WATER

TRANSLOCATION OF GROWTH REGULATORS IN CHARA VULGARIS A NONVASCULAR AQUATIC PLANT FOUND IN VIRGINIA'S WATERS

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PREFACE

Many cities and towns in Virginia depend in part on impounded waters for domestic and industrial uses. In addition there are in excess of 30,000 ponds constructed for a variety of purposes including stock watering, irrigation, fire protection, and recreation. Aquatic weeds and algae present imposing problems for the effective and efficient use of these waters for the purposes originally intended.

Chara vulgaris is the most prevalent problem plant in impounded waters in the hard water regions of Virginia. This initial research on C. vulgaris developed the techniques for intensive study of intercellular movement of herbicides which can lead to effective control of this plant and result in the better utilization of ponds, streams, and lake waters.

William R. Walker, Director

Water Resources Research Center
Virginia Polytechnic Institute
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INTRODUCTION

Vascular plants have been used in many experiments designed to show translocation patterns of foliar or root applied nutrients and herbicides. Chara (Chara vulgaris L.) is a nonvascular filamentous green alga which consists of a strand of alternating long and short cells, the short cells forming the nodes and the long cells (often five to ten centimeters long) forming the internodes. The chara thallus is usually completely submersed in water and is normally attached to a pond or stream bottom by a single celled hyaline "root-like" structure termed the rhizoid. For normal alga growth and development the rhizoids must be attached (44). It is therefore deemed that the rhizoids have functional capacities other than attachments.

Because of their long cells the Characeae have been used to study cell and membrane functions (30, 42). Detailed studies have been made with the aid of the electron microscope using genera of this plant family and plasmodesmata have been found between chara cells (9, 50, 59). Consequently the transportation or movement of inorganic nutrients and organic molecules must be across cell membranes, cell walls or through intercellular channels in order to be translocated in the nonvascular chara thallus.

^{45}Ca, ^{35}S, ^{32}P, and ^{14}C were chosen as nutrients in this study because of their essentiality, availability and utility (length of half life and energy emission).

Chara has become a nuisance plant in many ponds, streams and lakes in Virginia, particularly in the hard water regions. The effects of certain herbicides on this plant have been studied and the efficacy of 2-chloro-4, 6-bis(ethylamino)-s-triazine (simazine) and 2,4-dichlorophenoxyacetic acid (2,4-D) for controlling this plant has been reported (36, 55, 56). Simazine has been reported to effectively control chara at two to four ppm and 2,4-D is relatively uneffective as high as 100 ppm.

The translocation patterns of these two herbicides in vascular plants are quite different. Simazine moves apoplastically in the xylem and 2,4-D moves primarily down through the phloem (symplast) along with food material

but may be transported from the soil upward in the transpiration stream with the movement of water and soil nutrients through the xylem (apoplast). The objectives of the studies reported herein include: the identification of sugars in plant extracts of chara; the fate of ^{14}C when applied as $NaH^{14}CO_3$ and sucrose-^{14}C to the water medium surrounding chara which was exposed to periods of light and dark; the movement of ^{35}S, ^{32}P, and ^{45}Ca from treated areas in partitioned chara plants exposed to periods of light and dark; and the movement and fate of ^{14}C when it was supplied to the water medium surrounding the chara as 2,4-dichlorophenoxyacetic acid-1-^{14}C (2,4-D-1-^{14}C) and ring labeled 2-chloro-4,6-bis(ethylamino)-s-triazine-^{14}C (simazine-^{14}C).

REVIEW OF LITERATURE

Genera of the Characeae have been used by research workers for studying cell functions. In the early 1940's these plants were used by Jacques (30) and Osterhout (42) to study the separation of potassium ions by living membranes and plasmolysis due to salts. Hope et al. (26) studied the efflux of chloride on cells of Nitella and Chara. More recently Scott (48) used these same genera to study bioelectricity and its effects on protoplasmic surfaces. In 1964 Green (22) used Nitella cells with the aid of the electron microscope to show chloroplast division and spiral growth. Sievers (51) studied geotropical reactions; he described the ultrastructure of young normal rhizoids of chara which showed positive geotropic character. Barron (3) made electron microscopic studies of cell surface activity in Chara vulgaris. The reports of these workers and recent personal correspondence with others in this field (9, 50, 59) indicate that the presence of intercellular connections between chara cells exist. Shen (50) in 1963 made electromicroscopic studies on Chara zeylanica Klein ex Willd. and found plasmodesmata present in the cell wall.

Ruttner (47) believed aquatic vascular plants to be progeny of land flora in an aquatic habitat. They are plants which in their evolution came out of the water, developed as aerial organisms and then returned to the water. Aquatic microflora and the larger algae, including the Characeae, have never left the water medium in the course of their phylogeny; and are therefore nonvascular (47).

Early workers concluded that the nutrient elements required by algae were very similar to those for higher plants (8, 16, 33, 43, 45). In most lakes and streams all the major elements are present in excess with the exception of nitrogen and phosphorous (43). Forsbing (17) studied the nutrition of chara in sand cultures and found the growth of C. globularies Thuill. was stimulated by additions of extra bicarbonate to the medium. The chara showed a great tolerance to wide salt changes and very small amounts of phosphorous were required for normal growth (20µg/l was optimum).

The carbon cycle in natural waters has been studied and reported by several workers (28, 45, 46). Carbon for photosynthesis in water is derived from CO_2, carbonates, bicarbonates, or organic compounds. The equilibrium of each of these various forms depends on the hydrogen ion concentration, amounts of excess bases, the partial pressure of CO_2 in the atmosphere and the water temperature. Below pH 5 free CO_2 was the important source, but at the pH range of 7 to 9 bicarbonates became important and above pH 9.5 the important carbon source was carbonate.

Uptake of carbon by photosynthesizing plants is not significantly different between ordinary carbon dioxide ($^{12}CO_2$) and radioactive carbon dioxide ($^{14}CO_2$) (4). Many experiments have been made whereby plants exposed to $^{14}CO_2$ have incorporated the radioactive carbon into metabolites, being most helpful in determining the carbon reduction pathway.

Vernon and Arnoff (60) studied the process of translocation in the soybean plant using $^{14}CO_2$ photosynthesis by the first trifoliate leaf only, followed by qualitative and quantitative analysis of the plant for radioactive material. Within 20 minutes of exposure to $^{14}CO_2$ sucrose was found to be the main compound labeled in soybeans. Of the labeled compounds found sucrose was translocated most rapidly followed by glucose and fructose. Light had no effect upon translocation other than the formation of translocatable materials through photosynthesis. Tolbert et al. (57) in 1954 exposed excised tops of Sedum spectabile to $^{14}CO_2$ in a photosynthesizing chamber. Radioactive fructose, glucose and sucrose were found and all three were translocated basipetally in the plant.

Nelson et al. (41) reported on the uptake and translocation of ^{14}C labeled sugars in soybeans. In the light translocation of sucrose-^{14}C or glucose-^{14}C out of a treated primary leaf was very slow. Only one percent of the ^{14}C from sucrose was translocated after 14 hours. The actively growing stem tip accumulated very little ^{14}C in either light or dark from labeled sugars. In the light or the dark ^{14}C from a primary leaf that had been allowed to photosynthesize for 15 minutes in an atmosphere of $^{14}CO_2$

spread throughout the entire plant in three hours. In the light more ^{14}C from photosynthates accumulated in the growing stem tip than in the root. This situation was reversed in the dark. The minimum amount of ^{14}C translocated from sucrose-^{14}C treated leaves may be directly attributed to the reduced penetration and uptake of the sucrose molecule by the leaf.

Water analyses show clacium is the major cationic constituent of natural waters (27), it has been found to be abundant also in soft waters. The sulfate content of lake waters varies from a trace to rather large amounts but the ion ordinarily is the second or third most abundant anion in lakes (29). Phosphorous in natural waters is probably the most limiting element in aquatic plant growth. As phosphorous becomes available in a natural water medium it usually becomes incorporated into organic forms immediately (29).

In vascular terrestrial plants ^{35}S and ^{32}P are phloem mobile and move in the symplast but may be redistributed apoplastically through the xylem (11). Bukovac and Wittwer (7) placed a small drop of radioactive elements on the upper surface of one of the primary leaves of bean plants and grew them in nutrient solution. At various times different plants were harvested and autoradiographed. They classified Na, K, P, Cl and S as mobile elements and Ca, Sr, and Ba as immobile. Calcium was absorbed by the leaf but not exported; phosphorous moved more than sulfur; however, both were considered mobile. Biddulph et al. (5) studied the circulation patterns of phosphorous, sulfur and calcium in rad kidney bean plants. Plants were grown in a nutrient solution for ten days and then radioactive isotopes of calcium, sulfur, and phosphorous were added individually to the nutrient solutions. The bean plants were left in the normal solutions. At intervals of zero to 96 hours plants were harvested and autoradiographed. Phosphorous was found to be mobile throughout the entire experimental period; sulfur was mobile for a short period, but its translocation was reduced rapidly due to metabolic capture in developing leaves; and calcium did not move following initial delivery through the transpirational stream. Crafts (11) mentions that in studies

in which P, S and Ca have been exposed to roots of barley very little upward movement of the calcium was noted. However, there was upward movement of the phosphorous and sulfur.

Other workers (21, 39, 40, 61) also present evidence showing calcium to be immobile in vascular plants and that no redistribution takes place once the ion has been removed from the transpirational stream. They also show that phosphorous and sulfur are mobile and may be redistributed.

Kessler and Moscicki (32) investigated the effects of triiodobenzoic acid and 1,2-dihydropryidazine-3,6-dione (maleic hydrazide) upon the transport of foliar applied calcium and iron. They found polarity was associated with immobility of calcium in plants and movement of calcium could be obtained by suspension or reversing polarity.

Tolbert and Weibe (58) studied phosphorous and sulfur compounds in plant xylem sap. Up to this time it was assumed that inorganic phosphates and sulfates were the major, if not the only, forms in which these elements moved upward in the xylem of plant stems. However, data from their investigations using radiotracers and paper chromatographic analysis show that although inorganic phosphate is the major form of phosphorous transported, there are in the xylem sap two other phosphorous containing compounds, one of which may account for substantial portions of the transported phosphorous. Inorganic sulfates appear to be the only forms of sulfur transported.

The absorption and translocation of ^{32}P in Chara globularis was studied by Littlefield and Forsberg (37) in 1965. Their results show that all parts of the chara plant could absorb ^{32}P about equally well. Approximately the same portion of the absorbed ^{32}P was translocated from the apices or rhizoids to other parts of the plant. Round (45) reported that phosphorous depleted cells can absorb phosphorous in the dark, but replete cells of alga absorb phosphorous only in the light.

Sulfur is generally present in small quantities in all alga cells but it is probably not the limiting factor for alga growth under normal conditions (45).

In many fresh hardwater streams and ponds chara has become a nuisance plant and methods of chemical control have been studied. Rates of 2,4-dichlorophenoxyacetic acid as high as 100 ppm have not been effective in controlling it (36). Simazine has been shown to provide effective control at two to four ppm (36, 55, 56) when added to the water medium.

Many translocation studies have been made using terrestrial vascular plants and ^{14}C labeled 2,4-D and simazine. Generally it has been found that simazine moves apoplastically in the xylem and 2,4-D moves primarily down through the phloem (symplast) along with the plant food materials possibly as a 2,4-D metabolite. Translocation of 2,4-D from soil upward follows the transpiration stream with the movement of water and soil nutrients through the xylem (34).

Wort (63) in a review of enzyme studies discussed the influence of 2,4-D on enzyme systems. He reported that applications of 2,4-D resulted in an immediate increase in enzyme activity followed by a decrease and that the herbicide had effects on a number of plant enzymes. The effects differ from plant to plant and also in the various organs of a given plant. He pointed out that the age of the plant at the time of application is important physiologically since the cells and their enzyme systems change with age.

Fang et al. (15) in 1951 studied the absorption of radioactive 2,4-dichlorophenoxyacetic acid and the translocation of ^{14}C by bean plants. They showed that ^{14}C labeled 2,4-D is rapidly absorbed and that it moved in bean plants. Young plants absorbed and moved the indicator more rapidly than older ones; absorption and translocation were not correlated with dosage applied, and small amounts of the growth regulator were broken down and metabolized with a consequent loss of radioactive CO_2. Later Crafts and Yamaguchi (12) presented data to indicate that the export of food is the motivating force in moving 2,4-D. This movement is not brought about by simple osmotic forces but involves a metabolic component. Crafts (11) compiled research work of his own and that of other workers concluding

that 2,4-D diffuses into leaves, enters the symplast, migrates to the phloem, and moves along in the assimilate stream with the food materials in vascular plants.

Aldrich and Otto (1) used a thin latex membrane to partition roots and leaves of Potamogeton pectinatus L. They found movement of radioactivity was primarily basipetal in plants receiving 2,4-D-1-^{14}C via the leaves. In both root-fed and leaf-fed plants, accumulation of radioactivity was more extensive in the roots than in leaves. Funderburk and Lawrence (20) treated waterstargrass (Heteranthera dubia Small.), a submersed vascular aquatic plant, with ^{14}C labeled 2,4-D and simazine. Carboxyl labeled 2,4-D moved to a slight extent from shoot to root but no movement from root to shoot could be detected by autoradiography. However, counting data indicated that small amounts of ^{14}C from 2,4-D-1-^{14}C not recorded on the autoradiograph moved from the root to shoot. ^{32}Phosphorous was found to move in both directions in waterstargrass with greatest movement occurring in an acropetal direction. After the ^{32}P was translocated in either direction there appeared to be a definite accumulation in the nodes. In the same and in another experiment (19, 20) simazine or the labeled carbon atom from the chain labeled simazine moved from the roots to the shoots and vice versa. The workers point out that appreciable downward translocation of the herbicide had not been observed in terrestrial plants.

Simazine has been found to be readily absorbed by roots from culture medium (10); however, being low in water solubility (5 ppm at 20-22 C) it is not readily absorbed and translocated out of oat leaves via the phloem. In higher plants simazine penetrates the cuticle of a leaf and moves within the leaf acropetally. It appears to enter the mesophyll, diffuse along cell walls and does not enter the living symplast. This apoplastic movement is accelerated by the flow of water in the plant. Roots of barley seedlings were exposed to ^{14}C labeled simazine and it was found that enough simazine was absorbed in 30 minutes to exposure to the herbicide to produce a strong autoradiograph of the roots. Simazine moved into the plant tops

within two hours and continued to increase up to the eighth day of the experiment; concentrations increased in the roots also.

In potato tuber tissue tests (11) ^{14}C labeled simazine did not seem to penetrate the living cells but appeared to move along the cell walls.

Sheets (49) studied the toxicity of simazine and found it to be toxic to oats and soybeans in solution cultures. Gysin and Knüsli (23) reported on the activity and mode of action of triazine herbicides. Inhibition of sugar formations was noted when it was found that starch-free Coleus leaves which were kept in the dark in a sucrose solution could form starch in the presence of simazine. Later it was demonstrated that simazine inhibited the Hill reaction. A system was found present in corn plants that could convert the simazine molecule to the nonphytotoxic 2-hydroxy analog. A potential metabolite of simazine, 2-hydroxy-4,6-bis-ethylamino-S-triazine did not inhibit the Hill reaction and did not possess herbicidal activity.

Davis et al. (14) used radioactive simazine to study the absorption and translocation of this compound by roots and leaves of corn, cotton, and cucumbers grown in nutrient cultures and in soil. Simazine was found very toxic to cucumbers, intermediately toxic to cotton, and slightly toxic to corn. Simazine or biologically ^{14}C labeled degradation products accumulated at specific areas in cucumbers and cotton but in corn the radioactivity was rather uniformly distributed throughout the plant. Absorption and translocation of simazine from soil followed the same pattern as that from nutrient cultures.

Ashton et al. (2) ran detailed studies of $^{14}CO_2$ fixation by bean plants treated with triazine herbicides. They found that 0.25 ppm of simazine decreased $^{14}CO_2$ fixation by about 30%; however, at one ppm or above fixation was almost completely blocked. The effects of exposure time were also studied and at the end of three hours one ppm of simazine inhibited $^{14}CO_2$ fixation. Simazine drastically inhibited CO_2 fixation in light.

Jordan et al. (31) studied the effects of photosynthesis-inhibiting

herbicides on nonphotosynthetic tobacco callus tissue. Tobacco was grown in the dark and supplied with sucrose and nutrients. At the end of 21 days tobacco callus tissue growth was inhibited by simazine and the workers concluded that a process other than photosynthesis is also affected by this compound.

MATERIALS AND METHODS

I. Description of the Plant

Chara (Chara vulgaris L.) grows completely submerged in fresh standing, clear, hard water and is attached to the muddy or sandy bottom by rhizoids (52, 53), a nongreen single celled "root-like" structure (62). If the water surrounding the plant is saturated with $CaCO_3$ deposits of this material may encrust the plant (62).

The thallus or "plant" is a branched filamentous structure and consists of a central axis with whorls of laterals arising from the nodes at more or less regular intervals. The axis consists of alternating long and short cells, single long cells forming the internode and short cells forming the nodes. The short nodal cells divide periclinally producing a ring of peripheral nodal cells from which the lateral branchlets evolve (62). These branchlets also have nodes and internodes which are similar to the main axis. In this species the long internodal cells become overgrown by sheath cells. Several of these sheath cells are needed to span the length of the main internodal cell (25). The mature internodal cells contain a large central vacuole surrounded by a multinucleate cytoplasm (62). The layer of cytoplasm closest to the vacuole shows unidirectional cyclosis (62). When viewing under a microscope the streaming cytoplasm seems to flow up one side of the cell wall and down the other side being deflected and changing directions at the cell ends. Numerous small chloroplasts are embedded in a fixed layer of peripheral cytoplasm (25).

II. Preparation and Partitioning of Chara Plant Parts Prior to Treatment

A. Collection and Growing

Chara plants were collected from a local spring fed pond by scooping up a layer of pond bottom soil approximately three to six cm deep in which chara plants were growing. The soil along with the attached plants was placed in plastic buckets and set into a water bath where the light (350 ft-c) and temperature (12.5 C) were maintained. The temperature was used

because it was very close to the spring water temperature which the chara tolerated in nature and it retards growth and contamination by other algae. The chara maintained in these water baths was used as a source of supply and the plants were transferred to a Sherer Gro-Lab model 25-7HL for detailed studies. The controlled environmental chamber was programmed on alternating twelve hour cycles of full light intensity (1200 ft-c) at 15.5 C and complete darkness at 10 C throughout the experiment except where otherwise noted.

B. Partitioning of Plant Parts

The method for studying absorption and translocation in submersed aquatic plants as outlined by Frank and Hodgson (18) was used for part of the experiments. These were termed "funnel partitioned" plants. The procedure used for each partitioning procedure were as follows.

1. Funnel partitioning

Chara plants were selected for uniformity and strung individually through plastic funnels (Figure 1). Those used for rhizoid treatments were strung so that only the rhizoids extended below the funnel stem. Others were prepared so that a section of the morphological plant base extended below the stem. The funnel stem was tilled with melted technical grade eicosane which was cooled until it was still liquid but about to solidify. The hardened eicosane acted as a barrier separating the plant so that the portions above or below the barrier could be treated separately. The funnels containing the plants were then placed in large mouth quart jars which contained a sufficient amount of tap water so that portions of the plant extending below the funnel stem were completely submerged. The funnel top was filled with tap water also (Figure 1). The jars containing the plants and funnels were placed in the controlled environmental chamber and allowed to grow for 24 hours prior to treatment.

2. Trough partitioning

The plant separation method used in this part of the experiment was a modification of that outlined above. A thin band of

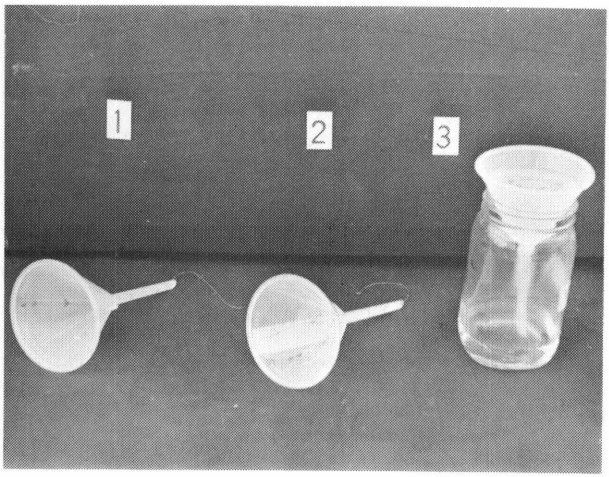


Figure 1. Funnel partitioning of chara. (1) Chara plant strung through plastic funnel. (2) Chara plant in funnel with eicosane wax barrier in funnel stem. (3) Funnel containing chara and eicosane barrier placed in quart jar ready for treatment.

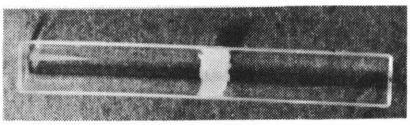
melted paraffin approximately one-half inch wide was poured in the center of a glass chromatograph trough and as the paraffin solidified semicircular, filter paper walls, which were pre-cut to the trough diameter, were inserted at each edge of the paraffin band (Figure 2). Prior to inserting the filter paper walls a slit with a pin hole at the end was made so that a chara plant could be strung through the paper walls and be suspended in air close to the bottom of the trough. After the paraffin hardened a chara plant was placed longitudinally in the trough with an internode extending through the filter paper and an eicosane barrier was built up to the top of the trough between the filter paper walls (Figure 2). Paraffin was used because previous results showed that it acted as a better seal against the glass than the eicosane and gave better support to the filter paper walls. Filter paper was chosen because when it became wet its swelling did not damage the chara cells. The melted eicosane was poured while it was still liquid but very close to solidifying (36 C). As it was added and hardening water was added to each side of the barrier. The troughs containing the chara plants prepared as described were placed in the controlled environmental chamber and allowed to grow for 24 hours prior to treatment.

III. Identification of Sugars in Chara

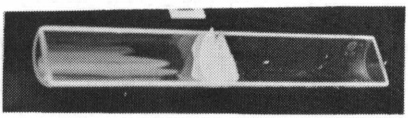
A. Chromatographic Studies

Fifty grams, wet weight, of chara were washed thoroughly in tap water and ground in an Omni-mixer for ten minutes with the Power Stat set at 120 volts. An ice bath was used throughout the grinding procedure. The ground plant material was then filtered through a double layer of cheesecloth to separate the pulp from the filtrate which was saved for chromatographing. Individual known solutions of glucose, fructose, and sucrose for chromatographing were made by dissolving one gram of the known sugar in five ml of distilled water. Ten microliter spots of each sugar solution were made on Whatman #4 chromatographic paper. A fifty microliter spot using the plant extract was also made. The paper was placed in a descending chromatography cabinet and 900 ml of a solvent containing n-butanol,

(1)



(2)



(3)

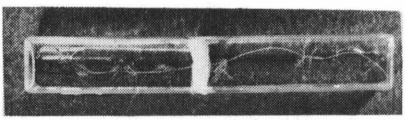


Figure 2. Trough partitioning of chara. (1) Glass chromatograph trough with a thin band of paraffin in the center. (2) Glass chromatograph trough with semicircular filter paper walls set into the paraffin band. (3) Glass chromatograph trough containing chara plant embedded in eicosane barrier ready for treatment.

acetic acid, and water (4:1:5, v/v/v) was added to the lower drawer of the Chromatocab and allowed to saturate the paper (6, 24). After saturation 100 ml of the n-butanol, acetic acid, and water solvent was poured into the chromatography trough and allowed to descend until the solvent front was two to three inches from the bottom of the paper. This solvent system was used to separate glucose from sucrose.

The chromatograph was then removed from the cabinet, dried overnight and cut into strips 2.5 cm wide (1.25 cm on either side of the original spot) the length of the paper. These strips were dipped into a freshly prepared aniline-diphenylamine solution which was made in the following proportions; one gram of diphenylamine, one ml of aniline, 98 ml of acetone and ten ml of 85% phosphoric acid (54). This locating reagent caused areas where sugars were located on the chromatograph to appear as a colored spot or spots (54). The color of the spot and its location aided in identification. After dipping, the strips were hung for four to five minutes in a drying oven which was preheated to 95 to 100 C. Comparisons of spots from the chara plant extract were made with spot locations and spot colors of known sugars.

Another chromatogram was prepared to separate fructose from glucose and sucrose using the procedure outined above except a phenol and water (160 gr:40 ml) solution was used as the solvent (54).

IV. ^{14}C Studies in Chara in Light and Dark Using $NaH^{14}CO_3$ and Sucrose-^{14}C

A. Autoradiographic Studies

Chara plants were selected and partitioned in funnels and troughs as outlined previously. Rhizoids and morphological plant apexes and bases were treated as shown in Table 1 using five μc of ^{14}C per 300 ml of distilled water. The ^{14}C was supplied as $NaH^{14}CO_3$ (specific activity 10 g/μc) or sucrose-^{14}C (specific activity 2.94 mc/mM). All carbons of the sucrose molecule were labeled. Three replications of each treatment were made.

Table 1. Method of partitioning, plant portions treated, and days of plant exposure to $NaH^{14}CO_3$ and sucrose-^{14}C.[1/]

Method of Partitioning	Plant Portions Treated	Days of Exposure to ^{14}C
Funnel	Morphological base	1
Funnel	Morphological base	4
Funnel	Rhizoid	1
Funnel	Rhizoid	4
Funnel	Morphological apex	1
Trough	Morphological apex	1
Trough	Morphological base	1
Trough[1/]	Morphological apex[1/]	1
Trough[1/]	Morphological base[1/]	1

1/ All treatments were carried out in the controlled environmental chamber under 12 hours alternating light and dark cycles except for the last two treatments which were conditioned by placing them in the dark for 24 hours prior to treatment and by remaining in the dark up to and including harvest.

At the end of the experiment periods the plants were harvested by first severing the plant portions on the untreated side of the eicosane barrier and then severing those plant parts on the treated side. The severed parts were individually washed in cold, running tap water. The parts remaining in the funnel stems were washed in hot tap water to loosen and melt the eicosane barrier. All portions were dried on paper towels, mounted on 10 x 12 Warren's Crumb Gloss paper, bundled, air dried for 24 hours and placed in a plant press for 24 additional hours after which they were exposed for three weeks to Kodak Royal Blue X-ray film for autoradiographing (13). After this time the film was placed in X-ray developer at 20 C for two and one half minutes, dipped in water and then in an acetic acid stop bath for 15 seconds. The film was placed in X-ray fixer for five minutes and then washed in running water for one hour.

B. Chromatographic Studies

Fifty grams of chara, wet weight, were placed in a one liter graduated glass cylinder to which tap water was added and placed in the controlled environmental chamber with full light intensity (1200 ft-c) for 24 hours. At the end of this period the tap water was poured off and replaced with a solution prepared by mixing five μc of ^{14}C per 300 ml of

distilled water. The ^{14}C was supplied as $NaH^{14}CO_3$. The chara was grown for another 24 hours in this solution under full light intensity. After this time the ^{14}C solution was poured off and the plants were washed under running, cold, tap water for two to three minutes. Plant extractions and paper chromatographs were made similar to those outlined under the sugar identification procedure.

The above procedure was followed during the dark part of the experiment except the chara was allowed to grow in the dark for 24 hours prior to treatment and then treated, harvested, and ground in the dark. All samples were frozen when they were not being used.

A descending chromatogram was prepared by spotting ten microliters of each of the previously prepared known sugar solutions and 50 microliters of each of the extracts prepared from the chara plants grown in light and dark. The solvent used was n-butanol-acetic acid-water (4:1:5, v/v/v). The chromatograph was removed from the cabinet, dried overnight, and cut into strips 2.5 cm wide (1.25 cm on either side of the original spot) the length of the paper. Spot locations and color comparisons were made by dipping the strips into a freshly prepared aniline-diphenylamine solution and placing in a preheated drying oven for four to five minutes at 90 to 100 C. The strips were then cut into 2.5 cm square sections starting at the spotting line, placed on individual planchets, and counted on a Beckman Low Beta II Counter. Each planchet was counted twice for ten minutes. Comparisons of spot locations, spot color, and high count areas on the chromatogram strips were made.

V. Movement of ^{35}S, ^{45}Ca, and ^{32}P in Chara

A. Autoradiographic Studies

Solutions containing radioactive ^{35}S, ^{45}Ca, and ^{32}P were prepared individually with distilled water using 50 μc of $H_2{}^{35}SO_4$ per 2000 ml of water, two mc of ^{45}Ca (99%) per 2000 ml of water, and ten μc of $H_2{}^{32}PO_4$ per 2000ml of water. Chara plants were selected and partitioned in funnels

and troughs as previously outlined. Rhizoid and morphological plant apexes and bases were treated with the radioactive solutions as shown in Table 2. After harvesting the plants were washed, dried, and pressed. The ^{45}Ca and ^{35}S treated plants were exposed to Kodak Royal Blue X-ray film for a period of two weeks. The ^{32}P treated plants were exposed to the film for one week only. Three replications were made of each treatment.

Table 2. Methods of partitioning, plant parts treated, and days of plant exposure to ^{35}S, ^{45}Ca, and ^{32}P.

Methods of Partitioning	Plant Portion Treated	Days of Exposure to Radioactive Solutions
Funnel	Morphological base	4
Funnel	Rhizoid	4
Funnel	Morphological apex	4
Trough	Morphological apex	1
Trough	Morphological base	1
Trough	Morphological apex	1 1/
Trough	Morphological base	1 1/

1/ All treatments were conducted in the controlled environmental chamber under 12 hour alternating light and dark cycles except the last two treatments which were conditioned by placing them in the dark for 24 hours prior to treatment and by remaining in the dark up to and including harvest.

VI. Movement of ^{14}C in Chara When Applied as 2,4-Dichlorophenoxyacetic acid-1-^{14}C

A. Autoradiography Studies

Chara plants were selected and partitioned in funnels following the procedure mentioned. Rhizoid and morphological plant apexes and bases were treated with 2,4-dichlorophenoxyacetic acid-1-^{14}C (specific activity 25.7 µc/mg) at different rates and periods of exposure to the herbicide as shown in Table 3. The plants were prepared for autoradiographing as previously mentioned and exposed to Kodak Royal Blue X-ray film for three weeks. Each treatment was replicated three times.

Table 3. Rate of 2,4-D-1-^{14}C, number of days of exposure to herbicide, and plant portions treated in funnel partitioned chara. 1/

Rate of 2,4-D in ppm	No. of Days Exposed to Herbicide	Plant Portion Exposed
1	1	Morphological base
1	2	Morphological base
1	4	Morphological base
1	8	Morphological base
1	4	Morphological base
2	4	Morphological base
4	4	Morphological base
1	4	Morphological apex
2	4	Morphological apex
4	4	Morphological apex
3	4	Rhizoid
3 1/	1	Morphological base
3 1/	1	Rhizoid
3 1/	1	Morphological apex

1/ All treatments were conducted in the controlled environmental chamber under alternating 12 hour light and dark cycles except the last three treatments which were conditioned by placing the plants in the dark 24 hours prior to treatment and by remaining in the dark up to and including harvest.

B. Chromatographic Studies

1. Plant extraction procedure

Approximately 50 grams of chara plants were transferred into a one liter graduated glass cylinder to which tap water was added completely covering the plants. The cylinders containing the chara were placed in the growth chamber for one day. The tap water was drained off and replaced with a distilled water solution containing three ppm of 2,4-D-1-^{14}C. At the end of four days the 2,4-D solution was poured off and the plants were washed in cold, running, tap water for five minutes. The chara plants were air dried and cut into pieces approximately 1.25 cm long. Plant extractions were prepared similarly to that outlined by Marquardt *et al.* (38) with modifications. The plants were ground in an Omni-mixer for one minute with the Power Stat set at 50 volts and then allowed to stand for five minutes at the end of which time the liquid was decanted and placed in a 500 ml flask. Fifty ml of distilled water was added and stirred until

evenly distributed. An ice bath was used throughout this procedure. One hundred and twenty-five ml of a solution containing ten ml of 10% sulfuric acid, 15 ml of 95% ethyl alcohol, 25 ml of petroleum ether, and 75 ml of ethyl ether was added and the mixture was allowed to stir for three hours on a magnetic stirrer. The mixture was centrifuged at 30,000 x g for ten minutes at the end of which time the liquid was decanted and filtered through Fisher 9-795 filter paper.

The residue was washed twice with 20 ml aliquots of a solution prepared from equal volumes of ethyl and petroleum ethers. The original filtrate and the washings were combined and placed in a freezer at -18 C until the bottom portion (water extract) was frozen. The liquid on top (ether extract) was then poured off. The ether extract was reduced to ten ml by evaporation. The frozen water extract was allowed to thaw at room temperature and reduced to 50 ml by bubbling air through it. Plant extractions from duplicate treatments and an untreated control (containing no 2,4-D) were made.

2. Chromatography

A descending paper chromatograph was prepared by spotting on Whatman #4 chromatography paper 30 microliters each of the ether and water extracts prepared from the 2,4-D-1-^{14}C treated plants, of the untreated control treatment, and of a distilled water solution containing three ppm 2,4-D-1-^{14}C. The paper was placed in a chromatography cabinet using n-butanol, acetic acid and water (4:1:5, v/v/v) as the solvent. When the solvent front was approximately two inches from the bottom edge of the paper, the chromatograph was removed, dried, and exposed for three weeks to Kodak Royal Blue X-ray film.

The plant extracts prepared above were also chromatographed with known solutions of glucose, fructose, sucrose (one gram per five ml distilled water), and a solution containing four ppm carboxyl labeled 2,4-D-1-^{14}C using phenol and water (160 gm:40 ml) as the solvent. Sixty microliters of the ether and 100 microliters of the water extracts were spotted

on Whatman #4 chromatographic paper along with ten microliters of each of the known sugar solutions and 20 microliters of the known 2,4-D-1-^{14}C solutions. After the solvent had descended it was removed from the chromatographic cabinet, dried, cut into strips 2.5 cm wide, (1.25 cm on each side of the original spot), and dipped in a freshly prepared aniline-diphenylamine (54) reagent to bring out sugar locations and colors. This part of the procedure was similar to that mentioned in the sugar identification procedure.

Another chromatogram was prepared in a similar manner. However, after dipping in the aniline-diphenylamine reagent the plant extracts and 2,4-D-1-^{14}C chromatograph strips were cut into 2.5 cm square sections starting at the spotting line, placed on individual planchets, and counted twice for ten minutes on a Beckman Low Beta II Counter. Each treatment and extractions of them were repeated twice.

The 2,4-D extraction procedure was performed using a 10% sucrose solution in place of the ground, filtered plant extracts. A descending paper chromatogram was prepared on Whatman #4 paper using 100 microliters of the water extract, 60 microliters of the ether extract, and ten microliters of each of the known glucose, fructose, and sucrose solutions. Phenol and water (4:1, w/v) was used as the solvent. When the solvent front neared the bottom of the paper the chromatograph was removed from the cabinet, dried, cut into 2.5 cm strips (1.25 cm on either side of the original spot), and dipped into the freshly prepared aniline-diphenylamine reagent for sugar identification.

VII. Movement of ^{14}C in Chara When Applied as Ring Labeled Simazine-^{14}C

A. Autoradiographic Studies

Chara plants were selected and partitioned in funnels following the procedure previously mentioned. Rhizoid and morphological plant apexes and bases were treated with ring labeled simazine-^{14}C (specific activity 6.7 μc/mg) at varying rates and periods of exposure as shown in Table 4.

The plants were prepared for autoradiography as previously mentioned and exposed to Kodak Royal Blue X-ray film for three weeks. Each treatment was replicated three times.

B. Chromatography Studies

1. Plant extraction procedure

Approximately 50 grams of chara (wet weight) was transferred into a one liter graduated glass cylinder to which tap water was

Table 4. Rate of ring labeled simazine-^{14}C, number of days of exposure to the herbicide, and plant portions treated in funnel partitioned chara.

Rate of Simazine-^{14}C in ppm	No. of Days Exposed To the Herbicide	Plant Portion Treated
1	1	Morphological base
1	2	Morphological base
1	4	Morphological base
1	8	Morphological base
1/4	4	Morphological base
1/2	4	Morphological base
1	4	Morphological base
2	4	Morphological base
4	4	Morphological base
1/4	4	Rhizoid
1/2	4	Rhizoid
1	4	Rhizoid
4	4	Rhizoid
1	4	Morphological apex
2	4	Morphological apex
4	4	Morphological apex
1[1/]	1[1/]	Morphological base
1[1/]	1[1/]	Rhizoid
1[1/]	1[1/]	Morphological apex

1/ All treatments were conducted in a controlled environmental chamber under alternating 12 hour light and dark cycles except the last three treatments which were conditioned by placing them in the dark for 24 hours prior to treatment and by remaining in the dark up to and including harvest.

added completely submerging the chara. The cylinder with the chara was placed in a controlled environmental chamber for one day at the end of which time the tap water was replaced with a distilled water solution containing three ppm of ring labeled simazine-^{14}C. After four days the

simazine solution was poured off and the chara plants were washed for five minutes in cold, running, tap water. Two replications and an untreated control (containing no simazine) were made. The extractions were prepared similar to that outlined by Knüsli *et al.* with modifications (35). The plants were cut into small sections and ground for ten minutes with 50 ml of chloroform in an Omni-mixer with the Power Stat set at 120 volts. An ice bath was used throughout the grinding procedure.

Using 50 ml of chloroform to rince out the Omni-mixer vessel, the ground plant material was transferred to an Erlenmeyer flask and stirred for 30 minutes using an ice bath. The sample was then filtered using a glass funnel fitted with Fisher #9-795 filter paper. The filtrate was washed twice in a separatory funnel with 25 ml of 2% NaOH. The NaOH washings were drained off and saved. The original filtrate was then washed in a separatory funnel with 0.5N HCl which was drained off and HCl washings was reduced to five ml by bubbling air through it. The NaOH and HCl separation washings were each condensed to five ml by heating in an oven at 50 C.

The plant residue that remained on the filter paper was washed into a beaker with 25 ml of methanol, stirred for four hours, and then refiltered. This methanol extract was reduced to five ml by bubbling air through it.

2. Chromatography

A descending paper chromatogram was prepared on Whatman #4 chromatography paper by spotting 50 microliters of each of the chloroform extract, NaOH washings, and HCl washing prepared above. These were chromatographed with a 50 microliter spot of four ppm ring labeled simazine-^{14}C and a 20 microliter spot of 333 ppm ring labeled hydroxysimazine-^{14}C. The simazine solution was prepared by dissolving the simazine first in chloroform and then bubbling off the chloroform through water. The hydroxysimazine was dissolved in ethanol. The solvent system used was isoaml alcohol saturated with 3N HCl. When the solvent front descended to within three

inches of the bottom of the paper, the chromatogram was removed, dried, and cut into 2.5 cm strips (1.25 cm on each side of the original spot). The strips were cut into square sections (starting at the spotting line), placed on individual planchets, and counted twice for ten minutes on a Beckman Low Beta II Counter. Two replications of the treatment and the extractions were made.

Another paper chromatogram was prepared using the identical extraction, chromatographic, and counting procedure mentioned above substituting a distilled water solution containing four ppm ring labeled simazine-^{14}C for the ground plant residue.

RESULTS

I. Preparation and Partitioning of the Chara Plant Parts Prior to Treatment

Chara plants were randomly selected at intervals from plastic buckets in the water baths in which they were growing and examined under a microscope for cyclosis, which if present was used as an indicator that the chara cells were alive. Eicosane partitioning of the chara was also studied using protoplasmic streaming as the indicator. In preliminary studies it was established that chara plants could be grown successfully while partitioned in the funnels and troughs. They were grown for periods up to eight days and examined under a microscope for cyclosis. No appreciable effects on the cells were noted which indicated that chara cells could exist under conditions of the experiment.

Other chara plants were subjected to periods of darkness up to 60 hours and during this period cells were checked for protoplasmic streaming periodically. Fresh chara cells could withstand periods of darkness as long as 60 hours without appreciable slowing down of the streaming. Cyclosis was more easily detected in the cells grown in darkness because these cells had less green pigment and were more translucent.

II. Identification of Sugars in Chara

A. Chromatographic Studies

Sucrose and glucose were found in chara with sucrose being predominant. Chromatograms prepared from the plant extracts and known sugars, using phenol and water as the chromatographic solvent, and dipped into the aniline-diphenylamine indicator solution, showed spots which matched in color and location to the known glucose and sucrose. No fructose was found. The Rg[1/] values for glucose and sucrose (100 vs. 107) using this solvent system were proximate to each other so that it was difficult to distinguish between these sugars. The n-butanol-acetic acid-water solvent and the

[1/] $Rg = \frac{\text{distance substance travels from origin}}{\text{distance glucose travels from origin}} \times 100$

aniline-diphenylamine dip were used to separate and confirm the previous mentioned results. Glucose and sucrose appeared, sucrose being predominant.

III. ^{14}C Studies in Chara in Light and Dark Using $NaH^{14}CO_3$ and Sucrose-^{14}C

A. Autoradiographic Studies

The images produced on the X-ray film by autoradiography showed that ^{14}C when applied in the water medium as $NaH^{14}CO_3$ moved both acropetally and basipetally in chara when the plants were subjected to periods of light. Four day exposure to ^{14}C solution produced darker images than those exposed for one day. Movement of ^{14}C from the treated rhizoid to untreated areas was not detected on the film even after four days of exposure to the solution containing $NaH^{14}CO_3$ (Figure 3). ^{14}C movement in trough partitioned plants occurred from morphological apexes and bases in light. However, when the plants were preconditioned by placing them in darkness for 24 hours prior to ^{14}C treatment and remained in the dark for an additional 24 hours no ^{14}C movement was detected in the untreated portions of the plant from the morphological apexes or bases (Figure 4).

When ^{14}C was supplied into the water medium as sucrose-^{14}C movement occurred both acropetal and basipetal in the light. Again four days of exposure produced darker images in the X-ray film than one day exposures. Slight movement out of the treated rhizoid was detected from the one and four day exposure periods (Figure 5). Movement of ^{14}C in trough partitioned plants occurred from morphological apexes and bases in light and dark (Figure 6).

B. Chromatographic Studies

When the chromatograph strips were dipped in the aniline-diphenylamine solution spots developed from extracts of chara grown in light and dark and then exposed to the $NaH^{14}CO_3$ solution. These spots matched in color and location to the spot produced by the chromatographed

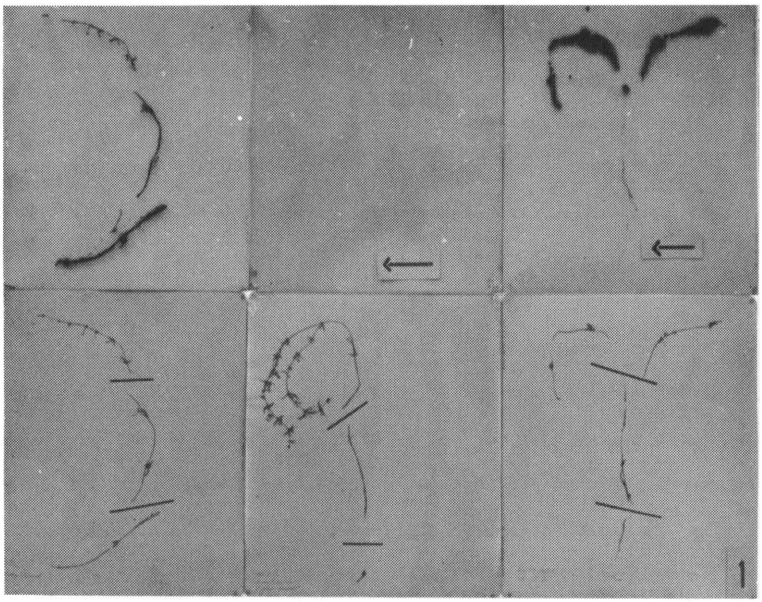


Figure 3. Autoradiographs prepared from funnel partitioned chara plants exposed for four days to a water solution of $NaH^{14}CO_3$. Plants below; autoradiographs above. Plant areas exposed to the tracer: (left) morphological base, (center) rhizoid only, (right) morphological apex. Arrow points to areas of faint image.

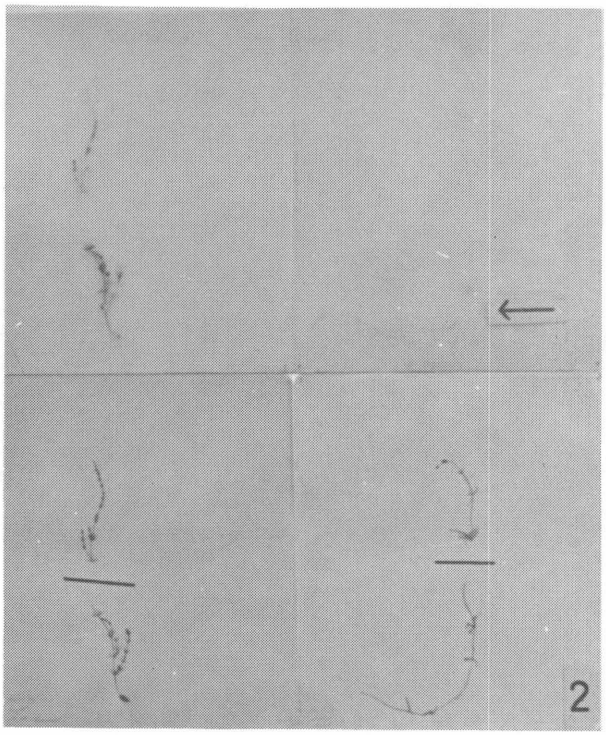


Figure 4. Autoradiographs prepared from trough partitioned chara plants exposed for one day to a water solution of $NaH^{14}CO_3$. Plants below; autoradiographs above. In both cases the morphological plant bases were exposed to the tracer. Left: plant was grown in light. Right: plant was preconditioned by growing in darkness for 24 hours prior to treatment. Arrow points to area of faint image.

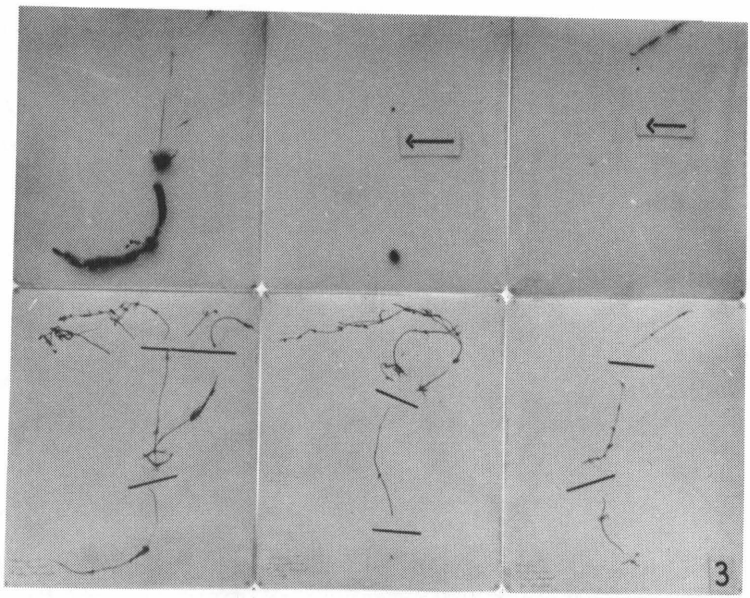


Figure 5. Autoradiographs prepared from funnel partitioned chara plants exposed for four days to a water solution containing sucrose-^{14}C. Plants below; autoradiographs above. Plant areas exposed to tracers: (left) morphological base, (center) rhizoid only, (right) morphological apex. Arrow points to area of faint image.

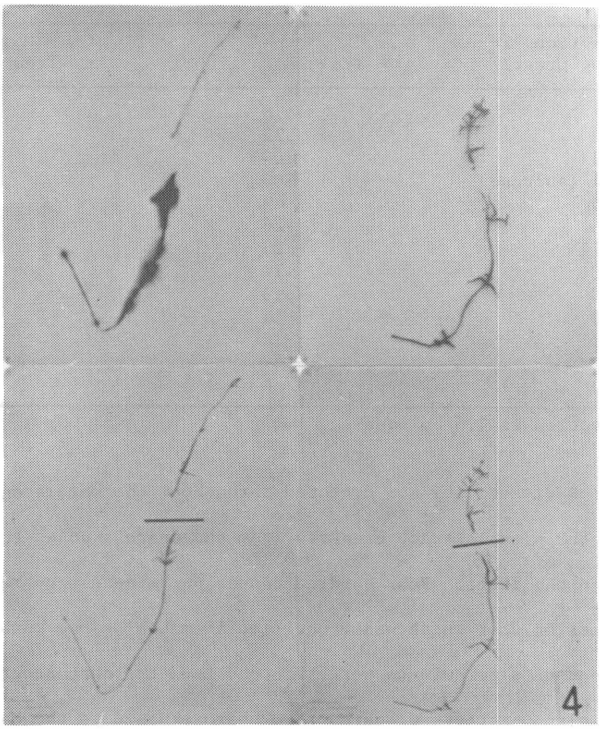


Figure 6. Autoradiographs prepared from trough partitioned chara plants exposed for one day to a water solution containing sucrose-^{14}C. The morphological base was treated in both cases. Left: the chara was grown in the light. Right: The plant was preconditioned by growing it in darkness for 24 hours prior to treatment.

known sucrose. The results of counting the radioactivity of these chromatographic strips are reported in Table 5.

Table 5. Counts per minute of chromatographed plant extracts prepared from chara conditioned in light and dark and exposed to $NaH^{14}CO_3$. Background subtracted. Average of three replications.[1]

Counted Sections in Descending Order[1]	Treated in Light	Treated in Dark
1	78	3
2	323	0
3	100	1
4 (sucrose)	400	1
5	42	1
6	26	2
7	23	0
8	12	7
9	13	0
10	14	2
11	16	0
12	47	0
13	6	0

[1] Each section is 2.5 cm square

Radioactivity was detected throughout the chromatographic strip made from the plant extract prepared from the chara exposed to the $NaH^{14}CO_3$ solution in the light. Four hundred counts per minute were recorded from counted section four which was identified as matching the known sucrose. Negligible counts per minute were recorded from the chromatograph strip made from plant extracts prepared from chara exposed to the $NaH^{14}CO_3$ solution in the dark. In the light fixation of ^{14}C into sucrose and other metabolites occurred when the $NaH^{14}CO_3$ was supplied to the water medium.

IV. Movement of ^{35}S, ^{45}Ca, and ^{32}P in Chara

A. Autoradiographic Studies

The images produced on X-ray film from partitioned chara plants exposed to ^{35}S, ^{45}Ca, and ^{32}P showed that these radiotracers move throughout the plant when they were applied in the water medium surrounding the partitioned plant parts. Acropetal and basipetal movement was detected in all cases. Rhizoids appeared to be functional in absorption of the tracers and images produced on the X-ray film indicated that movement out of the

treated rhizoid occurred (Figure 7, 8 and 9) even though the area treated was relatively small. The thallus was also fundtional in absorption and translocation of these elements from treated to untreated areas.

Movement of ^{32}P, ^{45}Ca, and ^{35}S in trough partitioned plants occurred from morphological plant apexes and bases in the light and the dark periods used in this experiment (Figures 10, 11, and 12). However, from the ^{32}P treated plants, lighter images were produced from the untreated portions of the autoradiograph made from plants preconditioned in dark than images from those grown in light.

V. Movement of ^{14}C in Chara When Applied as 2,4-Dichlorophenoxyacetic Acid-1-^{14}C

A. Autoradiography Studies

Slight upward movement of ^{14}C occurred in chara when 2,4-D-1-^{14}C was applied to the lower portions of the plant (Morphological base). The images produced on the X-ray film from the untreated plant parts were faint, however after periods of four and eight days of plant exposure to the radiotracer, ^{14}C movement was detected throughout the plants. Increasing the time of plant exposure to the radiochemical increased the intensity and length of the image; however, the movement was still considered slight (Figure 13). Increasing the rate of 2,4-D-1-^{14}C from one to four ppm did not appear to increase the movement of ^{14}C (Figure 13).

Basipetal movement of ^{14}C occurred in chara plants whose morphological apexes were treated with 2,4-D-1-^{14}C. This movement appeared to be greater than that in similar plants whose morphological bases received the radioactive herbicide (Figure 14). The absorption and translocation of ^{14}C out of the rhizoid of "rhizoid only" treated plants appeared to be greater than that from morphological base treated plants (Figure 14). More acropetal movement of ^{14}C occurred from morphological base, and rhizoid treated plants which were preconditioned in the dark than occurred from similar plants grown in the light (Figure 15).

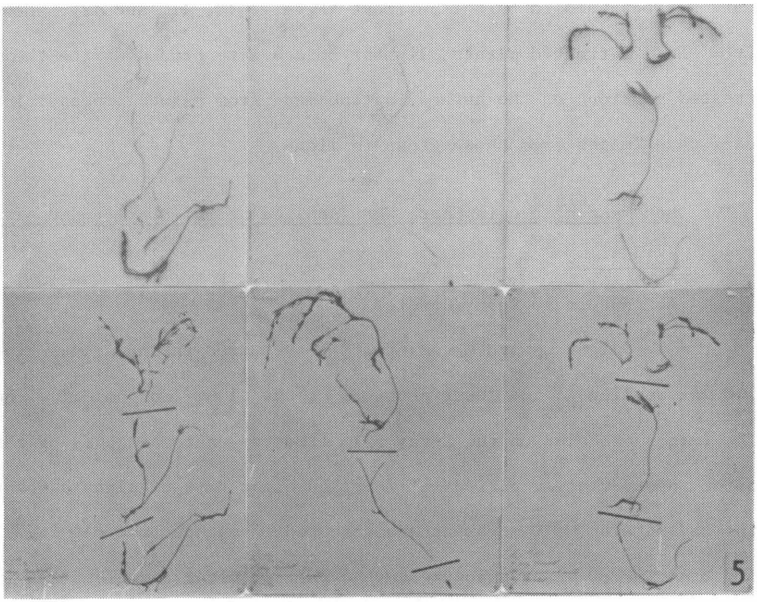


Figure 7. Autoradiographs prepared from funnel partitioned chara plants exposed for four days to a water solution containing $H_2{}^{35}SO_4$. Plants below; autoradiographs above. Plant areas exposed to the tracer: (left) morphological bases, (center) rhizoid only, (right) morphological apex.

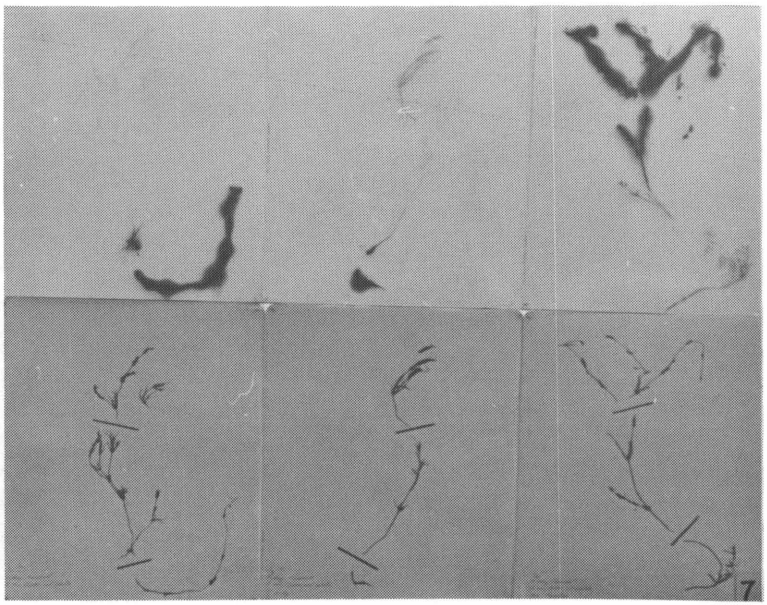


Figure 8. Autoradiographs prepared from funnel partitioned chara plants exposed for four days to a water solution containing ^{45}Ca. Plants below; autoradiographs above. Plant areas exposed to the tracer: (left) morphological base, (center) rhizoid only, (right) morphological apex.

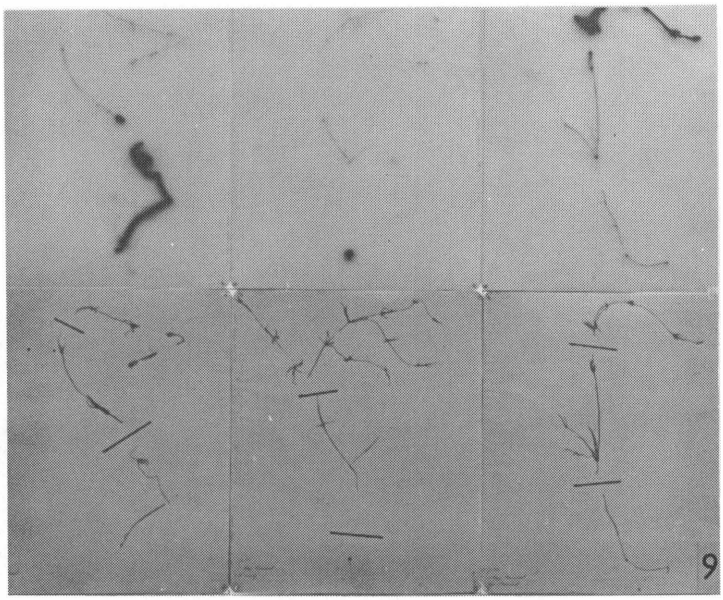


Figure 9. Autoradiographs prepared from funnel partitioned chara plants exposed for one day to a water solution containing $H_2{}^{32}PO_4$. Plants below; autoradiographs above. Plant areas exposed to the tracer: (left) morphological base, (center) rhizoid only, (right) morphological apex.

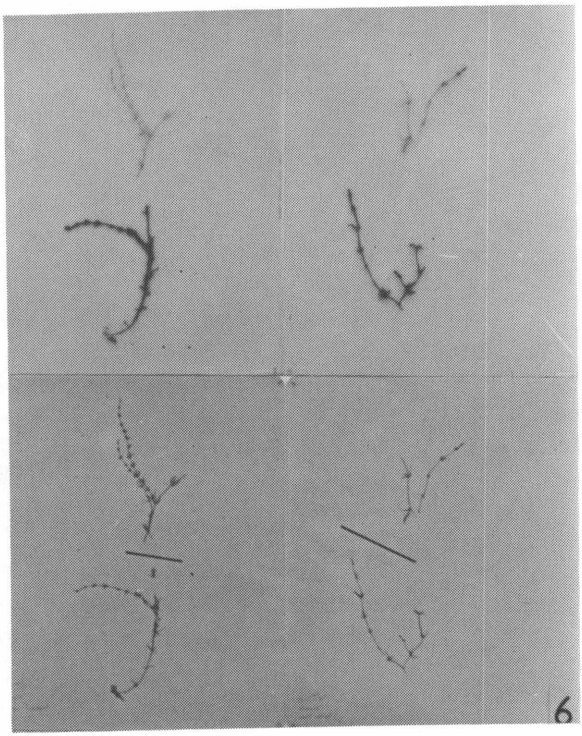


Figure 10. Autoradiographs prepared from trough partitioned chara plants exposed for one day to a water solution containing $H_2{}^{35}SO_4$. Plants below; autoradiographs above. The morphological plant bases were exposed to the tracer. Left: plant grown in light. Right: Plant preconditioned by growing it in darkness for 24 hours prior to treatment.

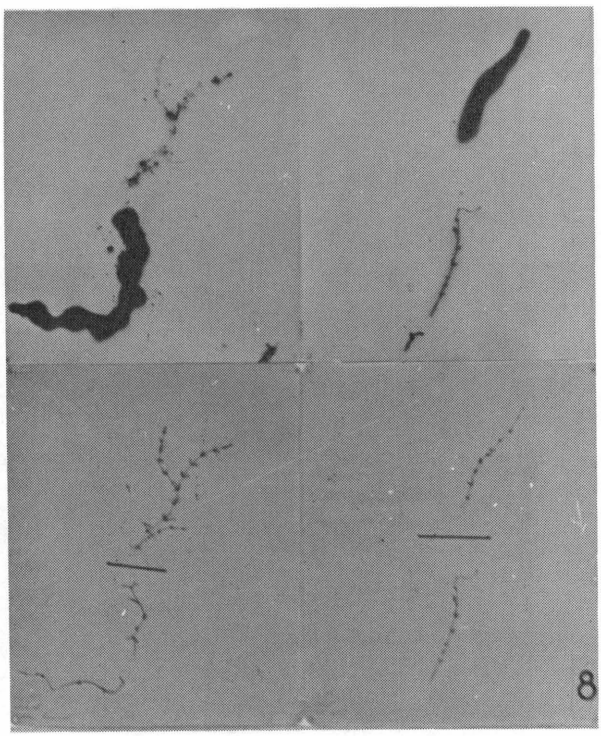


Figure 11. Autoradiographs prepared from trough partitioned chara plants exposed for one day to a water solution containing ^{45}Ca. Plants below; autoradiographs above. Left: plant was grown in the light and the morphological plant base was exposed to the tracer. Right: the plant was preconditioned by growing it in darkness for 24 hours prior to treating the morpholgical apex.

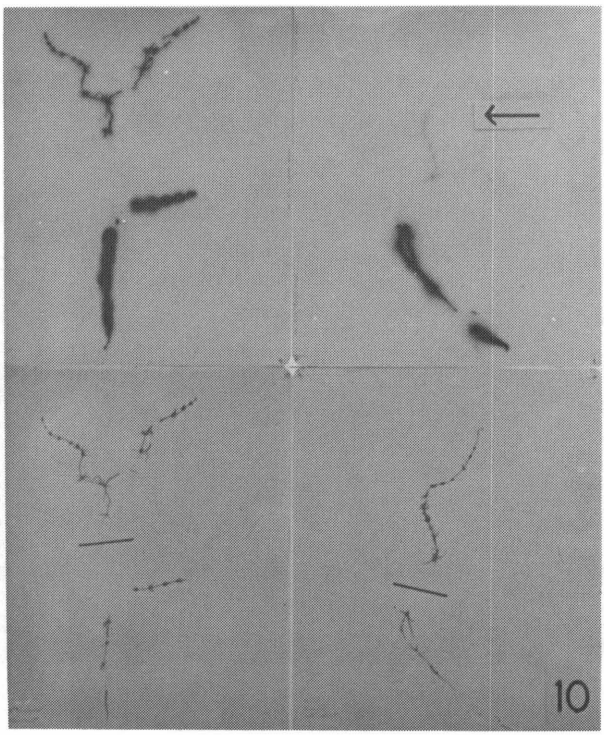


Figure 12. Autoradiographs prepared from trough partitioned chara plants exposed for one day to a water solution containing $H_2{}^{32}PO_4$. Plants below; autoradiographs above. The morphological plant bases were exposed to the tracer. Left: plant grown in light. Right: plant preconditioned by growing it in darkness for 24 hours prior to treatment. Arrow points to area of faint image.

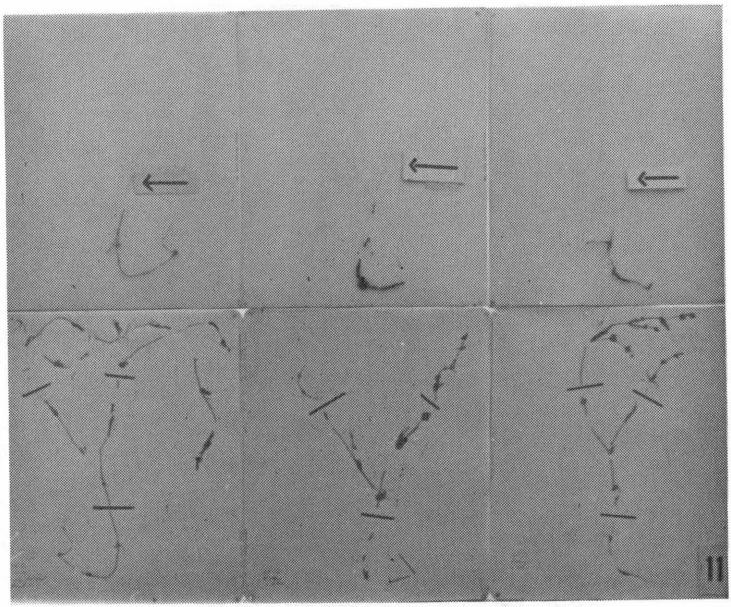


Figure 13. Autoradiographs prepared from funnel partitioned chara plants exposed to 2,4-D-1-^{14}C at varying rates and times of exposure. Plants below; autoradiographs above. The morphological plant bases were treated. Treatments: (left) one day exposure at one ppm; (center) four days exposure at one ppm; (right) four day exposure at four ppm. Arrow points to areas of faint images.

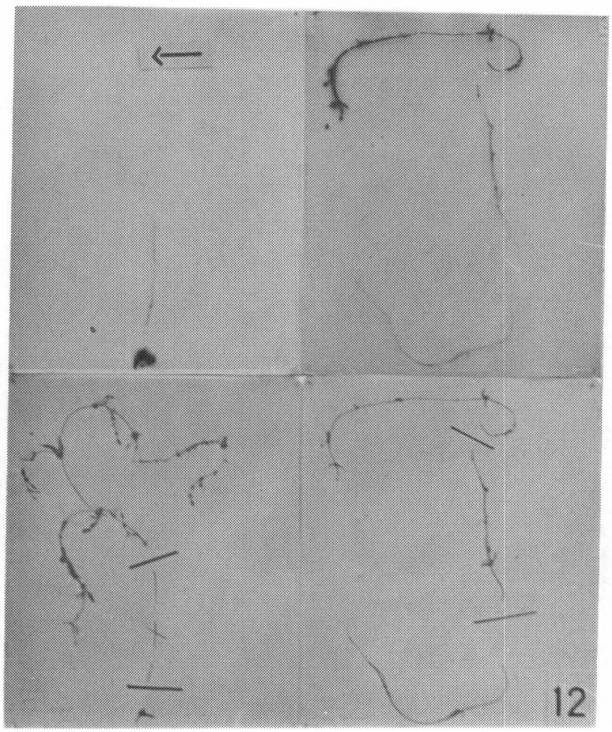


Figure 14. Autoradiographs prepared from funnel partitioned chara plants exposed to a water solution containing 2,4-D-1-^{14}C. Plants below; autoradiographs above. Treatment: (left) rhizoid only treated, four day exposure at three ppm; (right) morphological apex treated, four day exposure at one ppm.

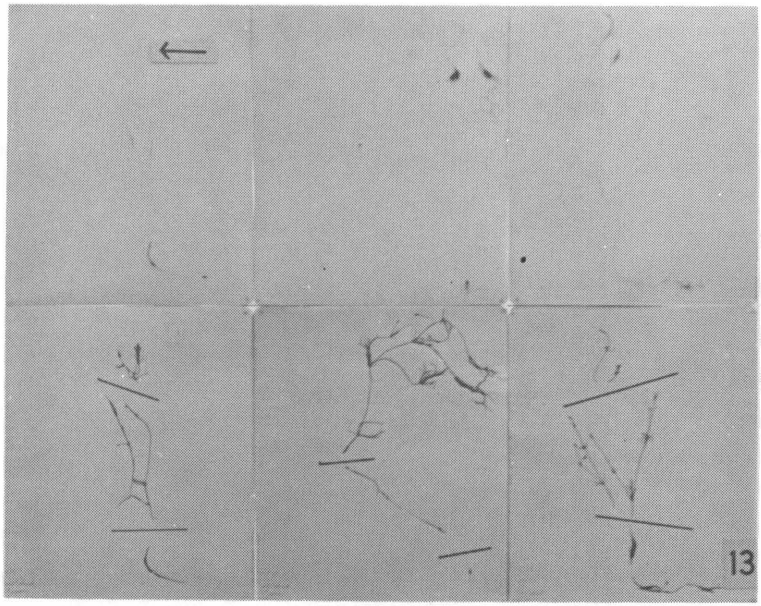


Figure 15. Autoradiographs prepared from funnel partitioned chara plants preconditioned by growing them in the dark for 24 hours prior to treatment with three ppm 2,4-D-1-^{14}C. Treatments: (left) morphological base treated, (center) rhizoid only treated, (right) morphological apex treated.

B. Chromatography

Two images from the water extract and one from the ether extract were produced on the autoradiograph made from the chromatogram prepared by spotting the water and ether extracts of plants treated with 2,4-D-1-^{14}C. The image produced by the ether extract appeared to descend with the chromatographed known 2,4-D-1-^{14}C water solution (Figure 16). Glucose and fructose were found when these same plant extracts were chromatographed with water solutions of known glucose, fructose, and sucrose. The spot locations (Rf) and spot colors were similar to those produced by the chromatographed known sugars after the chromatographic strips were dipped into the aniline-diphenylamine indicator solution (Figure 17). When a similar prepared chromatogram was cut into 2.5 cm sections and each section counted on a Low Beta II radiation counter radioactive sections were found on the water extract chromatographic strip which matched the spots produced by fructose and glucose (Table 6 and 7). The ether extract contained most of the radioactivity and a high count section was found further down the chromatograph strip than the high count area produced by the chromatographed known 2,4-D-1-^{14}C solution.

Results of the 2,4-D extraction procedure performed on a 10% sucrose solution used in place of the plant extract indicated that some of the sucrose was broken down to fructose and glucose by the procedure. Spots appeared after the chromatographic strips were dipped into the indicator solution which matched in location and color with those produced by the chromatographed known monosaccharides.

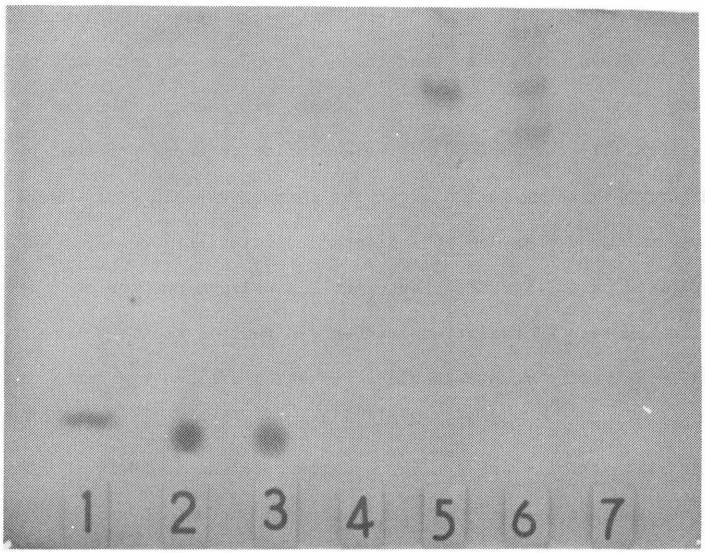


Figure 16. Autoradiograph made from the chromatogram prepared by spotting the water and ether extracts of plants treated with 2,4-D-1-^{14}C. Solvent used was n-butanol, acetic acid and water (4:1:5, v/v/v). (1) 2,4-D-1-^{14}C; (2), (3) ether extracts of chara plants exposed to 2,4-D-1-^{14}C; (4) ether extracts of untreated control plant; (5), (6) water extracts of chara plants exposed to 2,4-D-1-^{14}C; (7) water extract of untreated control plant. (3) and (6) are replicate treatments and extracts.

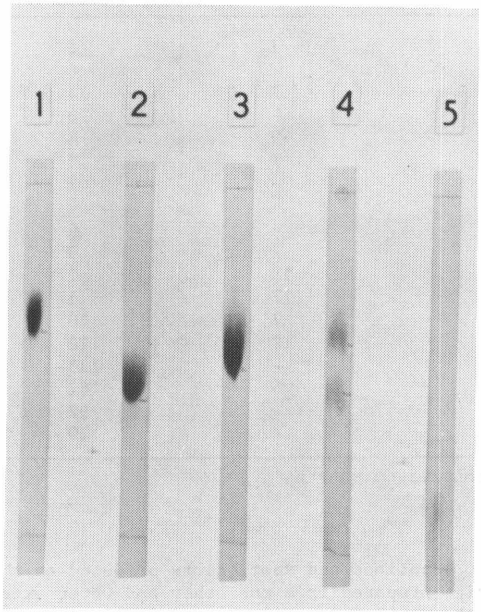


Figure 17. Descending paper chromatograph strips prepared from chara plants treated with three ppm 2,4-D-1-^{14}C for four days. Phenol and water (4:1, w/v) was used as the chromatographing solvent. The strips were dipped into an aniline-diphenylamine indicator solution. (1) known glucose (brown spot), (2) known fructose (reddish-brown spot), (3) known sucrose (brown spot), (4) water extract of 2,4-D-1-^{14}C treated plant (brown and reddish-brown spots), (5) ether extract of 2,4-D-1-^{14}C treated plant.

Table 6. Counts per minute from chromatographic strips prepared from ether and water extracts of chara treated with three ppm 2,4-D-1-^{14}C for four days. Average of two replications and two ten minute counts per replication.[1/] Background subtracted.

Counted Sections in Descending Order[1/]	Chromatographed Known 2,4-D-1-^{14}C	Ether Extract	Water Extract
1	1	2	24
2	-	6	28
3	1	11	17
4	-	16	35
5	49	4	37(fructose)
6	1	3	40(fructose)
7	-	5	32
8	-	4	34(glucose)
9	-	6	10
10	2	3	4
11	19	1	4
12	112	1	6
13	1	20	4
14	12	112	10
15	-	133	5
16	6	144	6
17	-	92	10
18	-	20	0

1/ Each section 2.5 cm square.

Table 7. Spot locations and spot colors produced on the chromatographic strips prepared from the ether and water extracts of chara treated with three ppm 2,4-D-1-^{14}C for four days.

Spot on Chromatogram	Spot Color	
	Reddish-Brown	Brown
Known 2,4-D-1-^{14}C water solution	none	none
Ether extract (Reps I and II)	none	none
Water extract (Reps I and II)	Section 8	Section 5 to 6
Known glucose	Section 8	none
Known fructose	none	Section 5 to 6

VI. Movement of ^{14}C in Chara When Applied as Ring Labeled Simazine-^{14}C

A. Autoradiographic Studies

Very little acropetal movement of ^{14}C was detected in chara exposed to periods of light when ring labeled simazine-^{14}C was applied to the morphological plant base and rhizoid portions of the plants. Increasing rates and increasing periods of exposure to the labeled herbicide did not appear to increase the translocation of ^{14}C from the treated to

untreated areas (Figure 18). Although still small more basipetal movement of ^{14}C appeared to take place when the ring labeled simazine-^{14}C was applied to the morphological plant apexes (Figure 19).

More acropetal and basipetal movement of ^{14}C was noted from plants preconditioned by placing them in the dark for 24 hours prior to exposure to the labeled herbicide (Figure 20).

B. Chromatography Studies

The chromatograms prepared by chromatographing known solutions of ring labeled simazine-^{14}C and hydroxysimazine-^{14}C with chara plant extracts prepared from plants treated with ring labeled simazine-^{14}C, produced radioactive areas in the chloroform, HCl, and methanol portions of the extracts. The radioactive areas in the chloroform and methanol extracts appeared to be very close to the radioactive area produced by the chromatographed known ring labeled simazine-^{14}C. The greatest radioactivity was found in the HCl portion of the extract and remained in the vicinity of the original spot at the top of the descending chromatogram. Very little radioactivity was found in the NaOH washing. Hydroxysimazine chromatographed with this solvent system produced radioactivity the length of the strip with a high radiation area in section seven. If this area were taken as the hydroxysimazine-^{14}C none was found in the plant extracts (Table 8).

Very little radioactivity was found in the NaOH and HCl washings prepared and chromatographed using the simazine extraction procedure on a distilled water solution containing four ppm ring labeled simazine-^{14}C. A radioactive spot was detected in the chloroform extract which matched closely with the radioactive area found on the chromatographed known ring labeled simazine-^{14}C strip. Again the ring labeled hydroxysimazine-^{14}C produced radioactivity the length of the chromatography strip with a high count area in section seven (Table 9).

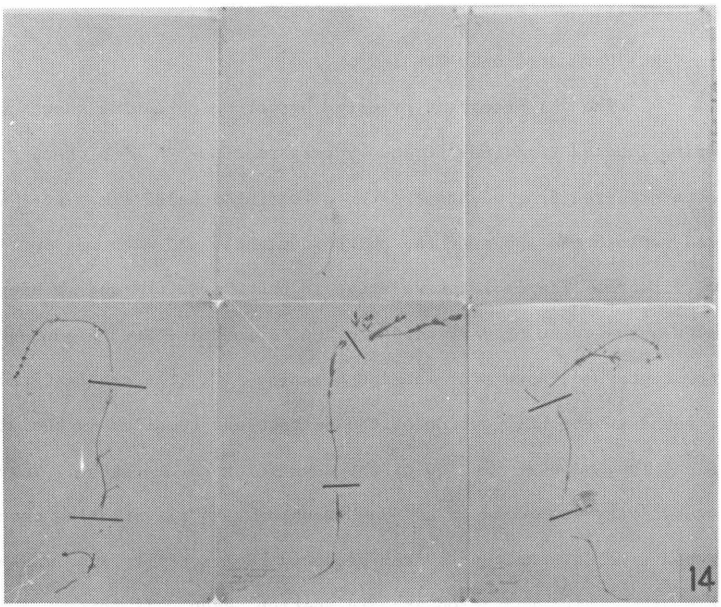


Figure 18. Autoradiographs prepared from funnel partitioned chara plants exposed to a water solution containing ring labeled simazine-^{14}C at varying rates and times of exposure. Plants below; autoradiographs above. The morphological plant bases were treated in each case. Treatments: (left) four day exposure at 1/4 ppm, (center) one day exposure at one ppm, (right) four day exposure at one ppm.

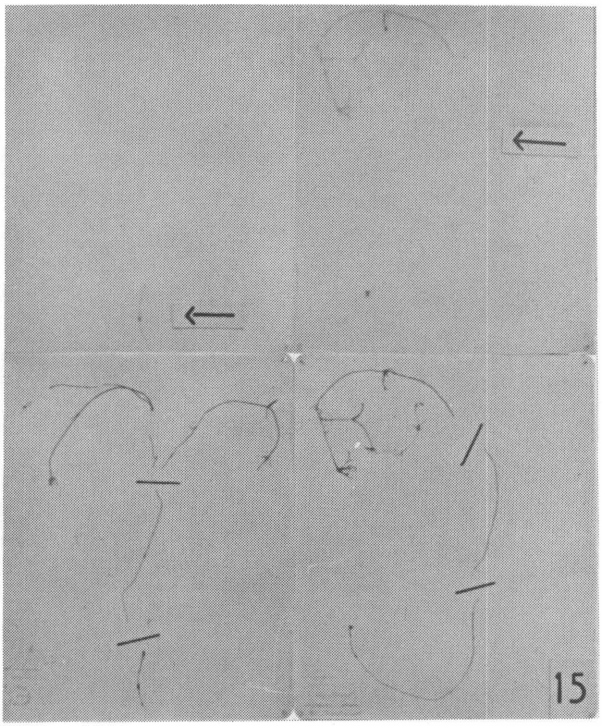


Figure 19. Autoradiographs prepared from funnel partitioned chara plants exposed for four days to one ppm ring labeled simazine-^{14}C. Plants below; autoradiographs above. Treatments: (left) rhizoid only treated; (right) morphological apex treated. Arrows point to areas of faint images.

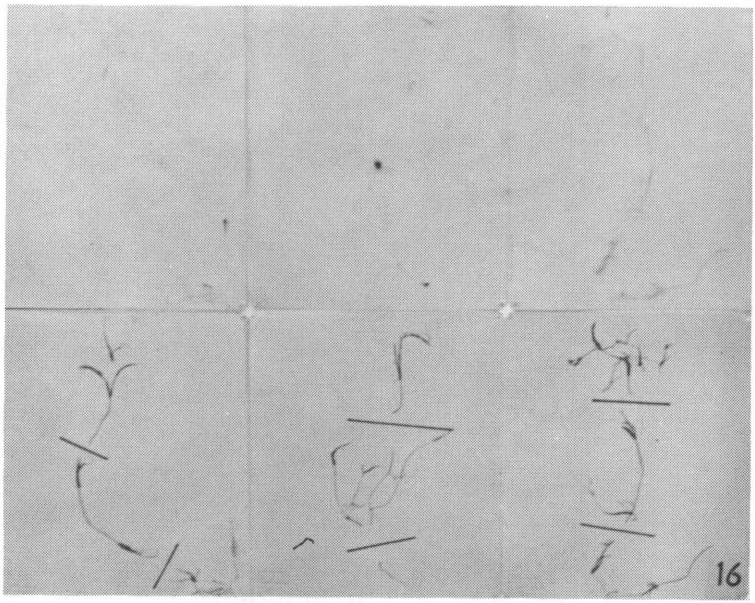


Figure 20. Autoradiographs prepared from funnel partitioned chara plants preconditioned by growing them in the dark prior to treatment with one ppm ring labeled simazine-^{14}C. Treatments: (left) morphological base treated, (center) rhizoid only treated, (right) morphological apex treated.

Table 8. Counts per minute from 2.5 cm square sections of chromatographic strips prepared from chara exposed for four days to three ppm of ring labeled simazine-^{14}C. Average of two replications, two ten minute counts per replication. Background subtracted.[1/]

Counted sections in Descending Order[1/]	Chloroform Extract	NaOH Washing	HCl Washing	Methanol Extract	Known Hydroxy-Simazine-^{14}C	Known Simazine-^{14}C
1	-	3	168	3	53	46
2	-	-	4	1	61	-
3	-	-	-	-	44	1
4	-	-	-	1	20	-
5	2	-	-	-	23	-
6	-	-	-	-	74	-
7	-	1	-	-	320	-
8	-	-	1	-	38	87
9	24	1	2	13	11	2
10	46	-	6	-	9	15
11	1	1	5	5	6	2
12	-	-	5	1	4	13

1/ Each section 2.5 cm

Table 9. Counts per minute from 2.5 cm square sections of chromatographic strips prepared by using the simazine extraction procedure on a distilled water solution containing four ppm of ring labeled simazine-^{14}C. Average of two ten minute counts. Background subtracted.[1/]

Counted Sections in Descending Order[1/]	Chloroform Extract	NaOH Extract	HCl Extract	Known Hydroxy-Simazine-^{14}C	Known Simazine-^{14}C
1	-	4	2	7	1
2	1	7	1	140	1
3	-	3	-	214	1
4	1	-	2	66	1
5	-	1	-	59	-
6	-	1	-	284	2
7	-	-	3	497	7
8	-	-	-	23	-
9	10	-	-	5	4
10	16	1	-	12	7
11	27	-	-	13	7
12	6	-	1	27	14
13	-	-	1	4	1

1/ Each section 2.5 cm

DISCUSSION OF THE RESULTS

The chara thalli and those sections of the thalli called rhizoids individually absorbed and translocated ^{45}Ca, ^{32}P, ^{35}S, and ^{14}C when the carbon was supplied as sucrose-^{14}C and 2,4-D-1-^{14}C. The movement of these isotopes occurred when the radioactive chemicals were supplied to the water medium surrounding the chara and the chara was subjected to periods of light or preconditioned by placing them in the dark for 24 hours prior to exposure to the tracers.

The distribution and movement of these compounds appeared to be non-polar as evidenced by their acropetal and basipetal movement.

When the ^{14}C was supplied as ring labeled simazine-^{14}C to chara grown in the light very little acropetal movement was detected by autoradiographing. Slightly more movement of ^{14}C occurred basipetally when the morphological apexes of the chara plants were treated with the ring labeled simazine-^{14}C or 2,4-D-1-^{14}C. This increased movement was attributed to the larger area of plant (morphological apexes) exposed to the chemical or to the movement of 2,4-D and simazine with metabolic transport since it was possible that the tops of the chara were photosynthesizing and exporting foods to other areas. Movement of the simazine with food transport, however, is contrary to the results received from similar work in vascular plants. In higher plants simazine has been found to penetrate the cuticle of a leaf, enter the mesophyll, and diffuse along the cell walls; it does not enter the living symplast. Cross sections made of chara show intercellular spaces between the main large internodal cell and the smaller sheath cells. Simazine movement similar to that in higher plants could occur along these interfaces.

When the ^{14}C was supplied to the water medium as $NaH^{14}CO_3$, in the light movement of the ^{14}C occurred from the treated thallus section. However, when the plants were subjected to preconditioning in the dark for a period of 24 hours, or only the hyaline rhizoids were treated, no movement

of ^{14}C was detected by autoradiography. The rhizoids are considered non-green and therefore probably nonphotosynthetic. Growing chara in darkness for 24 hours prior to treatment evidently was sufficient to deplete the stored radiant energy which was available for fixation of the ^{14}C into organic compounds. The chloroplasts of chara are embedded in a fixed layer of peripheral cytoplasm and therefore resist movement by cyclosis. The autoradiographs of the preconditioned dark $NaH^{14}CO_3$ treated plants showed images on the X-ray film only where the ^{14}C was supplied even if the eicosane barrier divided an internodal cell. The untreated portion of the internodal cell did not produce an image. This indicates then that ^{14}C when supplied as $NaH^{14}CO_3$ to the water surrounding the chara had to be converted to another form in order for it to move intracellularly. The possibilities existed however that in order to transport the ^{14}C from the $NaH^{14}CO_3$ solution across the cell wall to the chloroplasts, light was necessary, or that the images produced by the treated plant portions from preconditioned dark plants was from carbon or in the cell wall.

When the ^{14}C was supplied as sucrose-^{14}C or 2,4-D-1-^{14}C it traveled intra- and intercellularly in chara grown in the light, preconditioned in the dark, and from rhizoid treated sections. Movement of the ^{14}C when it was supplied in these organic molecules was not light dependent.

Chara, which is considered a nonvascular aquatic plant, transports ^{14}C intercellularly. Simple diffusion of carbon dioxide or a bicarbonate did not account for the movement. If it did, images would have been produced on the untreated side of the autoradiographs made from preconditioned dark plants. From the results it seems more likely that ^{14}C was converted to a metabolite and then transported.

Chromatographs prepared in this study indicated that sucrose and glucose were the most abundant sugars present in the simple water extracts prepared from chara, sucrose being predominant. Differences in radioactivity were found on the chromatographs made from extracts prepared from chara grown in the light and from chara preconditioned in darkness which

were then exposed to a solution containing $NaH^{14}CO_3$. Radioactive sucrose was identifided by chromatographing extracts from the plants grown in the light and counted on a radiation counter. Very little radioactivity was found in the extracts prepared from chara preconditioned in the dark. These results showed that the ^{14}C from the water solution of $NaH^{14}CO_3$ was entering into the chara plant and was synthesized into sucrose. Additional radioactive areas were found on the chromatogram prepared from extracts of the plants grown in the light which indicated the presence of other radioactive metabolites that were not identified.

The uptake and translocation of ^{45}Ca, ^{32}P, and ^{35}S may be light dependent also. It was possible that the 24 hour dark preconditioning period was not sufficient to deplete the energy or the action sites which were needed for fixation of these compounds into organic molecules. This may be particularly true of phosphorous since the images produced from the preconditioned chara plants on the autoradiographs were not as dark as those produced by chara grown in the light.

In these studies it was shown that the rhizoids of chara had functions other than attachment of the plant to the soil such as nutrient absorption. Whether they extract nutrients from the soil colloids has yet to be shown; however, in nature the rhizoids are usually located in the first few centimeters of the pond bottom soil which is normally saturated with water. Consequently, the rhizoids are bathed in a soil-water solution. The thallus section was also capable of absorbing nutrients directly from the water medium as shown by autoradiography. Whether the nutrients were thallus or rhizoid absorbed did not seem to make any difference because the evidence indicated that the Ca, P, or S moved throughout the plant in either case.

When the ^{14}C was supplied as ring labeled simazine-^{14}C very little movement out of the treated areas was detected by autoradiography. Slightly more basipetal movement of ^{14}C was noted than acropetal movement and more movement occurred in preconditioned dark plants than those grown in light. This increased mobility may be attributed to the fact that the light-

dependent chemical reaction in which simazine became involved did not operate in the chara after preconditioning in darkness for 24 hours and therefore the simazine was more free to move. Another probability for this increased mobility was that the chara plant had the ability to degrade the ring labeled simazine-^{14}C and that the degraded ^{14}C was moving in the dark as a metabolite.

Reports (36, 55, 56) of field studies showed that simazine effectively controlled chara at rates of two to four ppm applied to the water. The autoradiographs in this study indicated that simazine was not very mobile in chara; therefore, if effective control is to be achieved, simazine must be absorbed by practically every chamber or cell in the thallus. Applications of simazine during periods of darkness may increase the effectiveness of this herbicide because of its possible increased mobility in darkness.

Paper chromatograms prepared from plant extracts made from chara exposed to ring labeled simazine-^{14}C showed radioactive areas in the chloroform and methanol portions of the extracts which could have been simazine. The radioactivity was not great and appeared slightly below the chromatographed known ring labeled simazine-^{14}C. This could have been simazine which was not washed off of the plant surface, free simazine which was in the chara, or simazine which was separated from its metabolite complex by the extraction procedure. The extraction and chromatographic procedures used in this experiment did not reveal the presence of hydroxysimazine in the treated chara. Most of the radioactivity was found in the HCl portions of the extracts and was not soluble in isoamyl alcohol as evidenced by the fact that the radioactivity was located in the vicinity of the original spot on the chromatogram. Since simazine had been shown to affect chara growth it was possible then that simazine was bound in the chara plant as a metabolite which was soluble in HCl but not in isoamyl alcohol, 2% NaOH, or chloroform.

When a distilled water solution of ring labeled simazine-^{14}C was substituted in the extraction procedure in place of the simazine-^{14}C treated

plant extracts negligible radioactivity was found in the HCl portion of the extract. This was further evidence that the simazine in chara was converted to a metabolite more soluble in HCl than the other solvents used. The higher total activity recorded from the plant extracts indicated that chara accumulated simazine.

Chara was able to decarboxylate 2,4-D-1-^{14}C as evidenced by the isolation and identification of radioactive glucose and fructose by chromatographing with known sugars and counting the chromatogram in a Low Beta radiation counter. The 2,4-D extraction procedure used in this study was found to degrade sucrose to its monosaccharides: glucose and fructose. It was therefore probable that the radioactive glucose and fructose found by chromatographing the plant extracts were originally in the form of sucrose in the chara. The plant was able to decompose 2,4-D-1-^{14}C and the carboxyl carbon from the herbicide molecule was then available for conversion to sugars.

The detected movement of ^{14}C out of chara plant areas treated with 2,4-D-1-^{14}C may be attributed to the conversion of the carboxyl ^{14}C into sucrose and other metabolites. Radioactivity was found in the ether extracts on chromatograms prepared from plants treated with 2,4-D-1-^{14}C. These radiation areas moved further down on the chromatograph than the chromatographed known 2,4-D-1-^{14}C which may indicate that the 2,4-D molecule was metabolically bound in chara.

Reports indicated that chara was not susceptible to the effects of 2,4-D even when the herbicide was applied up to 100 ppm to the water medium surrounding the chara. The ability of chara to decompose the 2,4-D molecule may account for its resistence to the effects of this chemical.

Autoradiographs in this study indicated that more ^{14}C movement occurred in preconditioned dark plants than in plants grown in light when the ^{14}C was supplied as 2,4-D-1-^{14}C. This movement was detected throughout the plant. Increased movement of ^{14}C from 2,4-D-1-^{14}C preconditioned dark plants was attributed to the fact that decarboxylation may proceed in

the dark; or more likely that after a period of darkness the entire plant became a sink and the 2,4-D moved with food materials. This latter theory indicates that it may be easier to transport food to areas where it is needed than to convert it from storage in that area. It was also possible that 2,4-D-1-^{14}C did not get bound to its usual metabolite and the intact molecule was more free to move.

The overall results of this study indicated that chara can transport certain materials and that the rhizoids have functions other than attachment of the plant to the pond bottom. Because of its fairly complex sex organs, i.e. the gametangia being enclosed within a jacket of sterile cells, taxonomists consider this plant to be of the higher algae (62). Others believed it never left the water in its phylogeny (47). Therefore, chara may prove to be a transition species between vascular and nonvascular plants.

The recent discovery that plasmodesmata exist between chara cells coupled with the procedure developed in this study suggests that members of the Characeae would be excellent subjects for studying intra- and intercellular transport.

SUMMARY AND CONCLUSIONS

^{45}Ca, ^{35}S, ^{32}P, and ^{14}C as $NaH^{14}CO_3$, sucrose-^{14}C, 2,4-D-1-^{14}C, and ring labeled simazine-^{14}C were supplied to the water surrounding chara (Chara vulgaris L.). Autoradiographs were made of chara plants partitioned with an eicosane barrier which enabled certain portions of the plant to be treated. The transport of these chemicals within the chara thallus was nonpolar as evidenced by acropetal and basipetal movement. The vegetative thallus and the rhizoids absorbed and transported ^{45}Ca, ^{35}S, ^{32}P, and ^{14}C from sucrose-^{14}C, 2,4-D-1-^{14}C, and simazine-^{14}C when the plants were subjected to periods of light or preconditioned by placing them in the dark for 24 hours prior to exposure to the tracers. Movement in the thallus of ^{14}C when it was supplied to the water medium as $NaH^{14}CO_3$ was found to be light dependent; images were not produced from the untreated side on autoradiographs made from chara plants preconditioned in the dark. Movement of ^{14}C out of the hyaline rhizoids was not detected by autoradiography when only the rhizoids were exposed to a water solution of $NaH^{14}CO_3$.

Glucose and sucrose were found in simple water extracts of chara with sucrose being predominant. Descending paper chromatograms made from $NaH^{14}CO_3$ treated plants grown in the light and dark showed that ^{14}C can be fixed into sucrose and other metabolites in the light in chara plants; however, very little radioactivity was found in the water extracts prepared from chara plants preconditioned in darkness.

Slight movement of ^{14}C from 2,4-D-1-^{14}C was detected in chara. The plant decarboxylated the 2,4-D-1-^{14}C and the ^{14}C was available for fixation into fructose and glucose; which were probably found as the disaccharide, sucrose in the plant. Very little movement of ^{14}C from ring labeled simazine-^{14}C was detected by autoradiography. Extractions and chromatographs prepared from ring labeled simazine-^{14}C treated plants showed very little free simazine to be present in the chara; most of the radioactivity was

found in the HCl portion of the extract. The chara plant which is susceptible to the effects of simazine at two to four ppm can metabolize simazine into a form which is more soluble in HCl than in 2% NaOH or chloroform which was used for extractions and is not soluble in isoamyl alcohol which was used as the chromatographing solvent. Hydroxysimazine was not found.

More movement of ^{14}C was noted by autoradiographing plants preconditioned by growing them in the dark for 24 hours and then treating with 2,4-D-1-^{14}C or ring labeled simazine-^{14}C than in plants grown in light and then treating.

This study showed that chara can absorb and transport certain compounds from treated to untreated parts of the thallus and that the rhizoids have functions, such as nutrient absorption, other than attachment.

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