THE METABOLISM OF C^{14} LABELLED 2,4-DICHLOROPHENOXYACETIC ACID IN BEAN PLANTS

by

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TABLE OF CONTENTS

THE METABOLISM OF C^{14} LABELLED 2,4-DICHLOROPHENOXYACETIC ACID IN BEAN PLANTS

INTRODUCTION

The experimental evidence for the translocation and accumulation of growth substances in plants has been largely based on physiological responses brought about by the substance (8, pp.449-459; 19, p.339; 12, pp.393-407; 6, pp.51-68; 14, pp.301-314). In a review by Zimmerman and Hitchcock (20, p.618), it was stated that only a few papers reported detection or identification by direct methods of the hormone beyond the point of application. In using an isotopically labelled herbicide, Wood et al. (19, p.339) concluded that 2-iodo-3-nitrobenzoic acid labelled with iodine^{131} and applied to bean and barley plants was absorbed in molecular form and translocated as such. The work of Dhillon and Lucas (4, pp.198-207) presents a direct approach to this type of study by extraction of treated plants and identification of the growth substance applied. The identification of 2,4-D was made by means of a colorimetric test developed by Freed (7, pp.98-99) and by the production of a physiological response in Red Kidney Bean plants by the plant extract.

Holley et al. (9, pp.148-151) using 2,4-D labelled in the carboxyl group with C^{14} were able to demonstrate the presence of 2,4-D in bean plant homogenates as well as small amounts of at least two ether soluble radioactive organic acids other than 2,4-D by means of the isotope dilution method and counter current distribution. Of the radioactive material isolated from the stem, 2,4-D represented approximately 33%. Over half of the isolated material was not 2,4-D. It was water soluble and could not be extracted by ether from an aqueous solution at pH 8.0 or pH 1.5.

A paper from this laboratory by Fang et al. (5, pp.249-255) was concerned with the absorption and translocation of αmethylene C^{14} labelled 2,4-D in bean plants. Although the translocation of C^{14} could be reported, no evidence was available at that time with regard to the actual identity of the radioactive compound(s). It was to this end, that the present work was directed utilizing paper chromatography (3, pp.226-227) for the purposes of separation, isolation and identification.

It was of further interest to study the concentration change (percentage-wise) of 2,4-D and other radioactive compounds formed over a period of six weeks using both α methylene and carboxyl C^{14} labelled 2,4-D.

In this way, it was thought, a possible relationship could be found between 2,4-D and other radioactive compounds formed.

Several investigators (12, pp.393-407; 17, pp.514-516) reported that when 2,4-D was absorbed by roots it passed upward through dead segments of stems, but when absorbed by the leaves, it did not readily move downward through killed portions of stems and petioles. These observations have contributed to the conclusion that movement of the stimulus from the leaf took place only through living cells whereas movement of the stimulus from the roots took place through the xylem or dead cells. This being the case, it was felt of interest to determine whether a difference existed between the metabolism of 2,4-D when administered through the roots and when administered through the leaves.

Naturally, if diffusion of the 2,4-D from the xylem into other tissue were rapid, the metabolism would be expected to be quite similar to that found when application of the growth substance was made on the leaves.

In 1946, Mitchell and Brown had found that 2,4-D was not translocated from the leaves of young bean plants that had been depleted of carbohydrate in the dark. Rice (14, pp.301-314) was able to demonstrate that even though export from carbohydrate depleted leaves did

not occur, entry of the 2,4-D into the leaf did take place. It has been further shown (15, pp.86-88; 18, p.145) that carbohydrate depleted bean plants can be induced to move 2,4-D from the leaves of plants kept in the dark by applying sucrose, glucose, fructose, maltose, and galactose to the leaves.

The third section of the present work was therefore carried out in an effort to determine whether 2,4-D would be metabolized in etiolated and carbohydrate depleted plants and also to test the effect of glucose on its metabolism in etiolated plants.

I. THE METABOLISM OF 2,4-D IN BEAN PLANTS TREATED ON THE LEAVES

A. EXPERIMENTAL PROCEDURES

Synthesis of Carboxyl C^{14} Labelled 2,4-D

Carboxyl C^{14} labelled acetic acid was first made according to the method of Claycomb et al. (2, pp.38-48). The acetic acid was then brominated and coupled with 2,4-dichlorophenol according to the method of Holley et al. (9, p.150). The specific activity was 5.3×10^6 counts/minute/milligram.

The synthesis of α methylene C^{14} labelled 2,4-D has been presented elsewhere (5, p.250). The specific activity was 4.2×10^5 counts/minute/milligram.

Treatment of Bean Plants

The bean plants (Phaseolus vulgaris, var. Black Valentine) were grown under greenhouse conditions in potted soil. The plants were treated on each primary leaf along the midrib with 25 ug. of radioactive 2,4-D at the time they became fully unfolded. The solutions used contained 0.1% of α methylene C^{14} or carboxyl C^{14} labelled 2,4-D dissolved in 90% alcohol which contained 0.5% Tween-20.

The plants were harvested in groups of five or six at 1, 4, 7, 14, 28 and 42 days after treatment. Each plant was cut off level with the soil and sectioned into leaf and stem material. The material in each group was pooled, homogenized in a Waring blendor with 80% ethyl alcohol for one minute, transferred quantitatively to a 250 ml. volumetric flask and made to the mark with additional 80% alcohol.

Paper Chromatography

The solution was permitted to remain in contact with the residue for several days before samples were taken for counting and chromatographing. The solution was applied to strips of Whatman No. 1 filter paper (1 x 22 in.) by means of a fine drawn eye dropper, the diameter of the spot being maintained at approximately 0.75 cm. during application. Two strips were made in

each case. One was developed in phenol saturated with water for 24 hours, the other in butanol-propionic acid-water system for 12 hours (1, p.1711). After developing, the strips were air dried at room temperature for 24 hours and cut into 1 cm. sections. The first section included the region 0.5 cm. above and below the point of application. See appendix for schematic diagrams of the chromatographic chamber and a paper strip.

Radioactivity Measurements

The homogenates were filtered through weighed sintered glass crucibles to remove the alcohol insoluble residues. After thorough washing of the residues with 80% alcohol, they were dried in a vacuum oven at 50° C for twenty-four hours and then oxidized using the Van Slyke-Folch combustion solution (16, pp.511-512). The carbon dioxide was precipitated as $BaCO_3$, mounted on thin copper planchets, and counted with a "Tracerlab Autoscaler" (5, p.250).

The radioactivity of the extracts was determined by the direct plating method. Five-tenths ml. samples were evaporated to dryness in glass dishes and counted in a 64 scaler with a thin mica window tube (1.7 mgs/cm.^{2}). Aliquots of several extracts were evaporated to dryness in combustion flasks and oxidized

as described above. Filter paper was added as a diluent. By taking the ratio of activity determined as $BaCO_3$ to that determined by direct plating, a conversion factor of 2.58 was found. This factor was then employed to express all radioactivity as $BaCO_3$ in order to form a uniformly expressed balance sheet. Chromatograph sections from the experiment using carboxyl C^{14} labelled 2,4-D were counted with a "Tracerlab Autoscaler" containing a G.M. tube (1.7 mgs/cm.^{2}). The scale selector was set at 4096 counts which gives a one percent error for high activity samples, five percent error for medium ones and ten percent error for very low ones.

The chromatograph sections obtained from the experiment using α methylene C^{14} labelled 2,4-D were counted by means of a "Tracerlab Windowless Gas Flow Counter". The background count in this instrument was found to be consistently in the region of 14 to 16 cpm. and the sensitivity was approximately six times that of the autoscaler. This enabled the counting of very low activity samples with an error no greater than ten percent. Sections with an activity three or more times that of background gave a one percent error.

Treatment of Data

A graph of radioactivity versus R_f value indicated

an approximately normal type distribution in the vicinity of any particular radioactive compound. Similar results were obtained by Müller and Wise (13, pp.207-208) by means of an automatic scanning device which continuously recorded the activity of the strip as it passed by an ionization chamber. This method affords an accurate means of calculating R_f values for purposes of identification. Since R_f values are not always reproducible, particularly when using complex biological extracts, it was felt that larger increments of the strip could be counted and integrated with a reasonable degree of accuracy. It may be mentioned that due to the variability of R_f values, more emphasis should be placed on the relative position of the radioactive compounds found on the chromatograph rather than on their R_f values.

The activities of the sections of a given strip minus the background count were totalled and entered as total activity applied. The radioactivity of a given spot was also summed up and the relative percentage concentration of this compound in the 80% alcohol extract was calculated by the following expression:

$$\frac{\text{Total spot activity}}{\text{Total strip activity}} \times 100$$

Table I shows such calculations for the principal radioactive compounds chromatographed in phenol saturated

TABLE I

Reproducibility of the Distribution of the Principal Radioactive Compounds Chromotographed in Phenol Saturated with Water. Plants Harvested Two Weeks After Treatment.

		Percentage of Total Activity		
Compound	R_f	Run 1	Run 2	Run 3
Unknown 2	.33	8.7	9.5	8.0
Unknown 1	.57	65.1	64.5	66.7
2,4-D	.77	21.1	19.5	20.6

with water. This procedure therefore presents a means of expressing the relative concentrations of radioactive components in a particular solution with an accuracy limited generally by the error in counting.

B. RESULTS AND DISCUSSION

Pure radioactive 2,4-D was found to have an R_f value between .76-.78 in phenol saturated with water and .83-.86 in butanol-propionic acid-water. One of the radioactive compounds found in the 80% alcohol extract of the stems had an R_f value within these ranges. The portion of the strip containing this compound was eluted with 80% alcohol, mixed with pure radioactive 2,4-D and

rechromatographed. Only one spot of radioactivity was found in every case using two different solvent systems. Two other radioactive compounds were found in the 80% alcohol extract of the stems. The R_f values of Unknown 1 in phenol and in butanol-propionic acid-water were respectively .55-.59 and .29-.32. The R_f values of Unknown 2 were respectively .32-.35 and .66-.73 in the two solvent systems. In a few instances, the R_f values were found to differ by as much as 0.1 R_f units from the values reported above, but the relative positions of the three compounds were found to be approximately the same in any case.

In the experiment using carboxyl C^{14} labelled 2,4-D, the leaves were set aside and dried in the open at room temperature. Later, these leaves were homogenized and extracted with 80% alcohol. In the experiment using α methylene C^{14} labelled 2,4-D, the leaves were homogenized and extracted immediately following their removal from the plant. Chromatographic data of these extracts indicated that only two radioactive compounds were definitely present in the methylene C^{14} labelled 2,4-D treated leaves, whereas at least four radioactive compounds were present in the leaf extracts from the plants treated with carboxyl C^{14} labelled 2,4-D. The two major radioactive compounds in any case were found

to be free 2,4-D and Unknown 1. It should be mentioned, that the leaves obtained in the fourth week after treatment with α methylene C^{14} labelled 2,4-D were inadvertently dried before extraction. In this case two additional radioactive compounds were found with R_f values of 0.06 and 0.55 using butanol-propionic acid-water as the developing solvent. These two compounds represented two and five percent of the total radioactivity of the extract. Since it appears that more compounds were formed in the leaves which were dried before extraction, only the chromatographic data obtained from fresh leaves will be reported, with the exception of the four week extract of the α methylene C^{14} labelled 2,4-D treated leaves.

The percentage of the total radioactivity in the 80% alcohol extracts which each compound represents and their changes with time are presented in Tables II and IIa.

It is apparent from Tables II and IIa, that free 2,4-D may be found in relatively large concentration (5.4 ug/gm. fresh stem material) twenty-four hours after treatment, but this concentration drops quite rapidly reaching a minimum concentration in 28 days and 14 days with the two separate experiments. Perhaps the difference in the time taken to attain a minimum concentration of 2,4-D in the two experiments can be

TABLE II

Percentage of Total Radioactivity Represented By the Major Compounds Found in the 80% Alcohol Extract of Bean Stems and Bean Leaves from Plants Treated with 50 ug. of C^{14} Labelled 2,4-D.

Treatment	∝ methylene C^{14} labelled 2,4-D				
Days Elapsed After Treatment	1	4	7	14	28
Number of plants	6	6	6	6	5
Fresh wt. of stem material (gms)	7.89	12.38	12.98	13.89	12.72
Conc. of 2,4-D per gm. fresh stem (ug.)	5.0	1.2	0.44	0.16	0.44
Dist. (%) of radioactive cmpds in extracts					
1. Stem extracts					
a. 2,4-D	79	24	10.5	4.8	17
b. Unknown 1	19	60	61	74	56
c. Unknown 2	0	5.7	14	13	4.5
2. Leaf extracts					
a. 2,4-D	73	54	40	25	32*
b. Unknown 1	25	46	60	73	61*
Total radioactivity (cpm x 10^3)					
a. Stem extracts	54.2	64.5	59.3	50.3	36.1
b. Stem residues	1.1	4.4	9.4	7.1	8.9
c. Leaf extracts	63.6	34.1	27.8	21.4	18.1
d. Leaf residues	5.6	1.5	2.8	2.1	2.9
Total radioactive recovery (%)	99	83	79	64	62**

* Extract of dried leaves.

** Value corrected for loss of one plant.

TABLE IIa

Percentage of Total Radioactivity Represented By the Major Compounds Found in the 80% Alcohol Extract of Bean Stems and Bean Leaves from Plants Treated with 50 ug. of C^{14} Labelled 2,4-D.

Treatment	carboxyl C^{14} labelled 2,4-D					
Days Elapsed After Treatment	1	4	7	14	28	42
Number of Plants	5	5	5	5	5	4
Fresh wt. of stem material (gms)	7.42	10.45	11.98	12.74	15.13	13.53
Conc. of 2,4-D per gm.fresh stem(ug.)	5.4	2.4	1.1	0.82	0.08	0.71
Dist.(%) of radioactive cmpds in extracts						
1. Stem extracts						
a. 2,4-D	81	40	25	18	3	23
b. Unknown 1	18	59	66	67	68	55
c. Unknown 2	0	0	2.5	8.7	14	8.9
2. Leaf extracts						
a. 2,4-D	-	-	-	-	-	-
b. Unknown 1	-	-	-	-	-	-
Total radioactivity (cpm x 10^3)						
a. Stem extracts	495	603	556	579	410	373
b. Stem residues	10.6	34.1	58.5	101	92.5	87.6
c. Leaf extracts	625	438	310	209	270	135
d. Leaf residues	34.7	41.1	34.4	30.0	28.8	43.3
Total radioactive recovery (%)	88	84	73	70	61	60**

* Extract of dried leaves.
** Value corrected for loss of one plant.

explained by the fact that the experiment using carboxyl C^{14} labelled 2,4-D was conducted during the months of February and March while the other using methylene C^{14} labelled 2,4-D was carried out during April and May. The increased temperatures and total daylight hours in the later months could account for a more rapid absorption and translocation of 2,4-D. Rice has shown that the rate of absorption of 2,4-D is influenced by light (14, pp.307-308). It has also been demonstrated that the translocation of 2,4-D is related to photosynthesis (12, p.395; 11, pp.631-632; 17, p.516).

The results as plotted in Figure 1 and Figure 2 indicate a definite relationship between 2,4-D and Unknown 1. Although the data do not present an exactly quantitative relationship, the trend in both experiments is unmistakable. These results appear to agree favorably with the result recently published by Holley (10, pp.171-175). The inability to give a true quantitative representation of the radioactive compounds is caused by the presence of small areas of very low activity along the strips which may be caused by the formation of new compounds, tailing of the major radioactive constituents or incomplete resolution of these compounds. In the later stages of the experiments more compounds are definitely being formed, however difficulty is met in

Figure 1

Percent distribution of 80% alcohol soluble radioactive compounds present in the stems of bean plants treated with 50 ug of C^{14} carboxyl labelled 2,4-D. Plants harvested after varying intervals of time.

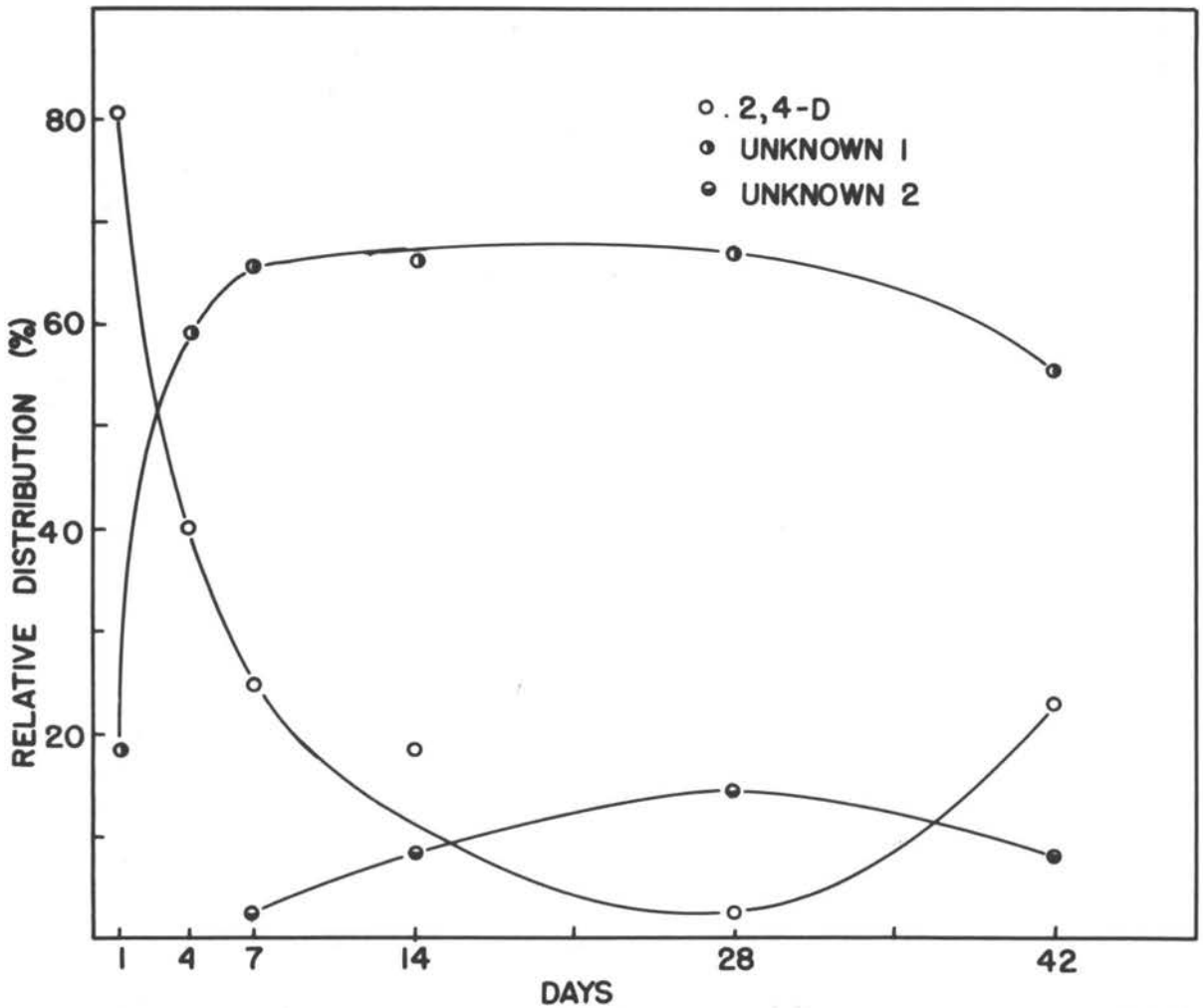
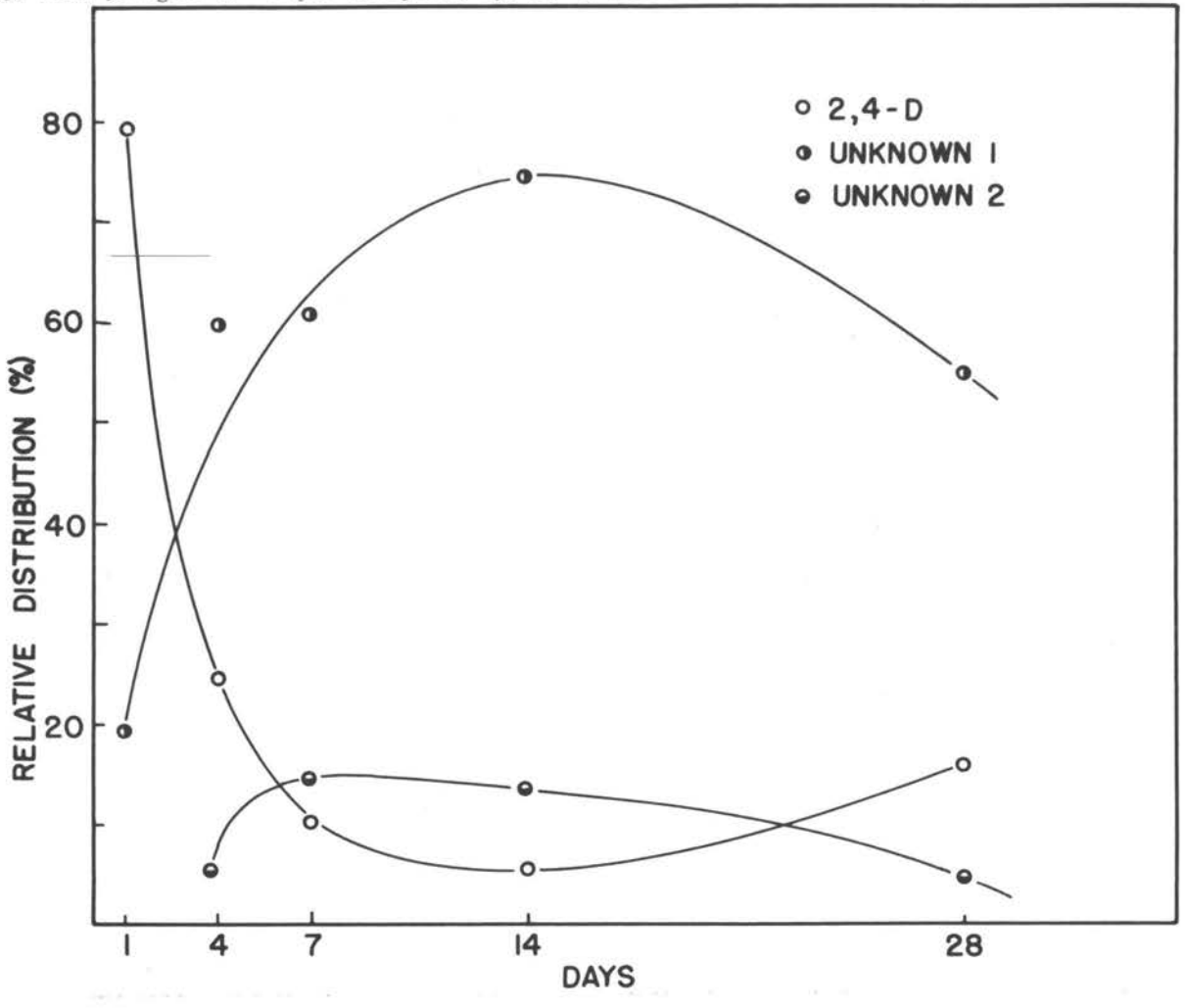


Figure 2

Percent distribution of 80% alcohol soluble radioactive compounds present in the stems of bean plants treated with 50 ug of C^{14} alpha methylene 2,4-D. Plants harvested after varying intervals of time.



attempting to designate a particular R_f value for them because of the low radioactivity and diffuse nature of these areas. It would appear that 2,4-D is converted to Unknown 1.

The data concerning the stem tissue as presented in Tables II and IIa, have reference to total stem tissue. In a separate experiment, thirty bean plants were treated as previously described and harvested five days later. The plants were sectioned into terminal buds, petioles, first internode, hypocotyl and roots. The sections of each plant were pooled and extracted with 80% ethanol. The chromatographic data of these extracts are shown in Table III. It suggests that either Unknown 1 can be formed anywhere within the plant or that a rapid relocation of Unknown 1 takes place after its formation at a given sight.

A comparison of the chromatographic data of the leaf extracts with that of the stem extracts shown in Table II indicates that the same reaction is operative in both leaf and stem tissue; namely, a direct conversion of 2,4-D to Unknown 1. That there is not simply a transport of Unknown 1 from the leaves to the stem is evidenced by the fact that although the total activity of the stem extract increases from 54,200 to 64,500 cpm. over a period from one to four days, the activity

TABLE III

The Percentage Distribution of Major Radioactive Compounds Found in 80% Ethanol Extracts of Bean Plants Treated With Carboxyl C^{14} Labelled 2,4-D. Plants Harvested Five Days After Treatment.

	Percentage of Total Activity				
Compound	Term. Buds	Petioles	First Internode	Hypocotyl	Root
2,4-D	11	9	12	30	19
Unknown 1	69	68	70	52	54
Unknown 2	3	13	10	4	5

represented by Unknown 1 in the same extract increases from 10,300 to 38,700 cpm. over this period. It may also be noted, that although the amount of Unknown 1 in the leaf extracts increases progressively with time on a percentage basis, the actual concentration of Unknown 1 based on radioactivity in counts per minute remains approximately constant with the exception of the four week sample. This would indicate that either the leaves have a limited capacity to form Unknown 1 or an equilibrium is established between its formation and metabolism. The possibility that some Unknown 1 is translocated cannot be entirely ruled out.

The identity of Unknown 1 has not been established. It is water soluble and ether insoluble, and would appear to be the same as one of the compounds reported by Holley et al. (9, p.150). Unknown 1 has been hydrolyzed with 2N HCl, takadiastase, and emulsin. Chromatographic analysis of the hydrolysates indicated that the decrease in concentration of Unknown 1 was accompanied by a corresponding increase in the concentration of 2,4-D. This would suggest that Unknown 1 may be a glycoside containing 2,4-D as the aglycon moeity and may arise through the detoxification of 2,4-D. Holley (10, p.174) states that the radioactive fragment from a HCl hydrolysate of Unknown 1 is not 2,4-D but some compound related to 2,4-D, as for example, a hydroxy derivative. The chromatographic techniques and the solvents used in the work presented here did not suggest this possibility. The answer to this problem must await further experimentation, however, attempted acetylation of the hydrolysate with acetic anhydride and pyridine followed by subsequent chromatographic analysis disclosed that the acetylation mixture and 2,4-D had the same R_f value.

The drop in the concentration of Unknown 1 and the increase in the concentration of 2,4-D in the last group of plants in both experiments would indicate a reversal of the suggested mechanism. The reason for such a reversal is difficult

to explain, but it could result through the inability of the plant to maintain the detoxification process after a certain degree of injury was incurred by the plant.

It is of interest to note that in the four week leaf extract of ∝ methylene C^{14} labelled 2,4-D treated plants, a decrease in the concentration of Unknown 1 and an increase in the concentration of 2,4-D occurred similar to that in the stem extract. It is not likely that this was the result of drying the leaves before extraction since the stems were extracted while fresh.

No information is available at present regarding the identity of Unknown 2. It is water soluble and ether insoluble.

Evidence indicating the liberation of radioactive CO_2 from bean plants when treated with carboxyl C^{14} labelled 2,4-D has been obtained by Holley et al. (9, p.149) and Fang et al. (5, pp.253-254). This would suggest several possibilities, two of which are worth while considering. One is that the carboxyl group of 2,4-D is directly removed. The other is that 2,4-D is cleaved at the ether linkage and the two carbon fragment formed is ultimately broken down to give off CO_2. It is not likely from the results presented in this thesis that the former possibility is valid, since, if it were true, one would not expect to find the same radioactive

compounds using carboxyl labelled 2,4-D for treatment in one case and α methylene C^{14} labelled 2,4-D in the other.

The balance sheet of radioactivity in Tables II and IIa indicates considerable loss of radioactivity over the period from one to twenty-eight days. Since the roots were not retained and since the soil in which the plants were grown was not analyzed for radioactivity, an exact accounting cannot be given for the activity not recovered. A consideration of the results obtained by Fang et al. (5, pp.252-253) would indicate that a small percentage of the activity was present in the roots. The main loss was probably due to the metabolism of 2,4-D resulting in the liberation of $C^{14}O_2$. Fang et al. (5, p. 254) state that in a period of three days after application of radioactive 2,4-D to bean plants, 17.5% of the 2,4-D applied was recovered as $C^{14}O_2$ in the atmosphere. The percent recovery levels off at the later stages of the experiments and is interpreted to indicate a general reduction and/or destruction of the plants metabolic activity.

Finally, it is noted that the total activities of alcohol insoluble stem residues increase rather rapidly in the first week or two followed by a decrease in the following weeks. The increase probably results during the proliferation of the stem tissue which reaches a

maximum in 72 to 96 hours after treatment. Later on a decrease would be expected on the basis of the dynamic state of metabolism; that is, the equilibrium between synthesis and hydrolysis would result in an exchange between a particular radioactive compound and its non-radioactive form causing in effect a decrease in radio-activity though not necessarily a decrease in the concentration of the compound. These activities amount to a rather small percentage of the total activity applied (approx. 8%) and could result through the incorporation of $C^{14}O_2$ and/or a two carbon fragment (resulting from the cleavage of 2,4-D at the ether linkage) into various metabolic systems.

II. THE METABOLISM OF 2,4-D ADMINISTERED THROUGH THE ROOTS OF BEAN PLANTS

A. EXPERIMENTAL PROCEDURE

Treatment of Bean Plants

Bean plants were grown in sand filled paper cups under greenhouse conditions. Thirty ml. of Hoagland's standard nutrient solution (see appendix) was supplied to each cup every other day. When the primary leaves of the plants were fully unfolded, they were removed from the cups. The root system was carefully washed free of sand on each plant and the plant was then placed in a 125 ml. Erlenmyer flask containing 25 ml. of a 4 p.p.m. 2,4-D (carboxyl C^{14} labelled) solution. The plants were randomized and removed from the flasks in groups of five after 1, 4, 10, 24, and 72 hours.

The roots of each plant were dipped in a beaker of distilled water and then sprayed with a wash bottle to remove as much as possible of the externally remaining 2,4-D solution. The plants were sectioned into leaves, petioles, first internode, hypocotyl, roots, and terminal buds, and the sections in each group were then pooled,

homogenized with 80% ethanol in a Waring blendor for one minute, and transferred quantitatively to volumetric flasks.

Radioactivity Measurements

The extracts were wet plated as described in part I and counted in a Tracerlab "Windowless Gas Flow Counter".

All chromatographs of the extracts were also counted in the above mentioned counter.

Chromatographic analysis was carried out as previously described.

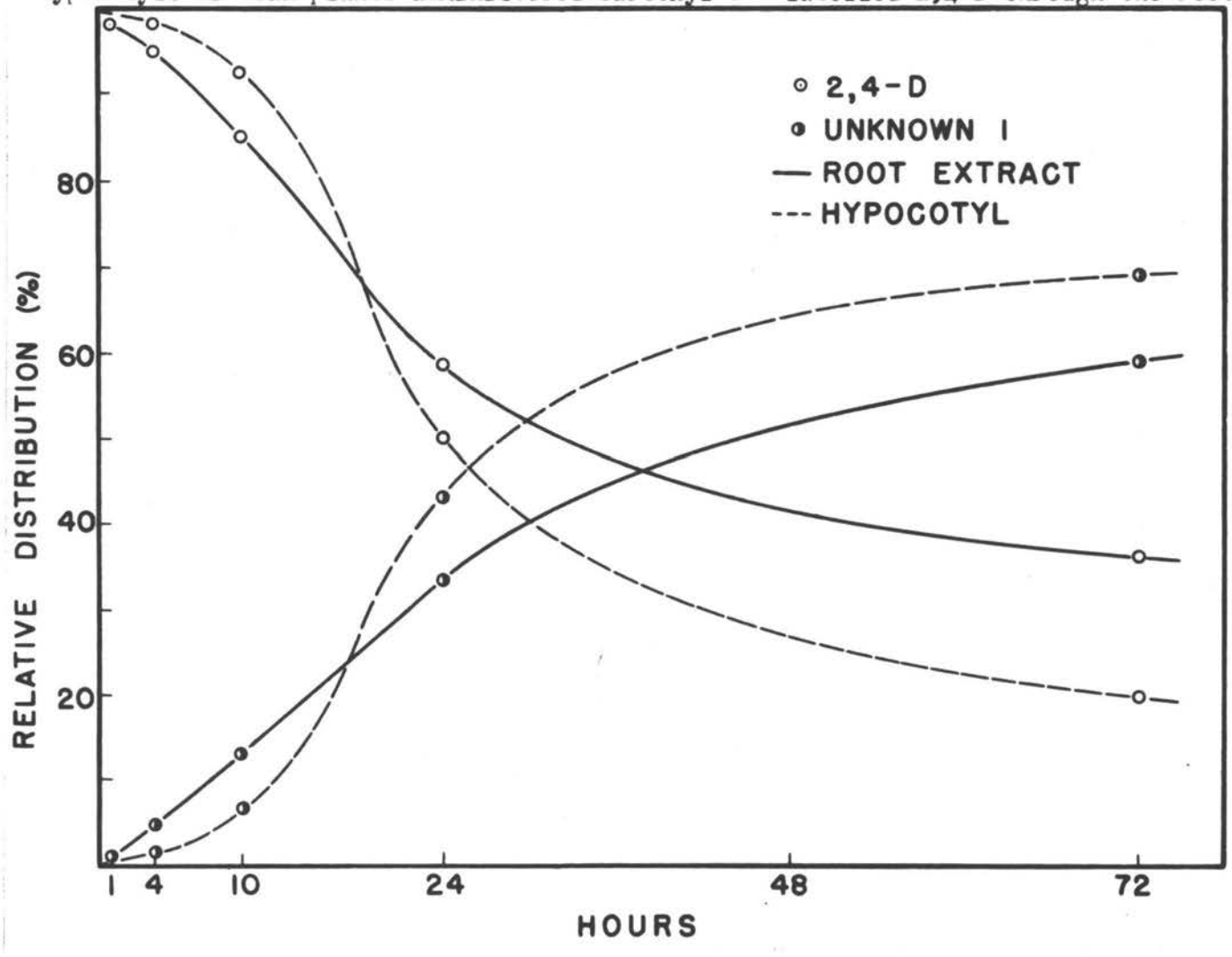
B. RESULTS AND DISCUSSION

No effort was made to make a careful balance sheet of the radioactivity in this experiment since the chief interest lay in determining whether 2,4-D would be metabolized in a manner similar to that observed when the 2,4-D was applied on the bean leaves.

It may be seen from Figure 3 that the relationship between 2,4-D and Unknown 1 in the roots and the hypocotyls is very similar to that demonstrated in the previous experiments,and it would appear that the hypocotyl is somewhat more active than the root in converting 2,4-D to Unknown 1. Chromatographic analysis of the remaining parts of the bean plants was not feasible

Figure 3

Percent distribution of 80% alcohol soluble radioactive compounds present in roots and hypocotyls of bean plants administered carboxyl C^{14} labelled 2,4-D through the roots.



because of the low radioactivity found in their extracts.

Unknown 2 which was not indicated in Figure 3 was formed in the roots after 24 hours and 3 days to the extent of 3% and 2.3% respectively. It was found in the hypocotyl after 3 days to the extent of 3%.

It is impossible to say whether the conversion of 2,4-D to Unknown 1 took place in the xylem or in the phloem.

The data in Table IV demonstrates the rapidity of movement of 2,4-D when given through the roots of bean plants. Although the movement was very rapid, the total amount of radioactive material moved to the upper portions of the plants was very small. This observation is contrary to results obtained by others (8, p.466; 4,p.199) and may be explained by the fact that during the period of the experiment, the weather was dark and rainy. This type of situation would be conducive to poor transpiration in plants. As mentioned by Weintraub and Brown (18, p.141), as well as Weaver and DeRose (17, p.516), weather conditions can influence to a considerable extent the amount of absorption and translocation of plant growth substances.

It may be seen that in general the activity of aerial parts of the plants rose with time while that of the roots progressively decreased with the exception of

TABLE IV

Distribution of radioactivity in 80% Ethanol Extracts of Bean Plants Following their Immersion in 25 ml. of a 4 ppm. Carboxyl C^{14} Labelled 2,4-D** Solution for Varying Intervals of Time. Five Plants used in Each Time Interval.

Plant Part	Time Hours				
	1	4	10	24	72
Root	455,000*	315,000	242,000	238,200	680,000
Hypocotyl	8,900	112,000	206,700	152,550	224,900
1st Internode	0	600	3,450	5,400	3,300
Petioles	0	100	400	850	500
Leaves	100	500	800	7,700	2,000
Terminal buds	0	20	30	260	160

* Data in counts per minute.

** Total radioactively available to plant in 25 ml. of solution was 4,850,000 cpm.

the 72 hour group. These data indicate that the movement of the growth substance from the roots to the aerial parts of the plant is much more rapid than the diffusion of the growth substance from the solution into the roots. The 72 hour discrepancy may have resulted from incomplete washing of the roots, however even this does not explain why the activities of the aerial parts should be less than at the 24 hour stage. It is not unlikely that

variation in the plants themselves, particularly with respect to root development, even though they were selected for uniformity and randomized, could bring about such a result.

III. THE METABOLISM OF 2,4-D IN ETIOLATED AND CARBOHYDRATE DEPLETED BEAN PLANTS

A. EXPERIMENTAL

Treatment of Bean Plants

Fifteen bean plants were grown in potted soil in a dark box for a period of twelve days, and a second group of 8 plants was grown under normal greenhouse conditions for the same length of time. The plants grown under normal conditions were fairly small, however the primary leaves were fully unfolded. The etiolated plants had extremely elongated stems, and their primary leaves were underdeveloped.

Five plants were selected at random to comprise a group for each of the first three treatments. Four plants were selected for treatments D and E.

Treatment A. Etiolated plants were treated with 20 ug. 2,4-D (carboxyl C^{14}) per plant on the primary leaves and kept in the dark for 4 days following treatment.

Treatment B. Etiolated plants were treated with 20 ug. 2,4-D (carboxyl C^{14}) per plant on the primary leaves, the leaves were then immersed in 1% glucose solution, and the plants kept in the dark for 4 days following treatment.

Treatment C. Etiolated plants were treated with 20 ug. 2,4-D (carboxyl C^{14}) per plant on the primary leaves and then subjected to normal light conditions for 4 days following treatment.

Treatment D. Plants grown under normal greenhouse conditions were placed in the dark for 48 hours to deplete them of carbohydrate. They were then treated on their primary leaves with 25 ug. 2,4-D (carboxyl C^{14}) per plant and harvested 4 days after treatment.

Treatment E. Plants grown under normal greenhouse conditions were treated on their primary leaves with 20 ug. 2,4-D (carboxyl C^{14}) per plant and harvested 4 days after treatment.

All treatments were carried out at night under diffuse red light. The treatment B was carried out by scarifying the leaf tips slightly with sand and then immersing them in 20 ml. beakers containing the glucose solution. The proper immersion of the leaves was obtained by scotch-taping the stems to the supports holding the beakers of glucose solution.

Four days after treatment, each group of plants was harvested by cutting the plants off level with the soil in their respective pots. The plants were sectioned into leaves and stems (including the petioles and terminal buds), homogenized in 80% ethanol for one minute and transferred quantitatively to suitable volumetric flasks.

Radioactivity Measurements

The extracts and chromatographs were prepared and counted in the manner previously described. The radioactivity of 100 ug. of 2,4-D (carboxyl C^{14}) as determined by the wet plating method in a 64 scaler was found to be 167,500 cpm.

B. RESULTS AND DISCUSSION

The greatest recovery of radioactivity as may be seen in Table V was obtained in Treatment A, as could be expected due to a reduced metabolic rate resulting from the lack of carbohydrates. The recovery appears to be greater than that applied, however it is within the experimental error of the method of application (2%). The data in Table V demonstrates that 2,4-D is translocated even in completely etiolated plants, although in such small concentration as to be unable to elicit a typical physiological response. That the material is

TABLE V

Radioactivity* of the 80% Ethanol Extracts of Bean Stems and Bean Leaves from Etiolated and Depleted Plants Treated with 20 ug. of Carboxyl C^{14} Labelled 2,4-D per Plant.
Five Plants in Treatments A, B, and C, and Four Plants In Treatments D and E Harvested 4 Days After Treatment.

Treatment	Leaves	Stems	Total	% Activity In Stems
A	166,600	1,500	168,100	0.9
B **	31,500	5,500	37,000	14.9
C	157,700	3,000	160,700	1.9
D ***	152,800	7,400	160,200	4.6
E	81,200	48,400	129,600	37.4

* Radioactivity expressed in counts per minute.

** One percent glucose solution supplied by dipping leaves in beaker of the solution following treatment with 2,4-D.

*** Plants placed in dark for 48 hours prior to treatment with 25 ug. 2,4-D per plant. The application of 25 ug. in treatment D was accidental.

in fact free 2,4-D has been demonstrated chromatographically as shown in Table VI.

It is apparent from Table V that both light and carbohydrate increase the translocation of 2,4-D. Glucose had a particularly strong effect even though a considerable portion of the 2,4-D applied was washed off by the glucose solution into which the leaves were dipped. As may be seen in the last column of Table V, on a percent basis it has by far the greatest effect on the translocation of the 2,4-D.

The data in Table VI suggest that both light and glucose are effective in increasing the rate of metabolism of 2,4-D both in the leaves and stems of bean plants. The lag in metabolism in the stems of treatment C as compared with treatment B was probably caused by the fact that the plants in treatment C first had to develop their chlorophyll and then carry out photosynthesis before they had a source of carbohydrate available whereas the plants in treatment B were furnished preformed and in large concentration the needed carbohydrate.

A comparison of the data from treatments D and E in Table VI demonstrates the rapidity with which carbohydrate depletion must occur in plants kept in the dark. The data again demonstrate the influence of carbohydrate on the formation of Unknown 1. The fact that in

TABLE VI

Percentage of Total Radioactivity Represented By the Major Compounds Found in the 80% Ethanol Extracts of Bean Stems and Bean Leaves From Etiolated and Depleted Plants Treated with 20 ug. of Carboxyl C^{14} Labelled 2,4-D per Plant.

Treatment	Leaf Extracts		Stem Extracts	
	2,4-D	Unknown 1	2,4-D	Unknown 1
A	85.5	13.2	100	0
B	62.5	35.4	57.6	37.8
C	40.5	47.4	85	15
D	82	17	39	54
E	54	43	20	77

treatment D the concentration of Unknown 1 in the leaves was much less than in the stems may be explained on the basis that during depletion, carbohydrates are rapidly translocated from the leaves to the stem of the plant. Thus the leaves would contain less soluble carbohydrate material than the stems following depletion and would therefore have a reduced capacity for metabolizing the 2,4-D. That soluble carbohydrates are needed for the reaction has already been demonstrated.

The influence of light upon the formation of Unknown 1 indicates that photosynthesis is required for

the formation, however, in view of the other data it would appear to be required only insofar as it supplies the carbohydrate and not some photosynthetic intermediate.

It is difficult to judge whether it is carbohydrate metabolism or the carbohydrate per se which is essential for the formation of Unknown 1 from 2,4-D.

SUMMARY

Three major radioactive compounds have been found in 80% alcohol extracts of bean stems from plants treated with either ∝methylene or carboxyl C^{14} labelled 2,4-D. One of the compounds was identified as 2,4-D.

The R_f values of these compounds were respectively: .76-.78 and .83-.86 for 2,4-D, .55-.59 and .29-.32 for Unknown 1, and .32-.35 and .66-.73 for Unknown 2 when chromatographs containing the extracts were developed with phenol saturated with water and butanol-propionic acid-water. Free 2,4-D and Unknown 1 were also found in the 80% alcohol extracts of leaves from bean plants treated with ∝methylene C^{14} labelled 2,4-D.

The concentration of 2,4-D in the stem extracts decreases to a minimum and then rises at a later date. A corresponding increase occurs in the concentration of Unknown 1. A drop in its concentration occurs at the time the concentration of 2,4-D begins to increase. A similar relationship is observed in the leaf extracts. The close relationship between Unknown 1 and 2,4-D, as well as the large quantity of Unknown 1 formed, and the relatively great stability of Unknown 1, suggests that a detoxification reaction may be operative in the plant. Unknown 1 may be a glycoside containing 2,4-D as the aglycon group.

The formation of Unknown 1 at the expense of 2,4-D has been demonstrated when the 2,4-D was administered through the root system. Although present evidence indicates that movement of 2,4-D takes place through the phloem when treatment is made on the leaves and through the xylem when treatment is made at the roots, it is not possible to ascertain in which tissue the actual conversion of 2,4-D to Unknown 1 takes place.

Both light and glucose were effective not only in increasing the amount of translocation of 2,4-D from leaves to stems but also in increasing the amount of Unknown 1 formed. Glucose was particularly effective in this respect. The data suggest that the formation of Unknown 1 is dependent upon glucose per se or at least on the metabolism of carbohydrates.

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APPENDIX

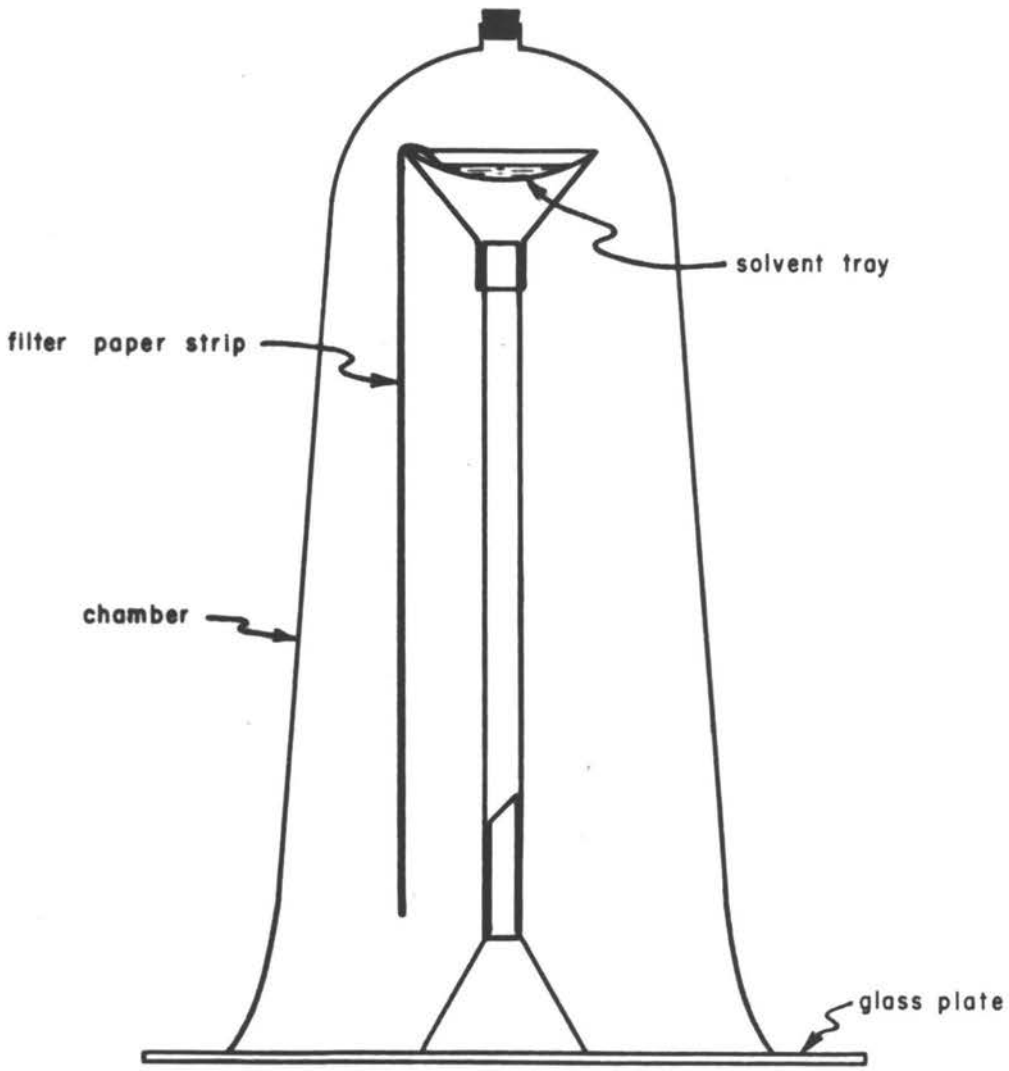
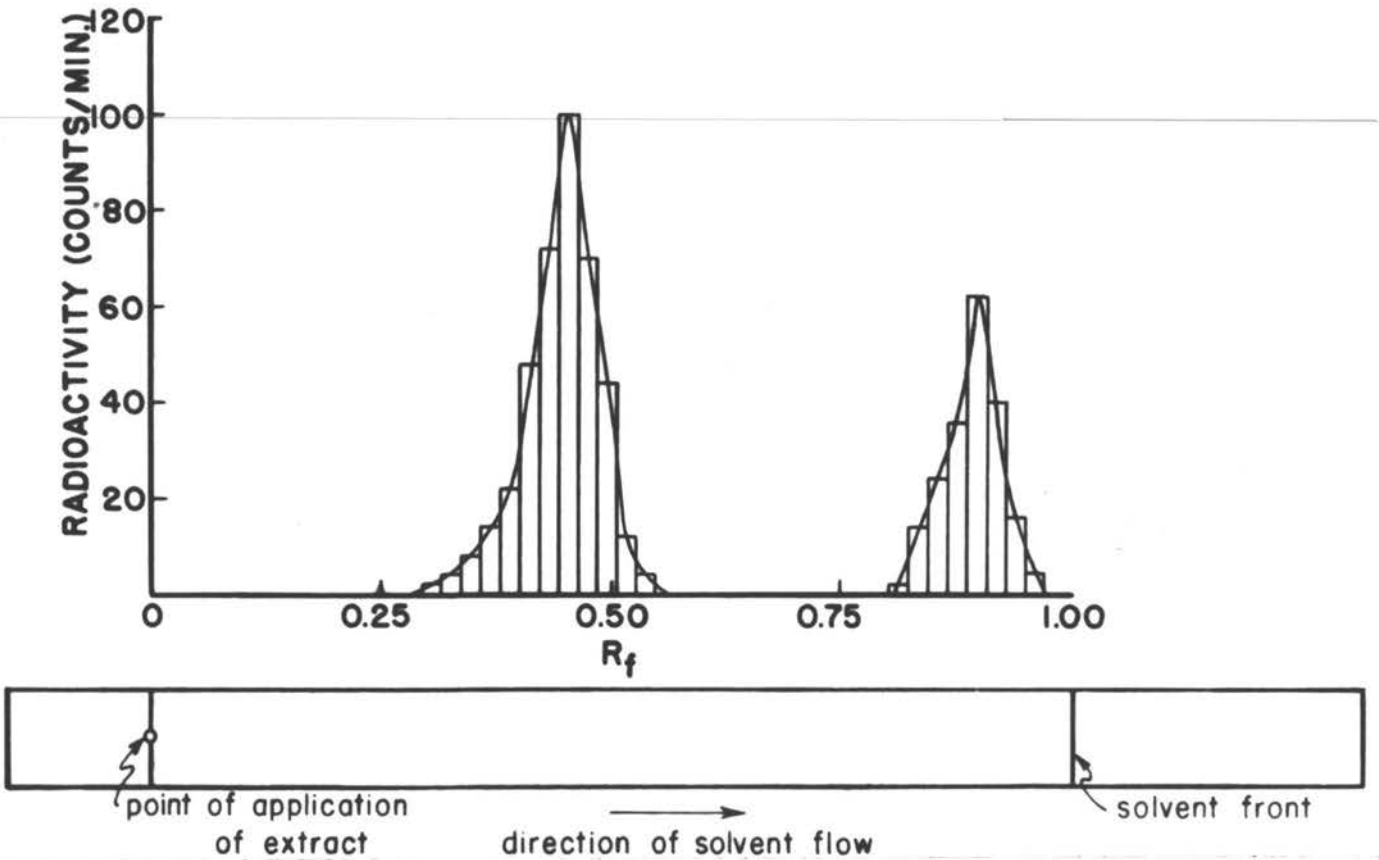


Figure 4. Paper Chromatography Apparatus

Figure 5

TYPICAL CHROMATOGRAPH WITH GRAPH ILLUSTRATING

THE DISTRIBUTION OF RADIOACTIVE COMPOUNDS



Hoagland's Nutrient Solution

Ions	P.p.m.	Ions	P.p.m.
K	190	PO_4	117
Ca	172	NO_3	700
Mg	52	SO_4	202