PLANT TISSUE CULTURES

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The term "tissue culture" is too well known to the scientific public through the work of Harrison (56–59), Burrows (10), Carrel (11–15), Fischer (31–36), Erdmann (25, 26), Lewis and Lewis (78–80), and others to need definition. In general, it has been used in such work to designate preparations in which somatic cells of a single type or a restricted number of types (fibroblasts, osteoblasts, epithelial cells, etc.), isolated from an animal body, have been kept growing for more or less extended periods of time, *in vitro.*

The concept of potentially unlimited growth as a *sine qua non* of a tissue culture is, in general, adhered to by Carrel and his school, but rejected by Lewis and others. Likewise, the concept that a tissue culture should be of a single type of cell and should undergo only limited differentiation is generally adopted by Carrel (13, 15), but must be rejected in the application of the term to the "organule" cultures of Fischer (31, 32, 34), Fischer and Parker (37), Fell (29, 30), *et al.* In view of this divergence of opinion, even in the animal field, as to just what constitutes a "tissue culture," and in view of the comparative newness of the term in the working vocabulary of most botanists, it seems desirable to begin by setting forth a definition for use in the following pages. A plant tissue culture will hereafter be considered to be *any preparation of one or more isolated, somatic plant cells which grows and functions normally, in vitro, without giving rise to an entire plant.* Under such a definition, spore cultures, cuttings, and cultures of whole embryos will be excluded, but a limited amount of differentiation will not be considered as automatically excluding a particular culture from this category. It is realized that such a definition may raise objections in some minds, and suggestions for its improvement will be welcomed. The author is inclined to concur in Carrel's concept that unlimited growth is a necessary criterion to be included in a proper definition, but since such an interpretation would restrict the literature of the field to only a very few papers, it will not be insisted upon at present. Nevertheless, it should be kept in mind that *unlimited survival* and growth has been the *aim*

of most of the work in both the animal and plant fields, and that one of Carrel's chief contributions to the field of animal tissue cultures was the development of a nutrient which would permit this aim to be attained (14).

Until recently, active interest in the subject of plant tissue cultures has been restricted almost exclusively to Germany, and publication has been mostly in non-botanical journals. For early discussions of the problem the reader is referred to the résumés of Börger (7), Küster (70, 71), Lamprecht (76), Schneider (107) and White (123), and the historical introductions to the memoirs of Gautheret (44) and Scheitterer (104).

Certain phases of botanical research not falling strictly in the category of tissue cultures have, nevertheless, been important in laying the foundations of technique and knowledge of tissue behavior necessary for success in the more specialized field, and should be briefly mentioned. Studies in tissue repair in injured plant organs (Vöchting, 119, 120; Hanstein, 55; Jaeger, 62; Küster, 68; Schilling, 105; La Rue, 72; Reiche, 98; Lopriore, 81), of callus formation and abnormal proliferation (La Rue, 73; Němec, 89; Stingl, 111; Haberlandt, 52, 53; Okado, 90; Dale, 20; Küster, 69; von Schrenk, 108; Winkler, 132), and of wound healing in general (Haberlandt, 52, 53; Brieger, 8; Lamprecht, 75; Miehe, 86; Olufsen, 91), have added considerable information to our store of knowledge of the conditions necessary for tissue proliferation. The cultivation of isolated embryos (Andronescu, 1; Arnaudov, 2; Tukey, 116, 117; Dietrich, 23; Essenbeck u. Suessenguth, 27; Buckner and Kastle, 9; Hannig, 54; Stingl, 110; White, 124) and the aseptic cultivation of whole plants (Combes, 18; McMillan, 85; Klein u. Kisser, 64; Bobko, 5; Hatch, 60; Weissflog, 122; Gerretsen, 45) have aided in determining the necessary environmental conditions and the experimental techniques adapted to such work. The extensive literature on the rooting of cuttings has likewise contributed much to our background.

While it is thus possible to mention a great deal of literature collateral to the subject, that referring strictly to plant tissue cultures, as such, as defined here, is quite meagre. Any treatment of the subject must begin with the work of Haberlandt (46–53), reported during the first third of the present century. Haberlandt clearly formulated the problem in 1902 when he said (46):* "So

* Reviewer's translation.

far as I know, there has been, up to the present, no well planned attempt to cultivate the isolated vegetative cells of higher plants in suitable nutrients. Yet the results of such cultures should throw many interesting side-lights on the peculiarities and capacities of the cell as an 'elementary organism'; they should bring into evidence the reciprocal relationships and many-sided influences to which the individual cells of a multicellular organism are subjected." This is, so far as the present writer is aware, the first formulation of the tissue culture idea, antedating by several years the first work in the field of animal tissue cultures (Harrison, 1907 (56), Burrows, 1910 (10), Carrel, 1911 (11)). Haberlandt attempted at the very outset to cultivate single cells, using palisade and medullary parenchyma, trichomes of various types (glandular hairs, stinging hairs, stamen hairs), epidermal cells, etc. (46). Although a perusal of this and subsequent papers makes it evident that he and his pupils grasped very well the nutritional problems involved, they seem not to have appreciated the important growth-restricting effects of differentiation, especially as regards the deposition of cell walls. The experimental results obtained were not encouraging. These investigations were continued in Haberlandt's laboratory over a long period of time, by Bobilioff-Preisser (4), Lamprecht (75), Thielmann (112–114), Thielmann and Bérziň (115), and others, and independently in other laboratories the same sort of approach has been used by Pfeiffer (95, 96), La Rue (72–74), Scheitterer (104), Úlehla (118), Schmucker (106), Kemmer (63), Börger (6), Czech (19), Winkler (132), and Kunkel (67). These authors never obtained definitely demonstrable, continued proliferation, and in 1927 Küster reviewed this work with the statement that (71)* "never to this day have isolated (plant) cells been brought to reproduce their tissues; all attempts have given the same negative results."

Molliard (88), Dauphiné (21), Rechinger (97), Scheitterer (104), and Smith (109) have attempted to grow small fragments of various tissues, especially pieces of embryos, with little success. The only work of this sort which appears to have been at all successful is that of Behre (3) with tissues of *Drosera* and certain unpublished work (1936) of La Rue (74), which cannot as yet be properly evaluated.

* Reviewer's translation.

From a careful study of these papers, the present reviewer feels justified in saying that up to 1936 only two groups of work in the field have given real promise of success. These two have taken their initial inspiration from the laboratories of Haberlandt (Berlin) and Molliard (Paris), and have dealt, respectively, with apical and with lateral meristems. Because it can be treated more briefly, the second mentioned work will be discussed first.

In 1935 Gautheret published an extensive memoir (44) entitled* "Researches on the cultivation of plant tissues," in which some 50 pages are devoted to a discussion of experiments on the cultivation of cambium removed from large trees of various species. The same work was summarized briefly in an earlier paper (43). Fragments of cambium a centimeter or more on a side were excised under aseptic conditions and placed on cotton or gelatin saturated with a nutrient solution. A slow proliferation was obtained giving rise to loose pseudo-parenchymatous outgrowths resembling the thalli of *Pleurococcus*. Growth continued over several months. These proliferations were similar to those described in regenerating tissues by Hanstein (55) in 1882. Histological preparations showed extensive modification of structure, not only of the surface layers but of more deep-seated tissues as well, with formation of an undifferentiated tissue resembling, at the exposed surface, that occurring in lenticels (Hanstein, 55) and, in the deeper tissues, that formed from proliferating pericycle and medullary rays or in the healing of internal wounds, as observed by Jaeger (62). Mitotic figures occurred frequently. The conditions employed did not permit indefinitely prolonged growth, so that this work does not fulfill that criterion of a tissue culture, and it is rather uncertain whether the changes occurring can be interpreted as those normal to a healthy tissue released from the influence of surrounding tissues, or merely those characteristic of injured cambium and cortex, left *in situ*. Moreover, only a small part of each explant took part in the growth, the major portion remaining dormant or becoming necrotic, so that it is uncertain if these cultures actually drew any of their material for growth from the nutrient supplied. The work will have to be greatly extended before it can be properly evaluated. Nevertheless, the result is promising and is important as representing the *only* case in the entire literature of the subject

* Reviewer's translation.

which would fulfill Carrel's second requirement, of *undifferentiated* active growth. In the strict sense, this is thus the only work reported to date that can even tentatively be called a "tissue culture" of plant material, and even this, as pointed out above, is a somewhat doubtful case. Further work in this direction will be followed with great interest.

This study of Gautheret represents the only work with the second category of tissues mentioned above, lateral meristems (cambium), which gave promise of success. The other, and in the present writer's opinion more promising and much more extensively developed approach to the tissue culture problem, has been that with isolated terminal meristems—stem-tips and root-tips.

The culturing of stem-tips is mentioned briefly by Robbins (100) and is treated in more detail by White (127). Using stem growing points of *Stellaria media,* the latter obtained considerable growth and differentiation, with apparently normal photosynthesis. The complex of conditions employed was not satisfactory for unlimited growth, and the normal mode of differentiation shown by these cultures suggested the behavior of cuttings rather than of tissue cultures, so that the work need not be treated in detail.

The cultivation of root-tips has been much more widely attempted and has given more satisfactory results. Heidt (61) and Felber-Pisk (28) studied the behavior of isolated roots in non-nutrient media or in air, and obtained only a very limited growth. Mayer (84) employed a great many different nutrient combinations, but obtained unsatisfactory results, probably due to excessive handling of the cultures with concomitant trauma. Really promising results with root-tips, however, were obtained by six authors, Kotte (65, 66), Robbins (100, 101), Robbins and Maneval (102, 103), Malyschev (82, 83), Gautheret (39–42), and White (124–126, 128–131). This work will require more extensive treatment.

In 1922 three papers on the cultivation of excised root-tips in nutrient media, representing independent work, appeared almost simultaneously: two by Kotte (65, 66) working under Haberlandt in Berlin, the other by Robbins (100) in the United States. Kotte grew apical and sub-apical fragments of *Pisum* and *Zea* roots on various media. He studied the nutrition of such root fragments, examining their requirements as regards sugars, organic nitrogen sources (amino-acids, peptones, tissue extracts), and inorganic

salts. He also studied their behavior toward light and gravity, the effect of size of initial fragment, of position of fragment in the root, of age of tissue, the phenomena of growth polarity, etc. He obtained considerable but not indefinite growth, some differentiation, but no suggestion of the formation of stem primordia. His best results were obtained with *Zea* roots in tubes of agar containing a Knop solution plus 1% dextrose with the addition of any one of four accessory materials which were, in descending order of satisfactoriness: 1) Liebig's meat extract; 2) α-alanine; 3) a mixture of peptone, asparagine, alanine, and glycine; 4) a pepsin-diastase digest of pea seeds. Such cultures grew well and branched actively for some time. Sub-cultures were not attempted and Kotte has published no further work on this subject.

In the same year Robbins, working independently, published (100) similar results. He grew roots of *Pisum, Zea* and *Gossypium.* He found a liquid medium somewhat more satisfactory than a solid one and studied the effects of several different sugars, concluding that dextrose was satisfactory for these plants. Roots were carried through three transfers (six weeks) but eventually died. In a second paper (101) the effects of various organic nitrogen sources were studied. The amino-acids were consistently found to be either detrimental or without effect. The best results were obtained with an autolyzate of yeast. This was studied at several concentrations. The same author and Maneval (102) extended the study to include tissue extracts and different concentrations of the nutrient ingredients, and in 1924 studied (103) the effect of light in the presence or absence of yeast extract. They concluded that moderate illumination was desirable. Many different sorts of plants were studied. All species, with the exception, curiously enough, of *Lupinus albus,* gave fairly satisfactory results. Some cultures were carried through ten passages (150 days), but eventually showed markedly decreased growth rates. These four papers (100–103), together with Kotte's two (65, 66), established a fairly secure basis on which to build, giving distinct promise of success.

Chambers' experiments (16, 17) in growing squash root-tips in hanging drops, in which he reported migration of cells as in animal fibroblast cultures, have not been confirmed, and were probably erroneously interpreted (see 123, 124).

In 1932 Malyschev, using a technique similar to that of Kotte, succeeded in carrying root cultures through 13 passages (83). He does not give details of growth rates, but made certain important observations on the differing carbohydrate requirements of different plants.

The major part of the history of this phase of the subject in the last ten years is, however, mostly the work of Gautheret and of White. In 1932 and 1933 Gautheret, who has already been mentioned in another connection, grew a number of sorts of roots and root fragments in various nutrients (39–42). An extensive account of this and other work was published in his memoir of 1935. All of his roots ceased to grow after a certain period varying from 10 to 100 days, and the author concluded that unlimited growth is not possible. Like many other authors, he has recourse, as an explanation for this failure of isolated tissues to grow indefinitely, to the theory of "hormones" provided by the parent plant, essential for growth and not formed in these tissues. Gautheret made a number of contributions to the technique of such cultures. In the opinion of the present reviewer, an important contribution was the demonstration that, although cystein-HCl is toxic at concentrations corresponding to those used by Robbins, Kotte, and other earlier workers in studying the effects of amino-acids, it is, nevertheless, markedly stimulating at lower concentrations, of 10^{-6} or less (44). Since neither unlimited growth nor undifferentiated growth was obtained, the results are, however, merely suggestive of certain potentially fertile methods of approach. Dauphiné (22) and Galligar (38) have made somewhat similar cultures.

In 1931 White began a series of studies in the general field of plant tissue cultures (123–130). In an introductory paper (123) the history of the subject was reviewed in detail. A brief paper (124) on the cultivation, in hanging drops, of three types of meristems: root-tips, embryos, and seed primordia, appeared in 1932. So far as the writer is aware, the only other paper in which attempts at cultivation of the last-named type of tissue are discussed is the unpublished dissertation of Moebius (Leipzig) (87). All three sorts of tissue gave distinct promise of success; only one, root-tips, has been followed up in detail, however. In 1933 appeared the paper on stem-tips (127) already mentioned. The cultivation of root-tips of *Triticum* was taken up in detail in 1932,

with a study of the effects of light, temperature, H-ion concentration, aeration, solution-volume, and presence or absence of dextrose or yeast or both (125). In 1933 the inorganic nutrient was carefully studied (126) with respect to each of its constituent ions, and a satisfactory formula developed. A very rapid and apparently normal growth was obtained for the short periods studied (two weeks only) in the best complex tested. The chief points of importance to be found in these papers are: the sharply-defined *maximum* temperature for growth (ca. 28° C.), the necessity for an *acid* solution (pH 5.0 to 5.5), in which respect these cultures differ from those of animal tissues which require a neutral or alkaline medium, the very low maximal as well as optimal concentration of phosphate (ca. 0.1 millimol as KH_2PO_4), the extreme sensitiveness of such cultures to absence or too low concentration of iron, the sharply-defined optima for calcium and potassium concentrations, and the relative indifference of the tissues studied to variation in anion concentrations. The nutrient developed in this work has been repeatedly compared with other well-known nutrients and found superior for such cultures (unpublished results). A brief note (128) on the effect of – SH compounds on root cultures, and one on the relative merits of liquid and solid media (129) likewise appeared in 1933.

Indefinitely-continued growth of such cultures was not obtained by Kotte, Robbins, Chambers, Malyschev, or Gautheret, and more or less elaborate theories, such as that of Miehe (86), have been evoked to explain this fact. The most widely accepted of these theories: that the plant must contain substances necessary for growth, which are irreplaceable and whose synthesis by isolated roots from the constituents of the culture medium is impossible, is perhaps best expressed by Gautheret (44, pp. 109–110, 1935). The arrest in development would, according to this theory, be the result of a disturbed equilibrium arising from the isolation and causing a complete change in the nutrition of the root. This has been the point of view of most of those who have worked in the field within recent years, and was justified by much of the data available until recently. In 1934, however, White (130), using tomato root-tips, a nutrient and culture method based on those developed in earlier papers (125, 126), with carbohydrate changed from dextrose to sucrose as required by tomato (unpublished re-

sults; compare Malyschev, 82), and employing certain slight modifications in method of preparing the nutrient, succeeded in carrying roots through more than 50 passages. A mean growth rate of ca. 5 mm. per culture per day (a quite normal value) was maintained, and a total *potential* tissue multiplication of the order of 10^{40} was obtained. The *actual* measured linear increment of a single isolated root-tip during one year of culture *in vitro* was 15,500 times the initial length, with 35,400 branches. This same root has now, at the present writing, been carried through about 160 passages (more than three years), has made a total measured increment exclusive of branches of nearly 2 miles, and is still growing entirely normally. The theory of irreplaceable essential substances expressed by Gautheret (44) and suggested as a possibility by Robbins (101) and others is evidently unnecessary, although "accessory substances" of an as yet unknown nature have been regularly supplied to these cultures in the form of a soluble extract of .01% of dried brewers' yeast (Harris). Since, however, Osborne and Wakeman (92) have shown that such an extract is free from demonstrable protein, these "accessory substances" cannot be very complex and should be capable of identification. In any case, they represent only a very minute fraction (less than 1/10,000) of the nutrient. An important feature of this work is that the medium used, unlike the media employed in animal tissue cultures, contains only this one unknown. All other constituents of the nutrient are known and can be varied at will.

Certain characteristics of these cultures are worthy of note. In routine work the initial explant has been regularly a piece 10–15 mm. long, including the apical meristem and some elongating tissue (130). This normally floats on the surface of the nutrient (125). Sometimes roots which at first sink to the bottom will subsequently come to the surface, but the growth rates and general appearance of the cultures are almost invariably better in those which have floated throughout the culture period (125). These must be considered to represent the normal type. This behavior of healthy tissues is probably due to occluded respiration gases. The excised root elongates as does a root in the soil, without any apparent abnormality except that, contrary to Kotte's findings (65, 66), it does not react geotropically. Five to 10 mm. of the tip of a growing culture may bend downward somewhat, but this curvature is

always removed in the region of elongation, so that a root grows horizontally throughout all but the terminal centimeter and continues straight as long as the restricting space allows, later curving around the inner surface of the flask. Whether failure to bend permanently geotropically is due to loss of reactivity, or merely to a balance between the downward geotropic tendency and the upward chemotactic reaction toward the oxygen gradient in the nutrient, or to the low specific gravity of the tissue which, in the absence of any firm point of attachment, prevents the geotropic reaction from being effective, cannot at present be said. Growth rates are somewhat variable but in general average an increase of about 5 mm. (one-half to one-third of the culture's original length) per day. A culture thus doubles in volume in the first 48 to 72 hours. This figure is comparable to that considered normal for animal tissue cultures (15). Individual growth rates as high as 40 mm. per day have occasionally been recorded, and one group of cultures under unusually satisfactory conditions averaged 13 mm. per culture per day for an entire week (unpublished data). Cultures usually begin to branch from the base on the third or fourth day, and at the end of a week will frequently have 30 or 40 branches, each one capable of being used as a sub-culture. The roots themselves are entirely normal in color; the root-cap is characteristic in its form and in its manner of sloughing off (124). The cells reported by Scheitterer (104) as simulating migrating cells (compare Chambers, 16, 17; Pfeiffer, 96; and White, 124) were apparently sloughed off root-cap and root epidermis cells. The main portion of the culture, contrary to the observations of Malyschev (82) (see Gautheret, 44, p. 99), maintains a constant diameter. If branch roots of small diameter are used as sub-cultures, they very quickly enlarge to this normal diameter as they grow. Because of this fact, linear increments are quite as satisfactory measures of tissue increase (125, 129, 130) as are dry weights. Cultured roots seldom exceed this norm, and when they do, are evidently unhealthy. Such excessively thick cultures do not grow satisfactorily. It is, therefore, clear that secondary thickening is not a characteristic feature of such isolated roots. Nevertheless, complete vascular strands containing all the primary elements are regularly formed (124. 130), although it would seem improbable that they perform either of their major normal func-

tions of conduction and support. No interfascicular cambium or pheloderm is laid down, but proliferation of the pericycle to form secondary growing points (branches) goes on normally. When a piece of root containing no apical growing point is cultured, callus is formed in considerable quantity at the distal end but not at the proximal (Kotte, 66; compare Vöchting, 120), and when branches are formed on such a piece their insertion on the root is characteristically oriented. The polarity of growth, as was pointed out long ago by Vöchting (120), is thus maintained. The root-cap cells contain normal quantities of starch and the older cortical cells at the base of cultivated roots, likewise, become loaded with starch. The development of these cultures, so far as it goes, appears to be quite normal both in quality and quantity. But, although *growth* is potentially unlimited (130), *differentiation* is not. These are typical rootlets, but in the absence of secondary thickening, cambium, pheloderm, etc., even in cultures maintained for months without transfer (130), they cannot be called "roots" in a complete sense. And since no stem-tips are formed, they are certainly not plants.

The question arises, are they "tissue cultures"? Strictly speaking, they are not. They are "organ cultures." Strictly speaking, "tissue cultures" of plants have never been obtained. Gautheret's cambium cultures (44), mentioned before, appear to have satisfied the criterion of undifferentiating growth. White's cultures have satisfied the criterion of unlimited growth. But both criteria have never been satisfied in any single instance reported to date. The consideration of these root cultures in a paper on tissue cultures must be justified on the basis of their aim, and the fact that the method of approach is certainly that which must be employed if tissue cultures are to be obtained. Furthermore, it is the writer's belief that there is a growing feeling among animal tissue culturists that "differentiation" is a matter of environmental conditions as much as it is of internal factors, and that the undifferentiating pure-line cultures of Carrel (13), Ebeling (24), Fischer (35, 36), Parker (94), and others have come to be considered the normal type only because of the aims in mind. Thus Parker, using a pure-line culture of blood monocytes, but replacing the usual solid medium by a liquid medium similar to that used by White and Robbins, caused these pure-line cultures to differentiate into

a capillarogenic tissue (93). The work of Carrel, Ebeling, and others has shown that heart fibroblasts, when grown in pure culture, maintain an undifferentiated behavior indefinitely. Cartilage cells derived from the embryonic eye of the chick, in pure culture, lose their ability to produce hyaline sheath substance, developing as an undifferentiated epithelium-like tissue quite different from normal cartilage (Fischer, 35). Cartilage from embryonic bone, freed from perichondrium, does not grow at all (34). But when a pure culture of heart fibroblasts and a pure culture of epitheloid tissue derived from cartilage are grown side by side in the same medium, at the point of contact a new tissue, actively growing typical cartilage, arises and can be carried on indefinitely (34). This is then no longer a "tissue culture" in the strict sense of being a pure-line culture of a single type of cell, and cartilage is not, strictly speaking, a "tissue." This point of view has been presented by Parker, Fischer and Parker, Fischer, Fell, and others, and shows the absurdities that result from trying to draw too sharp a line between what is and what is not a tissue culture.

Work with root cultures is being continued in an effort to simplify the method and make it more reliable, and these results are being applied to practical problems of physiology and pathology. One of the unfortunate features of the method is the rigid control necessary if satisfactory results are to be obtained. Such isolated plant organs are extraordinarily sensitive. Many of the important cultural variables have been studied in some detail (temperature, pH, etc.), but others have apparently even eluded identification. The sensitiveness of such roots is shown, for example, by the fact that increasing the total salt concentration of the solution used from 2.6 millimols (the optimal concentration) to 10 millimols, both extremely low absolute concentrations, will reduce the growth obtained by about 60% (unpublished results). A change of maintained temperature from 27° to 30°, a difference of only 3° but across a critical range, will reduce the growth rate of wheat root-tips from the normal average value of about 5 mm. per day to almost nil (125). Decreasing the Fe-ion concentration from .003 milliequivalents to .002 milliequivalents will decrease the tissue yield by 50% (126). The roots are even more sensitive to such variants as the cystein-HCl studied by Gautheret (44). Moreover, there appears to be marked specificity as regards carbohydrate re-

quirements, since tomato requires sucrose, being unable to utilize dextrose under the conditions studied, while wheat gives excellent results with dextrose. Efforts to increase the phosphate concentration so as to make possible buffering of the solution have regularly proved fatal. Failure to control properly some one or more of these and possibly unknown factors is doubtless responsible for the checks encountered by other workers, and it can not be overemphasized that the nutrient formulae, cultural conditions, etc., as published, can with certainty be applied *only* to the species of plant described.

As Haberlandt foresaw (46), the cultivation of excised plant tissues has thrown many interesting lights on the behavior of certain groups of cells even though single cells have not been successfully isolated. The *apparent* dependence of chlorophyll-free tissues on moderate illumination, as reported by Robbins and Maneval (103), White (125), and Felber-Pisk (28), but denied by Malyschev (82), is interesting and will require further verification. The necessary character of iron, usually thought of as merely a catalyst for chlorophyll formation, for the proper maintenance of a chlorophyll-free tissue (126), is likewise interesting, but quite in keeping with Warburg's concept of the relation of iron to respiration in general (121). The fact that tomato roots are able to utilize sucrose under the conditions studied, but can not utilize dextrose, indicates that either sucrose, contrary to earlier opinion, can enter the cells as such and is there utilized directly or broken down to materials other than dextrose and levulose, or else that roots secrete into the medium some hydrolyzing agent giving rise to materials other than these hexoses. The proved unlimited capacity for growth of such cultures (130) obviously sets aside the concept of indispensable and *specific* correlation hormones, since the only possible source of such hormones has been the thermo-stable, apparently non-protein (Osborne and Wakeman, 92) filtrate of a plant (yeast) taxonomically and physiologically widely separated from the cultured tissue. These and many other interesting facts have been brought to light by the studies made to date, and give some indication of the potential value of this method of approach. It is to be noted that every factor in the environment, with the exception of the yeast extract, is of known character and can be controlled and varied at will, so that all sorts of problems of nutrition

and of other phases of physiological behavior can be approached in this way. The method represents a powerful new tool for such studies. Unfortunately, perhaps because of the sensitiveness of such cultures and the need for rigorous control if results satisfactory for physiological studies are to be obtained, the method has not as yet taken a prominent place in the plant physiologists' armamentarium. It has received somewhat more attention from the pathologists and has been used as a means of maintaining pure-line cultures of obligate parasites (viruses, 131), and in the study of nodule-forming bacteria (Lewis and McCoy, 77). The latter work gave unsatisfactory results, probably due to faulty technique. Riker and Berge have suggested the use of the method in studies of the crown gall organisms (99). It is to be hoped that, with the method itself firmly established, its application will be extended to other fields.

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