543	530	1391
		8	660	513	728	590	1243
		14	671	660	616	660	1290
C	1 : 2	3	316	338	305	326	1290
		9	480	365	377	404	1253
		15	425	387	331	394	1320
D	1 : 4	4	207	196	192	200	1323
		10	338	169	159	225	1431
		16	235	164	219	196	1317
E	2 : 2	5	682	595	775	638	2509
		11	895	584	616	706	2610
		17	693	545	596	604	2513
F	4 : 4	6	1047	818	735	903	4860
		12	938	856	709	858	5008
		18	955	605	616	721	4955

* All radioactivity was converted to that when the plants were harvested.

\+ SEE Appendix Table X.

Appendix Table XII. Percentage Content of P^{31} in the Treatment Solution and Various Tomato Tissues in Experiment IV

Treatment	Unit+ ratio	Replicate	Leaves	Stem	Fruits	Whole top	Treatment solution
	P^{32}:P^{31}	Pot no.	%	%	%	%	ppm
A	1 : 0	1	0.455	0.530	0.640	0.518	0.2
		7	0.415	0.440	0.560	0.446	0.3
		13	0.490	0.530	0.580	0.527	0.6
B	1 : 1	2	0.675	0.675	0.680	0.676	62.5
		8	0.765	0.755	0.670	0.751	61.0
		14	0.750	0.775	0.680	0.757	65.0
C	1 : 2	3	0.715	0.795	0.700	0.754	110.5
		9	0.800	0.725	0.625	0.733	108.5
		15	0.780	0.800	0.705	0.779	115.0
D	1 : 4	4	0.715	0.750	0.655	0.729	230.0
		10	0.890	0.715	0.665	0.766	227.5
		16	0.650	0.805	0.740	0.740	245.0
E	2 : 2	5	0.700	0.735	0.710	0.720	113.5
		11	0.795	0.665	0.650	0.712	114.0
		17	0.740	0.730	0.670	0.726	120.5
F	4 : 4	6	0.775	0.795	0.690	0.780	225.0
		12	0.880	0.815	0.650	0.809	227.0
		18	0.865	0.745	0.650	0.762	237.5

+ See Appendix Table X.

Appendix Table XIII. Dry Weight* of Various Soybean Tissues in Experiment V

Treatment	Unit+ ratio	Replicate	Leaves	Stem	Pods	Top total
	P^{32}:P^{31}	Pot no.	gm	gm	gm	gm
A	1 : 0	1	52.3	63.5	62.4	178.2
		7	47.6	60.5	61.3	169.4
		13	45.8	54.2	64.0	164.0
B	1 : 1	2	50.8	59.9	60.9	171.6
		8	54.2	67.5	70.0	191.7
		14	36.1	40.6	63.5	140.2
C	1 : 2	3	41.5	49.9	49.2	140.6
		9	51.3	65.3	60.7	177.3
		15	38.2	42.3	63.3	143.8
D	1 : 5	4	40.3	47.8	44.0	132.1
		10	46.8	62.5	56.6	165.9
		16	39.2	44.1	67.4	150.7
E	2 : 2	5	42.8	48.8	50.9	142.5
		11	48.0	55.7	62.0	165.7
		17	37.3	35.0	49.7	122.0
F	5 : 5	6	47.3	56.0	50.4	153.7
		12	49.2	61.0	64.7	174.9
		18	49.8	61.5	81.3	192.6

* Total oven-dry weight of five plants in each pot.

\+ 1 unit of P^{32} = 0.156 mc. (assayed on August 16, 1954 but not converted to that at the time the isotope was used) in 14-liter mineral nutrient solution.
1 unit of P^{31} = a concentration of 45 ppm.

Appendix Table XIV. Radioactivity* of P^{32} in the Treatment Solution and Various Soybean Tissues in Experiment V

Treatment	Unit+ratio	Replicate	Leaves	Stem	Pods	Whole top	Treatment solution
	$P^{32}:P^{31}$	Pot no.	10^2c/m/g	10^2c/m/g	10^2c/m/g	10^2c/m/g	c/m/ml
A	1 : 0	1	203	165	259	209	---
		7	302	245	340	295	835
		13	509	538	533	528	915
B	1 : 1	2	991	571	641	720	1565
		8	991	557	712	736	1697
		14	1075	679	726	802	1581
C	1 : 2	3	1156	637	608	780	1649
		9	764	358	382	484	1693
		15	1212	575	552	734	1738
D	1 : 5	4	575	278	245	358	1623
		10	967	382	358	539	1642
		16	939	344	344	499	1716
E	2 : 2	5	2267	1108	1094	1451	3553
		11	2071	995	1113	1351	3137
		17	2844	1684	1288	1353	3620
F	5 : 5	6	4155	1712	1509	2397	8463
		12	5245	1792	1656	2713	8559
		18	5882	1755	1825	2852	8252

* All radioactivity was converted to that when the plants were harvested.
\+ See Appendix Table XIII.

Appendix Table XV. Percentage Content of P^{31} in the Treatment Solution and Various Soybean Tissues in Experiment V

Treatment	Unit+ ratio	Replicate	Leaves	Stem	Pods	Whole top	Treatment solution
	$P^{32}:P^{31}$	Pot no.	%	%	%	%	PPM
A	1 : 0	1	0.655	0.570	0.585	0.600	0
		7	0.885	0.660	0.630	0.712	0
		13	0.985	0.680	0.670	0.761	0
B	1 : 1	2	1.255	0.800	0.680	0.892	41.5
		8	1.335	0.750	0.700	0.897	45.5
		14	1.500	0.880	0.700	0.958	41.5
C	1 : 2	3	1.890	1.030	0.800	1.200	85.5
		9	1.575	0.780	0.700	0.983	89.0
		15	1.920	0.880	0.740	1.095	93.0
D	1 : 5	4	2.000	1.050	0.780	1.250	207.0
		10	2.850	1.020	0.930	1.506	210.0
		16	2.940	1.060	0.870	1.464	206.0
E	2 : 2	5	1.705	0.980	0.800	1.133	86.5
		11	1.770	0.970	0.790	1.134	85.5
		17	2.085	1.180	0.800	1.302	91.5
F	5 : 5	6	2.670	1.100	0.930	1.527	209.0
		12	3.000	1.050	0.900	1.543	209.0
		18	3.180	0.880	0.850	1.462	204.0

+ See Appendix Table XIII.

LITERATURE CITED

1. Arnon, D. I. and Hoagland, D. R. 1940 Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. Soil Sci. 50: 463-483.

2. Broyer, T. C. and Overstreet, R. 1940 Cation exchange in plant roots in relation to metabolic factors. Amer. J. Bot. 27: 425-430.

3. Douglas, D. L., Cooley, R. A. and Yost, D. M. 1949 Non-exchange of sulfur between carbon disulfide and hydrogen sulfide in benzene solution. Jour. Amer. Chem. Soc. 71: 3237-3238.

4. Gourley, D. R. H. 1952 Failure of phosphorus-32 to exchange with organic phosphorus compounds. Nature 169: 192-193.

5. Hevesy, G. 1923 The absorption and translocation of lead by plants. Biochem. Jour. 17: 439-443.

6. Hevesy, G. 1948 Radioactive indicators. Interscience Publishers Inc., N. Y.

7. Ishibashi, Masayoshi and Kusaka, Yuzuru 1953 Change of concentration of radioactive phosphorus 32 in solution during storage. Kagaku (Science) 23: 37.; Chem. Abst. 47: 3150 (1953).

8. Jenny, H. and Overstreet, R. 1938 Contact effects between plant roots and soil colloids. Proc. Nat. Acad. Sci. Amer. 24: 384-392.

9. Lindsay, J. G., Bourns, A. N. and Thode, H. G. 1951 C^{13} isotope effect in the decarboxylation of normal malonic acid. Canadian Jour. Chem. 29: 192-200.

10. Lukovnikov, A. F., Medvedev, V. P., Neiman, M. V., Mesmeyanov, An. N. and Shaverdina, I. S. 1950 Isotopic exchange of phosphorus between the phosphate ion and esters of phosphoric acid. Doklady Akad. Nauk S. S. S. R. 70: 43-45.; Chem. Abst. 44: 4315 (1950).

11. Martin, R. P. and Russell, R. S. 1950 Studies with radioactive tracers in plant nutrition II. The estimation of radioactive tracers. Jour, Expt. Bot. 1: 141-158.

12. McAuliffe, C. D., Hall, N. S., Dean, L. A. and Hendricks, S. B. 1947 Exchange reactions between phosphates and soils: Hydroxylic surfaces of soil minerals. Soil Sci. Soc. Amer. Proc. 12: 119-123.

13. Olsen, S. R. 1952 Measurement of surface phosphate on hydroxylapatite and phosphate rock with radiophosphorus. J. Phys. Chem. 56: 630-632.

14. Overstreet, R. and Broyer, T. C. 1940 The nature of absorption of radioactive isotopes by living tissues as illustrated by experiments with barley plants. Proc. Nat. Acad. Sci. Amer. 26: 16-24.

15. Szabo, A., Tudge, A., Macnamara, J. and Thode, H. G. 1950 The distribution of S^{34} in nature and the sulfur cycle. Science, 111: 464-465.

16. Thode, H. G., Macnamara, J. and Collins, C. B. 1949 Natural variations in the isotopic content of sulphur and their significance. Canadian Jour. Res. (Section B) 27: 361-373.

17. Tudge, A. P. and Thode, H. G. 1950 Thermodynamic properties of isotopic compounds of sulphur. Canadian Jour. Res. (Section B) 28: 567-578.

AUTOBIOGRAPHY

I, Tzu-liang Yuan, was born in Ningpo, Chekiang, China, on May 27, 1922. I received my junior and senior high school education in Ching-chung Junior High School and Kiangsu Provincial Shanghai High School respectively. My undergraduate training was obtained at the National University of Chekiang, China, from which I received the degree Bachelor of Science in Agricultural Chemistry in January, 1945. After graduation, I was appointed an assistant instructor in Soils in my Alma Mater and then instructor in the National University of Kweichow. In the spring of 1948, I accepted an appointment as assistant soil chemist in the Taiwan Sugar Experiment Station in Taiwan, China. In April, 1951, I was appointed University Scholar at the Ohio State University for the academic year of 1951-52 and started graduate studies in January, 1952. In December of that year, I received the degree Master of Science in Agronomy. I continued graduate work in the capacity of research assistant at the Ohio Agricultural Experiment Station while completing the requirements for the degree Doctor of Philosophy.