Effect of Deuterium Oxide on the Growth of Peppermint II

Histological Effects

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Representative sections of leaf, stem, and root of peppermint plants grown in 70 per cent D_2O nutrient were examined microscopically; comparison was made of the significant histological features with sections from plants grown in an aqueous nutrient. The major effect of deuterium on the growth of peppermint appears to be inhibition of cell divisions. Parenchyma cells are enlarged and vascular tissue appears to be reduced in the deuteriated plants. In general, the effects were more pronounced in growing tissues than in tissue exposed to deuterium after differentiation had occurred.

IN A PREVIOUS paper (1) the morphological effects of deuterium oxide on the growth of peppermint were reported. Peppermint plants were conveniently grown in 250-ml. Erlenmeyer flasks by liquid culture technique. The concentration of D_2O in the nutrient solution was varied from 0 to 100%. When the fully grown plants were harvested, samples of each plant part were killed in Randolph's modification of the Navashin fixing solution (2) and reserved for histological studies. While the histological effects were noted in all plants grown with D_2O concentrations in the nutrient up to 100%, comparison was made here only between the controls and the plants grown in 70% D_2O nutrient. Plants grown on intermediate D_2O concentrations showed effects in the same direction as the 70% plants, but to a proportionately lesser extent. Consequently, we compared the plants grown in the highest D_2O concentrations to the controls. Significant growth was not observed in plants grown in D_2O concentrations above 70% as reported earlier (1), and these plants were therefore not subjected to histological examination. It should also be noted that plants grown in nutrient containing 70% D_2O showed an uptake of 35% deuterium when the water obtained from combustion of the dried plants was analyzed for deuterium content. This represents organically bound exchangeable and nonexchangeable deuterium. The maximum histological effects described here thus result from the replacement by deuterium of about one-third of the hydrogen normally present in the tissue.

In this study representative sections of leaf, stem, and root of peppermint plants grown in 70% D_2O nutrient were examined microscopically and comparison of the significant histological features was made with comparable sections from plants grown in an aqueous nutrient.

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EXPERIMENTAL

Five peppermint plants (*Mentha piperita*) were grown on a nutrient containing 70% D_2O by the technique reported in the previous paper in this series. Three plants were grown in an aqueous medium and served as the controls. All plants were harvested after 50 days, at which time the controls went into the flowering stage. The plant parts studied for their histological response to D_2O in the nutrient included immature leaf (the smallest leaf

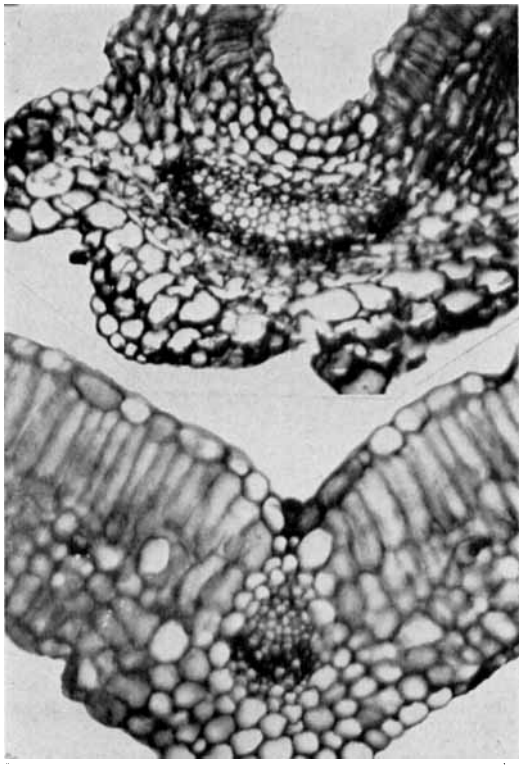

Fig. 1.—Transverse sections of immature leaves taken near apex of plants growing on (top) H_2O nutrient solution and (bottom) 70% D_2O nutrient solution. Both sections are taken from the midrib of the leaf. The magnification (× 100) is the same in both cases.

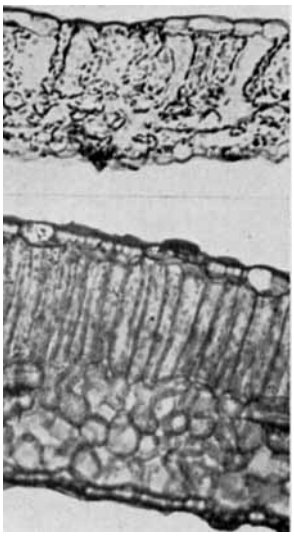

Fig. 2.—Transverse sections of mature leaves taken near the base of the primary stem of plants growing on (top) H_2O nutrient solution and (bottom) 70% D_2O nutrient solution. Magnification is the same in both cases (× 100).

at the apex discernible to the naked eye), mature leaf (near the base of the primary shoot), immature stem (directly below the pair of immature leaves under study), mature stem (directly above the mature leaf under study), and root (a section taken 2 cm. below the origin of the root on the stem base).

The tissues mentioned in the previous paragraph were dehydrated through an ethanol-xylol series and were imbedded in Tissuemat (55°) paraffin. They were sectioned at 20 μ thickness with a Spencer No. 820 rotary microtome. The sections were mounted on slides by Mayer's albumin fixative and were stained with Safranin O and Fast Green. They were permanently mounted in Canada balsam. The different histological features were measured in microns using an ocular micrometer for both the control and the 70% D_2O plant tissues. Photomicrographs of the sections of the plant parts were made with a Leitz Orthomat microscope camera and are shown in Figs. 1–5.

RESULTS AND DISCUSSION

Leaf.—Figures 1 and 2 show a photographic comparison of leaf sections of the control and the 70% D_2O plant. Table I summarizes measurements of the major histological features of representative sections of both young and mature leaves.

The young blade of the deuteriated plant is about twice as thick as the control. In the mature leaf this effect is much less pronounced, though still significant. The midrib shows little difference in thickness in response to deuteriation. However, there is a drastic reduction in midrib width and in the number of vascular elements, mainly xylem vessels and tracheids, which differentiated in the presence of D_2O. The dimensions of the tissues comprising the bulk of the leaf blade are greater in the deuteriated leaf—apparently the result of larger cells. Differences in all cases are more marked in the young leaf than in the mature leaf. Since the mature leaves had already gone through the earlier phase of their elongation cycle before the effect of D_2O was exerted on them, they do not exhibit differences so striking as those of the young leaf.

Deuterium oxide affects the kinetics of reactions involved in metabolism and thus interferes with the normal biochemistry of the plant. Of major significance is interference with cell division. Gross and Spindel (3) have recently treated at length the subject of inhibition of mitosis by deuterium. Stein and Forrester (4) studied the recovery of corn root cells after a 48-hour exposure to 80% D_2O solution and concluded that D_2O blocks cell division at interphase and metaphase. Flaumenhaft, *et al.* (5), Chorney, *et al.* (6), have observed that certain algae grown in a fully deuteriated medium are substantially larger than cells grown in water. Crespi, *et al.* (7), reported that algae transferred from an H_2O culture to 99.6% D_2O nutrient solution continued to grow, but cell division appeared to be strongly inhibited. Henderson (8) points out that one of the effects of D_2O on nucleic acids *in vitro* is impaired DNA strand separation which implies possible interference with DNA synthesis and cell division *in vivo*. We have observed that the growth of peppermint plants under the stress of D_2O is continuous, although it proceeds at a reduced rate compared to the controls. It appears that cell division is indeed severely impaired. The tissue cells tend to become greatly enlarged as a result of the inhibi-

TABLE I.—COMPARISON OF HISTOLOGICAL MEASUREMENTS IN LEAF FROM PEPPERMINT GROWN IN 70% D_2O NUTRIENT AND IN AQUEOUS NUTRIENT[a]

Feature	Young Leaf		Mature Leaf	
	H_2O	70% D_2O	H_2O	70% D_2O
Blade thickness	101.7 ± 0.1[b]	195.6 ± 0.4	161.0 ± 0.2	223.6 ± 0.6
Midrib thickness	235.7 ± 0.9	229.4 ± 0.3	367.3 ± 0.8	377.6 ± 0.1
Epidermal thickness				
Upper	12.9 ± 0.2	24.0 ± 0.3	14.4 ± 0.9	18.9 ± 0.4
Lower	10.2 ± 0.7	17.5 ± 0.6	8.8 ± 0.7	13.5 ± 0.6
Palisade layer				
Cell height	37.7 ± 0.4	69.0 ± 0.4	73.6 ± 0.4	85.8 ± 0.6
Cell width	8.9 ± 0.1	17.6 ± 0.8	15.5 ± 0.3	14.9 ± 0.4
Spongy mesophyll				
Cell diameter	44.3 ± 0.2	90.8 ± 0.2	57.0 ± 0.4	80.5 ± 0.5
Midrib vascular bundle				
Height	89.2 ± 0.9	82.1 ± 0.7	122.0 ± 0.9	127.9 ± 0.3
Width	207.4 ± 0.8	57.1 ± 0.6	218.0 ± 0.6	198.7 ± 0.4
No. of oil glands on surface[c]				
One-celled	13.0 ± 0.2	10.0 ± 0.1	3.2 ± 0.1	3.5 ± 0.1
Eight-celled	6.9 ± 0.2	5.5 ± 0.1	1.0 ± 0.1	5.0 ± 0.2

[a] All measurements are in microns, and each represents the average of at least 10 samples taken from not less than three different sections. [b] Standard deviation. [c] Based on a surface area of 6.60 × 0.02 mm. in all sections. Data are averages of at least 10 samples.

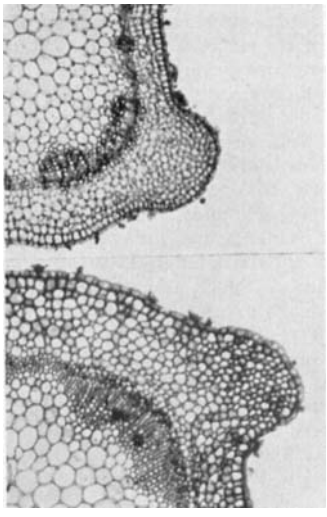

Fig. 3.—Transverse sections of immature stems taken near apex of plants growing on (top) H_2O nutrient solution, and (bottom) 70% D_2O nutrient solution. Magnification is the same in both cases (× 100).

tion of cell division. Impaired cell division and cell enlargement probably (but not necessarily) are directly related, since osmotic effects may also be important in cell enlargement.

The data in Table I indicate a marked increase in the size of palisade parenchyma and spongy mesophyll cells. This increased cellular volume has resulted in a decrease in the volume of intercellular atmosphere in the leaf. To compensate, the leaf has produced a greater number of stomata per unit area of leaf surface. Microscopic examination of the leaf surface indicated that in the deuteriated plant there were about 25% more stomata per unit area, thus permitting more rapid gas exchange at the epidermal surface.

The reduced rate of growth in the plant grown in D_2O nutrient was reflected in a greatly reduced rate of transpiration, evidenced by the smaller amounts of nutrient solution taken up by the plant as well as a lower requirement for tissue water to maintain the turgidity of the cells. A reduction in the development of vascular bundles in the deuteriated plant accompanied decreased transpiration of nutrient fluid.

It is apparent from Table I that in the young leaf there are fewer one-celled and eight-celled glandular trichomes in the deuteriated leaf. The number of trichomes is based on a count in an equal surface area in all sections. The glandular trichomes are produced at the apical meristem and develop while the leaves are still meristematic. Since it appears that D_2O interferes with cell division, a reduced number of presumptive glandular trichomes are initiated in the meristematic leaf. The effect is accentuated because the deuteriated leaves are substantially smaller than the corresponding control leaves. The effect on the glandular trichomes in the mature leaf is quite different. Since the mature leaves were meristematic prior to the exposure of the plant to D_2O, the number of presumptive glandular trichomes should have been the same in both the deuteriated and control mature leaf. Since the deuteriated leaves are smaller than the corresponding leaves of the control, the larger number of glands per unit surface area is to be expected. However, it is difficult to account for a fivefold increase in the number of eight-celled trichomes noted in the comparison of mature leaves.

Stem.—Figures 3 and 4 show typical sections of stem from the controls and plants grown in 70% D_2O nutrient. Both young and mature stems were compared. The histological measurements are shown in Table II.

The data in Table II indicate that the effect of D_2O in the young stem is essentially opposite to that found in the mature stem. In the young deuteriated stem, all of the features measured were larger than comparable measurements of the control stem. All growth occurred in the young stem after the plant was exposed to D_2O in the nutrient, whereas in the mature stem, most of the development took place prior to the exposure to D_2O.

The young stem section (cut a few millimeters below the shoot apex) from the plant grown in 70% D_2O nutrient was about twice the diameter of the control section measured in both concave and convex directions. The epidermis had more than twice the thickness, and large colenchyma bundles developed on all corners of the young deuteriated stem. A similar effect is evident with the large corner vascular bundles. Width of the cortex was doubled in the D_2O stem, and the width of the pith was much greater than in the control. Elongation of individual cells of pith and cortex occurred to a greater extent in the deuteriated stem.

The mature stem sections, obtained near the base of the erect stem, reflect growth responses to stimuli that exerted their influence mainly before the effect of D_2O could have operated to change the pattern of development. All cell divisions were complete, and the tissue was largely differentiated, except for that which occurred by way of further growth of the cambium in the vascular bundles. The cambium, a

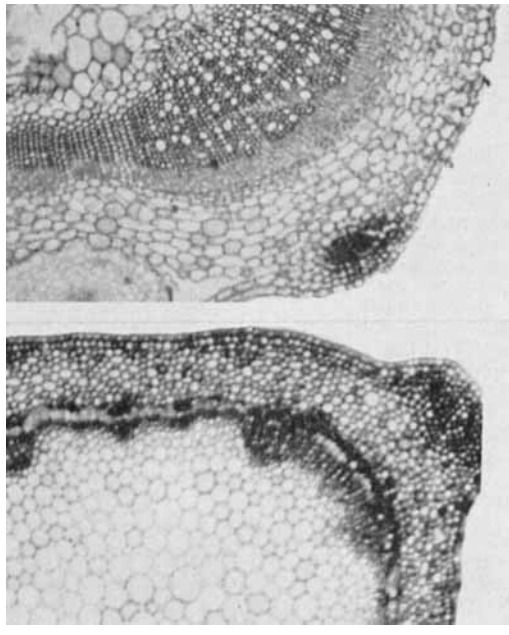

Fig. 4.—Transverse sections of mature stems taken near the base of plants growing on (top) H_2O nutrient solution and (bottom) 70% D_2O nutrient solution. Magnification is the same in both cases (× 100).

TABLE II.—COMPARISON OF HISTOLOGICAL MEASUREMENTS IN STEM FROM PEPPERMINT GROWN IN 70% D_2O NUTRIENT AND IN AQUEOUS NUTRIENT[a]

Feature	Young Stem		Mature Stem	
	H_2O	70% D_2O	H_2O	70% D_2O
Stem width				
Convex to convex	807.0 ± 5.2[b]	1437.5 ± 10.7	2728.7 ± 3.4	2050.0 ± 26.8
Concave to concave	587.0 ± 8.0	1247.3 ± 23.2	2699.0 ± 2.8	1782.0 ± 16.8
Epidermal thickness	9.3 ± 0.2	20.8 ± 0.2	20.9 ± 0.2	17.3 ± 0.3
Collenchyma ridge				
Length	242.0 ± 3.0	272.0 ± 3.5	313.0 ± 2.2	354.0 ± 4.0
Width	119.0 ± 2.7	207.6 ± 3.1	159.0 ± 1.6	177.4 ± 1.8
Corner vascular bundle thickness	83.1 ± 0.9	115.0 ± 0.9	496.2 ± 1.4	176.2 ± 1.7
Xylem thickness	53.7 ± 0.8	68.4 ± 0.6	367.5 ± 3.0	134.6 ± 1.5
Phloem thickness	29.4 ± 0.4	46.6 ± 1.2	128.7 ± 2.2	41.6 ± 0.4
Cortex width, convex side	112.6 ± 2.0	223.8 ± 2.0	261.8 ± 2.4	223.0 ± 2.4
Pith width	682.3 ± 2.5	890.0 ± 1.0	1758.0 ± 0.3	1397.2 ± 0.6
Single cortical cell				
Length	33.6 ± 0.4	35.7 ± 0.3	43.5 ± 0.7	42.0 ± 0.4
Width	27.7 ± 0.3	29.9 ± 0.4	66.0 ± 1.2	47.9 ± 1.1
Single pith cell				
Length	72.8 ± 1.0	86.4 ± 0.6	153.4 ± 4.7	124.0 ± 1.7
Width	62.8 ± 1.2	72.7 ± 0.8	122.4 ± 2.9	93.2 ± 1.6
No. oil glands on surface[c]				
One-celled	27.3 ± 0.6	21.6 ± 0.4	2.3 ± 0.1	6.0 ± 0.3
Eight-celled	16.0 ± 0.6	11.9 ± 0.3	1.5 ± 0.1	3.6 ± 0.2

[a] All measurements are in microns, and each represents the average of at least 10 samples taken from not less than three different sections. [b] Standard deviation. [c] Based on a surface area of 3.76 × 0.02 mm. in all sections. Data are averages of at least 10 samples.

layer of meristematic cells, continued to give rise to new vascular tissue in the control, while D_2O inhibited divisions in this lateral meristem as it did with primary meristematic tissue at the shoot apex. The corner vascular bundles in the mature D_2O stem were about one-third the size of that in the control. In fact, the control stem enlarged to greater dimensions than the D_2O stem in all features except the collenchyma ridge. The pith and cortical parenchyma of the D_2O mature stem were over 80% the dimensions of the control. Individual cells of the pith and cortex of the mature stem were also larger in the control than in deuteriated stems—more significantly in the pith.

Tissue sections selected for study from the control and deuteriated plants were taken at the same levels in relation to the apex and base of the stem. However, it should be noted that while on the average the control plants elongated about sixfold over a 50-day period, the D_2O stems at most doubled in size. Thus, the cells near the apex of the D_2O stem were further along in their pattern of differentiation than the comparable cells near the apex of the control stem. The lack of elongation of the stem in the deuteriated plant gives the impression that the control stem has been compressed to the length observed in the D_2O stem. This would, in effect, account for the larger dimensions reported in Table II for the young deuteriated stem and would also explain the greater amount of growth noted in the mature control stem compared with the mature D_2O stem. The young deuteriated stem section appears biologically older than the control section under study. The mature stem sections, however, are biologically similar in age, and this study demonstrates clearly the inhibitory effects of D_2O in the environment.

The collenchyma ridge in the D_2O mature stem was 10% greater in both dimensions than the corresponding control mature stem. Collenchyma serves a supporting function in the plant and develops by elongation from primary cells of the shoot apex along the corners of the square stem. Elongation of these cells is apparently not inhibited so much as other tissues by the presence of D_2O, and since no new cell divisions occur in this tissue once it is formed, the elongation rates mentioned above explain the increased size of the collenchyma ridge. Enlargement in the collenchyma ridge is due largely to cellulose thickenings laid down along adjacent cell walls. It appears that D_2O does not greatly inhibit the formation of cellulose; in fact, cell walls appear more rigid and distinct than the corresponding cell walls of control plant parts. However, it was noted that the cement-like intercellular carbohydrate material (calcium pectate) which imparts toughness and resiliency to the stem is produced in greatly reduced amounts. The deuteriated stem was very brittle and easily broken, whereas control plants could be handled without damage.

The number of volatile oil-containing glandular trichomes of the stem followed the pattern observed in the leaf. Since glandular trichomes are formed at the apical meristem and mature during the early differentiation of both leaf and stem, any factor such as D_2O which inhibits cell divisions at the meristem limits the formation of these epidermal cellular outgrowths. In the young D_2O stem there is a reduction in number per unit area of both one-celled and eight-celled glands when compared to the control stem. The effect is approximately the same in both types of glands. Once the stem has formed and developed, the spatial arrangement of the glands is a function of the degree of elongation of the stem. Since the D_2O stem did not elongate to the degree that its comparable control did, the glands were not as far apart, and the number of glands present in a comparable area was slightly greater, as would be expected. Again, the relative increase in both types of glands was about the same; the eight-celled gland count was not unusually high as observed in the leaf section.

Root.—Photomicrographs of root sections of control and plant grown in 70% D_2O nutrient are shown

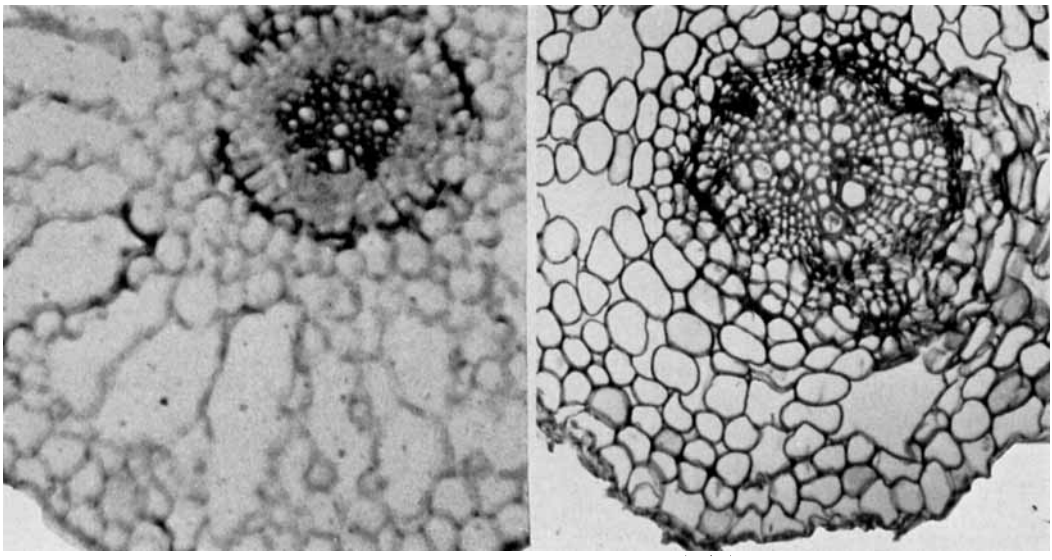

Fig. 5.—Transverse sections of mature roots taken 2 cm. below the junction of adventitious root and stem base of plants growing on (left) H_2O nutrient solution, (right) 70% D_2O nutrient solution. Magnification is approximately the same in both cases (× 100).

in Fig. 5. The histological measurements of the significant features are listed in Table III.

The root sections were cut from mature roots at a level 2 cm. below the point of origin from the stem base. Young root (from apex) was not studied because of problems in infiltrating and sectioning.

The diameter of the control root was significantly larger than that of the 70% D_2O root. However, the diameter of the stele in the two was almost identical. The cortex tissue in the control was over twice as thick as the deuteriated root. Cortical cells of the D_2O root had relatively thick cellulose walls that remained well organized during sectioning. The corresponding cells of the control had much thinner walls which ruptured easily during handling. The large intercellular air chambers between cortical cells appear to be a normal feature of peppermint roots, particularly when grown in liquid culture.

The number of primary xylem units in the stele was essentially the same; the wall thicknesses were not significantly different in the two cases. It is expected, therefore, that the endodermis, the single cellular layer marking the boundary of the stele, should also be the same in both cases.

There were a few cell layers of secondary xylem evident between primary xylem and phloem of the control root which had not developed in the D_2O grown root. This characteristic and the similar observation with the leaf and stem of the deuteriated plant indicate impairment in cell division, resulting in fewer cells in the cortex and in all the tissues arising from the cambium.

Table III.—Comparison of Histological Measurements in Root from Peppermint Grown in 70% D_2O Nutrient and in Aqueous Nutrient[a]

Feature	Mature Root	
	H_2O	70% D_2O
Root diameter	1087.5 ± 5.6[b]	716.5 ± 0.7
Stele diameter	336.2 ± 2.2	332.4 ± 2.6
Epidermal thickness	44.4 ± 4.1	38.8 ± 0.6
Cortex thickness	381.9 ± 3.7	187.8 ± 2.8
Endodermal thickness	24.5 ± 0.4	22.8 ± 0.5
No. of primary xylem units across stele	11.9 ± 0.1	13.1 ± 0.1
Phloem area		
Length	121.4 ± 2.8	156.4 ± 2.3
Width	48.2 ± 0.9	54.6 ± 1.4
No. of cells across cortex	9.6 ± 0.1	7.0 ± 0.0

[a] All measurements are in microns, and each represents the average of at least 10 samples taken from not less than three different sections. [b] Standard deviation.

SUMMARY

Deuterium oxide appears to exert its effect on the growth of peppermint, observed by histological examination, by inhibiting cell division presumably as a result of the disturbance of the rates of enzymatically controlled biosynthetic reactions. Parenchyma cells generally are enlarged with consequent changes in leaf and stem dimensions and other structural effects. Vascular tissue appears to be reduced in the deuteriated plants. The deuterium effects are more pronounced in growing tissues than in tissue exposed to deuterium after differentiation occurs.

Thus, deuterium is observed to exert a profound effect on both the macroscopic and microscopic morphology of the plant. The effects are complex and before generalizations can be profitably attempted, examination of other plant species is necessary. Such studies are now being conducted.

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