30 sec, had a pronounced effect on the crystallization temperature of the water. This water retained this property even after standing two months in a bottle.

Five measurements of crystallization temperature were made on this water, each measurement being made on a new surface using a new sample of water: (1) −38.5° C, (2) −28.7° C, (3) −28.5° C, (4) −30.0° C, (5) −29.0° C.

All these measurements were made with the water sample in contact with air, and in each test the temperature was checked by noting the melting point with rising temperature.

It was found that freezing took place in two different ways at low temperature. Often the water seemed to freeze with great rapidity from many locations to form an opaque, milky ice easily recognized by the naked eye. The freezing sometimes took place in an altogether different manner, however, and could be observed only through polaroids. In these cases, single, beautifully colored crystals could be seen to grow slowly out from widely separated nuclei. Sometimes only one crystal developed in a whole water sample.

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Internal Suberization of Plant Tissues

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Suberization of the internal surface, *i.e.* of the intercellular spaces, has been reported in the leaf of the Valencia orange, *Citrus sinensis* (*4*). Intercellular spaces in the mesophyll of the lamina and in the tissues of the petiole and of the basal and laminar abscission regions are lined with a thin film, ± 1 μ in thickness, which resembles the cuticle in its refringence. A similar but more tenuous pellicle lines the inner surface of the cell wall and resembles a tertiary lamella. The films were first identified during the investigation of plasmodesms and cell walls while using a standard cellulose test, 1KI followed by irrigation with H_2SO_4 (± 80%). Both films, extracellular and intracellular, stain yellow with 1KI and darken on irrigation with H_2SO_4. During and after the blue coloration, the swelling, and ultimate solution of the cellulose of the cell wall, the films remain more or less intact. The degree of persistence depends on film thickness and therefore on the age and, to some extent, on the region of the leaf, since the swelling of the cellulose naturally disrupts all except the heavier coatings and impregnations.

The results obtained in citrus and other plants with 1KI-H_2SO_4 may be confirmed by the use of other reagents. When sections of fresh material are mounted directly in glycerin, lactophenol, or similar media, suberin pellicles are clearly visible. Staining with Sudan III or Sudan IV proves effective in some cases, particularly if preceded by treatment either with phloroglucin and HCl or with chromic acid (± 50%). Irrigation of sections with strong chromic acid dissolves wall materials such as pectic substances, cellulose, and lignin, but leaves suberized membranes alone intact. In the examination of lignified tissues this method is therefore indispensable, since lignin is not broken down by H_2SO_4.

The leaves of sycamore, avocado, castor bean, and squash were examined at the same time and with the same techniques as used in citrus, and the results obtained were similar. It therefore appeared probable that suberization might occur generally in all tissues of vascular plants throughout the plant kingdom. The present survey of more than 50 species[1] confirms this surmise. The plants selected differ in habit and habitat, and incidentally in phylum and family, and include annuals and perennials, xerophytes, mesophytes and hydrophytes, pteridophytes and spermatophytes.

In this and in the previous paper the term suberin is used in preference to cutin, since by current microchemical methods these substances cannot be distinguished satisfactorily. Suberin is more widely distributed than cutin, and it is identifiable macrochemically in deep-seated tissues such as endodermis. It also occurs in the wound cork which may differentiate in parenchymatous tissues after deep or surface wounds, and it is in the principal component in the walls of normal cork cells (*1*, *2*).

Suberin appears first in the intercellular spaces of differentiating tissues such as leaf mesophyll, and the parenchyma, cortex, and pith of stem and root. When first observed the films are at the limit of visibility and thereafter, during tissue growth, increase in thickness. Suberization may extend sooner or later along the middle lamellae but does not interrupt the plasmodesms between adjacent cells.

Suberin films are not confined to intercellular spaces but occur also within the cell. A tenuous film which resembles a tertiary layer of the cell wall may be identified soon after the first appearance of the intercellular suberin. It is similar in translucence and in chemical

[1] Some of the species examined were: *Pteridium aquilinum, Selaginella Bigelovii, Araucaria imbricata, Pinus Coulteri, Potamogeton* sp., *Clivia miniata, Iris Pseudacorus, Musa nana, Eichhornia crassipes, Nymphaea alba, Macadamia ternifolia, Annona cherimola, Persea americana, Crassula arborescens, Bergenia cordifolia, Platanus racemosa, Vicia Faba, Cercidium floridum, Dalea spinosa, Citrus sinensis, Ricinus communis, Mangifera indica, Acer macrophyllum, Aesculus californica, Jussiaea repens, Primula polyantha, Asclepias subulata, Acanthus mollis, Cucurbita Pepo, Echinocystis macrocarpa, Venegazia carpesiodes, Cynara scolymus.*

reaction to the extracellular membrane and, like the latter, becomes more conspicuous in aging cells.

Suberin is observed in tissues of all types, parenchyma, and sclerenchyma, and also in the phloem and xylem of vascular strands. The differentiation of a secondary, border-pitted vessel segment in *Ricinus communis* may be taken as typical. The differentiating element begins to expand as soon as it is cut off from the parent cambial cell. At this stage the cell wall appears in surface view as a cellulose net, the roughly hexagonal meshes of which are perforated by closely set plasmodesms less than 1 μ in diameter. In the profile of the cell wall, the plasmodesms are observed connecting the protoplasts of the differentiating adjacent cells, vessel segments, fibers, xylem parenchyma, or rays. Deposition of the secondary wall becomes marked when the vessel segment reaches its maximum diameter. About this time extracellular and intracellular suberin layers are now visible. The former is evident in the middle lamella between the differentiating xylem element and the adjacent cells, whereas the latter forms a tertiary coating on the interpit reticulum of the secondary thickening of the cell wall. Although intercellular spaces are not obvious microscopically in differentiating vascular tissues, extracellular suberization appears to begin, as in the leaf mesophyll, at cell corners, presumably regions of growth strain, and extend therefrom along the middle lamella.

As wall growth continues, the mouths of the pits are narrowed by the overhang of the cell wall, and sooner or later lignification of the wall occurs. Meantime the intracellular suberin pellicle has increased considerably in thickness. As the end walls of the vessel segment disappear and the protoplast eventually dies, the plasmodesms withdraw from the pit areas, but leave behind a membrane which seals the base of the pit. Incidentally, this withdrawal presumably explains the absence of plasmodesms in the walls of lignified elements. The mature xylem vessel is thus completely lined throughout its length with a suberized membrane.

The differentiation and ultimate suberization of spiral vessels is essentially similar in pattern. Plasmodesms may be observed during early stages of growth (*3*), while the final suberization, extra- and intracellular, may be demonstrated by careful treatment with 1KI-H_2SO_4 (weak) or occasionally merely by staining fresh sections in Sudan III or Sudan IV.

The fact that suberin occurs apparently around and within all living cells in the numerous tissues so far examined appears to call for consideration in future discussions of such theories as transpiration, conduction, abscission, cellulose deposition, and permeability. A more detailed account of the work is in course of preparation.

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Effect of Coconut Milk on the Growth of Explants From Carrot Root

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Explants derived aseptically from the cambium and near cambial region of dicotyledonous plants have furnished a major source of material for growth experiments by the accepted techniques of tissue culture. In the hands of Gautheret and Nobécourt particularly (*7*, *11*), and with the use of indole-3-acetic acid, the indefinite culture of sterile explants from carrot roots became a demonstrable fact. These investigations following long-standing observations upon regeneration at cut surfaces exposed to air of many storage organs (*13*). This regeneration may take the form of periderm formation, as in the potato tuber, or a more diffuse proliferation stimulated in the region of the cambium (carrot, beet, etc.).

Our investigations stemmed from the desire to use sterile plant tissue cultures as experimental material with which to study the metabolism and behavior of growing cells. Several limitations of accepted plant tissue culture technique had to be overcome. As commonly grown, plant tissue cultures—even clones subcultured from the same source (*4*)—are very variable in growth rate. Secondly, though the growth is impressive, it is slow even when stimulated by certain growth substances like indole acetic acid, and therefore growth experiments have been continued for many weeks. These limitations can be overcome by the use of explants from carrot root, which can be made to grow at a rapid rate under controlled conditions; carrot tissue is also suitable for our special purposes. Another note (*5*) describes a new technique for the controlled growth of explants of carrot root in liquid media. The present note concentrates upon a striking nutritional effect of coconut milk on the growth of these cultures—an effect which may be demonstrated both in liquid media and on nutrient agar.

The stimulus which emanates from the buds to activate the vascular cambium in the stems of woody trees is commonly believed to be indole acetic acid (heteroauxin) (*3*), and the slow growth of the carrot cultures of Gautheret and Nobécourt required this substance. In our experiments the effect of a number of substances, added to White's nutrient medium (*16*) for plant tissue cultures, was investigated. These substances were additional to the usual nutrients (organic and inorganic, trace elements, and vitamin-like growth factors) and included indole acetic acid, growth substances like 2,4-D, and coconut milk. Attention here will be directed only to the relative effects of indole acetic acid and of coconut milk.

Coconut milk, the nutritive, fluid endosperm by which the developing *Cocos* embryo is nourished, has already found some application in the culture of bacteria (*2*), fungi and orchid embryos (*12*), and immature plant embryos (*15*). It has been claimed (*14*) that it contains

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