ascorbic acid oxidizing activity is contrary to the low capacity in vigorously growing tissue and thus must be due to an absence or inhibition of growth-promoting substances in cold treated tissue.

It has been reported by several investigators[8,9] that the biosynthesis of growth promoters in woody plants levels off during the growing season, and that the concentration of growth inhibitors increases toward autumn. It has also been reported that there was a significant increase of tryptophan in dormant tissue after low-temperature treatment[10]. As tryptophan is considered to be the precursor of indolyl-3-acetic acid[11] its accumulation would suggest an arrest of auxin synthesis. An actual decrease of auxin has been reported to occur in cold-acclimated plants[12].

Changes in growth regulators may influence many processes. It has been reported that they are able to affect the bioelectric potential of the plant[13] and thereby may change the cell membrane permeability[14] which is a prerequisite for the cold acclimation process[15]. They may also affect the properties of proteins[16] as well as the enzyme action already mentioned.

Growth inhibitors may affect the hardiness process as the synthetic growth inhibitor maleic hydrazide does[17], via the sulphydryl groups of the mitotic cell[18]. Several natural growth inhibitors have been reported to occur[19]; many are flavonoid-like structures[20,21]; others, such as β-inhibitor[22], have only been tentatively identified[23]. Flavonoids have been known to influence plant metabolism in several ways[24].

The phenomena in red-osier dogwood during autumn occur in the following order[25]: irreversible rest inducement, cold acclimation, and loss of rest. The sequence of this process could be in these steps. An inducement of rest is brought about by some kind of phytochrome system[26]. At this time, a hormone is produced which causes an increase in RNA content[27] and a subsequent increase of water-soluble proteins. Although the nature of these water-soluble proteins is obscure, some of them may represent an increase of enzymes which are responsible for the increase of carbohydrates and other organic products in cold acclimating plants.

R. B. Van Huystee*
C. J. Weiser
P. H. Li

Department of Horticultural Science,
University of Minnesota.

* Present address: Department of Horticulture, Purdue University, Lafayette, Indiana.

[1] Pilet, P.-E., in *Les Phytohormones de Croissance*, 485–514 (Masson et Cie, Paris, 1961).
[2] Levitt, J., *J. Theoret. Biol.*, **3**, 335 (1962).
[3] Boyer, P. D., *Brookhaven Symp. Biol.*, **13**, 1 (1960).
[4] Stern, H., *Science*, **124**, 1292 (1956).
[5] Marré, E., and Arrigoni, O., *Physiol. Plant.*, **10**, 289 (1957).
[6] Gunther, G., *Deutsche. Bot. Ges.*, **72**, 25 (1959).
[7] Waisel, Y., Kohn, H., and Levitt, J., *Plant Physiol.*, **37**, 272 (1962).
[8] Kawase, M., *Proc. Amer. Soc. Hort. Sci.*, **78**, 532 (1961).
[9] Doerffling, K., *Planta*, **60**, 390 (1963).
[10] Asen, S., and Stuart, N. W., *Proc. Amer. Soc. Hort. Sci.*, **71**, 563 (1958).
[11] Wightman, F., *Canad. J. Botany*, **40**, 689 (1962).
[12] Sulakadze, T. S., *Izvest. Akad. Nauk, S.S.S.R., Ser. Biol.*, **4**, 551 (1961).
[13] Key, J. L., and Wold, W., *J. Biol. Chem.*, **236**, 549 (1961).
[14] Fensom, D. S., *Canad. J. Botany*, **37**, 1003 (1959).
[15] Levitt, J., and Scarth, G. W., *Canad. J. Res.*, C, **14**, 285 (1933).
[16] Galston, A. W., Kaur, R., Maheshwari, N., and Maheshwari, S. C., *Amer. J. Bot.*, **50**, 487 (1963).
[17] Stewart, I., and Leonhardt, C. D., *Proc. Amer. Soc. Hort. Sci.*, **75**, 253 (1960).
[18] Hughes, C., and Spragg, S. P., *Biochem. J.*, **70**, 205 (1959).
[19] Giertych, M. M., *Bot. Rev.*, **30**, 292 (1964).
[20] Hendershott, C. H., and Walker, D. R., *Science*, **130**, 798 (1959).
[21] Furuya, M. A., Galston, W., and Stowe, B. B., *Nature*, **193**, 456 (1962).
[22] Doerffling, K., *Planta*, **60**, 412 (1964).
[23] Lane, F. E., and Bailey, L. F., *L. Physiol. Plant.*, **17**, 91 (1964).
[24] Stenlid, G., *Physiol. Plant*, **60**, 110 (1963).
[25] Van Huystee, R., Ph.D. thesis, University of Minnesota, St. Paul, Minnesota (1964).
[26] Wilson, J. R., and Schwabe, W. W., *J. Exp. Bot.*, **15**, 368 (1964).
[27] Siminovitch, D., *Canad. J. Bot.*, **41**, 1301 (1963).

Freeze-fixation, a New Method for Electron Microscopy

Freeze-fixation implies rapid freezing of small specimens followed by vapour fixation at a low temperature. This method differs from freeze-drying by not removing the water of the specimen before fixation, and from the freeze-substitution by not replacing the water during fixation. All these three methods work with frozen tissue, but since the mode of fixation varies the ultrastructure of the cellular constituents also varies. Therefore, further information of the cellular constituents might be obtained.

For freezing, small specimens are rapidly frozen in liquid nitrogen according to the usual technique for freeze-drying. The frozen specimens are collected in a small weighing bottle with grounded stopper. The bottle contains a substance suitable for vapour fixation and serves as a fixation chamber after freezing.

For fixation, the bottle containing specimens and fixation substance, and filled with liquid nitrogen, is placed in dry ice. At this temperature, the liquid nitrogen evaporates. The bottle is then firmly closed with its stopper and left in a freezer for 2–3 weeks for vapour fixation. The only fixation substance as yet tried in the experiment is crystalline osmium tetroxide. After the period in dry ice, the specimens are either left in the bottle for further fixation at room temperature or brought to the appropriate solution of the embedding chemicals used. In the present experiments, plastic embedding has been started in 70 per cent ethanol for the 'Epon' procedure or 25 per cent component '*A*' of the 'Durcopan' procedure.

The main drawbacks of freeze-fixation are the low vapour pressure of the fixation substance at −30° C and the slow penetration of the fixative into the frozen tissue. The specimens therefore must be small, preferably single cells. The morphological quality of the tissue is inferior to that obtained after freeze-drying. Thus, the several artefacts impede investigations of the whole cell, but they do permit analysis of, for example, the cell membrane.

This work was supported by "Riksföreningen mot Cancer", Sweden.

O. Nilsson

Department of Human Anatomy,
University of Uppsala,
Sweden.

MICROBIOLOGY

Endogenous Bud Formation in the Hypotrichida

As a general rule, ciliated protozoans reproduce asexually by transverse binary fission. Suctoria, however, characteristically produce unequal endogenous buds which develop into migratory ciliated larval forms. Endogenous budding has not been reported in the remaining subclasses of the Ciliatea. Observations made by Kent suggest that one member of the Hypotrichida has a larval stage in its life-cycle[1]. He observed the development of an embryonic hypotrich, which closely resembled *Glaucoma margaritaceum* Ehr., into *Aspidisca costata* Ehr. In the same treatise Kent considers the holotrich genera *Glaucoma* and *Microthorax* to be embryonic or transitional phases in the life-cycles of higher hypotrichous forms. No other reference to such life-cycles in the Hypotrichida have been found.

This communication presents preliminary observations made on monoxenic clonal cultures of the hypotrichous ciliate *Histriculus vorax* Corliss. *Bacillus cereus* was used as the sole food organism, and the ciliate culture was originally separated from activated sludge.

©1965 Nature Publishing Group