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.

References

1. PRIESTLEY, J. H., and NORTH, E. *New Phyt.*, 1922, **21**, 113–139.
2. PRIESTLEY, J. H., and WOFFENDEN, L. M. *New Phyt.*, 1922, **22**, 252–268.
3. SCOTT, F. M. *Bot. Gaz.*, in press.
4. SCOTT, F. M., SCHROEDER, M. R., and TURRELL, F. M. *Bot. Gaz.*, 1948, **109**, 381–411.

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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both a specific factor for plant embryo development and a thermostable general growth factor, probably indole-3-acetic acid. An isolated reference (*1*), unsupported by quantitative data, claims that coconut milk stimulated growth of pieces of tissue from below the shoot apex of *Lupinus* and *Tropaeolum*. However, the evidence for an unidentified active principle in coconut milk which can foster the rapid growth of carrot cultures seems to be more clear cut than anything hitherto reported.

Qualities which may promote the growth of carrot cultures can be demonstrated in heat-sterilized, filtered, water-clear preparations of coconut milk (pH 5.6–5.9) obtained from mature nuts. Experiments not here reported show that 1% by volume of coconut milk added to an otherwise complete organic and inorganic nutrient medium causes a marked stimulus to growth; at about 15–20% there is an optimum, with marked decrease in growth at still higher concentrations. No growth of carrot cultures occurs in coconut milk alone.

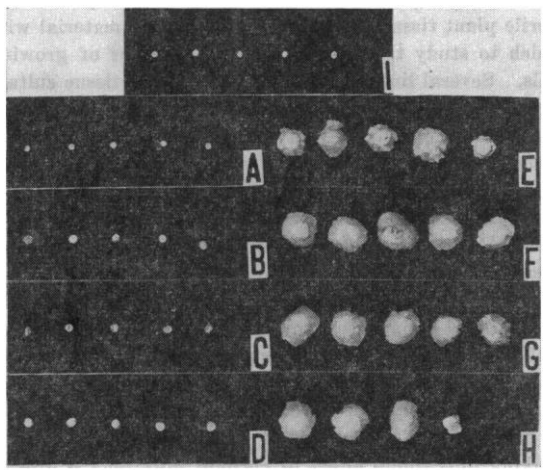

FIG. 1

The qualitative effect of coconut milk on the growth of carrot cultures is well shown in Fig. 1 (half reduction). This experiment was performed in test tubes on nutrient agar (0.8%) at a pH of 5.6. The basal medium contained the inorganic salts, trace elements, sucrose (2%), and the usual organic co-factors, as in White's nutrient solution. The 5 cultures at I show the size of pieces of phloem tissue removed from the carrot at a distance of 1 mm from the cambium and allowed to remain for 21 days on agar medium containing the basal nutrients, but without indole-3-acetic acid or coconut milk. No significant growth occurred. Series A, B, C, and D show the size of similar cultures grown for 21 days on the basal medium to which indole-3-acetic acid was added in the concentrations 10.0, 1.0, 0.1, and 0.01 mg/liter, respectively. Clearly, the cultures stimulated by small concentrations of indole acetic acid grow, but the comparison of E, F, G, and H with A, B, C, and D, respectively, shows the outstanding effect of 15% coconut milk in addition to all other nutrients and indole acetic acid.

In the presence of indole acetic acid the greatest fresh weight obtained in 21 days was 11.0 mg from 4 mg (mean, 8.2). With added coconut milk the best growth from 4 mg was 327 mg (mean, 184.0). This variability may be much reduced under controlled conditions to be described (*5*).

The growth rates of carrot cultures in the presence of indole acetic acid, but in the absence of coconut milk, which have been obtained in this laboratory are of the same order as those reported by Gautheret and others (*4*), but those now obtained with added coconut milk are of an entirely different order of magnitude.

The active principle of coconut milk[2] is not a constituent of the ash, nor is it any of the following vitamin-like growth factors: thiamin, niacin, pyridoxine, glycine. It is equally improbable that it is any of the normal constituents of yeast, malt, tomato, carrot, or liver extract when used at pH 5.6. (Carrot tissue responds slightly to a relatively high concentration of yeast extract, but this response is no greater than that due to indole acetic acid. Though coconut milk may contain indole acetic acid (*14*), this is clearly not the active substance in question.) The substance is stable to prolonged autoclaving and, since its activity decreases on dialysis, it must surely have relatively small molecules.

These experiments, however, point to much wider problems than those which merely concern carrots and coconuts. Pending the more detailed biochemical investigation which is to be made, the following points suggest themselves:

(1) An outstanding difference between the carrot cultures which do not grow in the absence of coconut milk (Fig. 1, I), or which sluggishly expand in the presence of indole acetic acid (Fig. 1, A, B, C, D), and those which burst into very rapid growth must surely be in their ability to harness their respiratory energy in protein synthesis, salt and water uptake, *i.e.* to maintain the processes in the cells which require the use of energy. It would be reasonable, therefore, to seek this coupling mechanism in some effect of the coconut milk which may promote not only carbohydrate breakdown but also the linkage of respiratory energy to useful work.

(2) The possibility exists, however, that the active principle in coconut milk is not peculiar to carrot tissue and is at least specific for cells which contain carotenoids. Preliminary trials with other plant tissues have been made. Though some have responded to coconut milk, potato cultures under similar conditions do not respond. It is of interest, therefore, that Lazar (*9*) claims that carotene supplied in the external medium can induce the formation of new roots as well as accelerate growth in both root and shoot of *Impatiens Balsamina* L.

The literature on vitamin A also suggests a possible connection between a constituent of the coconut and vitamin A or carotene (*8*, *10*). Apparently coconut

[2] This active principle has now been found in lyophilized preparations of corn in the milk stage. For access to these preparations we are indebted to Dr. S. A. Watson, of the Department of Agronomy, University of Illinois.

meal, as a partial source of protein in the basal vitamin A-deficient diet, causes the vitamin A to be more effectively used when it is supplied. It has been suggested that there is a factor in coconut meal which, combined with vitamin A, makes the vitamin more effective or which facilitates the transformation of carotene to vitamin A. So far as is known, vitamin A does not affect the growth of plants, but any connection between the physiology of carotene and of extracts of coconut is suggestive in the light of the results here reported. It is also interesting that coconut milk has been known to be used to supplement the nutrition of human infants in the tropics.

(3) The best growth of animal tissue cultures is well known to be fostered by a preparation of 9-day-old chick embryos (*6*). The effects due to the "embryo juice" in the culture of animal tissues is suggestive in view of the marked effect here reported by the use for plant tissue cultures of a nutritive fluid for a plant embryo.

References

1. Ball, E. *Amer. J. Bot.*, 1946, **33**, 301.
2. Blauvelt, L. *J. lab. clin. Med.*, 1939, **24**, 420.
3. Boysen-Jensen, P. *Growth hormones in plants.* New York: McGraw-Hill, 1936.
4. Caplin, Samuel M. *Bot. Gaz.*, 1947, **108**, 379.
5. Caplin, S. M., and Steward, F. C. *Nature, Lond.*, in press.
6. Carrel, A. *J. exp. Med.*, 1913, **17**, 14.
7. Gautheret, R. *C. R. Acad. Sci., Paris,* 1939, **208**, 118.
8. Gridgeman, N. T., Lees, H., and Wilkinson, H. *Soc. Chem. Ind. Lond.*, 1940, **59**, 120.
9. Lazar, O. *C. R. Soc. Biol., Paris,* 1936, **121**, 886.
10. Morgan, R. S. *Biochem. J.*, 1938, **28**, 1178.
11. Nobécourt, P. *C. R. Soc. Biol., Paris,* 1939, **130**, 1270.
12. Picado, T. C. *Arch. Hosp. Rosales,* 1943, **35**, 33.
13. Priestley, J. H., and Swingle, C. F. *USDA Tech. Bull. 151,* 1929.
14. Van Overbeeck, J. *Cold Spr. Harb. Symp. quant. Biol.*, 1942, **10**, 126.
15. Van Overbeeck, J., Conklin, M. E., and Blakeslee, A. F. *Science,* 1941, **94**, 350.
16. White, P. R. *A handbook of plant tissue culture.* Lancaster, Pa.: Jaques Cattell, 1943.

An Effective Depilatory Formula for Use on Laboratory Animals

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For a series of skin tests in the rabbit it was necessary to remove the hair from an extensive area of the abdomen quickly, completely, and with minimum trauma to the skin. The characteristically soft and luxuriant growth of hair in the rabbit quickly clogs mechanical clippers, whereas shaving is slow and traumatic. Chemical depilatories are frequently used, but the existing formulations either are intended for human use where only slight amounts of hair are to be removed or, when intended for laboratory use, are so caustic and vigorous in action that some burning of the skin ensues even if used cautiously. In an attempt to circumvent these disadvantages we have prepared a depilatory that has been successfully used on a large number of laboratory animals.

The most common depilatory agents are the inorganic sulfides. Of these, only barium and strontium sulfide are stable and active enough to be practical (*1, 3*). The barium salt is preferred because it is cheaper and more readily obtainable. The depilatory is prepared by triturating two-thirds by weight of purified yellow barium sulfide powder with one-third by weight of a commercial detergent. We have used "Tide"[1] exclusively, but other detergents such as "Dreft,"[1] "Orvus extra granules,"[1] and "Swerl"[2] appear to be equally effective. Three full teaspoons (25–35 gm) of the depilatory are mixed with 50 ml of a 10% glycerine-in-water solution until a smooth, creamy suspension is obtained. The area to be depilated is thoroughly wetted down with water. The depilatory is then applied with a wooden tongue-depressor blade and gently worked into the hair. The hair will be seen to gelatinize quickly and practically dissolve. The dissolved hair-depilatory mixture is carefully moved back and forth over the skin surface, particularly where the depilating action seems to be slower. Additional water may be sprinkled on to prevent drying. When the hair is completely removed, the area is rinsed off with a copious amount of water to insure complete removal of any sulfide residue. In this manner the abdomen and chest of a large rabbit can be depilated in approximately 5 min.

The action of the detergent in the mixture is fourfold. It enables the depilatory to come into intimate contact with the hair shaft; it produces a smooth, creamy suspension that is conveniently applied; it acts as a diluent; and it suppresses the odor of hydrogen sulfide that is associated with sulfide depilatories. Ordinary soap powder may be used, but the depilatory action is slower than when a detergent is employed. Water may be substituted for the 10% glycerine, but the latter is advantageous in that it produces a softer and smoother skin through its emollient action and it also retards the rate of drying. Bulfer, *et al.* (*2*) found that the monoethylether of diethylene glycol (Carbitol[3]) acts as a better stabilizer than glycerine, but this compound is not commonly found in the laboratory—a consideration that was used as a guide in formulating the depilatory.

The sulfide-detergent mixture has been used on various species of laboratory animals. In addition to its efficacy in rabbits, equally good results have been obtained with mice and in the preoperative preparation of dogs, cats, and monkeys, with no indication of secondary infections or delayed wound healing.

References

1. Atkins, F. *Perf. essent. Oil Rec.*, 1947, **38**, 231.
2. Bulfer, G., Boyle, A. J., and Baldinger, L. H. *J. Amer. pharm. Ass.*, 1936, **25**, 1104.
3. de Navarre, M. G. *The chemistry and manufacture of cosmetics.* New York: D. Van Nostrand, 1941.

[1] Procter and Gamble, Cincinnati, Ohio.
[2] Allied Chemical and Dye Corporation, New York City.
[3] Carbon and Carbide Chemicals Corporation, New York City.