STUDIES ON TRANSLOCATION

Thesis by

Vernon Douglas Burrows

In Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1958

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. James Bonner for suggesting the problem and for his helpful advice and encouragement throughout this study. I wish to also thank Dr. F. W. Went for his friendliness, constructive criticism and for the privilege of conducting the experiments in the Earhart Plant Research Laboratory.

I wish to express my gratitude to (a) Dr. S. Epstein and his staff of the Geology Department for making the O^{18} determinations reported in this study, (b) Dr. James Mead of the United States Atomic Energy Commission, Los Angeles, California, for allowing me to use his facilities for tritium determinations, and (c) Dr. S. C. Fang of the Department of Agricultural Chemistry, Oregon State College, Corvallis, Oregon, for providing the C^{14}-labeled 2,4,6-T used in this study.

The staff of the Earhart Laboratory will always be remembered for their kindness and willingness to cooperate in all phases of the work.

I also wish to thank the Division of Biology and the Institute for the financial support and research freedom. Without the financial aid of Institute tuition scholarships, a Lucy Mason Clark fellowship, and Arthur McCallum summer fellowships, it would not have been possible for me to continue graduate work.

Finally, the author wishes to express his sincere appreciation to his wife, Elizabeth, for her able assistance in preparation of the manuscript and for her constant encouragement.

ABSTRACT

Studies have been made on the translocation of C^{14}-labeled solutes (2,4-D, 2,4,6-T and sugar (s)) and labeled solvent (THO or H_2O^{18}) in the red kidney bean.

Transport of 2,4-D can be controlled by regulating the supply of carbohydrate in the leaves. For the first six hours following treatment of leaves with 2,4-D, the amount of 2,4-D transported to the epicotyl increases linearly with time. Over short time intervals the amount of 2,4-D transported is linearly related to the concentration applied. Over longer time intervals high concentrations of 2,4-D tend to depress transport somewhat. Transport of 2,4-D does not however saturate at concentrations which saturate the growth process of the plant.

Essentially the same amount of 2,4-D is transported to the epicotyl of plants grown under 1000 and 2000 f.c. of light. Growth of the epicotyls induced by equivalent amounts of 2,4-D is two to four times larger in plants grown under 2000 than in those grown under 1000 f.c. of light, however.

The compounds 2,4-D and 2,4,6-T are equally well absorbed by bean leaves and travel at the same speed in the phloem. The amount of 2,4,6-T which enters the phloem of the leaf, per unit time, is less than the amount of 2,4-D which so enters.

TIBA applied as a pre-treatment to petioles inhibits the transport of C^{14}-labeled 2,4-D, 2,4,6-T and sugars (predominantly sucrose). The inhibition of sugar movement may be used to interpret the inhibitory effect of TIBA on 2,4-D and 2,4,6-T transport.

Foliarly applied tritium labeled (THO) and O^{18}-labeled (H_2O^{18}) water are transported downward in bean seedlings. The carbohydrate status of the leaf does not govern the transport of labeled water in the same manner as it governs 2,4-D transport. The transport of THO takes place equally well or better in girdled as in normal plants. Movement of tritium apparently takes place in the xylem rather than in the phloem.

GLOSSARY OF SYMBOLS

2,4-D - 2,4-dichlorophenoxyacetic acid

2,4,6-T - 2,4,6-trichlorophenoxyacetic acid

TIBA - triiodobenzoic acid

THO - tritium labeled water

H_2O^{18} - oxygen labeled water

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

FIGURE PAGE

TABLE OF CONTENTS

INTRODUCTION AND REVIEW OF LITERATURE

The physiological process of translocation is of importance to higher plants throughout their entire development. The life cycle may be interrupted by periods of dormancy, but during all periods of metabolic activity, the translocation process functions to distribute substrates and chemical stimuli from regions of synthesis or storage to regions of utilization. Translocation provides a means of integrating and modifying the activities of all plant organs.

In spite of the large amount of effort devoted by numerous investigators to the study of this process, no single hypothesis has been proposed which explains, to the satisfaction of all plant physiologists, the mechanism of solute translocation in phloem tissue. The most controversial issue is whether the sieve tube plays a passive or active role in the transport of solute. As evidence of this controversy, at least four major hypotheses have been proposed.

Mass or pressure flow hypothesis

The mass or pressure flow hypothesis described by Münch (41) describes movement as a passive process in which solvent and solute move simultaneously along a pressure gradient from cells of high turgor pressure to cells of lower turgor pressure. Synthesis of sugars in the leaf causes an

increase in the osmotic pressure of the parenchyma cells. In response to the osmotic gradient, water from the xylem flows to these cells and their turgor pressure increases. As this continues, solution is pressed out of the leaf cells via the plasmodesmata and finally reaches the sieve tubes. Driven by the hydrostatic pressure gradient, solution passes through the sieve tubes to regions of sugar utilization and storage. Once the osmotic pressure of the sieve tube contents falls below that of the receiving cells, water leaves the sieve tubes and is diverted back into the xylem.

Crafts (13) has modified the original Münch hypothesis to make it conform to modern knowledge of anatomy and physiology. Münch pictured movement of solution through the open pores in the sieve plates, but these pores have since been shown to be overlain and filled with cytoplasm. Crafts believes the cytoplasm to be sufficiently permeable to allow the passage of solution. He also stresses the importance of metabolic activities of the parenchymatous cells throughout the plant in controlling the introduction and withdrawal of osmotically active material from the sieve elements.

Protoplasmic streaming hypothesis

De Vries (17) was first to use the phenomenon of protoplasmic streaming as an explanation for the rapid movement of solutes. In more recent times, Curtis (15) has been the

main advocate of this hypothesis. He defined the term protoplasmic streaming to encompass a mass movement of granular material, less granular hyaline layers, invisible mono- and polymolecular layers, and more or less fluid parts not ordinarily visible. Solute was thought to be carried from one end of the sieve tube to the other by the streaming cytoplasm and then to move through the pores of sieve plates either in the cytoplasm or by diffusion. This hypothesis is frequently referred to as the cyclosis diffusion theory.

Translocation along protoplasmic interfaces

Van den Honert (55), in 1932, described a model system which demonstrates that transport of potassium oleate along a water-ether interface is a very rapid process. The speed was approximately 68,000 times faster than diffusion and agrees well with Mason's and Maskell's figure for the velocity of carbohydrate transport in the cotton plant (44,000 x diffusion). The motive force is the concentration gradient of the substance being transported. It is inferred from the model that solutes, in vivo, move at certain interfaces in the sieve tube.

Activated diffusion or activated transfer hypothesis

Several investigators (28,37) have placed special emphasis on the absolute requirement of living phloem for transport. They believe that the experimental data demonstrate that the sieve tube participates in solute transport

by the expenditure of metabolic energy. The manner in which sugar is converted into a form of energy capable of providing the motive force for transport of solutes is unknown.

Some of the pertinent literature on various aspects of the translocation process will be reviewed.

Role of carbohydrates in phloem translocation

A positive correlation exists between the transport of carbohydrates and the transport of a wide variety of different compounds including certain viruses (3,4), flower inducing hormones (10,51), P^{32} (1), Co^{60} (20), and various growth regulators (58).

The systemic herbicide 2,4-D has played a major role in the establishment of this relationship. Mitchell and Brown (39) first reported that 2,4-D is absorbed but not translocated from destarched bean leaves kept in darkness. Exposure of treated plants to light induces movement of 2,4-D from the leaf to other regions of the plant (58). Light in the absence of CO_2 is ineffective (58), but, in the presence of CO_2, the amount of 2,4-D transported is increased as light intensity is increased up to 900 f.c.(49). Removal of part of a treated leaf results in a reduction in the amount of transport. All this information points to the importance of photosynthate in the translocation process.

The effect of light in affecting translocation through the synthesis of sugars was tested directly by Rohrbaugh and Rice (50). They found that applied sugar is a suitable replacement for light in inducing 2,4-D transport in de-starched plants. Several sugars (glucose, fructose, sucrose, maltose, lactose and galactose) are effective in inducing transport (50, 58), but two (arabinose and mannitol) are ineffective (23). It is possible that some of these sugars are converted into other sugars which provide the motive force for 2,4-D transport. The failure of arabinose and mannitol to induce 2,4-D transport may be due to the lack of such a chemical conversion.

Further evidence of the direct relation between sugar movement and 2,4-D movement is furnished by the work of Gauch and Dugger (19) and of Mitchell et al. (40). Boric acid increases rate of sugar (19) and of 2,4-D movement (40). The manner in which boron brings about this effect is unknown but a recent speculation by Dugger et al. (18) is of interest. They suggest that boron may decrease the rate of enzymatic conversion of glucose-1-phosphate to starch. The increased steady state concentration of glucose-1-phosphate may then permit a greater rate of synthesis and hence of translocation of sucrose. Other hypotheses have been propounded to explain the role of boron in increasing the rate of sugar transport. Gauch and Dugger (19) suggest that boron enters into a sugar-borate complex which moves

through cellular membranes more readily than non-borated, non-ionizable sugar molecules. Nelson and Gorham (42) agree that boron increases the rate of absorption of sugars but present evidence which disagrees with the hypothesis that a sugar-borate complex is involved. It is conceivable that boron may play some role in the polar transport of sugar in the leaf. This phenomenon is described in the next section.

Solute concentration gradients

Phillis and Mason (47) have stated that sugar is transported from the mesophyll to the phloem tissue against a concentration gradient. They found a higher concentration of sugar in the veins of the cotton leaf than in the mesophyll. The concentration gradient may be more apparent than real as shown by the results of Went and Engelsberg (61) with the tomato plant. These workers made use of the fact that the tonoplast restricts the passage of sucrose into the vacuole and calculated a value of 14-20% for the concentration of sucrose in the cytoplasm of the mesophyll cells. This corresponds to a concentration of 15-20% sucrose in the sieve tubes. They conclude:

> ". . . it is quite possible that the sieve tube contents are pure cytoplasmic sap, and that sucrose is not concentrated as it enters the sieve tubes."

Leonard (30) observed polar transport of sugar in mature sugar beet leaves although not in younger leaves. In young leaves, sugar derived from older leaves moves out of the phloem into the mesophyll cells. As the young leaf expands the sugar concentration gradient ultimately reverses as the polar mechanism is established and as the rate of photosynthesis increases. Movement of sugar from the veins in the leaf to the rest of the plant follows a positive concentration gradient (13, 36, 41). This positive gradient determines the direction of flow in the sieve tubes. Sucrose contributes the major part of the osmotic pressure of the sieve tube sap. The transport of nitrogen also follows a positive concentration gradient but the nature of the compound(s) (residual nitrogen) which contribute to this gradient is obscure (35). The flow of solute from some leaves can be reversed experimentally by placing them in darkness. These leaves then behave as "sinks" rather than sources of carbohydrates. Bauer (2) has shown that the direction of movement of fluorescein can be controlled by regulating the diffusion pressure deficits of solutions applied to the ends of excised petioles of Pelargonium.

The transport of solute in the sieve tubes may appear to be against a concentration gradient. Loomis (33) has shown that the concentration of sucrose in the leaves of maize (0.3%) is lower than that in the stems (7-8%). The

sugar beet is another example in which the concentration of sugar in the leaf is lower than that in the root tissue which receives the sugar. Went and Engelsberg (61), however, state that the concentration of sucrose in the storage tissue of sugar beet or sugar cane never exceeds 20% and this is the upper limit for the concentration of sucrose in the cytoplasm of the mesophyll and sieve tubes of the phloem. Crafts (13) is also of the opinion that the concentration gradient in the sieve tubes of these plants is positive and that a negative gradient is located between the sieve tubes and the storage parenchyma.

Phloem exudation

The phenomenon of phloem exudation is often used to demonstrate that the motive force for translocation is pressure flow (13). When active phloem is severed, solution is exuded from the cut surface. Exudation can sometimes be demonstrated in a few, but not in all, herbaceous (12) and woody species (65) and is employed in the collection of sieve tube contents for chemical analysis. Because the volume of exudate is too large to have come from only the sieve tubes adjacent to the cut surface, those at a considerable distance must also contribute to the exudate. This argues against the contention that the protoplasmic connections in the sieve pores present a barrier to the rapid movement of solution (13).

Bidirectional movement of solutes

The simultaneous movement of compounds in opposite directions in bark has been used as evidence against the mass flow hypothesis. Chen (11) prepared willow plants with two leaves joined by a section of stem which had a portion of the bark separated from the wood. He applied P^{32} to the upper and $C^{14}O_2$ to the lower leaf or the reverse. In both cases, the bark flap was analyzed and found to contain both tracers. Similar results were obtained by Phillis and Mason (48) and taken as indicating the bidirectional movement of carbohydrates and organic nitrogen.

Bauer (2) has observed that fluorescein moves in opposite directions in individual vascular bundles of Pelargonium. He concludes that the opposite movements of fluorescein occur in different sieve tubes.

This evidence does not refute the hypothesis of mass flow. The hypothesis of mass flow allows bidirectional movement of solutes in the same vasculature but not in the same sieve tube at the same time.

Unequal rates of solute movement

Unequal rates of solute movement in the phloem have been observed and are also used as evidence against the mass flow hypothesis of translocation. Vernon and Aronoff (57) determined in soybeans the distance traveled by C^{14}-labeled sucrose, glucose and fructose 20 minutes

after administration of $C^{14}O_2$ to a trifoliate leaf. Sucrose traveled faster (84 cm./hr.) than either glucose or fructose. Biddulph and Cory (6) compared the speed of transport of $C^{14}O_2$ (sucrose), tritiated water (THO) and P^{32} in red kidney beans. Again sucrose traveled faster (86-106 cm./hr.) than either THO (66 cm./hr.) or P^{32} (60 cm./hr.). Swanson and Whitney (53) studied the ratios of $\frac{K^{42}}{P^{32}}$ and $\frac{Cs^{137}}{P^{32}}$, in the stem at various distances from the point of application on the leaves. Because the ratios changed with distance they concluded that each tracer is transported independently and at different speeds.

It is evident that any chemical contained within a sieve tube will move out into the surrounding tissue. This leakage will act to decrease the apparent rate of movement of the material. The apparent rates of movement of substances to which the sieve tube is differentially permeable will therefore be necessarily different. A more general consideration of the apparent rates of transport of materials in the sieve tube will be presented later in this thesis.

Metabolic inhibitors and phloem translocation

The dependence of translocation on the activity of living cells has been known for a long time. Mason and Phillis (37) demonstrated this relationship in cotton by showing that translocation is inhibited by anaerobic condi-

tions. They covered part of the stem with vaseline or plasticine and found the degree of inhibition to be proportional to the length of covered stem. Bauer (2) observed a similar inhibition by an atmosphere of nitrogen.

Placing sugar beet petioles in an atmosphere of carbon monoxide prevents the export of C^{14}-labeled sugars from the leaves (28).

Kendall (27) found that injection of bean petioles with dinitrophenol or sodium fluoride inhibits P^{32} transport, whereas, IAA, 2,4-D and TIBA are ineffective. Bauer (2) found that phloridzin, a compound which inhibits phosphate transfer, interferes with uranin transport if applied to the leaf of Pelargonium but not to the petiole.

Nelson and Gorham (43) found that KCN (10^{-2} M) applied to soybean leaves inhibits the transport from the leaf of applied C^{14}-labeled sucrose but not of C^{14}-labeled glucose or fructose. They concluded that sucrose travels in living tissue, whereas, glucose and fructose travel in dead tissue.

Rote of temperature in phloem translocation

Most investigators who have studied the matter agree that changes in temperature effect translocation, but there is disagreement over the sign of the response of the process. The Q10 values reported for transport vary from less than one to greater than one.

Swanson and Böhning (52), Kendall (26), and Böhning et al. (8) have shown with beans, and Böhning et al. (7)

with tomato plants, that both excessively low and excessively high temperatures retard the transport of sugar from treated leaves. By the use of temperature jackets, the temperature of the petiole (26, 52) or of the hypocotyl (8) was varied, the other parts of the plant remaining at a moderate and constant temperature. The optimum temperature for translocation (in the translocating organ itself) is between 20°-30°C., and the Q10 for sugar transport in tomatoes is 1.5 between the range of 12°-24°C.

The method used by these investigators to assay sucrose transport was as follows. Sucrose solution was applied to carbohydrate-depleted leaves in darkness. At intervals over a period of several days, elongation of the apical internode and leaf (8, 26, 52) or internodes (7) was measured and compared with that of the untreated controls. Growth of the apical region under these conditions is proportional to the concentration of sugar (between 0-0.4 M) applied to the leaf (7).

Kendall (26) subjected petioles to fluctuating temperatures and found a greater retardation of transport by an intermittent exposure to low to medium temperature than by exposure to continuous low temperature. Continuous high temperature treatment retards transport more than an intermittent medium to high temperature treatment. The effect of hypocotyl temperature on transport from the leaf to the apical region is explained as due to a transmittance

of the temperature change throughout the system. From their description of the experiment, part of the epicotyl was enclosed in the temperature jacket and this may be the reason for the observed inhibition by low temperature. Sucrose may not pass from the petiole to the apical region directly but may pass downwards through the phloem to the cotyledonary node before it travels upward to the apical region. The results of this thesis (page 57) support this view.

A more direct approach to the study of the effect of temperature on translocation was presented by Vernon and Aronoff (57). They administered $C^{14}O_2$ to soybean leaves and determined the distance traversed by radioactive sugars past a zone of the stem held at 0°C. or 30°C. by means of a temperature jacket. The activity front traveled 24 cm. at 30°C. and 8 cm. at 0°C. after a 20 minute translocation time. Swanson and Whitney (53) studied the effect of petiole temperature on the translocation of foliarly applied P^{32}, K^{42}, Ca^{45} and Cs^{137}. The maximum amount of transport occurred at 30°C. but at 5°C. the transport of all tracers was inhibited approximately 85%.

Several investigators (25, 59, 62) have shown that sugar transport decreases progressively as the temperature is increased (Q10 for translocation less than one). A review of this subject and its implications for plant development is given by Went (60). Went (59), Went and Hull (62),

and Hull (25) used a variety of techniques on tomato plants to establish the relationship of transport to temperature. They measured, at various night temperatures, (a) the loss of sugar from leaves, (b) the amount of sugar accumulated above a stem girdle, (c) the transport of C^{14}-labeled sugar, and (d) the rate of bleeding from the stump of decapitated plants after treatment of the leaves with sucrose. In all cases, the results were essentially the same and showed that sugar transport is greater at low (approx. 5°-8°C.) than at high temperatures (approx. 26°C.).

These experiments have been criticized by Hewitt and Curtis (24) and by Crafts (13). Hewitt and Curtis believe that the lower rate of transport at high night temperatures is due to the fact that less sugar is available for transport because of higher leaf respiration rate. Went (60) agrees that the respiration rate is higher at elevated temperatures but disagrees that respiration plays as important a role as translocation in lowering the carbohydrate of the leaf during darkness. The sugar content of leaves is higher in plants after warm than after cool nights. He states,

> "Therefore, we can expect that the sucrose content of the leaves at the end of the night mainly reflects the amount translocated during night."

Crafts (13) criticizes the bleeding experiments of Went and Hull (62) as being too indirect to measure the effect of temperature on transport. He suggests that the effect of temperature is on the process of bleeding rather than on transport. He attempts to explain the lower rate of bleeding when the stem is placed in a high temperature jacket by assuming that the tissue respires rapidly and the CO_2 descends the stem and interferes with the metabolic processes responsible for bleeding. He states,

> "Such an explanation seems at least as logical as any that proposes a negative Q10 for a process of the nature of translocation."

This is an indication of the controversy which exists over the role of temperature in translocation.

MATERIALS AND METHODS

All of the experiments reported in this thesis were conducted in the Earhart Plant Research Laboratory, California Institute of Technology, Pasadena, California, U.S.A.

Choice of plant material

The red kidney bean Phaseolus vulgaris L.* was used exclusively in these investigations. It was chosen because several other investigators have used it successfully and it has become a standard plant for the study of translocation.

Selection of seed

Seeds were selected for uniformity of size and color. To obtain uniform germination it was found important to remove all seed with cracked coats since they have a high proportion of cracked or broken cotyledons. This condition impairs the growth of seedlings either by limiting the food supply or by providing avenues for infection by microorganisms. Damaged seed coats were detected by placing seeds in tap water for five minutes after which time seeds with damaged coats became wrinkled. The damaged seeds were then removed. The remainder were planted in crushed stone

---

*Seed obtained from Ferry Morse Co., Los Angeles, California.

which was kept moist for the first day. If too much water is available during this period, it is imbibed rapidly by the seeds and a large percentage of them develop cracked cotyledons. On the second day a one-half inch layer of vermiculite was placed on the top of the crushed stone and was washed into the air spaces surrounding the stones. It is important to give sufficient water to the germinating seeds after the first day, since failure to do so results in poor development of the primary leaves. These leaves are small, cup-shaped, slightly chlorotic and very irregular in size.

Growth of plants

After emergence of secondary roots, uniform seedlings were transplanted to plastic cups containing an equal mixture of vermiculite and crushed stone. The plants throughout their entire development were grown at 23°C. and watered each day with Hoagland's solution.

From emergence until the day of treatment with test solution, the plants were grown on a regime of 16 hours light (1,000 f.c.) and 8 hours dark. In most experiments, the plants were treated with test solution when they were ten days of age. At this time the primary leaves were at least two thirds expanded and the apical bud was beginning to elongate. Any deviations from this procedure are noted in the descriptions of the individual experiments.

Selection of plants for experimentation

Prior to use plants were selected for uniformity as to height, color and size of leaves, and as to freedom from any type of deformation. An aid in selection was found in the following empirical observation: it was found that if one easily measured part of the plant is selected for uniformity of size (i.e. the epicotyl length) the other parts are also uniform. The number of plants used in each experiment varied but in general each treatment included ten plants.

Treatment of plants

1. 2,4-D and 2,4,6-T

In general, the following method was used to treat bean plants with 2,4-D and 2,4,6-T. Aliquots of solutions of either radioactive ($-C^{14}OOH$) or nonradioactive 2,4-D or 2,4,6-T were placed on the upper epidermis of the primary leaves with a micropipette (10 μl). The drop was usually placed directly above the mid-vein at a point one cm. from the junction of lamina and petiole. Treated plants were then transferred to darkness to allow even absorption of the applied chemical. After this period the plants were either kept in darkness or transferred to light. At various time intervals after treatment, groups of plants were dissected and equivalent parts combined. The combined portions were then assayed for 2,4-D or 2,4,6-T. In many of

the experiments, however, only the epicotyls were assayed. The criteria used to measure the amounts of 2,4-D and 2,4,6-T transported were either growth of the epicotyl or the C^{14} content (cpm) of the tissue.

Two types of growth measurements were used in these studies. The first measure used was that of the degree of epicotyl curvature caused by placing 2,4-D on one of the primary leaves. Epicotyls of treated plants were shadowgraphed and the degree of curvature of the epicotyl determined from the silhouettes with a protractor. An easier, and more sensitive method consisted in measurement of change in length of the epicotyl, at various time intervals, after a drop of 2,4-D solution had been placed on each of the primary leaves.

For the assay of C^{14}-carboxyl labeled 2,4-D and 2,4,6-T the plant parts were first ground in warm acetone. The ground material was filtered and washed several times with acetone and 80% alcohol. The combined filtrate was then made up to a specified volume. Aliquots of this filtrate were then spotted on sand blasted aluminum planchets and allowed to dry. The planchets were placed in ring holders which were then placed in a model 3037 automatic sample changer. This instrument was connected to a model D-47 gas flow counter, a model D-181 decade scaler and a model C-111 printing timer. All of these instruments are constructed by Nuclear-Chicago Instrument Co.

The unit measures the time taken to count a prescribed number of disintegrations. This value was converted to the number of counts per minute (cpm) above background per unit plant part.

Two separate experiments were conducted to determine if the C^{14} which is translocated and measured is in the form of 2,4-D or 2,4,6-T. In the first experiment, two groups of 5 plants were treated with 2,4-D ($-C^{14}OOH$) and 2,4,6-T ($-C^{14}OOH$) respectively. They were placed in darkness for three hours and then returned to light for four hours. The epicotyls were extracted and a sample of the extract placed together with reference spots of 2,4-D and 2,4,6-T on Whatman no. 3 paper. The chromatogram was developed in isopropanol-NH_4OH-H_2O (10:1:1) at room temperature. The paper after drying was cut into strips and the position of the labeled material determined with the aid of a strip counter.

The operation and construction of the strip counter were as follows. The paper was first secured to a thin metal plate. This plate was placed into a model C-100 Actigraph strip feeder connected to a model D-47 gas flow counter. These instruments were in turn connected to a model 1620 analytical count rate meter and an Esterline-Angus Graphic Ammeter-model AW, which records radioactivity as the paper moves automatically past a slit under the Geiger tube. The graph is matched to the paper strip, with the aid of

reference spots, and the centers of the peaks of radioactivity marked on the paper. The Rf value is then calculated.

In each case, the radioactivity was found in a single spot with Rf values corresponding to those of the reference spots of 2,4-D and 2,4,6-T. These values are given in Table I.

In the second experiment, similar procedures were used except that the plants were left in light for six hours before dissection. Extracts of the petioles, epicotyls, hypocotyls and roots were chromatographed. The conclusions reached in this experiment are the same as those of the previous experiment although the Rf values are larger (Table I). This difference is probably due to a difference in room temperature during development of the chromatograms.

These results demonstrate that (a) the 2,4-D and 2,4,6-T used in these studies are radiochemically pure, and (b) within the time intervals of these experiments, the C^{14} content of the tissue extracts is a measure of the 2,4-D and 2,4,6-T which it contains. If the C^{14}-labeled 2,4-D or 2,4,6-T is transformed by the plant to other compounds, the concentrations of these materials are too low for detection by the present methods.

TABLE I

Rf values of 2,4-D, 2,4,6-T and of the radioactive materials present in extracts of plants treated with C^{14}-carboxyl labeled 2,4-D and 2,4,6-T

		Rf values			
		Extracts of leaf treated with C^{14}-labeled		Pure	
Experiment number	Part of plant extracted	2,4-D	2,4,6-T	2,4-D	2,4,6-T
1	Epicotyl	0.55	0.55	0.56	0.58
	"	0.55	0.58	0.53	0.61
2	Petiole	0.68	0.63	0.67	0.66
	Epicotyl	0.67	0.65	-	-
	Hypocotyl	0.62	0.68	-	-
	Root	0.70	*	-	-

* Insufficient C^{14} present on the paper to detect.

2. TIBA (triiodobenzoic acid)

A weighed amount of recrystallized TIBA was first dissolved in 95% alcohol. The petiole of each primary leaf was treated with a 10 microliter aliquot (50 μg of TIBA) of this solution. The solution was applied as a circular band to the surface of the petiole. The concentration used is identical with that used by Hay (21).

3. $C^{14}O_2$

In certain experiments plants were supplied with $C^{14}O_2$ and allowed to photosynthesize. Ten day old bean plants were placed at random in a large (20" x 20" x 3') plastic growth chamber specifically designed and built for radioactive studies. This chamber, which could be tightly sealed to prevent the escape of $C^{14}O_2$, was equipped with an internal fan to circulate air and a thermostat and cooler to maintain the desired temperature. A weighed amount of $BaC^{14}O_3$, in a dish, was placed in the chamber directly beneath a supply of H_2SO_4. The flow of H_2SO_4 could be controlled from outside the chamber. The acid was added to the $BaCO^{14}O_3$ and the $C^{14}O_2$ liberated. After a definite exposure time the excess $C^{14}O_2$ was removed from the chamber and trapped in three absorption towers.

Immediately after and at various time intervals after exposure to $C^{14}O_2$, groups of plants were dissected and the parts assayed for C^{14}. The methods of extraction and

counting were similar to those described for 2,4-D and 2,4,6-T.

Identification of the principal labeled compound (sucrose) in the extract was determined by paper chromatography.

4. Oxygen labeled water*

Tween 80 was added to isotopically labeled water (1.4% atoms excess O^{18}) to give a final concentration of surface active agent of 0.8%. This was done to ensure an even coverage of the leaves with labeled water. The primary leaves of ten day old bean seedlings were then immersed and excess water allowed to drain from the tips. Care was taken to avoid the spreading of any water to the surface of the petioles. The plants were then placed in darkness to allow equilibration of the applied and the endogenous water in the leaf. They were then either returned to light or kept in darkness.

At various time intervals, groups of three plants were dissected and the epicotyls placed in glass vials which were then sealed and frozen. The frozen epicotyls were next lyophylized for 12-15 hours and the water collected in vessels immersed in a dry ice-methyl cellosolve freezing mixture. From each sample of three epicotyls a volume of

---

*Obtained from the Stuart Oxygen Co., San Francisco, California.

approximately 3 ml. was collected. The O^{18}/O^{16} ratio of each sample was then determined by mass spectrometry.

Because it is undesirable to introduce water directly into the mass spectrometer, a standard but indirect method was employed to assay O^{18} content. The water in each sample was placed in a separate equilibration flask, frozen, and the air and dissolved CO_2 removed by evacuation. Carbon dioxide of known isotopic composition was then bled into each flask and the oxygen atoms of the CO_2 and the water allowed to equilibrate at 25°C. for 3-5 days. The reaction follows the equation

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

Because this reaction is reversible, oxygen in the water exchanges with oxygen of the CO_2. After equilibration the CO_2 was removed and trapped for use in the mass spectrometer. An enrichment of O^{18} in this CO_2 was detected by comparison with the original CO_2 standard used in equilibration.

5. Tritium labeled water*

The method for application of THO differed from that used to administer H_2O^{18}. THO was applied as a drop to each leaf in a manner similar to that described for 2,4-D

---

*Obtained from Isotopes Specialties Co., California.

and 2,4,6-T. The method of extraction of water from the epicotyls of treated plants was similar to that described for H_2O^{18}. The amount of activity in the epicotyl water was measured in a liquid scintillation counter. This instrument was connected to a Coincidence Amplifier and Autoscaler. All these instruments are constructed by the Tracerlab Incorporated.

A 0.5 ml. aliquot of water was placed in a glass bottle containing 50 ml. of solution consisting of the following ingredients:

2,5-Diphenyl oxazole	-	4 g.
Popop (1,4-bis-2(5-phenyloxazolyl)-benzene)	-	15 mg.
Toluene	-	770 ml.
Ethanol (absolute)	-	230 ml.

The light given off per unit time by the phosphor in darkness is a measure of the number of disintegrations due to the presence of tritium.

RESULTS AND DISCUSSION

Comparison between the uptake of 2,4-D K and 2,4-D acid

The first matter considered in the present investigation is that of the factors which govern uptake of 2,4-D K (pH 4.5), as compared to 2,4-D acid (pH 2.9).

Twenty plants were treated, each with 2×10^{-2} uM of 2,4-D (K salt of 2,4-D). To ten of these plants, 2,4-D was applied (with a micropipette) as a drop to the upper surface of one of the primary leaves. The other ten plants were treated by smearing the same volume of 2,4-D solution along the length of one side of the epicotyl. At intervals, the angle of curvature of the epicotyl was determined (Materials and Methods). The results are shown in Table II. The curvature of the epicotyls of plants whose primary leaf was treated with 2,4-D was very small and very inconsistent. The epicotyls are sensitive to 2,4-D, however, since strong curvatures are obtained when 2,4-D is applied directly to the epicotyl.

Results similar to the above are obtained when different concentrations (4×10^{-2}, 8×10^{-2} and 16×10^{-2} uM per plant) of 2,4-D are applied to either the primary leaf or to the epicotyl (Table III).

It is concluded, from these experiments, that the potassium salt of 2,4-D is not readily translocated from

TABLE II

Angle of curvature of epicotyls, at various time intervals, after treatment of the leaf or epicotyl with 2×10^{-2} μM of 2,4-D K

Time (min.)	Curvature in degrees: Leaf treated	Curvature in degrees: Epicotyl treated
0	0	0
62	-0.3	-
77	-	9.1
122	0.7	-
137	-	21.8
182	-1.3	-
197	-	32.7
300	1.4	-
317	-	36.5
420	3.1	-
437	-	32.6

TABLE III

Angle of curvature of epicotyls, at various time intervals, after treatment of the leaf or epicotyl with 4×10^{-2}, 8×10^{-2}, and 16×10^{-2} μM of 2,4-D K solution

Concentration of 2,4-D K applied/plant

4×10^{-2} μM			8×10^{-2} μM			16×10^{-2} μM		
Time (min.)	Leaf treated	Epicotyl treated	Time (min.)	Leaf treated	Epicotyl treated	Time (min.)	Leaf treated	Epicotyl treated
0	0	0	0	0	0	0	0	0
68	1.1	10.3	60	-0.5	9.6	56	0.1	11.1
141	0.6	36.6	136	0.3	36.6	138	0.9	33.2
217	1.7	51.6	199	0.4	55.8	209	0.2	45.8
276	1.1	59.4	260	-0.3	64.2	277	0	48.0
366	0.6	60.0	346	0.4	63.2	359	0	55.3

the primary leaf of red kidney bean to the epicotyl. Since Day (16) obtained large curvatures with an alcoholic solution of 2,4-D acid, it was thought that either the potassium salt is not absorbed or translocated as well as the acid, or alcohol increases the amount of 2,4-D absorbed by the leaf.

In an attempt to find the barrier to transport of the potassium salt, the following experiment was performed. Two solutions, of equal 2,4-D K concentration were prepared. They differed, however, in that one contained alcohol (2% of final volume), whereas, the other contained only water. Four groups of plants were selected and each member in a group was treated with one of these two solutions. The plants in three of the groups were treated with the alcohol free solution. The first received an application to one of the primary leaves, the second to the epicotyl, and the third was treated like the first but differed in that the epidermis underlying the treated zone was scraped gently with a needle. This operation was performed to determine whether the cuticle prevents the absorption of 2,4-D K. Plants in the fourth group were treated (leaf application) with an alcoholic solution of 2,4-D. Epicotyl curvatures were determined and the results are presented in Table IV.

The addition of alcohol (2%) did not increase the amount of 2,4-D K transported to the epicotyl. Scraping

TABLE IV

Angle of curvature of epicotyl, at various time intervals, after placement of 2,4-D (4×10^{-2} μM/plant) on the leaf or epicotyl of bean plants. The 2,4-D K solutions were applied in the presence and absence of alcohol (2%)

Angle of curvatures in degrees

	2,4-D K (alcohol free)			2,4-D K (alcohol present)
Time (min.)	Leaf treated	Epicotyl treated	Leaf treated (cuticle scraped)	Leaf treated
0	0	0	0	0
67	-	5.6	-	-
161	0	27.0	30.8	0
230	-	36.8	-	-
286	0	41.2	62.2	0

of the epidermis underlying the site of 2,4-D K application, however, resulted in large curvature of the epicotyl. Therefore, the failure of the epicotyls to display curvature, in response to leaf application of 2,4-D K, is located at the cuticle and not in the transport system. Crafts (14) has shown that the sodium salt of 2,4-D is not absorbed as well as the acid.

The superiority of the acid over the K-salt in inducing curvatures of the epicotyl, is demonstrated in the next experiment. Three solutions of different 2,4-D concentration (1×10^{-3}, 2×10^{-3} and 4×10^{-3} M) but equal alcohol concentration (2%) were prepared. Ten µl of each concentration was applied to two groups of ten seedlings. The treatment was applied to the leaves of one group and to the epicotyl of the other group of plants. Curvature of the epicotyl was measured at various time intervals and the results are shown in Table V.

Comparison of the curvatures which result from leaf treatments with the potassium salt (Table III) with those of Table V reflect the greater amount of absorption of the acid by the leaf in the latter case.

It may be inferred that the cuticle of the leaf offers little resistance to the absorption of the acid form of 2,4-D. It does, however, offer some resistance, since when leaves treated with 2,4-D acid were scraped at the site of application, the amount of chemical transported to the

TABLE V

Angle of curvature of epicotyls, at various time intervals, after treatment of the leaf or epicotyl with 2×10^{-2}, 4×10^{-2}, and 8×10^{-2} μM of 2,4-D acid solution containing 2% alcohol

Concentration of 2,4-D acid applied/plant

2×10^{-2} μM			4×10^{-2} μM			8×10^{-2} μM		
Time (min.)	Leaf treated	Epicotyl treated	Time (min.)	Leaf treated	Epicotyl treated	Time (min.)	Leaf treated	Epicotyl treated
0	0	0	0	0	0	0	0	0
100	0.2	22.0	95	1.5	19.1	90	-	17.8
185	-	48.7	175	-	40.8	95	0.4	-
190	5.2	-	185	19.0	-	170	-	18.2
285	25.1	73.0	270	59.2	45.2	180	14.0	-
360	-	80.4	360	85.5	-	275	52.5	11.6
400	34.1	-	390	-	40.7	390	-	7.5
-	-	-	-	-	-	420	80.1	-

epicotyl was greater than if the leaves were not scraped. The criterion used to measure the amount of 2,4-D translocated in this experiment was increase in length of the epicotyl over that of the untreated controls (Materials and Methods).

The experiment which demonstrates this point was conducted as follows. Two groups of ten plants each were treated in the light (1000 f.c.) with 2,4-D acid solution containing 2% alcohol. The amount of chemical applied to each of the primary leaves was 4×10^{-2} µM. After application the cuticle beneath the treated zone, in one group of plants, was scratched with a needle. The cuticle of plants in the second group was not altered. Ten additional plants were left untreated and served as controls. At various time intervals after treatment, the length of the epicotyls was measured. The results are shown in Table VI.

Even though the cuticle offers some resistance to the penetration of the acid, a substantial amount of the material is none the less absorbed from alcoholic solution. 2,4-D acid dissolved in aqueous ethanol was used in all subsequent experiments.

TABLE VI

2,4-D induced growth (% increase in length over controls) of epicotyls, at various time intervals, after treatment of both primary leaves with 8 x 10^{-2} μM of 2,4-D acid (2% alcohol)

Time (min.)	Growth	
	Normal leaves	Pricked leaves
120	1.82	7.36
210	5.63	14.66
300	9.58	19.82
480	19.58	28.21

Translocation of 2,4-D

Role of light and dark in 2,4-D transport

The role of carbohydrates in the translocation of 2,4-D, and several other compounds, has already been described (Review of Literature). 2,4-D is absorbed but not translocated in destarched plants kept in darkness. The addition of certain sugars to the leaves or transfer of treated plants to light results in transport of 2,4-D to other portions of the plant. The amount of transport is proportional to the light intensity but light in the absence of CO_2 is ineffective. Translocation of 2,4-D from leaves appears, therefore, to be controlled by the size of the photosynthate or carbohydrate pool.

One of the great difficulties encountered in translocation experiments is the inability of the investigator to separate the processes of absorption from those of translocation. This separation is especially important when comparisons between different compounds are to be made. The ideal situation would be to have the compound absorbed but not translocated from the leaf until such transport is initiated by the addition of carbohydrate. The separation of absorption from translocation has been achieved in the present work. Plants are treated in darkness and absorption allowed to proceed to conclusion. They are then transferred to light for the initiation of translocation.

To determine if 2,4-D is translocated in the dark, the following two experiments were conducted. The plants used in each experiment were grown for nine days in the Green greenhouse (23°C. day - 17°C. night) of the Earhart Plant Research Laboratory (60). They were then placed in a dark room (23°C.) for 8 hours. In experiment 1, the plants then received three hours of light (1000 f.c.). The primary leaves were then treated with 4×10^{-2} μM of C^{14}-labeled 2,4-D. In experiment 2, the plants received an additional day (16 hours light and 8 hours dark) plus 3 hours of light (1000 f.c.) prior to treatment with 2,4-D. After treatment, in both experiments, one set of 20 plants was placed in darkness and the other set in light. Ten plants from each set were dissected at the times shown in Table VII and the 2,4-D content of the epicotyls determined by radioactivity. It may be concluded from the data of Table VII, that little 2,4-D is translocated to the epicotyls in the dark.

The following experiment shows that the carbohydrate level of the leaf does affect and control the amount of transport of 2,4-D in the dark.

One hundred plants were grown in Yellow greenhouse (23°C. day - 17°C. night) for 9.5 days. At the end of this period, fifty plants were placed in darkness while the remainder were placed in light (1000 f.c.) for 20 hours. Both groups were then placed in light (1000 f.c.) for three hours

TABLE VII

Amount of 2,4-D (cpm) in the epicotyls of light and dark grown bean plants, at various time intervals, after treatment of the primary leaves with 4×10^{-2} μM of C^{14}-labeled 2,4-D

Experiment 1			Experiment 2		
Time (min.)	cpm/epicotyl		Time (min.)	cpm/epicotyl	
	Light	Dark		Light	Dark
190	-	5	175	190	-
200	210	-	185	-	10
470	-	10	485	755	-
480	840	-	490	-	25

prior to treatment. Each of the 100 plants was then treated with 4.7×10^{-2} μM of C^{14}-labeled 2,4-D (5% alcohol) and placed in darkness for three hours. At the end of the dark period, ten plants from each group were assayed for 2,4-D by the extraction method. The remaining plants were returned to light and the epicotyls assayed for 2,4-D at the various time intervals shown in Figure 1.

The data demonstrate that plants which received 23 hours of light transported 2,4-D during the dark period. Conversely, plants which received 20 hours of dark followed by three hours of light did not transport 2,4-D during the dark period. 2,4-D was not detected in the epicotyl until more than one hour in light. This shows that the three hour light period prior to treatment is not sufficient to initiate transport in the dark.

By regulating the duration of light received by the plants prior to treatment, the time of arrival of the first amounts of 2,4-D in the epicotyl can be controlled with reasonable accuracy. This is shown in the following experiment where the objective was to have 2,4-D absorbed in the dark but not translocated until the plants were placed in light.

Ninety plants were grown for nine days on a regime of 16 hours light (1000 f.c.) and 8 hours dark. At the end of the last eight hour dark period, they were given an exposure to three hours of light, treated with C^{14}-labeled

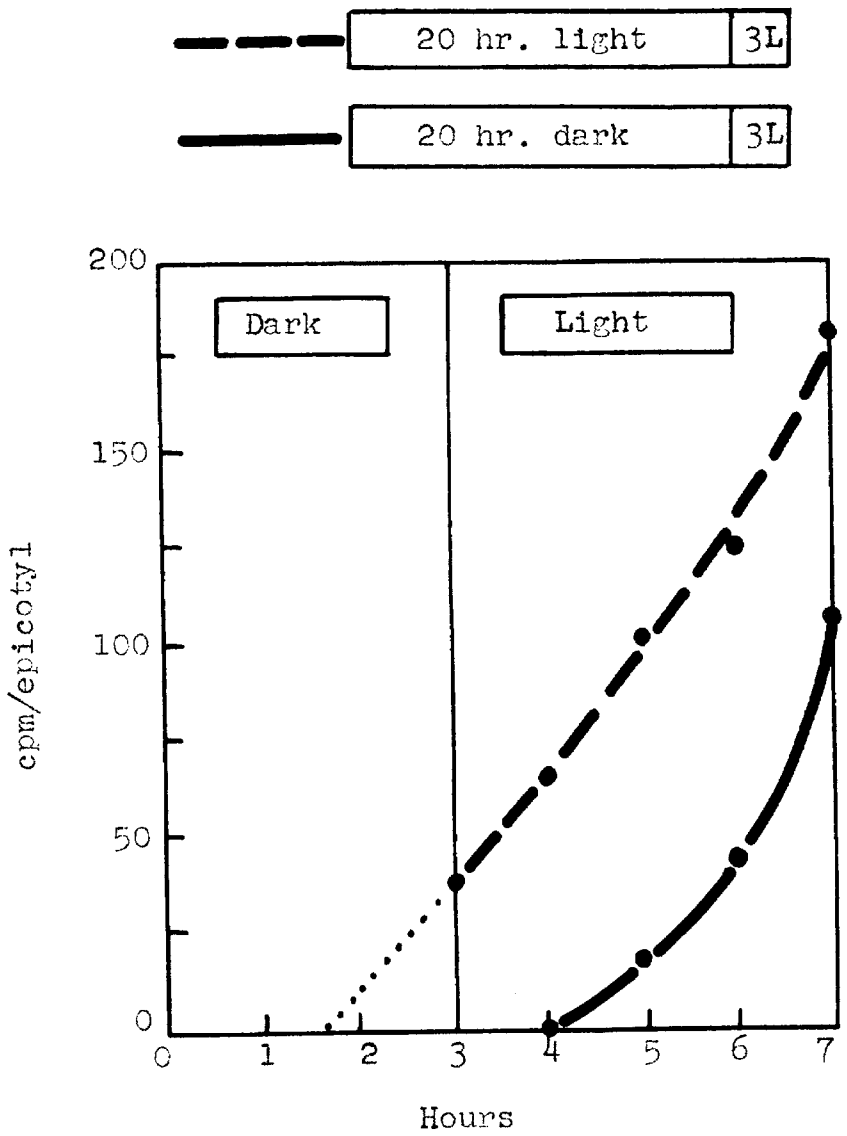

Figure 1. Amount of 2,4-D (cpm), at various time intervals, in epicotyls of plants which received 23 hours of light (upper curve), or 20 hours of dark followed by 3 hours of light (lower curve), prior to treatment of the laminae with C^{14}-labeled 2,4-D.

2,4-D (4.7×10^{-2} μM per plant) and placed in darkness for three hours. They were then returned to light and at various time intervals (3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, and 9.0 hours after treatment) the epicotyls assayed, in groups of ten, for 2,4-D. Growth of the epicotyl induced by 2,4-D was also measured. The results are shown in Figure 2.

With this regime of treatments plants are obtained which exhibit little if any translocation of 2,4-D to the epicotyls during the dark absorption period. When the plants are returned to light, the amount of 2,4-D in the epicotyl increases linearly with time up to six hours.

Growth of the epicotyl induced by 2,4-D began one hour after the first amounts of 2,4-D arrived in the epicotyl. This time lag between the arrival of 2,4-D and the appearance of 2,4-D induced growth is of the same order as that found by Day (16) by an independent method.

Concentration dependence of 2,4-D translocation

The purpose of the following experiments was to determine the time course curves of transport when different concentrations of 2,4-D are applied to the leaves.

Two hundred uniform plants were grown and subjected to the conditions described in the previous experiment except the light intensity was changed from 1000 to 2000 f.c. The plants were divided into four groups. The plants in each

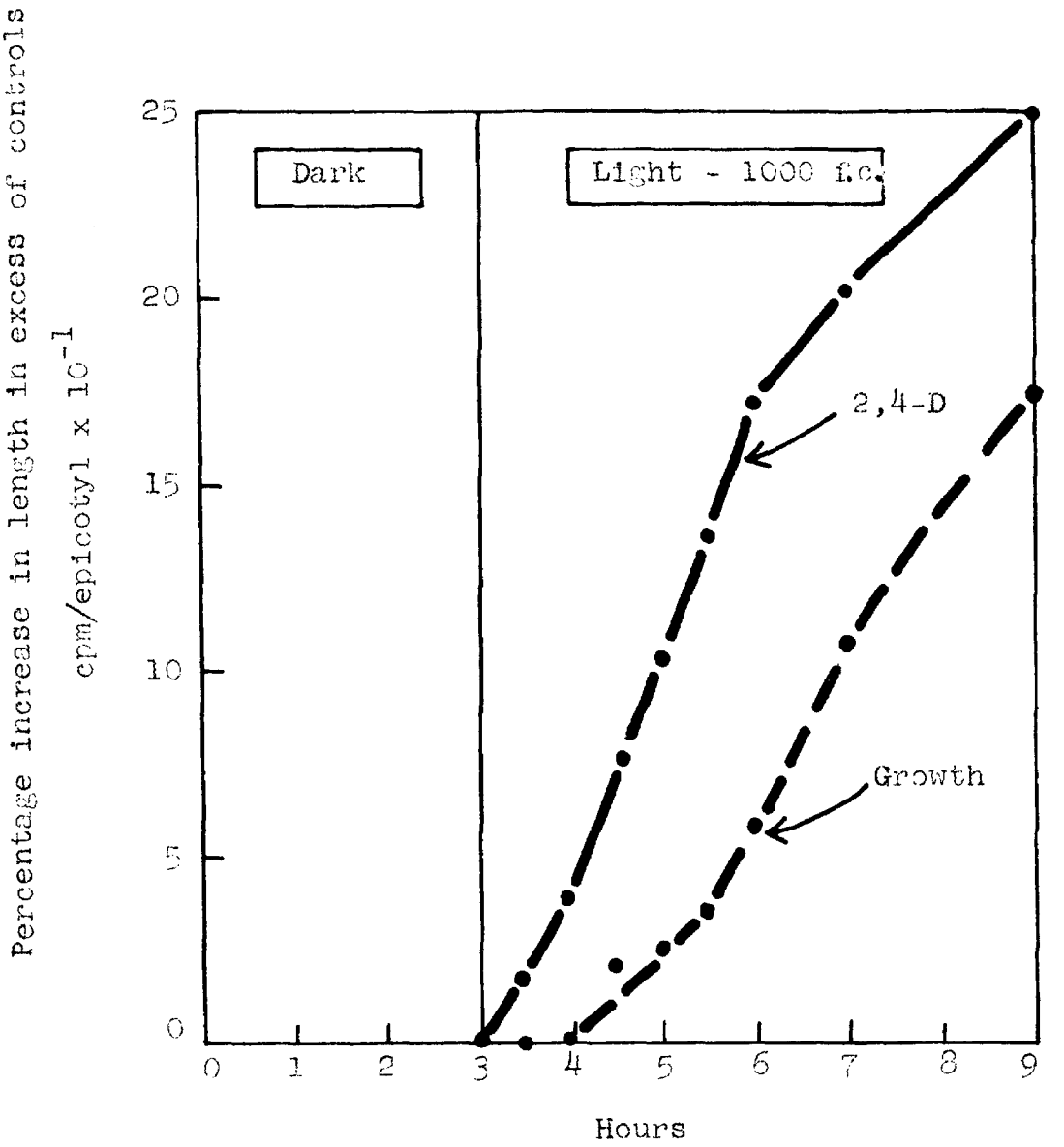

Figure 2. Transport of 2,4-D from the laminae to the epicotyl as measured, at various time intervals, by C^{14} (cpm) and growth determinations.

group received a different concentration (0.5, 1.0, 2.0, 4.0 x 10^{-2} μM) of 2,4-D upon the leaves. The epicotyls of ten plants from each group were sampled, at various time intervals (3.00, 4.00, 4.75, 6.50 and 9.00 hours) after treatment, to determine the amount of 2,4-D transported from the leaves. Since the change in light intensity resulted from an oversight when growth facilities were changed, the experiment was repeated using plants grown at 1000 f.c. The concentrations of 2,4-D (1, 2, 4, and 8 x 10^{-2} μM per plant) used in the second experiment were doubled. The results of the two experiments are shown in Table VIII and the average values plotted in Figure 3.

From Figure 3 it is clear that the amount of 2,4-D in the epicotyl increases linearly during the first six hours after treatment. This permits ready extrapolation of the different concentration time curves to the abscissa. Such extrapolation reveals that the first amounts of 2,4-D arrive in the epicotyl at the same time for each of the treatment concentrations.

The curves showing the relationship between the concentration of C^{14}-labeled 2,4-D applied to the laminae and the amount of 2,4-D (cpm) translocated to the epicotyl are plotted, for the various sampling times, in Figure 4. These curves do not resemble those which relate applied auxin concentration to growth of tissue immersed in auxin solution. The transport curves of Figure 4 tend to be almost linear

TABLE VIII

Amount of 2,4-D (cpm) in epicotyls of plants grown under 1000 and 2000 f.c., at various time intervals (3, 4, 4.75, 6.5, and 9 hours), after treatment of the primary leaves with different concentrations (0.5, 1, 2, 4, and 8 x 10^{-2} μM) but equal specific activities of 2,4-D

Time (hr.)	Light intensity (f.c.)	μM of 2,4-D applied per plant				
		0.5 x 10^{-2}	1 x 10^{-2}	2 x 10^{-2}	4 x 10^{-2}	8 x 10^{-2}
3.00	1000	-	3	13	34	75
	2000	3	8	27	52	-
4.00	1000	-	18	28	120	231
	2000	5	17	37	121	-
4.75	1000	-	24	43	228	382
	2000	6	29	86	211	-
6.50	1000	-	58	105	390	753
	2000	15	55	124	299	-
9.00	1000	-	40	149	555	994
	2000	23	68	196	528	-

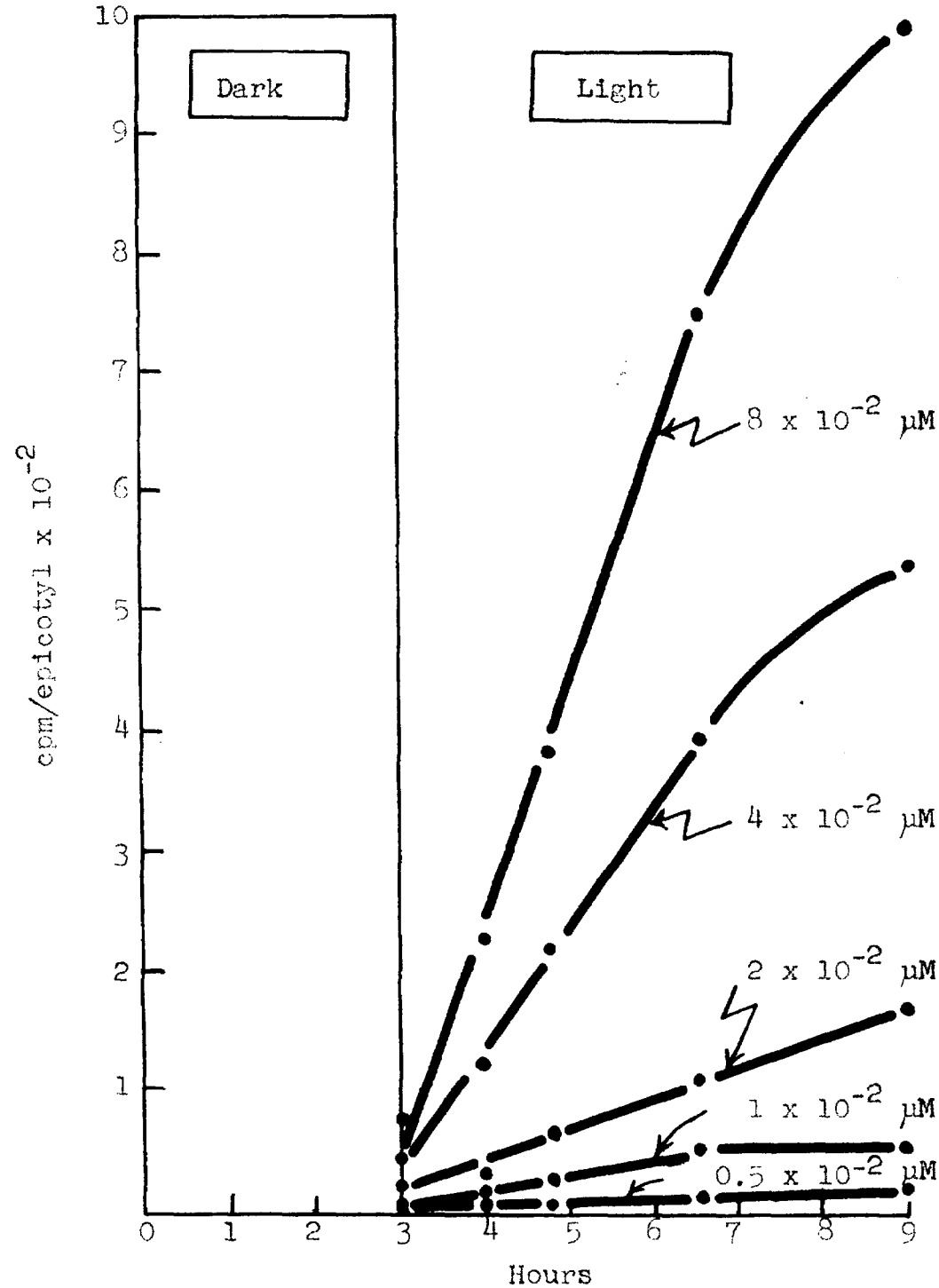

Figure 3. Amount of 2,4-D (cpm) in epicotyls, at various time intervals, after treatment of the primary leaves with different concentrations (0.5, 1, 2, 4, and 8 x 10^{-2} µM/plant) but equal specific activities. The plotted values are averages of data obtained in two experiments, one conducted under 1000 and the other under 2000 f.c. of light.

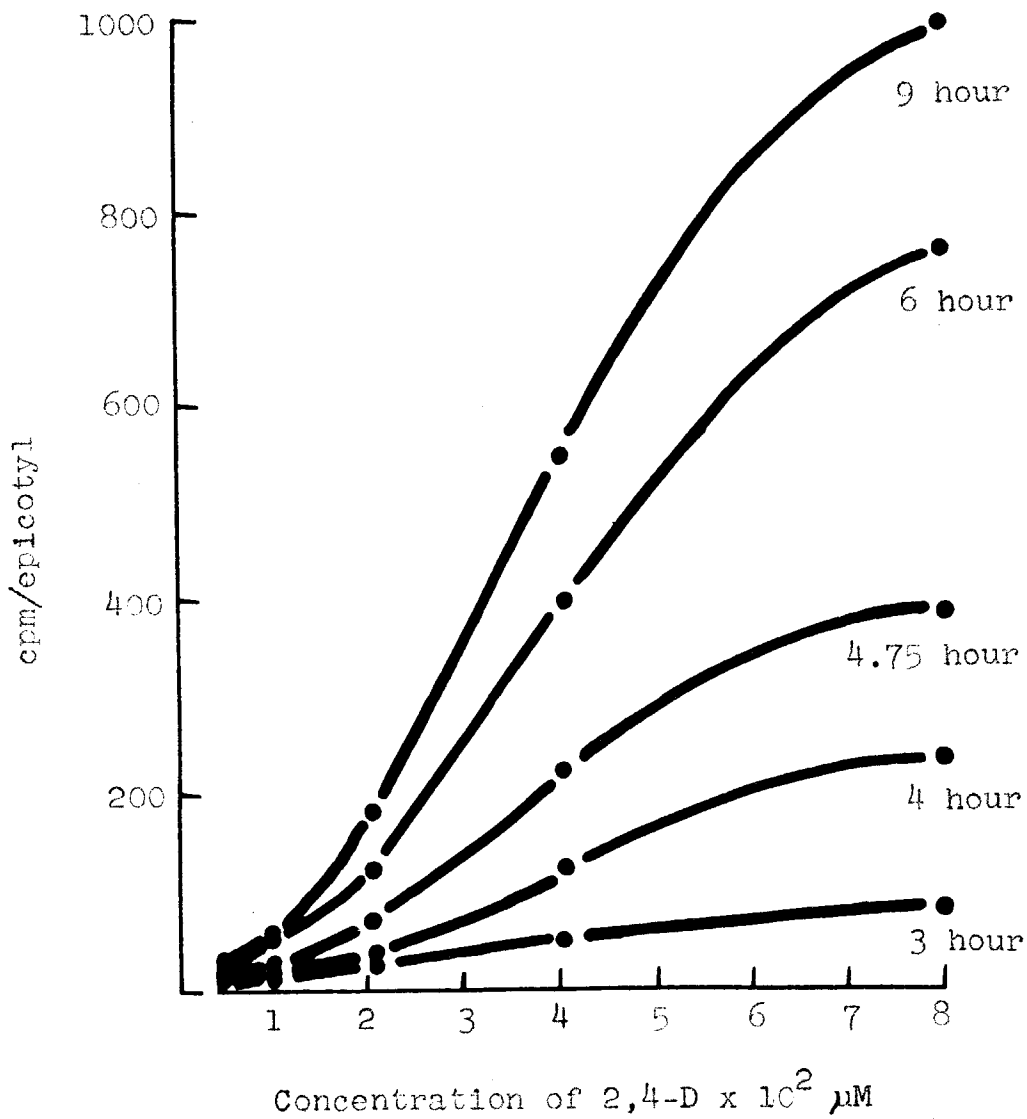

Figure 4. Plot of the amount of 2,4-D (cpm) versus concentration of C^{14}-labeled 2,4-D applied to the laminae, at 3, 4, 4.75, 6.5, and 9 hours after treatment.

at short time intervals rather than hyperbolic as are growth curves. Over longer time intervals the transport curves tend to become slightly sigmoid, indicating some decrease in efficiency of 2,4-D transport at higher concentrations. It appears, therefore, that there is a saturation of the transport mechanism at elevated 2,4-D concentrations. The mechanism responsible for the transport (cell to cell) of indoleacetic acid (63), and indolebutyric, naphthaleneacetic or anthraceneacetic acid (64), in Avena coleoptiles becomes saturated at elevated concentrations. It will be shown in a later section of this thesis (Interaction of 2,4-D and 2,4,6-T in transport) that high concentrations of non-radioactive 2,4-D or 2,4,6-T are capable of inhibiting the transport of C^{14}-labeled 2,4-D in the phloem. This finding agrees with the results of Hay and Thimann (23) who have shown recently that the transport of 2,4-D is inhibited at high concentrations of 2,4-D (100 µg per plant).

The data in Table VIII show that the rate of delivery of 2,4-D to the epicotyl is essentially the same (except for the discrepancy at 2×10^{-2} µM) for plants subjected to 1000 f.c. as for plants subjected to 2000 f.c. This is true for the whole range of 2,4-D concentrations. Photosynthesis of seedling plants is, in general, saturated at light intensities of 1000 f.c. or lower and it is probable, therefore, that the higher light intensity does not in fact elicit any increased rate of sugar transport.

Growth of the epicotyl induced by 2,4-D was also measured in both of these experiments. The results are shown in Table IX and Figures 5 and 6. Figure 5 shows 2,4-D induced growth of the epicotyl of plants (grown under 1000 and 2000 f.c. of light) as a function of the amount of 2,4-D (cpm) extracted from the epicotyl, nine hours after application of different concentrations of C^{14}-labeled 2,4-D to the laminae. Figure 6 is plotted to show 2,4-D induced growth of the epicotyl as a function of of the time (hours) after treatment of the plants (grown under 1000 and 2000 f.c. of light) with C^{14}-labeled 2,4-D.

Growth of the epicotyl induced by equivalent amounts of 2,4-D in the epicotyl (Table VIII) is 2-4 times greater in plants grown under 2000 f.c. than in plants grown under 1000 f.c. of light (Figure 5). The amount of growth of the control epicotyls is only slightly greater for plants grown under 2000 f.c. than it is for plants grown under 1000 f.c. of light. The curves of Figure 5 resemble those which relate applied auxin concentration to growth of tissue immersed in auxin solution. These results suggest (a) the growth of untreated plants is limited by auxin concentration, and (b) the efficiency of applied 2,4-D in inducing growth is greater in plants grown under 2000 f.c. of light because of the presence in the stem of a factor(s) which complements its action. The presence of small quantities of 2,4-D in the epicotyl (Table VIII), after treatment of

TABLE IX

2,4-D induced growth (mm and %) of epicotyls of plants grown under 1000 and 2000 f.c., at various time intervals, after treatment of the leaves with different concentrations of 2,4-D

Time (hr.)	Light intensity f.c.	μM 2,4-D applied per plant: 0.5×10^{-2} mm	%	1×10^{-2} mm	%	2×10^{-2} mm	%	4×10^{-2} mm	%	8×10^{-2} mm	%	Controls mm	%
3.00	1000	-	-	.13	4.36	.12	4.46	.14	5.29	.12	4.23	.11	4.02
	2000	.07	2.95	.10	5.02	.14	6.59	.21	9.84	-	-	.12	5.85
4.00	1000	-	-	.17	5.90	.16	5.68	.20	6.53	.20	7.00	.16	5.80
	2000	.13	5.90	.16	7.47	.20	8.88	.28	12.54	-	-	.15	7.38
4.75	1000	-	-	.16	5.63	.17	6.33	.22	7.20	.23	8.02	.17	5.87
	2000	.15	6.83	.25	11.84	.23	10.66	.34	15.88	-	-	.16	7.52
6.50	1000	-	-	.24	8.10	.23	8.40	.31	10.37	.45	15.82	.20	7.04
	2000	.20	9.39	.23	11.34	.36	17.13	.57	25.53	-	-	.20	9.63
9.00	1000	-	-	.27	9.33	.34	12.00	.54	21.23	.71	24.11	.25	9.13
	2000	.19	8.36	.32	14.21	.55	26.25	.91	39.73	-	-	.22	10.37

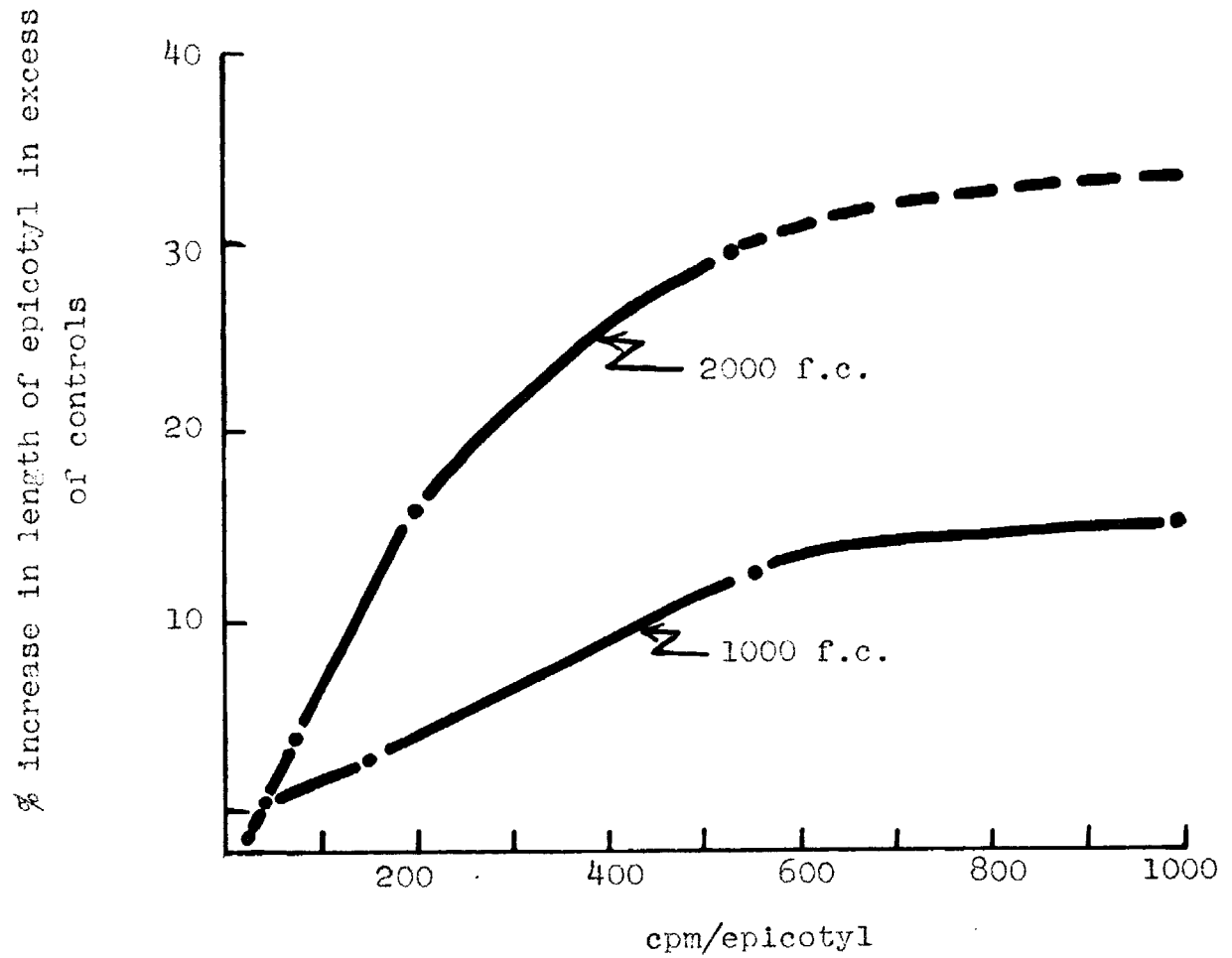

Figure 5. 2,4-D induced growth of the epicotyl of plants, grown under 1000 and 2000 f.c. of light, as a function of the amount of 2,4-D (cpm) extracted from the epicotyl, 9 hours after treatment of the laminae with different concentrations of C^{14}-labeled 2,4-D.

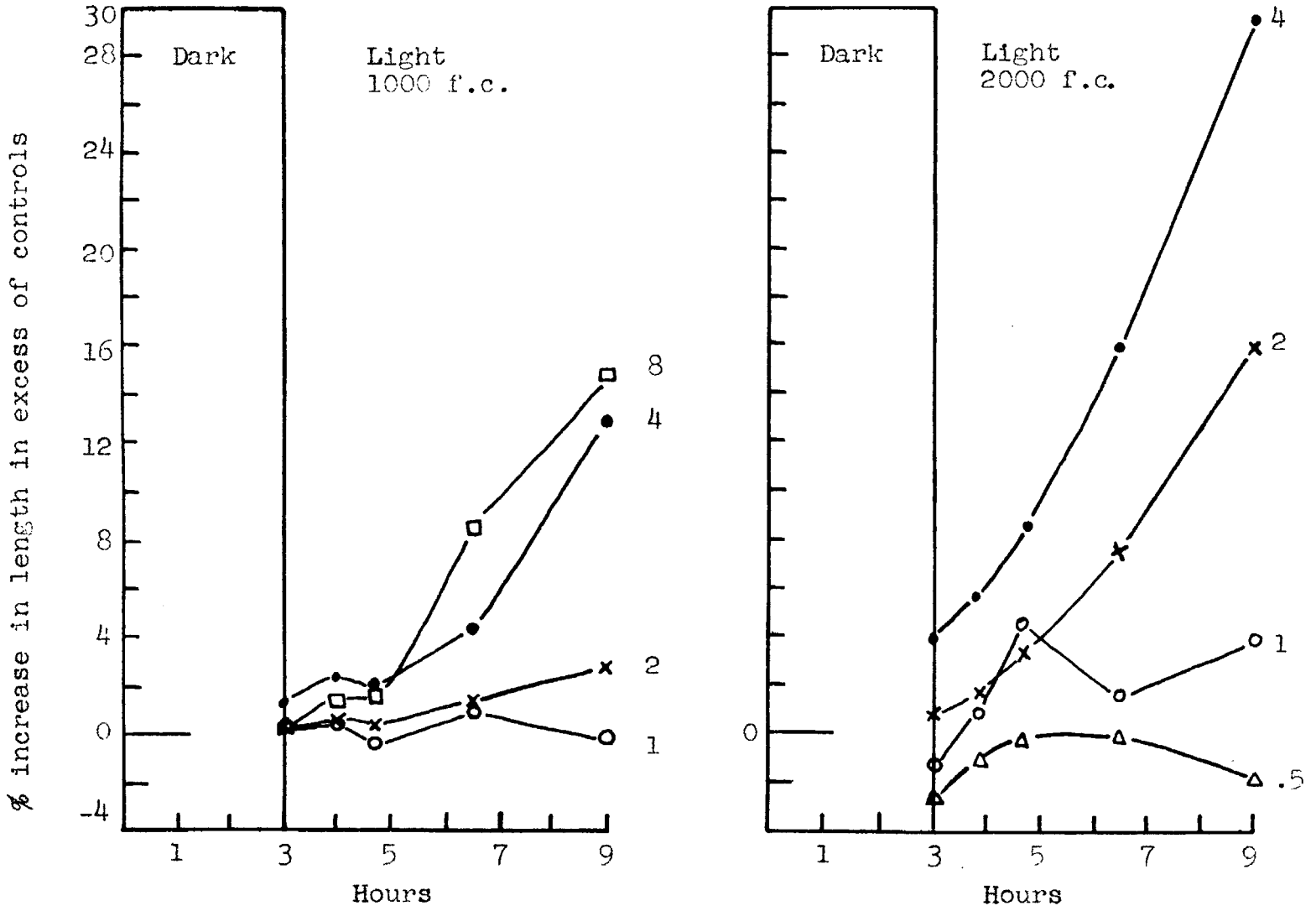

Figure 6. 2,4-D induced growth of epicotyls of plants, grown under 1000 and 2000 f.c. of light, at various time intervals, after treatment of the leaves with different concentrations (0.5 x 10^{-2} µM △ , 1 x 10^{-2} µM ○ , 2 x 10^{-2} µM **X**, 4 x 10^{-2} µM ● , and 8 x 10^{-2} µM □ /plant) of C^{14}-labeled 2,4-D.

the laminae with 0.5 x 10^{-2} μM of C^{14}-labeled 2,4-D/plant, does not increase the growth of the epicotyl (Table IX); in fact, there may even be a slight reduction in growth of the epicotyl. Similar inhibitions have been observed by McRae (38) with Avena coleoptile sections. The basis of the response is unknown. The presence of a greater concentration of a factor(s), which complements 2,4-D action, in the stems of plants grown under 2000 f.c. of light, is further indicated by the shorter time lag between the arrival of 2,4-D in the epicotyl and the induction of growth of plants treated with 4 x 10^{-2} μM of 2,4-D (Figure 6).

It may be argued that the increased efficiency of 2,4-D in inducing epicotyl growth at 2000 f.c. may have been due to a higher plant temperature rather than to an increased supply of a factor(s) which complements 2,4-D action. Although the air temperature (23°C.) was the same, in both experiments, the plants which received 2000 f.c. of light may have been warmer due to their closer proximity to the lights. However, if the temperature was elevated high enough to give a 2 - 4 fold increase in the effectiveness of 2,4-D, such a temperature would probably have decreased the amount of 2,4-D translocated to the epicotyl (Review of Literature). The data in Table VIII show very clearly that the amount of 2,4-D transported to the epicotyl is essentially the same for each treatment concentration.

The results of these two experiments demonstrate the merit of using radioactivity rather than growth measurements to assay the translocation of 2,4-D in plants grown under different environmental conditions. The use of radioactivity measurements (cpm) to assay 2,4-D translocation has been questioned by Hay and Thimann (22). They state that the presence of C^{14} in tissue, remote from the site of C^{14}-labeled 2,4-D application, may represent a chemical degradation product rather than the applied intact C^{14}-labeled 2,4-D molecule. The data of Table I demonstrate that, for short term experiments, radioactivity measurements can be safely used to assay 2,4-D transport.

In the next section, a comparison is made between the translocation of 2,4-D and 2,4,6-T. The following experiment demonstrates that these two chemicals are translocated in the phloem. Twenty uniform plants, grown on the regime described in Materials and Methods, were divided into two groups of ten plants. Twenty-one hours before treatment, the petioles of five plants in each group were girdled with steam. The five normal and five girdled plants in each group were treated with C^{14}-labeled 2,4-D or 2,4,6-T (equal specific activities) and placed in darkness for one hour* after which they were returned to light for five hours.

---

*The dark period was shortened from three hours to one hour because both chemicals were dissolved in 95% alcohol and the treated zones were dry at this time. The alcohol

All the plants were then dissected and the epicotyls assayed (cpm) for C^{14}-labeled 2,4-D or 2,4,6-T. Each determination was performed in duplicate so that a total of 40 plants were used in the entire experiment. The results are shown in Table X. Very little if any 2,4-D or 2,4,6-T is translocated past a steam girdle. It is concluded, therefore, that both of these chemicals are transported in the living phloem. The difference between the amount of 2,4-D and 2,4,6-T transported in normal plants will be described in the next section.

Comparison between the translocation of 2,4,6-T and 2,4-D

From the work of Osborne and Wain (46), who studied the ability of certain synthetic compounds to induce morphogenic responses in the tomato plant, Leopold (31) concludes that chlorination of the phenyl ring in the phenoxy series of growth regulators appears to increase their translocation. Both 2,4-D and 2,4,5-T are translocated and are used as systemic herbicides. In contrast to this conclusion, Bonner (private communication) has found that the antiauxin 2,4,6-T does not appear to be readily translocated in Xanthium pennsylvanicum. The same has been found for the antiauxin p-chlorophenoxy isobutyric acid in bean plants

---

concentration had to be raised above 5% to facilitate solution of the 2,4,6-T. A separate experiment (not reported here) revealed that the alcohol concentration may be raised to 95% without inhibiting the translocation of 2,4-D (10 µl/lamina).

TABLE X

Amount (cpm) of 2,4-D or 2,4,6-T in the epicotyls of girdled* and normal plants 6 hours after treatment of the primary leaves with 4×10^{-2} μM of C^{14}-labeled 2,4-D or 2,4,6-T of equal specific activities

Condition of plants	Sample number	cpm/epicotyl	
		2,4-D	2,4,6-T
Normal	1	604.5	82.7
	2	596.3	99.9
Girdled	1	0.3	0.0
	2	1.1	1.1

*The petioles of the primary leaves were girdled with steam.

by McRae (38). The compound 2,4-D is of course remarkable by virtue of its ready transportability within the plant. It is of interest therefore to determine what structural and physiological features of the molecule are associated with its systemic properties. To this end a series of time course experiments was conducted to compare the translocation of C^{14}-labeled 2,4,6-T with that of C^{14}-labeled 2,4-D.

One hundred uniform plants, grown on a regime of 16 hours light (1000 f.c.) and eight hours dark for nine days, were selected for the first experiment. After the last eight hour dark period, they were placed in light for three hours. One half of these plants were next treated with 2,4-D, the other half with 2,4,6-T. The test solutions were of equal specific activity and the leaves of each plant received an application of 4×10^{-2} μM. After treatment the plants were placed in darkness. The dark period was shortened from three hours to one hour because both compounds were dissolved in 95% alcohol and the treated zones were dry at this time. At intervals of 1, 3, 6, 9 and 12 hours, groups of ten plants treated with 2,4-D and 2,4,6-T were dissected and similar plant parts combined, and the amount of 2,4-D and 2,4,6-T determined by the extraction method. The results are shown in Table XI.

From Table XI it can be seen that the amount of C^{14}

TABLE XI

Amount of 2,4-D and 2,4,6-T (cpm) in different portions of bean seedlings, at various time intervals (1, 3, 6, 9, and 12 hours), after treatment of the leaves with 2,4-D or 2,4,6-T

Time (hr.)	Treatment	Portion of plant					
		Laminae	Petioles	Epicotyl	Hypocotyl	Bud	Roots*
1	2,4-D	18,450	17	19	10	4	0
	2,4,6-T	18,472	19	8	6	10	0
3	2,4-D	17,414	269	218	198	5	396
	2,4,6-T	-	190	165	106	16	-
6	2,4-D	14,946	921	561	874	80	1118
	2,4,6-T	17,552	360	375	165	41	22
9	2,4-D	14,217	427	761	1034	115	1946
	2,4,6-T	16,756	510	394	214	144	497
12	2,4-D	10,502	1222	1005	1203	154	4414
	2,4,6-T	13,962	506	432	449	215	2951

*Calculated values for 2,4-D or 2,4,6-T (total cpm extracted after one hour—total cpm extracted after 3, 6, 9, or 12 hours).

present in and on the laminae (whole laminae assayed) one hour after treatment is the same for 2,4-D and for 2,4,6-T treated plants. The loss of 2,4-D from the laminae, at successive time intervals, is greater than that of 2,4,6-T. This difference is reflected in essentially all the petiole, epicotyl, and hypocotyl determinations. 2,4-D appears to travel at a faster rate than 2,4,6-T. However, the amounts of 2,4-D and 2,4,6-T present in the petiole, epicotyl, hypocotyl and bud, at the one hour sampling time are approximately the same. This suggests that the first amounts of the two compounds travel at the same speed, but, that in the case of 2,4,6-T,there is some barrier present either in or on the leaves which reduces the supply of 2,4,6-T to the transport tissues.

Another experiment was performed for verification of this result. The plants were grown and treated in the same manner as in the previous experiment. Sampling was, however, more frequent (1, 2, 4 and 5.5 hours) and five plants rather than ten were used for each treatment. The epicotyls were assayed for 2,4-D and 2,4,6-T as described previously. The results are shown in Figure 7. Extrapolation of the two progress curves to the abscissa reveals again that the first amounts of the two compounds arrive in the epicotyl at the same time. However, as in the previous experiment, the amount of 2,4,6-T delivered to the epicotyl, per unit of time, is less than the amount of 2,4-D

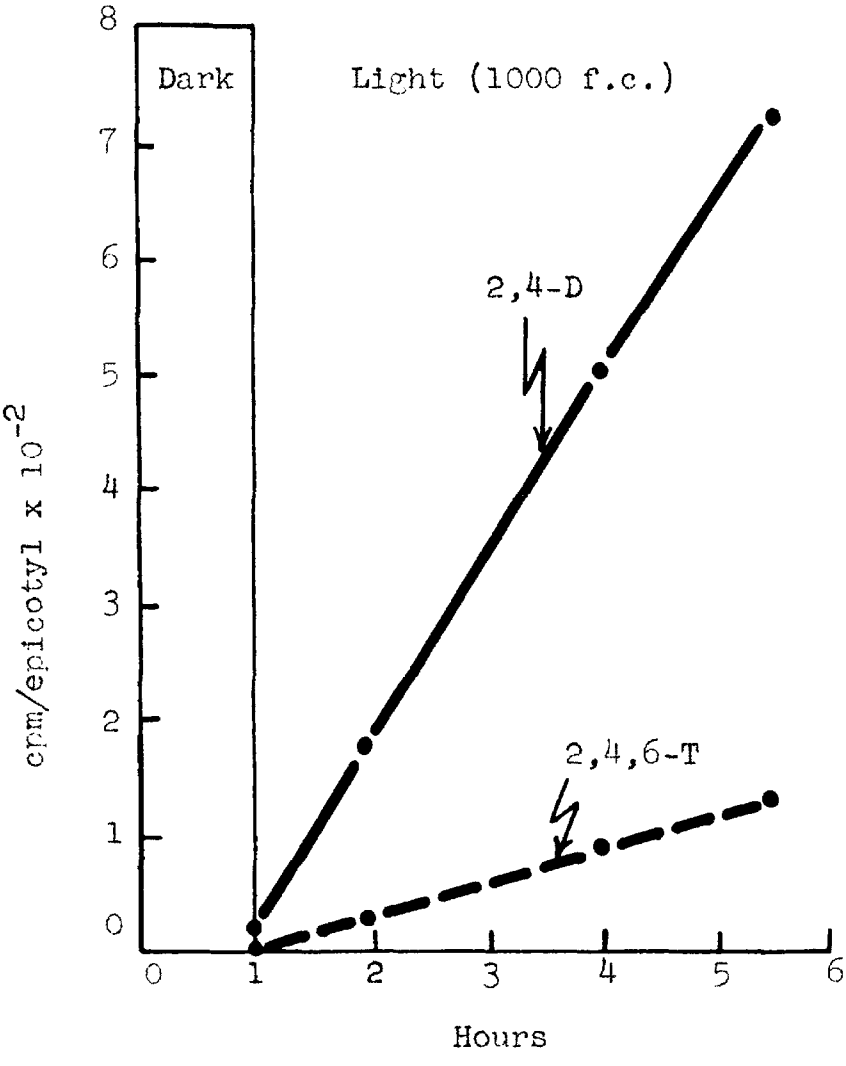

Figure 7. Comparison of the amounts of 2,4-D and 2,4,6-T (cpm) present in the epicotyls, at various time intervals, after treatment of the leaves with 2,4-D and 2,4,6-T of equal specific activities.

so delivered. Since the first amount of both compounds travels at the same rate, a common mechanism of transport is suggested.

An experiment was also conducted to determine if 2,4,6-T is absorbed by leaves as well as is 2,4-D. Five plants were treated with 2,4-D and five with 2,4,6-T. The solutions used were the same as those used in the previous experiment. After treatment, the plants were placed in darkness for one hour. The leaves were then washed with a 5% alcohol solution and the amount of activity in the washings determined. From a knowledge of the amounts of activity applied and recovered, the amount of absorption may be determined. The results are shown in Table XII.

It may be concluded from these data that 2,4-D and 2,4,6-T are equally well absorbed by bean leaves. The reason for the observed difference in the transport of 2,4-D and 2,4,6-T appears to lie in the leaf. Evidently more 2,4-D is delivered to the conducting tissue per unit of time than is delivered of 2,4,6-T. This may be a reflection of molecular weight or of the nature of the substituted atom. It would be of interest to know how labeled 2,4,5-T compares with 2,4-D and 2,4,6-T in this respect.

Crafts (14) compared the epicotyl curvature of plants treated with equal concentrations of 2,4-D and 2,4,5-T acid, each mixed with wetting agent. The curvatures induced by 2,4,5-T were less than those induced by 2,4-D. He concludes

TABLE XII

Percentage absorption of 2,4-D and 2,4,6-T by red kidney bean leaves

	Chemical	
	2,4-D	2,4,6-T
cpm applied to leaves	92,714	93,802
cpm recovered in leaf washings (5% alcohol)	68,704	69,355
cpm absorbed by leaves	24,010	24,447
Percentage uptake of label by leaves	25.9	26.0

that more 2,4-D than 2,4,5-T is translocated to the epicotyl. If this interpretation is correct, and the reduced curvature is not due to a difference in the efficiency of the two chemicals in inducing growth, the results reported here for 2,4,6-T agree with those of Crafts for 2,4,5-T. This would indicate that the addition of a chlorine atom to the 2,4-D molecule, at either the five or six position, reduces the amount of chemical delivered to the phloem. Crafts states the following when comparing the transport of 2,4-D and 2,4,5-T:

> "Theoretically, the chlorine substitutions in these molecules are lipophilic, and the third chlorine might hinder the partition of the molecule from the lipid phase of the leaf or cell surface into the aqueous medium of the living cell."

It may be concluded, therefore, that the concentration of a chemical (i.e. 2,4-D) in a leaf determines the amount of this material transported, but that when comparisons are made between compounds, the concentration required to obtain the same amount of transport depends on the composition of the molecules.

This molecular specificity in transport seems to be especially true of certain metabolites, and indicates the presence of some type of screening mechanism which determines the nature and volume of the substances leaving the laminae.

A good example is found in the work of Vernon and Aronoff (57). As mentioned previously they subjected leaves of soybean to $C^{14}O_2$ and studied the transport of C^{14}-labeled compounds after 20 minutes. They were able to extract C^{14}-labeled sucrose, glucose, fructose, raffinose, triose, succinic acid, citric acid, alanine, glutamic acid and aspartic acid from the leaf, but only sucrose, glucose and fructose from the stem. The other compounds were not transported from the leaves during the experimental period. It would be very interesting to know where the barrier is located which opposes the movement of these chemicals from the laminae.

The fact that labeled compounds containing amino nitrogen were not transported out of the leaf during the experimental period is particularly interesting. It is possible that the experiment was too short to obtain the incorporation of a significant amount of C^{14} into a translocatory form of nitrogen, but it is also possible that a translocatory form of nitrogen is not synthesized in light. This is in agreement with the findings of Maskell and Mason (34) who found that the total nitrogen in the leaf of cotton increases during the day and diminishes at night.

Interaction of 2,4-D and 2,4,6-T in transport

If the mass flow hypothesis describes the translocation of solutes in plants, different molecules, regardless of their size (within limits) and configuration, should move in the sieve tubes of the phloem at the same rate. The data of the previous section comply with the former criterion, because the first amounts of 2,4-D and 2,4,6-T travel to the epicotyl at the same speed. On the basis of this result, it was inferred that the two compounds are transported by a common mechanism. Even if this inference is correct, however, it does not help in elucidation of the nature of the transport mechanism. Similar molecules might travel at equal rates by either active or passive mechanisms.

The following experiments were conducted to determine if the transport of 2,4,6-T and 2,4-D are independent, or if 2,4,6-T inhibits the transport of 2,4-D. In the first experiment, the primary leaves of one group of plants were treated with 4×10^{-2} µM of 2,4-D ($-C^{14}OOH$). A second group was treated with 1 µM of 2,4,6-T, a third group with a mixture of 4×10^{-2} µM of 2,4-D ($-C^{14}OOH$) plus 1 µM of 2,4,6-T. A fourth group was left untreated. The epicotyls were measured and the 10 plants were then dissected, the epicotyls measured and their 2,4-D (cpm) contents determined. The results are given in Table XIII (Experiment 1).

Both the data on growth and on radioactivity show that

TABLE XIII

Effect of 2,4,6-T on the transport of C^{14}-labeled 2,4-D

	Treatment	% increase in length of epicotyls	cpm/ epicotyl
Exp. 1	Control	4.3	-
	2,4-D*	10.6	119
	2,4,6-T+	6.0	-
	Mixture (2,4-D* + 2,4,6-T+)	7.1	61
Exp. 2	Control	5.6	-
	2,4-D*	15.0	227
	Mixture (2,4-D* + 2,4-D+)	20.8	150
	Mixture (2,4-D* + 2,4,6-T+)	14.5	189
Exp. 3	Control	4.8	-
	2,4-D*	17.5	227
	2,4,6-T+	3.9	-
	Separate spots (2,4-D* + 2,4,6-T+)(a)	10.5	140
	Separate spots (2,4,6-T+ + 2,4-D*)(a)	13.9	172
	Mixture (2,4-D* + 2,4,6-T+)	11.0	164

*C^{14}-labeled 2,4-D (4×10^{-2} µM/plant).

+Non-radioactive 2,4,6-T or 2,4-D (1 µM/plant).

(a)The first chemical in parenthesis was applied one cm. and the second chemical three cm. from the junction of the lamina and the petiole.

2,4,6-T inhibits the amount of 2,4-D transported to the epicotyl. This experiment does not demonstrate whether the lack of transport of 2,4-D in the presence of 2,4,6-T is due to competition in transport or whether 2,4,6-T inhibits the absorption of 2,4-D into the leaves.

A second experiment was conducted to determine if the addition of non-radioactive 2,4-D is as effective as 2,4,6-T in preventing the transport of radioactive 2,4-D. Three of the treatments were the same as those of experiment 1. The fourth group of plants was treated with a mixture of 4×10^{-2} μM of 2,4-D ($-C^{14}OOH$) and 1 μM of non-radioactive 2,4-D. Measurements were taken after the plants had been in light for five hours. The results are shown in Table XIII (Experiment 2).

The addition of either non-radioactive 2,4-D or 2,4,6-T to the radioactive 2,4-D decreased the amount of label transported to the epicotyl. The inhibition by 2,4,6-T is therefore not due to its anti-auxin properties.

A third experiment was performed in which 2,4-D ($-C^{14}OOH$) and 2,4,6-T were both applied to each leaf but as separate drops rather than as a mixture in one drop. This was done to prevent any competition in absorption. In one case, C^{14}-labeled 2,4-D (4×10^{-2} μM/plant) was applied to the upper surface of the leaf over the mid vein at a point one cm. from the junction of lamina and petiole. 2,4,6-T (1 μM/plant) was applied in the same manner at a distance

of three cm. from the junction point. In another set of ten plants, the positions of the two compounds were reversed. Four other groups of plants were included. One group was treated with C^{14}-labeled 2,4-D (4×10^{-2} μM/plant), another with 2,4,6-T (1 μM/plant) and a third with a mixture of C^{14}-labeled 2,4-D (4×10^{-2} μM/plant) and 2,4,6-T (1 μM/plant). The fourth group of plants was used as a control (untreated). The results are shown in Table XIII (Experiment 3).

As in the previous two experiments, 2,4,6-T in mixture with 2,4-D exerts an inhibitory effect on the movement of 2,4-D. A similar degree of inhibition is obtained, however, when the two chemicals are applied separately to different regions of the lamina. Therefore, 2,4,6-T at the concentration used in these experiments (1 μM/plant) appears to inhibit the transport and not the absorption of 2,4-D. We have already seen (Figure 4) that there is a decrease in the efficiency of C^{14}-labeled 2,4-D transport when a high concentration (8×10^{-2} μM/plant) of 2,4-D is applied to the laminae. Hay and Thimann (23) have shown that treatment of bean plants with 2,4-D inhibits the transport of a second application of 2,4-D made 24 hours later. It may be concluded that sufficiently high concentrations (1 μM/plant) of 2,4,6-T or 2,4-D are capable of inhibiting the transport of C^{14}-labeled 2,4-D.

The mechanism of this inhibition is not clear either

from the results reported here or from those reported by Hay and Thimann. Hay and Thimann (23) suggest that 2,4-D inhibits its own transport by damaging the transport mechanism in the stem. This judgment is based upon the fact that when leaves are treated with a dosage of 100 µg/plant of 2,4-D the amount transported to the stem is less than when the leaves receive an application of 75 µg/plant. The amount of 2,4-D which entered the leaf from the 100 µg application is, however, greater in absolute amount than that which entered from the lower dose. It would be very interesting to know how much of the applied 2,4-D entered the phloem in the two treatments.

There is another possible explanation for the observed inhibition of transport of 2,4-D by high concentrations of 2,4,6-T or 2,4-D. The two compounds may interfere with the rate of entry of sugar into the phloem. Any chemical which interferes with or augments (boron) the transport of sugars will affect the rate of 2,4-D translocation. Vernon and Aronoff (57) conclude from their studies that 2,4-D, applied to soybean leaves 24 hours before exposure of the leaves to $C^{14}O_2$, acts to decrease the rate of diffusion of sugars into the sieve tubes. Their experiments were not designed to determine how rapidly the inhibitory effect of 2,4-D is realized. If the time involved is short, and if 2,4,6-T is equally capable of inhibiting sucrose movement into the sieve tubes, this may be the reason for the

inhibitory effect of 2,4,6-T on the transport of 2,4-D.

A third possibility is that the capacity of the transport system is saturated at high concentrations of 2,4-D or 2,4,6-T.

The results of the last two sections show that 2,4-D and 2,4,6-T are translocated at the same speed in the phloem of the red kidney bean. However, 2,4-D is transported with greater facility than is 2,4,6-T. Since the two compounds are absorbed equally, the additional Cl atom in 2,4,6-T, because of its lipophilic nature (14), reduces the mobility of 2,4,6-T within the lamina. An elevated concentration (1 μM/plant) of unlabeled 2,4,6-T inhibits the transport of C^{14}-labeled 2,4-D, but no more so than an equal concentration of unlabeled 2,4-D. The mechanism(s) of the inhibition is not known but it is suggested that these compounds may either (a) damage the sieve tube cells, (b) interfere with the transport of sugars or (c) saturate the transport mechanism at elevated concentrations.

Effect of TIBA on transport

Application of triiodobenzoic acid (TIBA) to plants alters their normal development. Treated plants may display, (a) a loss of apical dominance, (b) a loss of geo- and photo-tropic sensitivity, (c) fasciation, (d) formative effects on leaves, (e) initiation and enhancement of flowering, and (f) increased abscission of plant parts. TIBA is

a sulfhydryl inactivating compound and has been found to affect auxin action. It is synergistic at low concentrations and inhibitory at higher concentrations in the Avena curvature test (54).

The papers of Niedergang-Kamien and Skoog (45) and of Niedergang-Kamien and Leopold (44) reveal that the abnormal symptoms displayed by plants treated with TIBA can be explained by a failure of auxin to be transported in the characteristic polar manner (63). Several other compounds, which interfere with respiratory processes, have also been shown to inhibit polar auxin transport. Dinitrophenol inhibits the process at concentrations which both stimulate and inhibit respiration. KCN is also an effective inhibitor of polar transport. In addition to TIBA, other sulfhydryl inactivating compounds such as iodoacetate, p-chloromecuribenzoic acid, phenyl mecuric chloride, and n-ethylmaleimide appear to interfere specifically with the polar transport of auxin.

Kuse (29) has demonstrated that TIBA, applied in a lanolin ring around a petiole, prevents the normal inhibition of the lateral bud by the leaf blade as well as by IAA applied to the cut end of the petiole. This demonstrates that TIBA inhibits transport of IAA through the petiole.

The work summarized above all concerns the cell to

cell polar transport of auxin and does not bear on the problem of transport of auxin in the phloem. The experiments reported here were designed to determine if TIBA, applied to the petioles of bean seedlings, inhibits the translocation of 2,4-D, 2,4,6-T or $C^{14}O_2$.

Effect of TIBA on the translocation of 2,4-D

The eighty plants used in this experiment were grown at 23°C. for ten days on a regime of 16 hours light (1000 f.c.) and eight hours dark. At the beginning of the last eight hour dark period, three groups of plants (A, B, and C) were selected for experimentation. Those of A were treated with TIBA solution at the rate of 100 µg/plant and those of group C were treated with alcohol (Materials and Methods). All three groups of plants were then placed in darkness for eight hours after which they received three hours of light (1000 f.c.). Radioactive 2,4-D solution (4×10^{-2} µM/plant) was then applied to the upper side of each primary leaf in the manner described previously (Materials and Methods). Plants in group B received, in addition, a treatment of TIBA as described for the plants of group A. To summarize, plants in all groups were treated at the same time with 2,4-D solution but those in group A received a pre-treatment with TIBA, those in group B were given TIBA at the same time as they were given 2,4-D, and those in group C were left untreated. After a

one hour dark period for absorption of the applied chemicals, the plants were returned to light.

The epicotyls of five plants in each group were sampled periodically to determine the amount of 2,4-D translocated from the leaves. The method of assay has already been described (Materials and Methods).

The data are given in Table XIV and Figure 8 and are expressed as the amount of radioactivity (cpm) present in each epicotyl. Comparison of columns A and C reveals that TIBA, applied as a pre-treatment, inhibited the amount of 2,4-D transported to the epicotyl. Column B, which represents those plants treated simultaneously with 2,4-D and TIBA, demonstrates that 2,4-D transport was unaffected by this TIBA treatment. This difference might be explained by assuming that 2,4-D is absorbed more rapidly than TIBA so that it is transported through the treated region before TIBA becomes effective. While this possibility cannot be neglected, it cannot be clarified from this experiment. Absorption of TIBA should have been complete at the end of the first hour, since the petiole was dry at this time. It must take at least five hours for TIBA to become inhibitory to transport. These results suggest that TIBA does not compete directly with 2,4-D in transport.

TABLE XIV

Effect of TIBA (100 µg/plant), applied to the petioles, on the amount of 2,4-D (cpm) present in the epicotyls of bean plants, at various time intervals, after treatment of the primary leaves with C^{14}-labeled 2,4-D (4 x 10^{-2} µM/plant).

	cpm/epicotyl		
Time (hr.)	A	B	C
1	45	-	61
2	118	269	211
3	171	416	620
4	313	836	830
5	480	1016	1263
6	-	1926	1790

A-plants pre-treated with TIBA.

B-plants treated simultaneously with TIBA.

C-plants untreated.

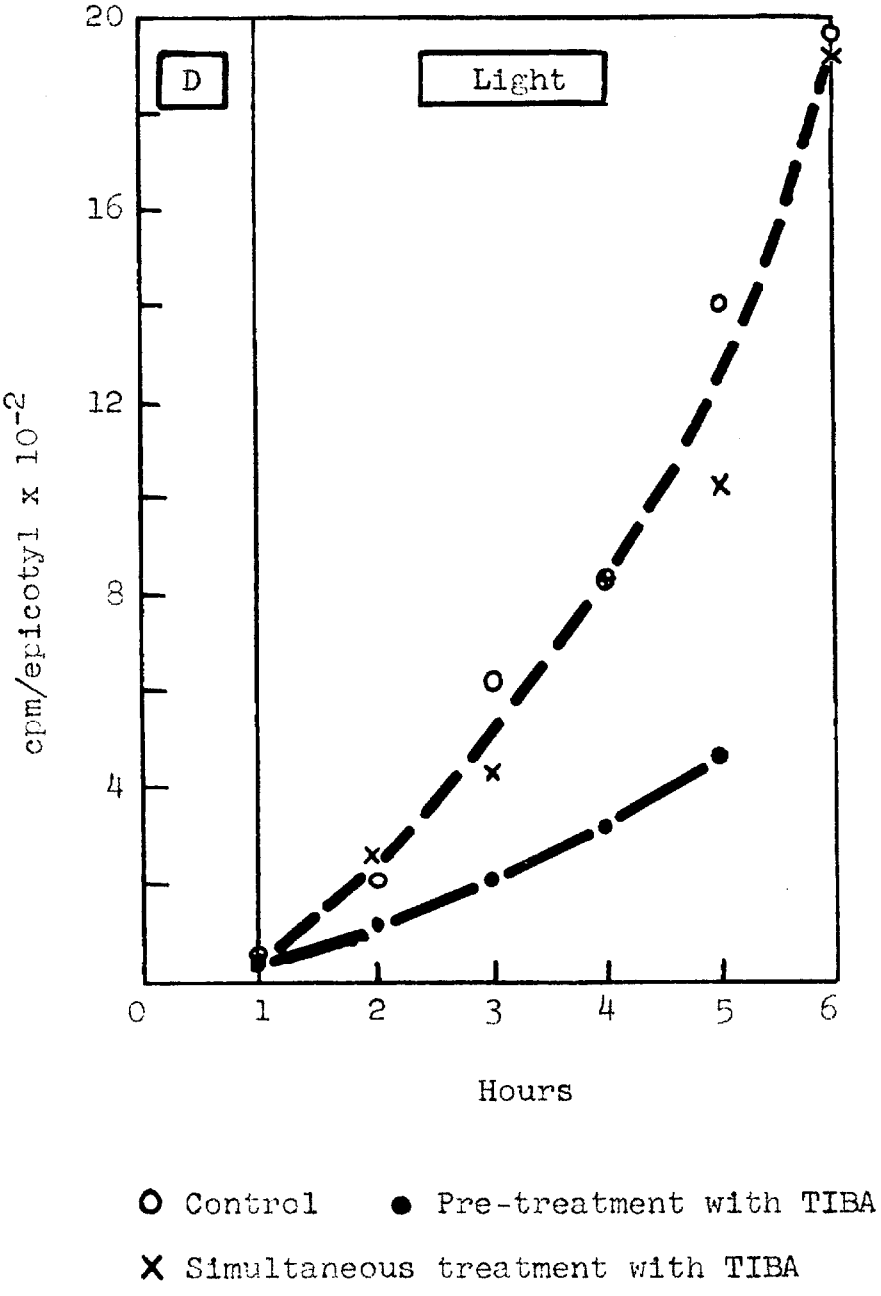

Figure 8. Effect of TIBA (100 µg/plant), applied to the petioles, on the amount of 2,4-D (cpm) present in the epicotyls, at various time intervals, after treatment of the primary leaves with C^{14}-labeled 2,4-D (4 x 10^{-2} µM/plant).

Effect of TIBA on the translocation of 2,4,6-T

The methods of culture and treatment of plants were the same as those just reported for the effect of TIBA on the translocation of 2,4-D. However, two rather than three groups of plants were used in this experiment. Plants in each group were treated with radioactive 2,4,6-T (4×10^{-2} μM/plant). One group was pre-treated with TIBA (100 μg/plant) eleven hours prior to the application of 2,4,6-T, and the other group served as a control (alcohol treated). The data are given in Table XV and Figure 9. The results are essentially similar to those found with the 2,4-D experiment. Pre-treatment of the petioles with TIBA inhibited the amount of 2,4,6-T translocated to the epicotyl.

The possibility that TIBA inhibits the amount of 2,4,6-T absorbed by the leaves was investigated by determining the amount of activity recovered in the leaf washings of TIBA treated and untreated plants. The results, shown in Table XVI, demonstrate that approximately the same amount of activity is recovered from both groups of plants.

It has been shown that pre-treatment of bean petioles with TIBA leads to a decrease in the amount of 2,4-D or of 2,4,6-T translocated to the epicotyl. Inhibition of 2,4-D transport was not found when TIBA was applied simultaneously with 2,4-D. The next question to be answered is that of whether TIBA is specific in inhibiting the transport of

TABLE XV

Effect of TIBA (100 μg/plant), applied to the petioles, on the amount of 2,4,6-T (cpm) present in the epicotyls of bean plants, at various time intervals, after treatment of the primary leaves with C^{14}-labeled 2,4,6-T (4×10^{-2} μM/plant)

	cpm/epicotyl	
Time (hr.)	2,4,6-T	2,4,6-T and TIBA*
1	13	-
3	289	200
6	1021	582
9	1293	962

*TIBA applied 11 hours prior to 2,4,6-T treatment.

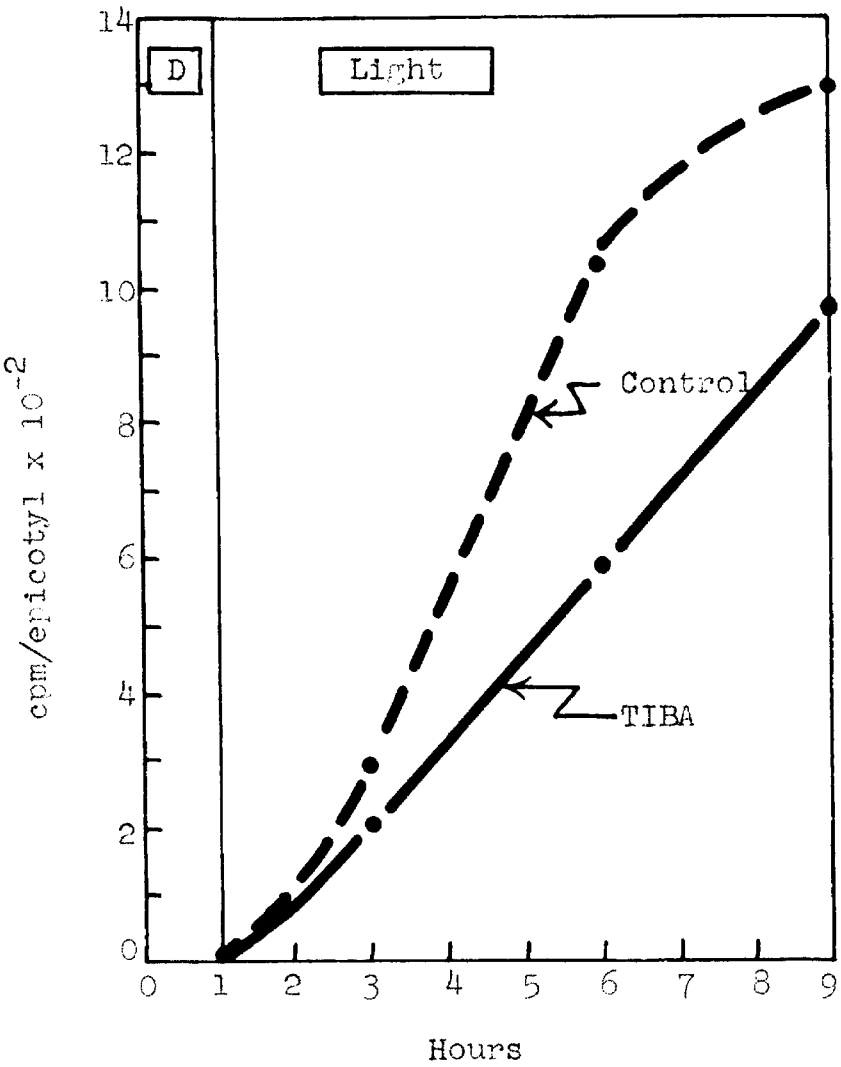

Figure 9. Effect of TIBA (100 µg/plant), applied to the petioles, on the amount of 2,4,6-T (cpm) present in the epicotyls, at various time intervals, after treatment of the primary leaves with C^{14}-labeled 2,4,6-T (4 x 10^{-2} µM/plant).

TABLE XVI

Recovery of 2,4,6-T (cpm) in leaf washings of TIBA pre-treated and untreated plants 6 and 9 hours after application of C^{14}-labeled 2,4,6-T (4×10^{-2} μM/plant)

Time (hr.)	2,4,6-T	2,4,6-T and TIBA	Diff.
6	85,178	86,275	1,097
9	87,781	87,937	156
Sum	172,959	174,212	1,253
Ave.	86,480	87,106	626

compounds of the phenoxy series or whether it affects the transport mechanism. It will now be shown that the latter is in fact the case.

Effect of TIBA on the translocation of sugars

An experiment was performed in which randomly labeled sucrose was applied to the leaves of TIBA pre-treated and untreated plants. Despite the fact that the sucrose offered contained 86,000 counts per minute, no significant amount of label could be detected in the epicotyl at any time interval up to ten hours. This was probably due to a lack of sufficient absorption into the leaves. Recently Nelson and Gorham (42) have reported a similar problem with sugar absorption by the soybean leaf. They were able to overcome this difficulty by adding a surface active agent and boric acid to the sugar solution.

Vernon and Aronoff (57) have shown that sucrose is the principal labeled product translocated from soybean leaves which have photosynthesized in the presence of $C^{14}O_2$. This indirect method was used to administer sucrose in the present case. It was first demonstrated that Vernon's and Aronoff's finding with soybean is true also of the red kidney bean. In passing it may be noted that sucrose was found to be the principal labeled product translocated from leaves of different ages so that the age of the leaf is not a complicating factor.

TIBA pre-treated and non-treated (alcohol treated) plants were allowed to photosynthesize in the presence of $C^{14}O_2$ for ten minutes (Materials and Methods). The chamber was then evacuated, excess $C^{14}O_2$ trapped and the plants removed. A total of 20 minutes elapsed between the time $C^{14}O_2$ was released until the plants were removed. Some of the plants, in each group, were dissected immediately and the various parts (leaves, petioles, buds, epicotyls, hypocotyls and roots) extracted and the C^{14} content of the extract determined (Materials and Methods). The remainder were dissected 80, 140 and 260 minutes after the beginning of C^{14} administration.

Since the entire plant was exposed to $C^{14}O_2$, and because the object of the experiment was to determine how much transport occurs from the leaves, some plants from which all leaves had been removed were also exposed to $C^{14}O_2$. These plants were used to correct the data since they revealed the amount of C^{14} taken up directly by the various plant parts as distinct from the amount transported to them from the leaves. The amount of $C^{14}O_2$ absorbed by these defoliated plants was only 0.1-0.2% of that absorbed by plants possessing leaves. The corrected values showing the distribution of C^{14} (cpm and %), in the various plant parts, at 20, 80, 140 and 260 minutes after exposure to $C^{14}O_2$ are presented in Table XVII. The percentage values are plotted in Figure 10.

TABLE XVII

Distribution of C^{14} (cpm and %) in normal and TIBA pre-treated plants, at various time intervals, after a 20 minute exposure to $C^{14}O_2$

		20 min.		80 min.		140 min.		260 min.	
Plant part		TIBA	NORMAL	TIBA	NORMAL	TIBA	NORMAL	TIBA	NORMAL
Bud	cpm	0	110	2,745	1,603	2,700	4,530	2,430	2,050
	%	0	0.04	1.28	0.69	1.84	2.02	1.95	1.56
Petiole	cpm	1,637	3,664	11,806	14,077	8,733	12,529	8,210	8,445
	%	0.85	1.28	5.51	6.06	5.94	4.60	6.58	6.44
Epicotyl	cpm	539	1,691	11,372	15,405	1,715	10,130	5,750	7,163
	%	0.27	0.59	5.30	6.63	1.17	4.52	4.61	5.47
Hypocotyl	cpm	222	726	40,206	35,356	25,122	31,152	21,942	28,016
	%	0.11	0.25	18.75	15.21	17.10	13.91	17.60	21.38
Roots	cpm	132	300	44,475	47,575	44,475	70,425	39,825	39,975
	%	0.07	0.11	20.74	20.47	30.28	31.46	31.94	30.51
Total transport	cpm	2,530	6,491	110,604	113,976	82,745	128,766	78,157	85,639
	%	1.31	2.27	51.60	49.04	56.34	57.42	62.68	65.36
Leaves	cpm	191,250	278,625	103,785	118,447	64,132	95,115	46,538	45,390
	%	98.69	97.73	48.40	50.96	43.66	42.48	37.32	34.64
Total	cpm	193,780	285,116	214,389	232,423	146,877	223,881	124,695	131,029

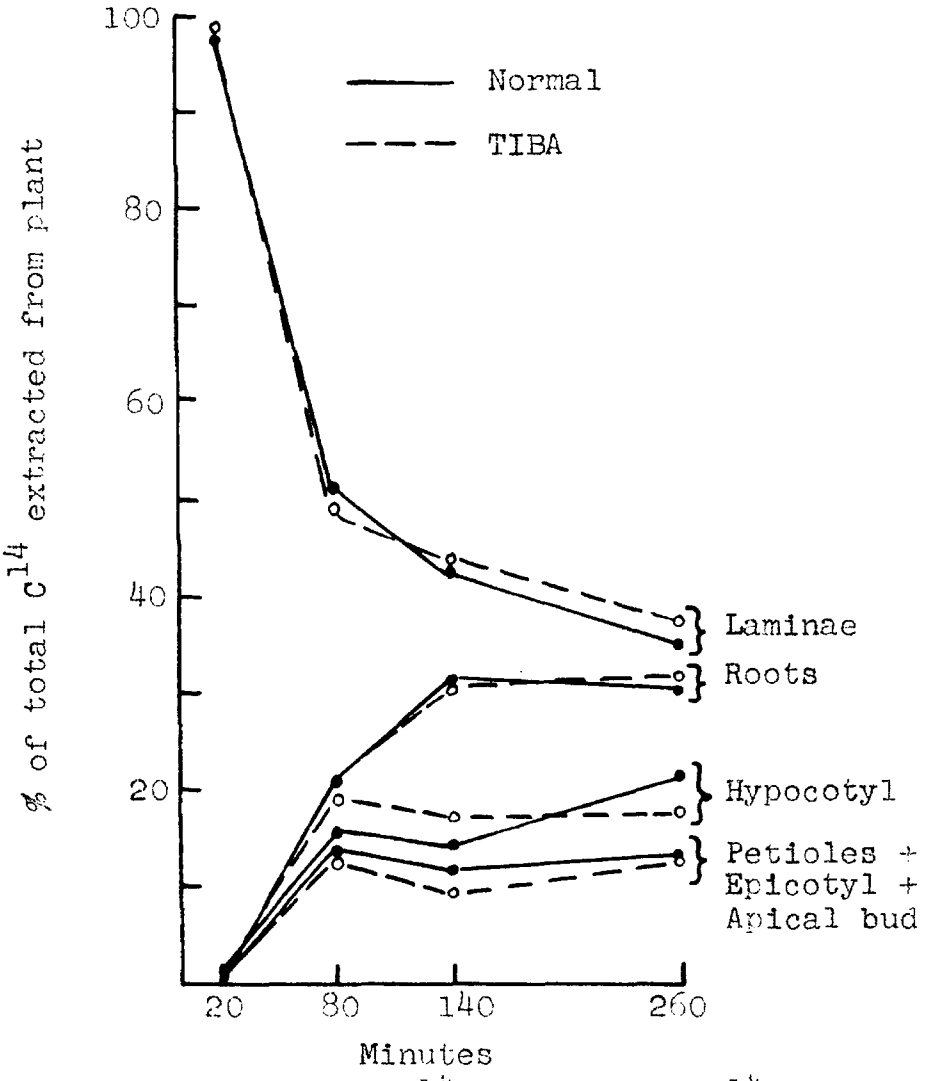

Figure 10. Distribution of C^{14} (% of total C^{14} extracted from plant) in the laminae, roots, hypocotyl and remaining portions (petioles, epicotyl and apical bud) of normal and TIBA pre-treated plants, at various time intervals, after exposure to $C^{14}O_2$ for 20 minutes

Before discussing this experiment, the data from a second experiment will be presented. Instead of determining separately the amount of C^{14} in the petioles, epicotyl, hypocotyl, roots and apical bud, these parts were combined and assayed for C^{14} as a single unit. The laminae were analyzed separately for their C^{14} content. By this method, the partition of C^{14} (cpm and %) between the laminae and the rest of the plant was determined, at various time intervals, and used to describe the transport of C^{14}. The methods used to culture and treat the plants with TIBA and $C^{14}O_2$ were the same as those described in the previous experiment. Each determination except one (see Table XVIII), was performed in duplicate.

The data in Tables XVII and XVIII show a difference between the amount of C^{14} extracted from normal and TIBA pre-treated plants. In the first but not in the second experiment, there is also a decline in extractable C^{14} with time (Appendix A). The absorption of different quantities of activity by plants makes direct comparisons between treatments difficult. This difficulty can be overcome by expressing the amount of C^{14} activity in each of the various parts as a percentage of the total extractable C^{14}. When this method is used, the results of the two experiments are in reasonably good agreement. The time course curves prepared by averaging the results of the two experiments are shown in Figure 11. The data will be discussed under the

TABLE XVIII

Distribution of C^{14} (cpm and %) in control and TIBA pre-treated plants, at various time intervals, after a 20 minute exposure to $C^{14}O_2$

Plant no.	Plant part		Time of dissection: 20 min. TIBA	20 min. Control	80 min. TIBA	80 min. Control	140 min. TIBA	140 min. Control	260 min. TIBA	260 min. Control
1	Laminae	cpm	140700	115500	97900	116100	61400	69000	44200	46508
		%	98.26	95.90	65.40	62.27	42.86	46.05	37.62	32.63
	Rest of plant	cpm	2485	4825	51800	70350	81850	80850	73275	96030
		%	1.74	4.01	34.60	37.73	57.14	53.95	62.38	67.37
	Total	cpm	143185	120325	149700	186450	143250	149850	117475	142538
2	Laminae	cpm	115600	114300	87800	79700	-	46200	50250	40400
		%	98.97	96.31	61.72	59.77	-	41.40	43.12	34.85
	Rest of plant	cpm	1230	4375	54450	53650	-	65400	66275	75530
		%	1.30	3.69	38.28	40.23	-	58.60	56.88	65.15
	Total	cpm	116830	118675	142250	133350	-	111600	116525	115930
Ave.	Laminae	cpm	128150	114812	92850	97900	61400	57600	47225	43454
		%	98.57	96.15	63.61	61.22	42.86	44.06	40.36	33.62
	Rest of	cpm	1857	4600	53125	62000	81850	73125	69775	85780
		%	1.43	3.85	36.39	38.78	57.14	55.94	59.64	66.38

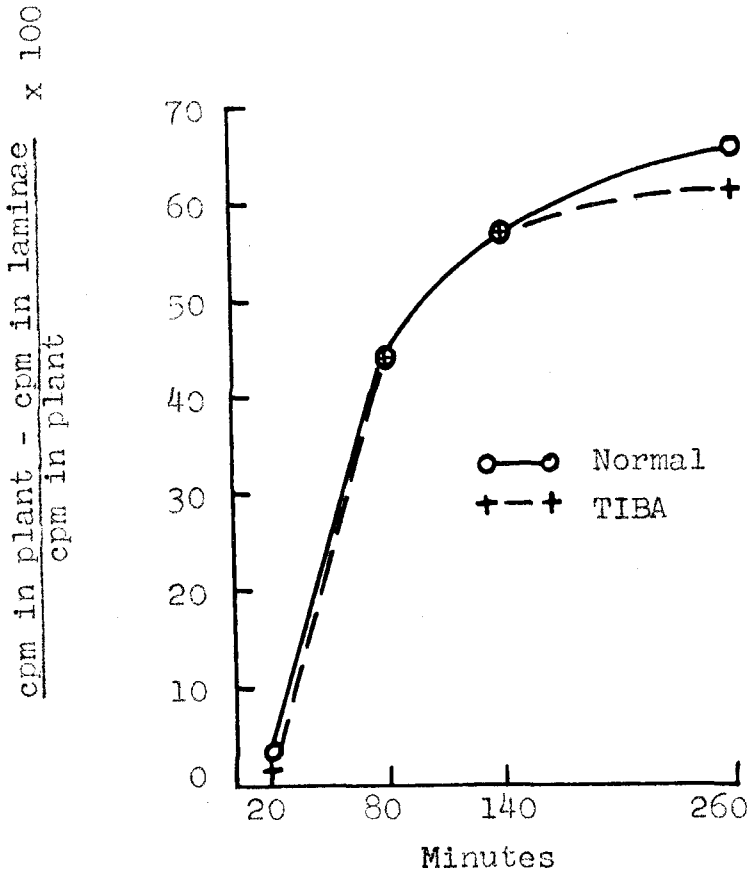

Figure 11. Transport of C^{14} (% of total extractable C^{14} in the plant which is in the stem and root), at various time intervals, after exposure of normal or TIBA pre-treated plants to $C^{14}O_2$ for 20 minutes.

following headings:

Distribution of C^{14} (%) in the petiole, apical bud, epicotyl, hypocotyl and roots at 20, 80, 140, and 260 minutes after exposure of the plants to $C^{14}O_2$

Figure 10 shows, at the different sampling times, the distribution of C^{14} in the different portions of normal and TIBA pre-treated plants. The data for apical bud, petioles and epicotyl (Table XVII) are combined to clarify the figure.

During the first 20 minutes after exposure to $C^{14}O_2$ only a very small portion of the C^{14} fixed by the leaves is transported to the rest of the plant. This time lag is probably required for the incorporation of C^{14} into sucrose and, more important, the accumulation of the latter into the veins.

During the next hour a very large portion of the C^{14} in the leaf is translocated. Accompanying this loss from the laminae is a concomitant increase of C^{14} in all of the remaining portions of the plant.

Transport of C^{14} continues from the leaf during the following hour (80 - 140 minutes) but at the 140 minute sampling time the relative distribution of label in the root and stem has changed. The percentage of extractable C^{14} in the root has continued to increase, whereas, the percentage of C^{14} in the portions of stem (except for the apical bud of normal and the apical bud and petioles of

TIBA pre-treated plants) has declined.

Measurement of the distribution after 260 minutes reveals (a) the laminae have continued to lose C^{14}, (b) the percentage C^{14} in the roots is essentially the same as at the 140 minute sampling time, and (c) the percentage C^{14} in the different portions of the stem has increased.

Comparison between the transport of C^{14} in normal and TIBA pre-treated plants

Tables XVII and XVIII show that during the first 20 minutes, less C^{14} is transported from the laminae of TIBA pre-treated plants than from normal plants. The difference is large and occurs in each of the three cases where comparisons can be made. The apical bud data (Table XVII) show very clearly the slower rate of C^{14} transport in TIBA pre-treated plants. Similar inhibitions were detected at the other sampling times in six out of the eight possible cases. Even a small difference in the amount of label transported probably represents a considerable difference in the total amount of sugar transported.

Three suggestions may be offered in an attempt to explain the inhibitory action of TIBA on sucrose transport.

1. TIBA may impair transport across the treated region of the petiole.
2. TIBA may interfere with the synthesis or the accumulation of sugar into the phloem.

3. TIBA may interfere with the utilization of sugar in the sieve tubes by inhibiting the interconversion of carbohydrates.

If the first suggestion is correct, one might expect an accumulation of C^{14} in the petiole above the treated region. Comparison of the petiole data in Table XVII (20 and 80 minute sampling times) shows this not to be true.

From the available data, it is impossible to evaluate the importance of each of the other two suggestions. This is due to the fact that TIBA is probably transported to both the stem and laminae after application to the petioles. The experiments of Hay (21) demonstrate that TIBA is translocated downward from the leaf to the epicotyl where it exerts an inhibitory effect on the transport of auxin. Epicotyls dissected from plants pre-treated (24 hours) with TIBA transported less IAA from donor to receiver blocks of agar than epicotyls from untreated plants. Whatever the chemical action of TIBA, the net effect appears to be a reduction in the amount of sugar which leaves the laminae per unit time.

These results may be used to explain the inhibitory effect of TIBA on the transport of C^{14}-labeled 2,4-D and 2,4,6-T. It is suggested that TIBA inhibits 2,4-D and 2,4,6-T transport by lowering the rate of sugar movement which in turn is positively correlated with transport. The

data shown graphically in Figure 9 support this conclusion. The upper curve (untreated) shows that the rate of arrival of 2,4,6-T in the epicotyl begins to decline six hours after treatment, whereas, 2,4,6-T transport in TIBA pretreated plants (lower curve) is essentially linear up to nine hours. Presumably if this experiment had been conducted over a longer period of time the same amount of 2,4,6-T would have been delivered to the epicotyl in both cases.

The results of this section demonstrate that pretreatment of bean petioles with TIBA (100 µg/plant), which inhibits the polar transport of auxin, also inhibits the translocation in the phloem of 2,4-D, 2,4,6-T and the C^{14}-labeled sugars derived from photosynthesis in the presence of $C^{14}O_2$. The inhibition of sugar transport is used to explain the inhibition of 2,4-D and 2,4,6-T transport, although it is realized that TIBA probably affects several physiological processes and cannot be expected to be specific in its action.

Translocation of labeled water

Up to this point, we have discussed the characteristics of solute translocation. These results, however, aid neither the investigator nor the reader in reaching a decision regarding the nature of the mechanism of translocation. Bonner and Galston (9) have made the following proposal in their book:

> "It would seem that a clear decision between pressure flow and diffusion as the motive force of translocation might be made on the basis of experiments designed to determine whether or not solvent and solute flow together in the sieve tube. According to the concept of pressure flow, solvent and solute must flow together as a solution. According to the concept of diffusion, on the other hand, solutes diffuse through the solvent, and the movements of the two are independent. The critical experiment, which might be done by following simultaneously the movements of isotopically marked water and sugar within the phloem, has not been done."

The first investigators to conduct and report on such an experiment were Biddulph et al. (5) and Biddulph and Cory (6). They applied three tracers P^{32}, THO and $C^{14}O_2$ simultaneously to bean leaves and studied their distribution in the stem 15 minutes later. The C^{14} traveled almost

exclusively as sucrose and at a speed of 106 cm. per hour. P^{32} and T moved at speeds of 60 and 66 cm. per hour, respectively. These speeds are based on attempted measurements of the moving front of each tracer. In the words of the investigators,

> "It is necessary to assign sucrose movement a status of its own in translocation, independent of water movement, or to condemn THO as a tracer for water movement in the phloem."

Preliminary experiments

The experiment which is to be done consists in discovering whether labeled water moves down the plant despite the fact that there is a mass of water moving up the plant in the transpiration stream. The first experiments to be reported were done with O^{18}-labeled water. This choice was made partly on the basis of available facilities and partly on the basis of Biddulph's and Cory's skepticism of the use of tritium as a tracer for water translocation. In later experiments, however, tritium labeled water (THO) was also used to measure water transport.

The method of growing bean plants was as described earlier. The water transport experiments are, therefore, similar to those on 2,4-D, 2,4,6-T and sucrose transport and comparisons may be made between solute and solvent transport.

Two groups of plants were treated with O^{18}-labeled water and then placed in darkness for three hours. One group was removed to light (800 f.c.) while the other was kept in darkness. At intervals, plants from light and from dark were dissected and the O^{18} content of the total water of the epicotyls determined (Materials and Methods). Two sets of untreated plants were dissected at the beginning of the experiment to determine the natural O^{18} content of the epicotyl water. The values presented (Table XIX) represent enrichment of O^{18} in the sample over that of the untreated control sample.

The data of Table XIX indicate that O^{18} is translocated downward from the leaves and that this translocation appears to be dependent upon light.

In a second larger scale experiment, the technique of the experiment was modified in that (a) two rather than three hours in the dark were allowed for equilibration (this is the time which it takes treated leaves to dry), (b) the plants received 1000 rather than 800 f.c. after the dark equilibration period, and (c) C^{14}-labeled 2,4-D (4×10^{-2} μM/plant) was applied as a drop (10 μl) to each leaf immediately after labeled water was administered. This was done to determine if water and 2,4-D move simultaneously from the leaves to the epicotyl.

The comparison between 2,4-D and H_2O^{18} transport is, however, not actually valid since two different methods were

TABLE XIX

Enrichment (% over controls) of O^{18} in epicotyl water, of light and dark grown plants, at various time intervals (2, 3, 5.5, and 7.8 hours), after treatment of the leaves with H_2O^{18}

Time (hr.)	Percent enrichment of O^{18}	
	Light	Dark
2.0	-	.08
3.0	-	.09
5.5	.31	.10
7.8	1.19	-.31*

*This value is suspected to be incorrect, since technical difficulties were encountered in determining the O^{18} content.

employed for application of the two tracers. A separate experiment was therefore performed under exactly the same conditions except that 2,4-D ($-C^{14}OOH$) was added to the water before application to the leaves. H_2O^{16} was used instead of H_2O^{18} because only a limited number of O^{18} determinations could be made. The results of the two experiments are shown in Table XX and are discussed under the following heading.

Comparison between transport of 2,4-D ($-C^{14}OOH$) and H_2O^{18} in plants placed in light after a two hour dark equilibration time

Except for the high value of 1.55% O^{18} enrichment at the three hour sampling time, the data from this experiment can be interpreted to mean that 2,4-D (cpm) reached the epicotyl in detectable quantities before H_2O^{18}. Enrichment of O^{18} in the epicotyl water did not occur, to any considerable extent, until approximately 3 - 4 hours after the arrival of 2,4-D. This delay in O^{18} transport immediately suggests that transport of water and 2,4-D have different properties.

It is also evident that in this experiment water moved from leaf to epicotyl in plants in light. Between four and five hours after treatment, the O^{18} content of the water in the epicotyl began to increase. It then rose abruptly, passed through a maximum between five and seven hours, and

TABLE XX

Amount of 2,4-D (cpm) and H_2O^{18} (% enrichment over controls) in epicotyl of light (1000 f.c.) and dark grown beans, at various time intervals, after treatment of the primary leaves.

		2,4-D (cpm)				H_2O^{18} (% enriched)	
		A*		B**			
	Time (hr.)	Light	Dark	Light	Dark	Light	Dark
DARK	0	-	-	0	0	-	-
	1	-	-	-	-	-	-
	2	-	9	36	14	-	0.01
LIGHT	3	39	24	97	67	1.55	0.12
	4	42	-	131	163	0.11	-
	5	80	82	199	157	0.75	0.19
	6	154	-	297	269	4.52	-
	7	238	131	359	331	2.87	4.00+
	8	275	-	603	248	0.08	-
	9	274	215	563	180	-0.02	0.35

*2,4-D applied to primary leaves as a drop immediately after treating the whole leaf with H_2O^{18}.

**2,4-D applied to primary leaves by dipping in H_2O^{16} containing 2,4-D.

+This value is thought to be correct because of the reproducibility found in the control plants dissected both at time 0 and at the end of 9 hours. At time 0 duplicate samples differed by only 0.006% O^{18} and at 9 hours by 0.015% O^{18}. The average difference between the two sets of values (0.015% O^{18}) is an indication of the constancy of the normal O^{18} content of epicotyl water.

again declined to approximately background level eight and nine hours after treatment. Therefore, under the conditions employed in this experiment, a single application of labeled water resulted in what appears to be a peak of O^{18} activity in the epicotyl approximately six hours after treatment. In light grown plants, there is also an indication of an earlier peak of activity. These peaks were not apparent in the first experiment because the number of O^{18} determinations was insufficient.

Comparison between transport of 2,4-D ($-C^{14}OOH$) and H_2O^{18} in plants kept in darkness after treatment

It is evident from the data of Table XX that in this type of experiment, both water and 2,4-D move from the leaf to epicotyl even in the dark. That the amount of 2,4-D transported in darkness was larger than in earlier experiments may have been due, in part, to the use of relatively large volumes of water containing wetting agent. Water may increase 2,4-D transport by (a) increasing the absorption of 2,4-D (56), or (b) increasing the turgor pressure in the leaves (see mass or pressure flow hypothesis in Introduction and Review of Literature). That much movement of water in the dark was not evident in the first experiment is evidently due also to an insufficient number of samples.

These results demonstrate that, with the present tech-

nique, labeled water and 2,4-D are both transported in dark as well as in light although the amount of transport is greater in the light.

If O^{18} is a suitable label for tracing the downward transport of water, the same time course curves should be obtained if tritium labeled water (THO) is substituted for H_2O^{18}. The results from such an experiment are reported in the next section.

Transport of THO

The primary leaves of 54 plants were treated with THO (100 μC/plant) and then placed in darkness for one hour. At the end of the dark period (a) two groups of three plants were dissected and the epicotyls saved for tritium determinations, and (b) 24 of the remaining 48 plants were returned to light. At intervals of 2, 3, 4, 5, 6, 7, 8, and 9 hours after treatment, samples of three plants from light and dark were dissected and the water of the epicotyls combined and assayed for tritium (Materials and Methods). The results are shown in Table XXI.

For purposes of comparison, the results of this experiment and those of the last experiment are plotted adjacent to one another in Figure 12. Figure 12 (b) shows that the time course curve for plants treated with THO and grown in light (after one hour in dark) is very similar to the one obtained for plants treated with H_2O^{18}. The fact that the

TABLE XXI

Amount of THO (cpm) in epicotyls of light and dark grown plants, at various time intervals, after treatment of the primary leaves with THO (100 μC/plant)

Time (hr.)	cpm/epicotyl	
	Light	Dark
1	4200	3745
2	356	1854
3	3	1960
4	171	1880
5	2000	912
6	2870	203
7	3650	674
8	42	2965
9	790	1585

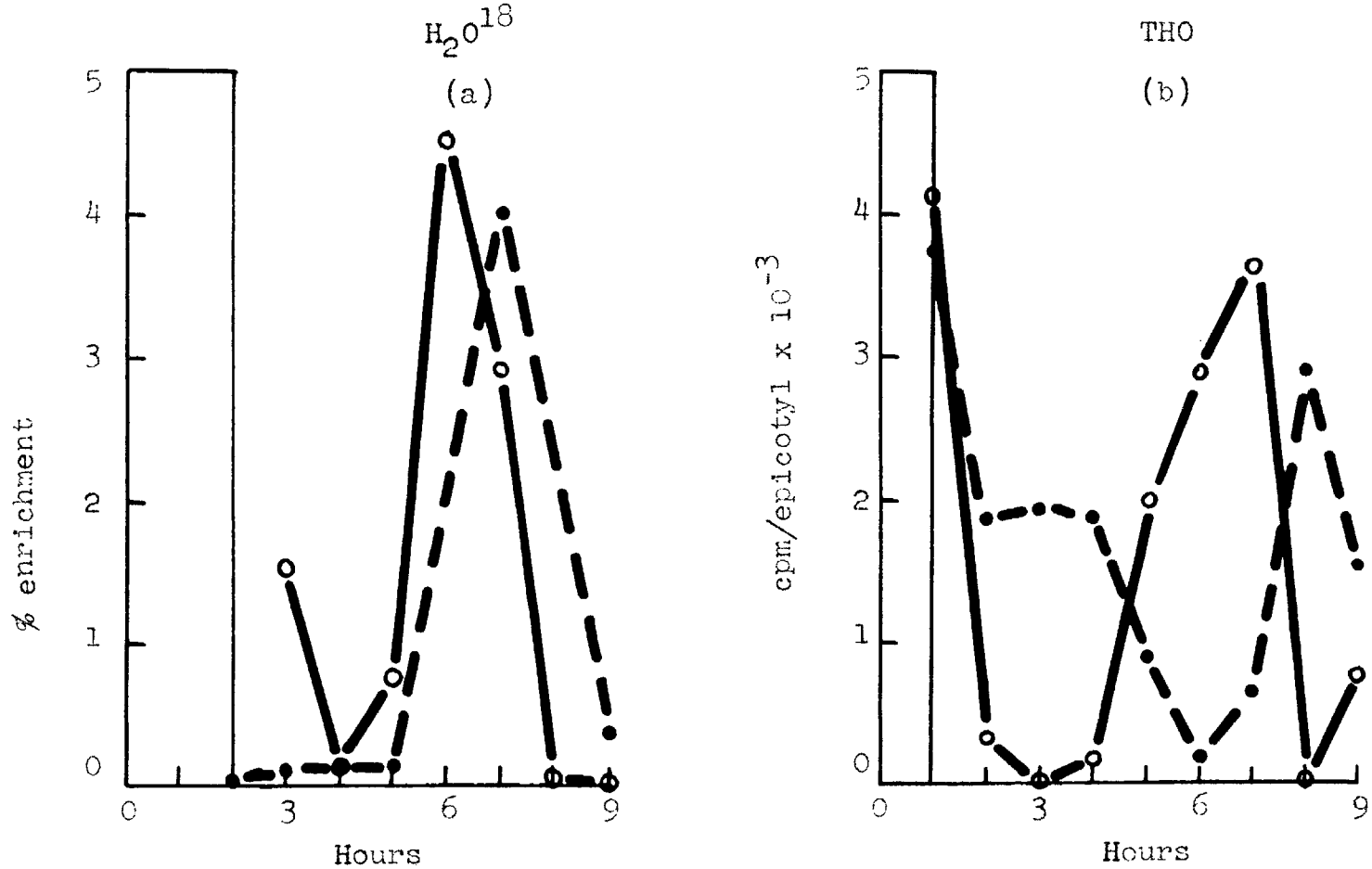

Figure 12. Amount of (a) O^{18} (% enrichment) and (b) T (cpm) in water extracted from epicotyls of light (—o—) and dark (— — • — —) grown plants, at various time intervals, after treatment of the laminae with H_2O^{18} or THO.

tritium level is high at the end of the first hour indicates that a considerable amount of label was transported from the leaf during the dark period. Therefore, the high value for O^{18} (Table XX), three hours after treatment, is probably correct and is not due to a sampling error.

The time course curves for dark grown plants treated with H_2O^{18} (Figure 12 (a)) and THO (Figure 12 (b)) are quite different. In H_2O^{18} treated plants, the O^{18} content of the epicotyl water is not increased until five hours after treatment, whereas, the tritium level is almost as high, at the end of the one hour dark period, as in light grown plants (duplicate samples). This shows that the transport of water measured by the technique used with tritium is different from the transport measured by the technique used in the O^{18} water experiments.

The tritium level in the epicotyls of dark grown plants does not decline as rapidly as it does in light grown plants, and does not reach a minimum until six hours after application of THO to the leaves. After six hours the level again increases and passes through a maximum after eight hours. It is interesting that the peaks for O^{18} and T, in light and dark, bear the same relationship to one another (i.e. the dark peak occurs approximately one hour after the light peak).

The discrepancy in the early parts of the O^{18} and T time course curves are probably to be understood on the basis

of the different techniques used in the application of the labeled water to the leaves.

Before the results of this experiment were obtained, two experiments were conducted to determine if the transport of labeled water (THO and H_2O^{18}) is dependent upon a supply of carbohydrate in the leaves.

Transport of THO, H_2O^{18} and C^{14}-labeled 2,4-D in carbohydrate-depleted plants

(a) Transport of H_2O^{18} and 2,4-D ($-C^{14}OOH$)

Sixty uniform plants were placed in darkness for 30 hours to lower the carbohydrate level of their leaves. Fifty four of these plants were then treated with H_2O^{18} containing 2,4-D ($-C^{14}OOH$) and returned to darkness for two hours. The other six plants were divided into two groups of three, dissected and the O^{18} content of the normal epicotyl water determined (Materials and Methods). Twenty seven of the treated plants were then placed in light (1000 f.c.) and the remainder left in darkness. At various time intervals (3, 5, 7, 8, 9, 10, 11, 12, and 14 hours after treatment), groups of three plants were taken from both light and dark, dissected and the O^{18} content of the epicotyl water and 2,4-D content (cpm) of the dry epicotyl (extraction method) determined. The results are shown in Table XXII and are graphed in Figure 13.

Figure 13 (a) shows that 2,4-D is transported in light

TABLE XXII

Amount of 2,4-D (cpm) and H_2O^{18} (% enrichment of O^{18} over that of the untreated controls) in epicotyls of light and dark grown carbohydrate-depleted plants, at various time intervals (3, 5, 7, 8, 9, 10, 11, and 14 hours) after treatment of the laminae with H_2O^{18} containing 2,4-D ($-C^{14}OOH$)

Time (hr.)	2,4-D (cpm)		H_2O^{18} (% enrichment)	
	Light	Dark	Light	Dark
3	10	0	-.05	.07
5	57	10	.01	.01
7	247	0	.66	.08
8	134	0	.27	.21
9	270	0	.08	.84
10	349	0	2.04	1.32
11	518	0	2.07	.28
12	415	0	*	.46
14	483	0	1.00	3.10

*Sample lost during equilibration.

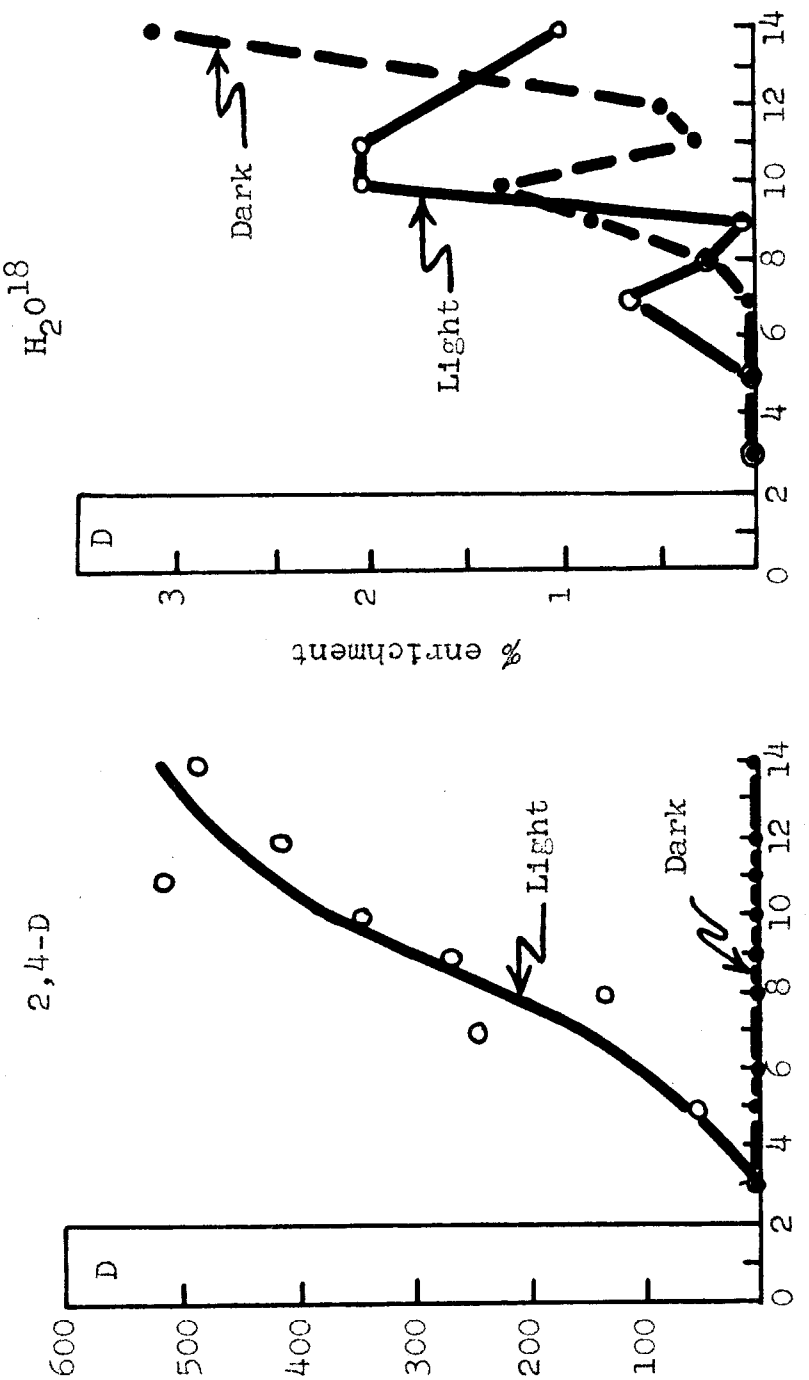

Figure 13. Amount of 2,4-D (cpm) and H_2O^{18} (% enrichment of O^{18} over that of the untreated controls) in epicotyls of light and dark grown carbohydrate-depleted plants, at various time intervals, after treatment of the laminae with H_2O^{18} containing 2,4-D ($-C^{14}OOH$).

but not in dark grown plants. The transport of H_2O^{18} is seen to be fundamentally different from 2,4-D movement by virtue of its movement in both light and dark grown plants. It is interesting to note that the water in the epicotyl of light grown plants did not become enriched in O^{18} until approximately seven hours after treatment, whereas, 8 - 10 hours elapsed before the water became enriched in O^{18} of dark grown plants. This may be a significant finding in view of the results obtained in the following section.

(b) Transport of THO and 2,4-D ($-C^{14}OOH$)

Thirty uniform plants, kept in darkness for 30 hours, were treated with THO containing 2,4-D. After treatment they were kept in darkness for two hours and then 15 of them were placed in light (1000 f.c.). At intervals of 3, 5, 7, 9 and 12 hours after treatment, groups of three plants from light and dark were dissected and the epicotyls assayed for 2,4-D and THO. The results are presented in Table XXIII and Figure 14.

The data for 2,4-D transport confirm those of the last experiment. The experimental values are not as variable as those of the last experiment, and the slope of the curve is greater, because the 2,4-D was applied as a drop (definite concentration) to the mid-vein instead of to the entire surface of the lamina. When labeled solution is applied to the entire surface, more time is required to accumulate

TABLE XXIII

Amount of 2,4-D (cpm) and THO (cpm) in epicotyls of light and dark grown carbohydrate-depleted plants, at various time intervals (3, 5, 7, 9, and 12 hours) after treatment of the laminae with THO (100 μC/plant) containing 2,4-D ($-C^{14}OOH$)

Time (hr.)	cpm/epicotyl			
	2,4-D		THO	
	Light	Dark	Light	Dark
3	0	0	2,813	4,757
5	151	0	622	640
7	317	0	8,500	3,100
9	320	0	8,367	0
12	341	0	4,427	1,360

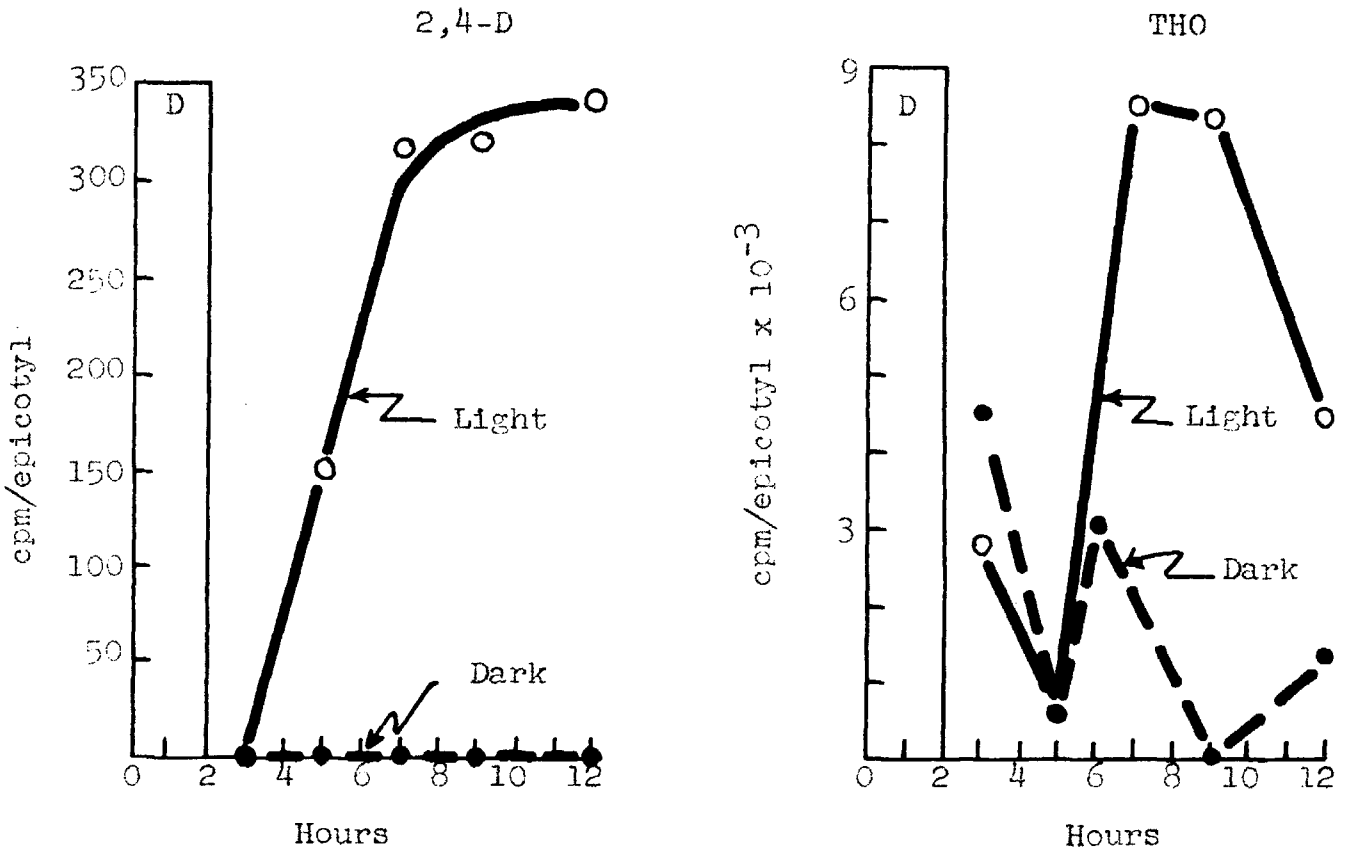

Figure 14. Amount of 2,4-D (cpm) and THO (cpm) in epicotyls of light and dark grown carbohydrate-depleted plants, at various time intervals, after treatment of the laminae with THO containing 2,4-D ($-C^{14}OOH$).

the label in the main veins.

The characteristics of THO transport are different from those of 2,4-D. Tritium travels more rapidly than 2,4-D to the epicotyl and moves equally well (3 and 5 hr. sampling times) in plants exposed to light or darkness after treatment. The amount of THO in the epicotyl from 6 - 12 hours after treatment, however, is less in dark than in light grown plants.

The experiments reported up to this point indicate that the transport of water (THO or H_2O^{18}) is fundamentally different from that of 2,4-D. The most striking difference between 2,4-D and labeled water movement, in carbohydrate-depleted plants, occurs in dark grown plants. This result immediately suggests that the downward movement of labeled water occurs in the xylem rather than in the phloem as reported by Biddulph et al. (5) and Biddulph and Cory (6).

Translocation of tritiated water in normal and girdled plants

The following experiment was designed to test whether THO travels in the phloem or xylem, and also to determine the distribution of label, in the various plant parts, at successive time intervals, after treatment of the leaves with THO. It was hoped that the data would reveal the peak of activity suspected, from the previous experiments, to occur in the epicotyls from 0 - 2 hours after treatment of

the leaves. From an analysis of all the plant parts, the source of label contributing to the peak of activity in the epicotyls of plants possessing starch 5 - 8 hours after treatment, should become evident. This second peak is particularly interesting because its occurrence could indicate (a) a slower type of transport in one conducting tissue, (b) downward movement at different rates in separate conducting tissues, or (c) a return of the first suspected peak of activity from the roots.

Eighty-four uniform plants were selected for this experiment. Twenty-four hours prior to treatment, the petioles of forty-two of these plants were girdled with a jet of steam. After steaming the petioles collapsed so the laminae had to be supported. They were placed at right angles to the incident light (1000 f.c.). Two hours before application of THO, the plants were watered with nutrient solution and the girdled plants inspected to make certain the steaming operation had been successful. The girdled petioles were completely shrunken and each appeared as a fine thread connecting the lamina and epicotyl. The water conducting properties of this steamed petiole did not appear to be impaired, however, because the laminae did not wilt at any time prior to or during the experiment. As evidence of the efficiency of girdling, neither 2,4-D nor 2,4,6-T was transported past the girdled zone in plants comparable in every respect to those used in this experiment.

After application of 10 µl of THO (10 mC/ml.) to each of the primary leaves, the plants were placed in darkness for one hour after which they were returned to light (1000 f.c.). Groups of three plants from girdled and normal plants were dissected, at various time intervals (0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 hours), after treatment into five portions (laminae, petioles and apical bud, epicotyl, hypocotyl and roots) and comparable parts combined, frozen, lyophylized and the water in all but the laminae assayed for tritium (Materials and Methods). The data are shown in Table XXIV and again in graphic form in Figures 15 and 16.

Figure 15 shows the distribution of tritium in the petiole and apical bud, epicotyl, hypocotyl and roots of normal plants, at various time intervals, after treatment of the leaves with THO. Inspection of these four graphs and the three in Figure 16 representing the tritium concentration in the epicotyls, hypocotyls and roots of girdled plants reveals the undulating nature of the time course curves.

Before commenting on these undulations, some general conclusions may be drawn from the data shown in Figures 15 and 16. Probably the most interesting result obtained from this experiment is the fact that as much or more THO is transported from the leaves of girdled as from the leaves of normal plants. Girdling of the phloem is completely in-

TABLE XXIV

Amount of tritium (cpm) in the petioles and apical bud (P + B), epicotyl (E), hypocotyl (H), and roots (R) of normal (N) and girdled (G) plants, at various time intervals, after treatment of the laminae with THO

		cpm x 10^{-1}							
	Time (hr.)	P + B		E		H		R	
		N	G*	N	G	N	G	N	G
DARK	0.25	1086	-	578	618	1216	1844	860	1026
	0.50	2100	-	1128	496	1344	1004	836	1360
	0.75	1698	-	1420	3422	1572	3656	830	838
	1.00	1218	-	1040	2820	1158	2746	682	317
LIGHT	1.50	2694	-	1556	3360	1072	2648	567	803
	2.00	1014	-	492	1538	580	3260	96	804
	2.50	516	-	242	550	694	870	669	291
	3.00	880	-	426	712	1332	846	536	279
	4.00	366	-	180	200	822	1148	381	464
	5.00	450	-	188	228	836	588	71	140
	6.00	216	-	848	288	362	1476	0	503
	7.00	498	-	158	210	728	1174	367	129
	8.00	144	-	130	220	536	672	342	233
	9.00	408	-	234	424	714	1390	0	14

*Not enough water could be extracted from girdled petioles for analysis.

Distribution of THO in Normal Plants

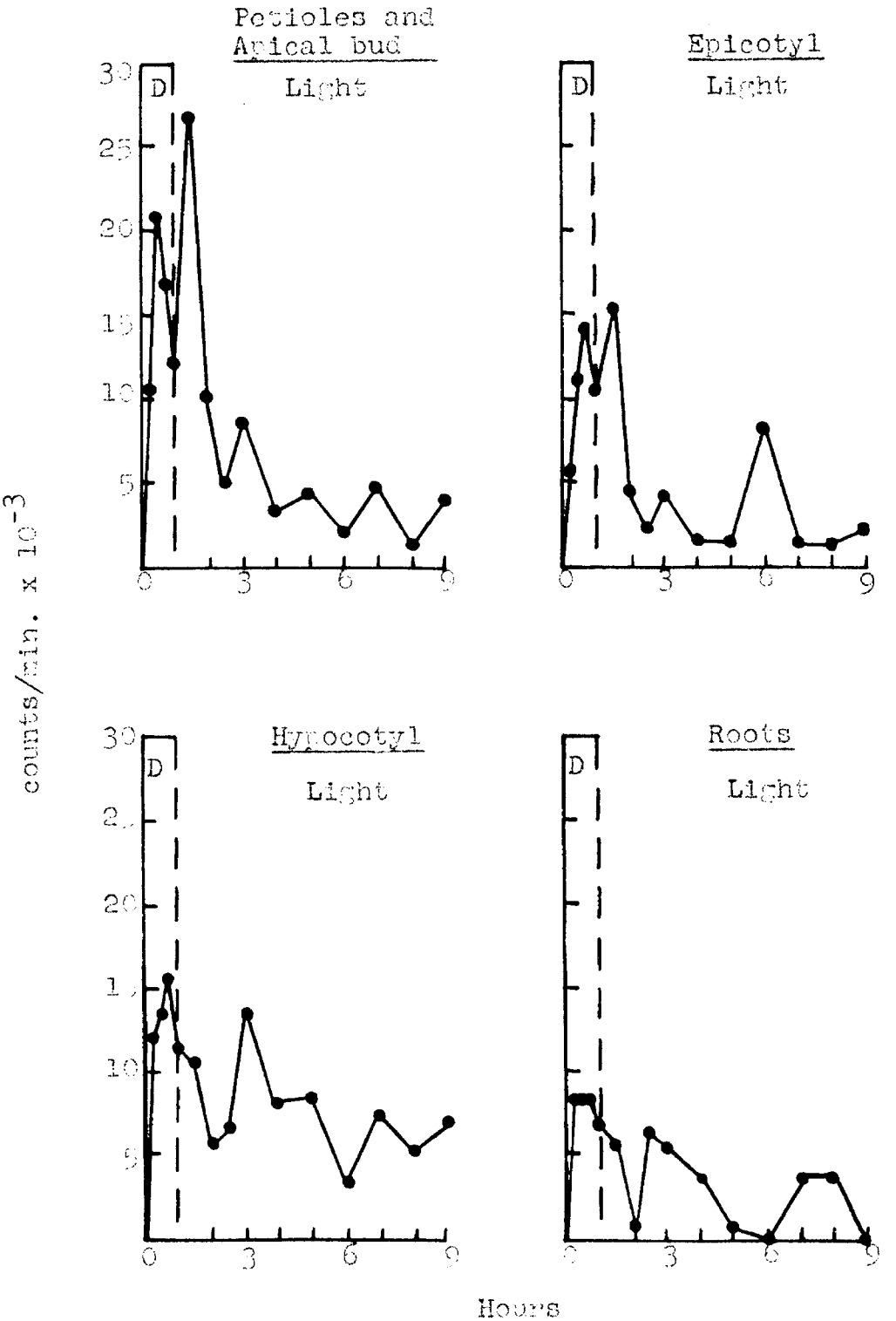

Figure 15. Amount of THO (cpm) in the water extracted from the petioles, epicotyl, hypocotyl and roots of normal plants, at various time intervals, after treatment of the laminae with THO.

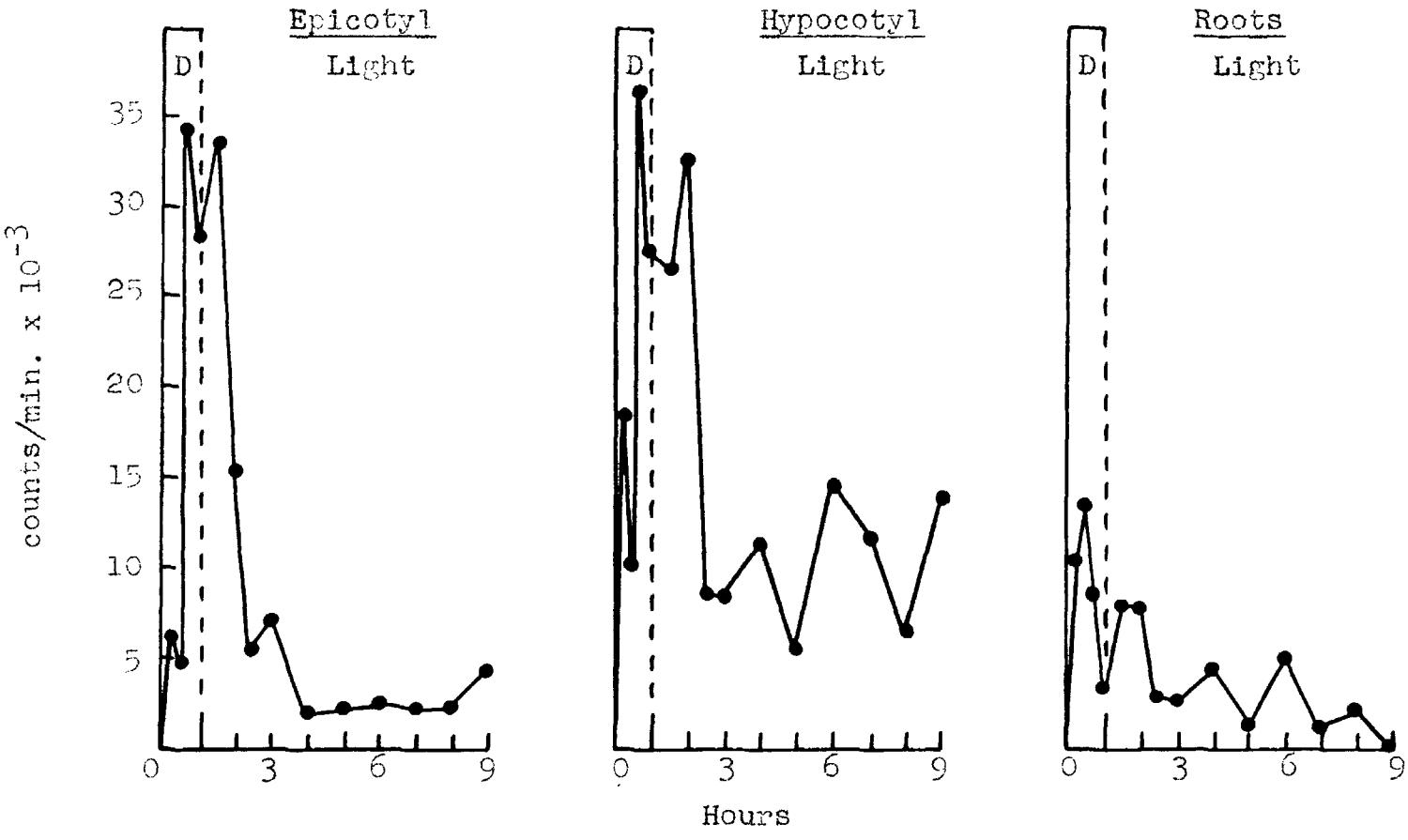

Figure 16. Amount of THO (cpm) in the water extracted from the epicotyl, hypocotyl, and roots of girdled plants, at various time intervals, after treatment of the laminae with THO.

effective in preventing the transport of tritium labeled water. Because the cells in the region of the girdle were killed with steam, it is concluded that downward movement of THO occurs in dead tissue presumably the xylem. If transport of THO occurs in the phloem it is completely masked by transport in the xylem. This is a particularly interesting observation for it demonstrates that the xylem is capable of transporting simultaneously in both directions. The evidence for the upward movement of water resides in the fact that the leaves were not wilted and inspection of the stomates of comparable plants by infiltration (alcohol) and the ultrapak microscope revealed that some of the stomates were open. Therefore, transpiration must have been operative. Some recent results of Nelson and Gorham (43) are in agreement with these results. They found that KCN did not prevent the downward movement of C^{14}-labeled glucose and fructose but prevented the transport of C^{14}-labeled sucrose. They concluded that glucose and fructose are capable of traveling in dead tissue, whereas, sucrose is transported in the living phloem.

The downward transport of THO in the xylem is very rapid and must be at least as fast or faster than the transport of some solutes (sucrose) in the phloem. An accurate estimate of the speed cannot be ascertained from this experiment, but a rough estimate would be 1 - 3 cm/minute. Biddulph et al. (5) report that tritiated water moves in

the phloem at a speed in excess of 3 cm./minute.

The major portion of the THO is transported within the first two hours after treatment. This result was suspected from the earlier experiments. It is interesting to note that tritium does not accumulate in the root. This must mean that THO is lost from the root by upward movement in the transpiration stream where it is presumably lost to the atmosphere. Another avenue of loss may be through equilibration with the outside nutrient solution. If the major portion is lost through transpiration, it is further evidence for a bidirectional movement of water in the xylem. This two way movement of THO in the plant is probably the reason, at least in part, for the undulating nature of the time course curves.

There exists the possibility that the undulations are due to the absorption and transport of unequal quantities of THO, by the different groups of plants. If this is the case the experimental points should be fitted to a smooth curve. While this may be the correct method of handling the data, there are some indications that at least some of the peaks of activity are real. For example, the shape but not the magnitude of the transport curves are approximately the same for normal and girdled plants during the first 4 - 5 hours after treatment. Three peaks of tritium activity appear to occur in the stem and root during this time interval. The first peak occurs during the one hour

dark period and the second upon returning the plants to light. The third suspected peak occurs within 2 - 5 hours after treatment. From the available information it is difficult to propound a plausible explanation for these apparent peaks of activity. A proper explanation will have to await the results of future experiments.

One of the objectives of this experiment was to find the source of label contributing to the peak of activity in the epicotyls 5 - 8 hours after treatment. Unfortunately, this peak is represented, in normal plants, by only one value. It should be noted, however, that this increase in activity is associated with a drop in activity of the root, hypocotyl and possibly the petiole. There is a complete absence of a peak of activity in the epicotyls of girdled plants although there is a peak in the hypocotyls. The significance of these apparent peaks will have to be tested in future experiments.

This investigator wishes the reader to know that the plants used in this experiment were very uniform. Therefore, the undulating transport curves cannot be ascribed to differences between groups of plants but must be ascribed to the complexities involved in the downward transport of labeled water.

The presence of more THO in the epicotyls and hypocotyls of girdled plants than in those of normal plants cannot be explained fully but the following suggestion is

offered. If it is assumed that the tritium activity of the water leaving the laminae, in girdled and normal plants, is the same, then there must be a greater loss of T in normal plants. Where could this preferential loss occur? If water travels in both directions in the xylem and at the same rate in normal and girdled plants, then the loss of label through equilibration would be expected to be the same in both cases. Equilibration of label with the surrounding tissue in the petiole, however, would be much greater in normal than in girdled plants. The net effect would be a preferential dilution of label in the petioles with the result that the activity of the water reaching the epicotyl, hypocotyl and roots in girdled plants would be greater than normal plants. Unfortunately, not enough water can be extracted from girdled petioles to determine their tritium content.

Before arriving at this interpretation, it was suspected that the greater carbohydrate supply trapped in the leaves of girdled plants may have led to the greater export of THO. This supposition was proved incorrect in the following interesting experiment where the transport of THO in plants possessing starch was compared with the transport of THO in carbohydrate-depleted plants. The experiment is presented to demonstrate an important pitfall which may be encountered in studies of translocation.

The 72 plants used in this experiment had the following

history. Fifty four plants were grown for 24 days at 23°C. on a regime of 16 hours light (1000 f.c.) and eight hours dark. After four hours of light on the twenty-fifth day, they were divided into three groups of 18 and placed in darkness for 47 hours. The remaining group of 18 plants was grown for an additional two days on 16 hours light and eight hours dark and on the twenty-seventh day they received three hours of light prior to treatment with THO. The petioles of nine plants from each of the four groups were girdled with steam 21 hours before treatment with THO.

One hour before studying the transport of tritium, the leaves of one group of carbohydrate-depleted plants were sprayed with a 5% sucrose solution containing 5 ppm. of boric acid and 0.1% Tween 80. The boron was added to increase the absorption of sugar (42), and Tween 80 to reduce the surface tension of water. Treatment of carbohydrate-depleted plants in darkness with sugar solution has been used frequently to demonstrate the direct participation of sugars in initiating transport in the phloem. As a control for this treatment, a group of carbohydrate-depleted plants were sprayed with a water solution containing only boric acid and Tween 80. The third group of carbohydrate-depleted plants and the one group of plants possessing starch were not pre-treated.

The primary leaves of all of the 72 plants were treated in subdued light with THO (200 μC/plant). They were then

placed in darkness and three girdled and three non-girdled plants from each group dissected after 20, 40 and 60 minutes. The epicotyls and hypocotyls from these three plants were combined, frozen, lyophylized and the extracted water assayed for tritium (Materials and Methods). The results are shown in Table XXV and Figure 17.

Plot 1 of Figure 17 shows that tritium transport in carbohydrate-depleted plants is very small during the first hour after treatment with virtually no difference between girdled and normal plants. Plot 4, however, shows that tritium is transported readily in plants possessing starch and the increased transport in girdled plants is a confirmation of previous results. Pre-treatment of the leaves of carbohydrate-depleted plants with a 5% sucrose solution (Plot 2) brings about a transport of THO comparable to that in plants possessing starch. If sugar is omitted from the pre-treatment solution, however, the same results are obtained (Plot 3). This control eliminates, therefore, the direct participation of sugar in the downward transport of THO in the xylem. It also demonstrates the importance of having a water control in experiments which are designed to test the relationship between the transport of a particular compound and the transport of sugars. It may not be important to have such a control if it is first proven that the compound under investigation travels in the phloem.

The following experiment was performed in an attempt

TABLE XXV

Activity (cpm) of water extracted from the stems (epicotyl + hypocotyl) of three groups of carbohydrate-depleted and one group of plants possessing starch. The leaves of one group of carbohydrate-depleted plants were treated with 5% sucrose solution, another with water, and the third left untreated (0) prior to treatment with THO. The plants possessing starch were not pre-treated. The experiment was conducted in the dark and in each group the transport of THO is compared at 20, 40, and 60 minutes, in girdled (G) and normal (N) plants

	cpm/epicotyl + hypocotyl							
	carbohydrate-depleted plants						plants possessing starch	
	0		5% sugar		water		0	
Time (min.)	G	N	G	N	G	N	G	N
20	2,204	2,970	16,840	6,320	21,940	8,400	15,560	2,850
40	3,900	1,760	21,184	6,800	23,100	9,200	27,000	5,700
60	3,420	1,792	10,400	10,800	10,106	9,790	20,320	10,880

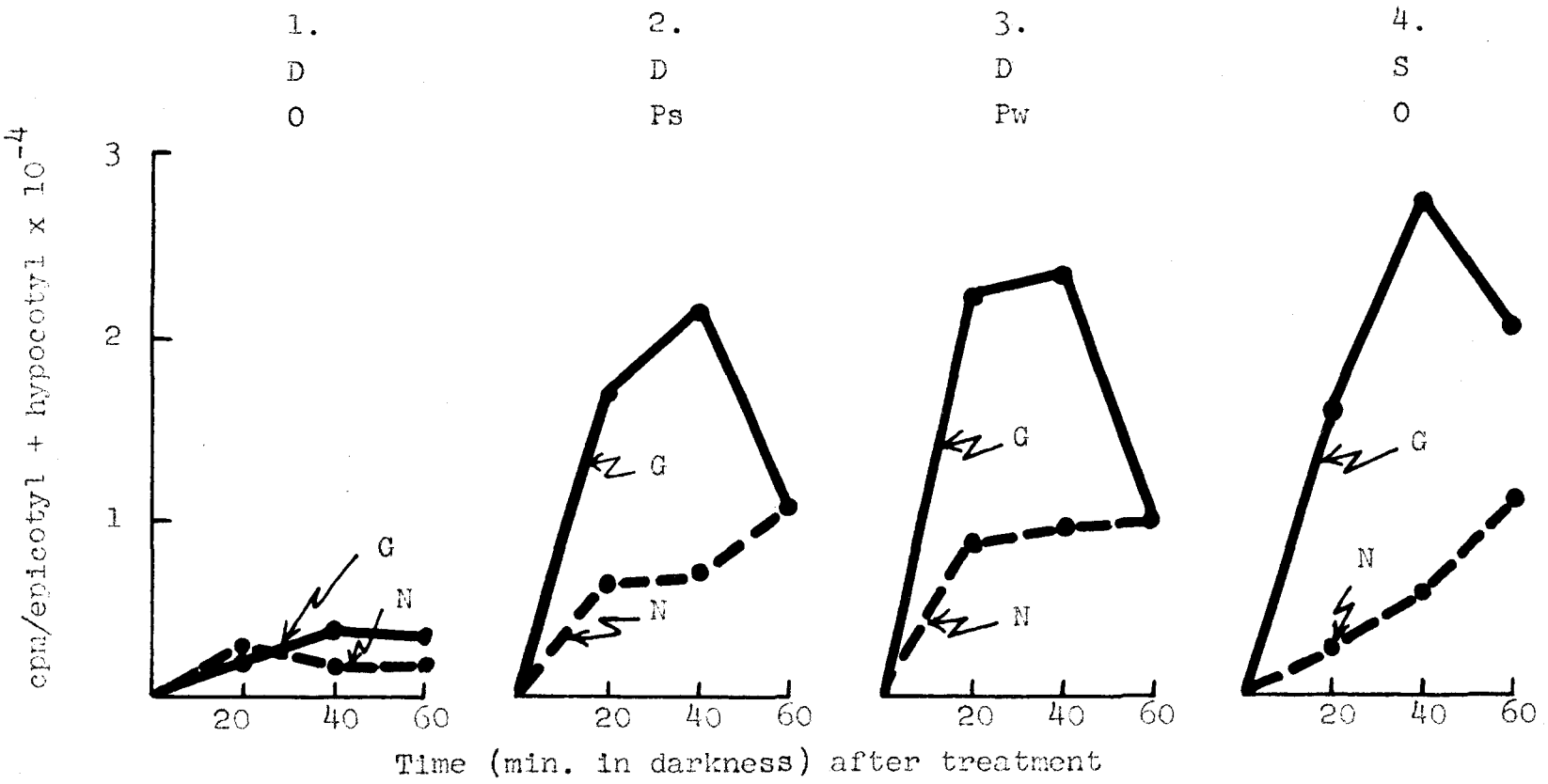

Figure 17. Activity (cpm) of water in stems (epicotyl + hypocotyl) of three groups of carbohydrate-depleted (D) and one group of plants possessing starch (S). The leaves of one group of D plants were treated with 5% sucrose solution (Ps), another with water (Pw) and the third left untreated (O) prior to treatment with THO. The plants possessing starch were not pre-treated. In each group, the transport of THO is compared in girdled (G) and normal (N) plants.

to determine which ingredient in the spray mixture is active in inducing the transport of THO in carbohydrate-depleted plants. The plants used in this experiment were grown under the same conditions as the carbohydrate-depleted plants in the last experiment. Forty uniform plants were divided into five groups (four girdled and four non-girdled) and each group received a pre-treatment with a different solution. The first group was pre-sprayed with distilled water, the second with water containing Tween 80 (0.1%), the third with water containing boric acid (5 ppm.), the fourth with water containing a mixture of Tween 80 (0.1%) and boric acid (5 ppm.), and the fifth was not pre-treated. One hour later, at which time the leaves were dry, the laminae were treated, in subdued light, with THO (200 μC/plant) after which they were returned to darkness. Forty minutes after application, the plants were dissected in groups of two and the stems (epicotyl + hypocotyl) collected for THO assay (Materials and Methods). Each determination was performed in duplicate. The results are shown in Table XXVI.

Table XXVI shows that there is a large variability between duplicate samples. This suggests that a factor(s) which influences the downward transport of tritium was not under control in this experiment. These results may lead the reader to seriously question the significance of the results reported in the previous experiments because of the

TABLE XXVI

Amount of tritium (cpm) in the water extracted from the stems (epicotyl + hypocotyl) of normal and girdled carbohydrate-depleted plants, 40 minutes after treatment of the leaves with THO (200 μC/plant). One set of plants was pre-treated with distilled water, the second with water containing boric acid (5 ppm), the third with water containing Tween 80 (0.1%), the fourth with water containing boric acid and Tween 80, and the fifth set left untreated

Laminae pre-treated with	Sample No.	cpm/(epicotyl + hypocotyl) Normal	Girdled	Ave.
0	1	2,670	4,116	
	2	2,564	2,676	
	Ave.	2,618	3,396	3,006
H_2O	1	11,802	12,034	
	2	12,790	22,900	
	Ave.	12,296	17,468	14,882
H_2O + Boric acid	1	20,460	45,588	
	2	15,884	13,310	
	Ave.	18,172	29,448	23,810
H_2O + Tween 80	1	35,910	23,336	
	2	68,484	98,214	
	Ave.	52,196	60,776	56,486
H_2O + Boric acid + Tween 80	1	14,180	18,840	
	2	84,744	10,820	
	Ave.	49,462	14,830	32,146

lack of replication. However, if the transport of tritium is as variable in different groups of plants, as that shown in Table XXVI, one would have expected the time course curves shown in the previous experiments to have been more variable and less reproducible. For example, Figures 15, 16 and 17 show clearly the difference in tritium transport in girdled and normal plants. The peak of activity occurring in the stem within the first hour is also the same in the two experiments. The similarity in the peaks of activity in Figures 15 and 16 and those shown in Figure 17 (plots 2 and 3) also indicate a general consistency in the results. Plots 2 and 3 may be considered as replicates because sucrose did not affect directly the transport of tritium.

The difference between the amount of tritium transport in plants possessing starch and carbohydrate-depleted plants (Figure 17, plots 1 and 4) is substantiated from the results of this experiment and from those of a separate experiment (not reported here in detail). The amount of activity transported in girdled carbohydrate-depleted plants was 4,116 and 2,676 cpm., whereas, the amount transported in duplicate sets of girdled plants possessing starch was 18,810 and 21,210 cpm.

The only general conclusion which can be drawn from the results shown in Table XXVI is that water appears to be the ingredient in the spray mixture which is mainly responsible for the increased transport of tritium in

carbohydrate-depleted plants. The additional increase with Tween 80 may be due to its ability to increase the efficiency of water in the induction of tritium transport.

The reason for the increased transport of tritium in carbohydrate-depleted plants pre-treated with water, to a level comparable with that of plants possessing starch (not pre-treated), is not known. It is possible that the addition of water to the leaves satisfies a condition of "incipient drying" (32) which results in a greater absorption or equilibration of labeled water. The added water, although it is absent from the surface of the leaf at the time of application of tritiated water, may provide a medium for equilibration to take place. If this hypothesis is true, only the portion of the laminae which is to be treated with THO need be pre-treated with water. This hypothesis assumes that plants possessing starch are not in a state of "incipient drying" at the time of application of THO.

CONCLUSIONS

The experiments reported in this thesis were designed to investigate specific questions regarding the transport of C^{14}-labeled 2,4-D, C^{14}-labeled 2,4,6-T, $C^{14}O_2$, H_2O^{18} and THO in the red kidney bean. It was hoped that the results would help to clarify the controversy which exists over the mechanism(s) of solute translocation in the phloem (Review of Literature). The principal results of these studies will now be summarized.

The cuticle of the bean leaf almost completely prevents the absorption of the potassium salt of 2,4-D but offers only moderate resistance to the absorption of the free acid of 2,4-D. This is in general accord with the findings of others who have found non-ionized auxin molecules to permeate the lipoidal cuticle more readily than ionized forms of the auxin (56).

After a compound has been absorbed, its mobility within the leaf depends upon its chemical constitution. For example, the amount of 2,4,5-T (14) or of 2,4,6-T which enters the phloem and is translocated to the rest of the plant is less per unit time than the amount of 2,4-D which so enters. It is suggested by Crafts (14) that the additional Cl atoms in 2,4,5-T and 2,4,6-T, reduce the mobility of these compounds within the leaf by making them more lipophilic.

The transport of foliarly applied 2,4-D to the epicotyl can be controlled by regulating the carbohydrate supply within the leaf by exposure of the plants to specific regimes of light and dark prior to treatment. Since the absorption of 2,4-D into the laminae is greater in darkness than in light (49), plants are treated in dim light and placed immediately in darkness until the treated zone is dry. If the treated leaf has an abundant supply of carbohydrate because of previous long exposure to light (Figure 1), 2,4-D is translocated during the dark period. However, if the treated leaf is low in available carbohydrate (Figures 1, 13 and 14), 2,4-D is not translocated until caused to do so by the addition of carbohydrate through photosynthesis. For carbohydrate-depleted plants, the first amounts of 2,4-D arrive in the epicotyl one hour after exposure of the plants to light (1000 f.c.). The carbohydrate level in the leaf may be regulated so that the first amounts of 2,4-D arrive in the epicotyl at the end of the dark period (Figure 2). This method (Materials and Methods) may be used in studies where the investigator wishes to compare the rates of transport of different compounds in the phloem. By regulating the carbohydrate supply in the leaf and by placing treated plants in darkness for a sufficient length of time, differences in rates of absorption into leaves of applied chemicals may be overcome. A difference in the time of arrival of the chemicals

at a site remote from the point of application may then be ascribed to a difference in speed of translocation.

The amount of 2,4-D translocated from the laminae to the epicotyl is dependent upon the concentration applied to the leaf (Figure 3). For each concentration, the first measurable amounts of 2,4-D arrive in the epicotyl at the same time. The amount of 2,4-D in the epicotyl then increases linearly during the following six hours with the slopes of the curves being greater as the concentration applied to the leaf increases.

The relationship between amount of 2,4-D transported to the epicotyl and concentration of 2,4-D applied to the laminae is essentially a linear one, and thus does not resemble the hyperbolic relation between 2,4-D concentration and growth. Over longer time intervals the transport curves tend to become slightly sigmoid indicating a decrease in the efficiency of 2,4-D transport at higher concentrations.

Plants grown under 1000 and 2000 f.c. of light translocate equal amounts of 2,4-D (Table VIII) presumably because of a saturation of the photosynthetic mechanism at 1000 f.c. However, the growth response to equivalent concentrations of 2,4-D is two to four times greater in plants grown under 2000 f.c. of light. It is suggested that plants grown under the higher light intensity possess a greater concentration of a factor(s) which complements 2,4-D action.

The presence of this factor is also indicated by the shorter time lag between the arrival of 2,4-D in the epicotyl and its inducement of growth. For plants grown under 1000 f.c. of light, 2,4-D induced growth of the epicotyl begins approximately one to two hours after the arrival of the growth substance (Figures 2 and 6), whereas, for plants grown under 2000 f.c. of light, the growth response is almost immediate especially for plants treated with the higher concentrations of 2,4-D (Figure 6).

The first amounts of 2,4-D and 2,4,6-T translocated from the laminae arrive in the epicotyl at the same time. However, the amount of 2,4-D which is delivered to the epicotyl, per unit time, is greater than the amount of 2,4,6-T so delivered. Movement of 2,4-D and 2,4,6-T at the same speed, suggests a common mechanism of transport. High concentrations of 2,4,6-T do inhibit somewhat the transport of C^{14}-labeled 2,4-D just as do high concentrations of 2,4-D itself. There does not appear to be any specific competition between 2,4,6-T and 2,4-D in their transport. The inhibition of C^{14}-labeled 2,4-D movement produced by high concentrations of these chemicals may be due to (a) damage to the sieve tube cells, (b) a reduction in the rate at which sugar is accumulated by the veins, or (c) a saturation of the transport mechanism (Results and Discussion).

TIBA inhibits the transport of C^{14}-labeled 2,4-D and 2,4,6-T if it is applied to the petioles of bean leaves,

11 hours prior to treatment of the laminae with 2,4-D or 2,4,6-T. TIBA does not inhibit 2,4-D transport if applied simultaneously with 2,4-D. The inhibition of C^{14}-labeled 2,4-D transport by TIBA is different from the inhibition imposed by 2,4,6-T because time is required for its development. The inhibitory effect of TIBA on the transport of labeled sugars may be used to interpret the inhibition of TIBA on the transport of 2,4-D and 2,4,6-T.

In further experiments it was attempted to test directly the two main hypotheses of solute transport by studying the downward transport of labeled water. If the pressure flow hypothesis describes transport, solute (C^{14}-labeled 2,4-D) and solvent (H_2O^{18} and THO) should be transported simultaneously in the phloem. If the activated diffusion hypothesis describes transport, labeled water should remain in the leaf and labeled solute should diffuse to the rest of the plant.

Both tritium and O^{18} are translocated downwards in the bean plant. This result is in complete agreement with the statement made by Biddulph et al. (5) and of Biddulph and Cory (6) that tritium, administered to the leaf as THO, moves down the plant to the root at a rapid rate (approximately 1-3 cm./minute). Despite this fact, the transport of labeled water (THO or H_2O^{18}) appears to be fundamentally different from the transport of 2,4-D. Biddulph and Cory (6) found that the transport of THO is different from the trans-

port of labeled sugar.

The most prominent difference between 2,4-D movement and water movement occurs in dark grown carbohydrate-depleted plants (Figures 13 and 14). In such plants, there is transport of labeled water but a complete absence of 2,4-D transport. It appears, therefore, that the carbohydrate status of the leaf does not govern the downward transport of labeled water in the same manner as it governs that of 2,4-D. This conclusion was tested directly and found to be correct (Figure 17).

These results imply that movement of labeled water does not occur in the phloem as reported by Biddulph et al. (5) and Biddulph and Cory (6). The possibility that tritium is translocated from the leaf to the root in dead tissue (presumably the xylem) was tested by following its movement in steam girdled (petiole) and normal plants. As much or more tritium is translocated in girdled as in normal plants (Figures 15, 16 and 17). The girdled regions completely prevented the transport of 2,4-D and 2,4,6-T, however (Table X). It is concluded that all of the tritium which is transported in normal plants can be attributed to movement in the xylem. If movement of labeled water takes place in the phloem, it is completely masked by movement in the xylem. It appears to be impossible to determine, by present techniques, whether water moves together with solute in the phloem.

Under a given set of experimental conditions, the time course curves describing the transport of tritium and O^{18} should be the same if these two labels describe accurately the transport of water. In general, there is a similarity between the curves describing the transport of T and O^{18}. There are indications from some of the experiments reported in this thesis, however, that the transport of tritium is different from the transport of O^{18}. A comparison of Figures 13 and 14 shows that the transport of tritium in dark grown plants, is much faster than the transport of O^{18}. This is further indicated in Figure 12 a and b. For plants grown in the light, the curves showing the transport of O^{18} and T agree more closely. At present, the simplest explanation for the apparent difference in T and O^{18} transport resides in the different techniques used to administer labeled water to the laminae in the two cases. H_2O^{18} was applied by immersing the entire lamina into labeled water, whereas, THO was applied as a drop to the mid vein of the lamina. The transport of a mixture of H_2O^{18} and THO in the same plant will have to be studied before this question may be answered with certainty, however.

The amount of THO transported in plants which have been kept in darkness for a considerable length of time (carbohydrate-depleted plants) is not as large as in plants which have been exposed to periods of light (plants possessing starch). Pre-treatment of the leaves of carbohydrate-

depleted plants with either sugar solution or water increases the amount of tritium transport to a level approximately equal to that which occurs in plants possessing starch. The fact that water alone is capable of inducing THO transport is evidence that carbohydrate does not provide the motive force for the transport of tritium.

It is suggested that pre-treatment of carbohydrate-depleted plants with water corrects a condition of "incipient drying" in the leaves. As a result of this correction, there is an increased absorption or equilibration of labeled water by the laminae.

The mechanism responsible for the downward transport of tritium is not known. The rate is much too rapid (1-3 cm./min.) for a diffusion process, and an active mechanism cannot be invoked because the label must, in girdled plants; pass through a two to three cm. dead zone (girdled region). Transport of tritium occurs through girdled petioles which are placed in a vertical or in a horizontal position. At present, the simplest explanation for the downward movement of tritium (THO) is that there is bidirectional movement of solution in the xylem which occurs in separate xylem elements.

The fact that tritium is translocated in dead tissue makes it difficult to come to a decision regarding the mechanism of solute transport in the phloem. Unless a

method of culture is found which will eliminate the downward movement of label in dead tissue, it will be very difficult to prove conclusively that there is movement of water in the phloem of the red kidney bean.

APPENDIX

The data in Table XVII show that (a) less $C^{14}O_2$ was fixed by TIBA pre-treated plants than by the controls, and (b) the amount of C^{14} extractable from both groups of plants decreased as time progressed. A separate experiment (Table XVIII) failed to confirm these results. The amount of $C^{14}O_2$ absorbed by control and TIBA pre-treated plants was variable. The two experiments were consistent, however, in detecting the inhibitory effect of TIBA upon the transport of $C^{14}O_2$.

The following question may be asked. Are the differences in C^{14} absorption due (a) to differences in leaf size, or (b) to an unequal distribution of $C^{14}O_2$ in the growth chamber because of poor circulation of air? If the decline in total extractable C^{14} with time, in the first experiment, is ascribed to a loss through respiration or a conversion into non-extractable compound(s), the data of the second experiment should also show this decline with time since they were subjected to the same growth conditions. However, even if the data of the second experiment are expressed as the total cpm/plant/gr. fresh weight of leaf there is still no consistent decline of C^{14} with time (Table XXVII). Unfortunately leaf weights were not recorded in the first experiment so the data cannot be corrected.

TABLE XXVII

Total cpm/plant/gram fresh weight of leaf, in control and TIBA pre-treated plants, at 20, 80, 140, and 260 minutes after exposure to $C^{14}O_2$

	Total cpm/plant/gr. fresh weight of leaf		
Time (min.)	Plant No.	Control	TIBA
20	1	66,500	74,200
	2	60,900	55,600
	Ave.	63,700	64,900
80	1	116,500	67,400
	2	66,000	73,600
	Ave.	91,250	70,500
140	1	73,100	90,600
	2	56,400	
	Ave.	64,750	90,600
260	1	75,000	58,700
	2	55,200	60,100
	Ave.	65,100	59,400

The failure to observe the downward trend, in the second experiment, suggests the possibility that the plants, in the first experiment, were of unequal leaf size and that a systematic bias entered the experiment. This was possible (within reasonably narrow limits) because of the manner in which the experiment was conducted. The plants were divided into two groups (TIBA pre-treated and normal) placed into the growth chamber, and exposed to $C^{14}O_2$. After exposure they were sampled and assayed for C^{14}. This is where the bias could have entered because it is possible that plants which were slightly larger were unconsciously selected first. In the second experiment, all of the plants were selected, matched and tagged prior to treatment with TIBA and $C^{14}O_2$.

For an explanation of the differences in the total uptake of C^{14} by TIBA pre-treated and normal plants at each sampling time, a reason other than leaf size has to be invoked because correction of the data (Table XXVII) failed to erase these differences. It is possible that the $C^{14}O_2$ was not distributed equally in the growth chamber.

LITERATURE CITED

1. Barrier, G. E. and Loomis, W. E. Absorption and translocation of 2,4-dichlorophenoxyacetic acid and P^{32} by leaves. Plant Physiol. 32: 225-231. 1957

2. Bauer, L. Zur Frage der Stoffbewegungen in der Pflanze mit besonderer Berücksichtigung der Wanderung von Fluorochromen. Planta. 42: 367-451. 1953.

3. Bennett, C. W. Correlation between movement of the curly top virus and translocation of food in tobacco and sugar beet. Jour. Agr. Res. 54: 479-502. 1937.

4. Bennett, C. W. Relation of food translocation to movement of virus of tobacco mosaic. Jour. Agr. Res. 60: 361-390. 1940.

5. Biddulph, O., Biddulph, S., Cory, R. and Koontz, H. Investigations on water movement in phloem. Abstracts of Papers, Amer. Soc. of Plant Physiologists, Western Section. June, 1954.

6. Biddulph, O. and Cory, R. Translocation in phloem using P^{32}, THO, and C^{14} simultaneously. Abstract of Papers, Amer. Soc. of Plant Physiologists, Western Section, Seattle. June, 1956.

7. Böhning, R. H., Kendall, W. A. and Linck, A. J. Effect of temperature and sucrose on growth and translocation in tomato. Amer. Jour. Bot. 40: 150-153. 1953.

8. Böhning, R. H., Swanson, C. A. and Linck, A. J. The effect of hypocotyl temperature on translocation of carbohydrates from bean leaves. Plant Physiol. 27: 417-421. 1952.

9. Bonner, J. and Galston, A. W. Principles of Plant Physiology. W. H. Freeman and Co., San Francisco. Chapter 7. 1952.

10. Carr, D. J. On the nature of photoperiodic induction IV. Preliminary experiments on the effect of light following the inductive long dark period in Xanthium pennsylvanicum. Physiol. Plantarum. 10: 249-265. 1957.

11. Chen, S. L. Simultaneous movement of P^{32} and C^{14} in opposite directions in phloem tissue. Amer. Jour. Bot. 38: 203-211. 1951.

12. Crafts, A. S. Further studies on exudation in Cucurbits. Plant Physiol. 11: 63-79. 1936.

13. Crafts, A. S. Movement of assimilates, viruses, growth regulators, and chemical indicators in plants. Bot. Rev. XVII, 203-284. 1951.

14. Crafts, A. S. Translocation of herbicides. 1 The mechanism of translocation: Methods of study with C^{14}-labeled 2,4-D. Hilgardia 26: 287-334. 1956.

15. Curtis, O. F. and Clark, D. G. An Introduction to Plant Physiology. McGraw-Hill. 1950.

16. Day, B. E. The absorption and translocation of 2,4-dichlorophenoxyacetic acid by bean plants. Plant Physiol. 27: 143-152. 1952.

17. De Vries, H. Uber die Beutung der Circulation und der Rotation des Protoplasma für den Stofftransport in der Pflanze. Bot. Zeit. 43: 1-6 and 17-26. 1885.

18. Dugger, W. M., Jr., Humphreys, T. E. and Calhoun, B. The influence of boron on starch phosphorylase and its significance in translocation of sugar in plants. Plant Physiol. 32: 364-370. 1957.

19. Gauch, H. G. and Dugger, W. M., Jr. The role of boron in the translocation of sucrose. Plant Physiol. 28: 457-466. 1953.

20. Gustafson, F. G. Absorption of Co^{60} by leaves of young plants and its translocation through the plant. Amer. Jour. Bot. 43: 157-160. 1956.

21. Hay, J. R. The effect of 2,4-dichlorophenoxyacetic acid and 2,3,5-triiodobenzoic acid on the transport of indoleacetic acid. Plant Physiol. 31: 118-120. 1956.

22. Hay, J. R. and Thimann, K. V. The fate of 2,4-dichlorophenoxyacetic acid in bean seedlings. I. Recovery of 2,4-dichlorophenoxyacetic acid and its breakdown in the plant. Plant Physiol. 31: 382-387. 1956.

23. Hay, J. R. and Thimann, K. V. The fate of 2,4-dichlorophenoxyacetic acid in bean seedlings. II. Translocation. Plant Physiol. 31: 446-451. 1956.

24. Hewitt, S. P. and Curtis, O. F. The effect of temperature on loss of dry matter and carbohydrate from leaves by respiration and translocation. Amer. Jour. Bot. 35: 746-755. 1948.

25. Hull, H. M. Carbohydrate translocation in tomato and sugar beets with particular reference to temperature effect. Amer. Jour. Bot. 39: 661-669. 1952.

26. Kendall, W. A. The effect of intermittently varied petiole temperature on carbohydrate translocation from bean leaves. Plant Physiol. 27: 631-633. 1952.

27. Kendall, W. A. Effect of certain metabolic inhibitors on translocation of P^{32} in bean plants. Plant Physiol. 30: 347-350. 1955.

28. Kursanov, A. L. Analysis of the movement of substances in plants by means of radioactive isotopes. Proc. Int. Conf. on Peaceful Uses of Atomic Energy. 12: 165-169. 1956.

29. Kuse, G. Effect of 2,3,5-triiodobenzoic acid on the growth of lateral bud and on tropism of petiole. Memoirs Coll. Sci., Univ. Kyoto, Series B. 20: Art. 12. 1953.

30. Leonard, O. A. Translocation of carbohydrates in the sugar beet. Plant Physiol. 14: 55-74. 1939.

31. Leopold, A. C. Auxins and Plant Growth. Univ. of California Press. Chapter VI. 1955.

32. Livingston, B. E. and Brown, W. H. Relation of the daily march of transpiration to variations in the water content of foliage leaves. Bot. Gaz. 53: 309-330. 1912.

33. Loomis, W. E. Translocation of carbohydrates in maize. Science 101: 398-400. 1945.

34. Maskell, E. J. and Mason, T. G. Studies on the transport of nitrogenous substances in the cotton plant. I. Preliminary observations on the downward transport of nitrogen in the stem. Ann. Bot. 43: 205-231. 1929.

35. Maskell, E. J. and Mason, T. G. Studies on the transport of nitrogenous substances in the cotton plant. II. Observations on concentration gradients. Ann. Bot. 43: 615-652. 1929.

36. Maskell, E. J. and Mason, T. G. Studies on the transport of nitrogenous substances in the cotton plant. III. The relation between longitudinal movement and concentration gradients in the bark. Ann. Bot. 44: 1-29. 1930.

37. Mason, T. G. and Phillis, E. Further studies on transport in the cotton plant. V. Oxygen supply and the activation of diffusion. Ann. Bot. 50: 455-499. 1936.

38. McRae, D. H. Studies on the kinetics of auxin-induced growth. Ph.D. Thesis. Cal. Institute of Technology. 1953.

39. Mitchell, J. W. and Brown, J. W. Movement of 2,4-dichlorophenoxyacetic acid stimulus and its relation to the translocation of organic food materials in plants. Bot. Gaz. 107: 393-407. 1946.

40. Mitchell, J. W., Dugger, W. M., Jr. and Gauch, H. G. Increased translocation of plant growth modifying substances due to application of boron. Science. 118: 354-355. 1953.

41. Münch, E. Die Stoffbewegungen in der Pflanze. Gustav Fischer, Jena. 1930.

42. Nelson, C. D. and Gorham, P. R. Uptake and translocation of C^{14}-labelled sugars applied to primary leaves of soybean seedlings. Can. Jour. Bot. 35: 339-347. 1957.

43. Nelson, C. D. and Gorham, P. R. Translocation of radioactive sugars in the stems of soybean seedlings. Can. Jour. Bot. 35: 703-713. 1957.

44. Niedergang-Kamien, E. and Leopold, A. C. Inhibitors of polar auxin transport. Physiol. Plantarum. 10: 29-38. 1957.

45. Niedergang-Kamien, E. and Skoog, F. Studies on polarity and auxin transport in plants. I. Modification of polarity and auxin transport by triiodobenzoic acid. Physiol. Plantarum. 9: 60-73. 1956.

46. Osborne, D. J. and Wain, R. L. Studies on plant growth-regulating substances. II. Synthetic compounds

inducing morphogenic responses in the tomato plant. Jour. Hort. Sci. 26: 60-74. 1950.

47. Phillis, E. and Mason, T. G. Studies on the transport of carbohydrates in the cotton plant. III. The polar distribution of sugar in the foliage leaf. Ann. Bot. 47: 585-634. 1933.

48. Phillis, E. and Mason, T. G. Further studies on transport in the cotton plant. IV. On the simultaneous movement of solutes in opposite directions through the phloem. Ann. Bot. 50: 161-174. 1936.

49. Rice, E. L. Absorption and translocation of ammonium 2,4-dichlorophenoxyacetic acid by bean plants. Bot. Gaz. 109: 301-314. 1948.

50. Rohrbaugh, L. M. and Rice, E. L. Effect of application of sugar on the translocation of sodium 2,4-D by bean plants in the dark. Bot. Gaz. 111: 85-89. 1949.

51. Stout, M. Translocation of the reproductive stimulus in sugar beets. Bot. Gaz. 107: 86-95. 1945.

52. Swanson, C. A. and Bŏhning, R. H. The effect of petiole temperature on the translocation of carbohydrates from bean leaves. Plant Physiol. 26: 557-564. 1951.

53. Swanson, C. A. and Whitney, J. B., Jr. Studies on the translocation of foliar-applied P^{32} and other radioisotopes in bean plants. Amer. Jour. Bot. 40: 816-823. 1953.

54. Thimann, K. V. and Bonner, W. D. The action of tri-iodobenzoic acid on growth. Plant Physiol. 23: 158-161. 1948.

55. van den Honert, T. H. On the mechanism of the transport of organic materials in plants. Proc. Kon. Akad. Wetensch. Amsterdam. 35: 1104-1111. 1932.

56. van Overbeek, J. Absorption and translocation of plant regulators. Ann. Rev. Plant Physiol. 7: 355-372. 1956.

57. Vernon, L. P. and Aronoff, S. Metabolism of soybean leaves. IV. Translocation from soybean leaves. Arch. Biochem. and Biophysics. 36: 383-398. 1952.

58. Weintraub, R. L. and Brown J. W. Translocation of exogenous growth-regulators in the bean seedling. Plant Physiol. 25: 140-149. 1950.

59. Went, F. W. Plant growth under controlled conditions. III. Correlation between various physiological processes and growth in the tomato plant. Amer. Jour. Bot. 31: 597-618. 1944.

60. Went, F. W. The Experimental Control of Plant Growth. Chronica Botanica Co., Waltham, Mass. 1957.

61. Went, F. W. and Engelsberg, R. Plant growth under controlled conditions. VII. Sucrose content of the tomato plant. Arch. Biochemistry. 9: 187-200, 1946.

62. Went, F. W. and Hull, H. M. The effect of temperature upon translocation of carbohydrates in the tomato plant. Plant Physiol. 24: 505-526. 1949.

63. Went, F. W. and Thimann, K. V. Phytohormones. The Macmillan Co., New York. 1937.

64. Went, F. W. and White, R. Experiments on the transport of auxin. Bot. Gaz. 100: 465-484. 1939.

65. Zimmerman, M. H. Translocation of organic substances in trees. I. The nature of the sugars in the sieve tube exudate of trees. Plant Physiol. 32: 288-291. 1957.