BIOSYNTHETIC POTENTIALITIES OF PLANT TISSUE CULTURE – A REVIEW

by

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The potential of isolated cells of higher plants for the biosynthesis of useful constituents—primary and secondary metabolites—was recognized in the 1950's but at that time culture techniques were too exacting and uncertain for general use. Recently, however, rapid advances have led to the development of techniques which allow the routine large scale cultivation of plant cells and thus product biosynthesis has become of considerable contemporary interest. Several publications have appeared emphasizing the potentials of plant tissue cultures, but only a few have critically evaluated the problem and the difficulties related to the growth and metabolism of tissue in vitro (Carew & Staba 1965, Nickell 1962, Staba 1963, Street, Henshaw, Buiatti 1965 Tulecke 1961, Puhan & Martin 1971, Constabel, Gamborg, Kurz and Steck 1974).

Biochemical and physiological studies of tissue culture and the plant parts from which they are derived have been studied by many workers (Weinstein, Nickell, Laurencot jr., and Tulecke 1959, Weinstein, Tulecke, Nickell and Laurencot. jr., 1962, Tulecke, Weinstein, Rutner and Laurencot jr., 1962). The comparison of the biochemistry of the plant tissue cultures with the biochemistry of the plant parts from which they were initiated affords an opportunity to study gross differences in cellular physiology in an intact, slow growing non-sterile, relatively mature organ and an analogous rapidly proliferating tissue grown aseptically. Growth may be visualized in terms of progressive formation of chemical compounds. If divisions from the zygote are strictly equational, then all its living derivative cells should have all the genetic information to achieve at any time all or any part of the biochemistry of the whole plant. Living cells of higher plants are in the morphogenetic sense potentially totipotent. And, therefore, it is assumed they should also be biochemically fotipotent. With the mounting evidence of "totipotency" in the cultured cells of higher plants (Steward, Mapes, Kent and Holsten 1964, Steward, Kent and Mapes 1966, Steward, Mapes and Mears 1958, Kent 1966), there is every possibility that use of the synthetic abilities of cultured cells and tissues could be exploited in the same way as those of microorganisms (Barker, 1956, Rau 1967).

Efforts in the research of product biosynthesis by plant cell cultures have been directed largely towards establishing that plant cells grown in isolation are capable of producing products which are produced by the parent plant. Callus and suspension cultures derived from plants which produce particular compounds have been shown, in many instances, also to produce the same compounds, however, the quantity is well below that in the intact plant. Among the products identified in plant cell cultures are: enzymes, growth regulators, organic acids, phenolics, pigments proteins, amino acids, alkaloids, glycosides, saponins, steroids, tannins, antibiotics, carbohydrates terpenoids and flavonoids.

Primary Plant Products: Cell mass

As soon as large-scale production of plant cells in suspension became feasible, the possibilities of using cell mass as a food source were considered. A number of workers (Byrne 1962, Byrne & Koch 1961, Mandels, Jeffers and El-Bisi 1968) have studied the mass culture of plant cells as an unconventional food source for feeding man.

Vasil and Hilderbrandt (1966) suggested the isolation of green, autotrophic strains of callus tissue and the use of such artificially grown tissues as sources of food for astronauts undertaking long journeys in space Mandel et al. (1968) have studied the production of cell mass by tissue cultures isolated from a number of edible plants and concluded that a considerable improvement in the growth rate is required before use of plant cell cultures as food can be realized economically. Plant tissue culture systems have been considered as a food and oxygen source for closed ecological systems (Tulecke 1963, 1963) and as a food source in unfavourable environs (Matthern 1962). Plant tissue culture cells are probably more nutritious on g dry-weight basis than most plant tissues.

A number of publications have appeared reporting the production of carbohydrates and organic acids by static plant tissue cultures (Alleweldt & Radler 1962, Koblitz & Koblitz 1962, Matthern 1962, Mohan Ram & Steward 1964, Norstog 1956, Routien & Nickell 1956, Scott, Burris & Ricker 1955). Starch grains are known to accumulate in some plant tissue culture cells (Torrey 1957). Norstog (1956) observed starch grains to accumulate in rye grass endosperm cultures during the first 25 days of growth and to disappear by the 30th day of growth. Steward et al. (1961) observed starch to disappear from potato tuber tissue culture during their active growth phase.

Amino acids

The cultured plant tissues synthesize usual amino acids e.g. protein (Warwick & Hilderbrandt 1966 Wickremasinghe & Swain 1965) Weinstein et a, (1962) reported the presence of high amount of hydroxyproline in Rosa sp. tissue cultures. This was in agreement with the report (Steward, Thompson and Pollard 1958) that hydroxyproline is a major constituent of rapidly growing, proliferating tissues. Tissue cultures of Citrus limonum were found to contain aminobutyric acid, proline, citric acid in higher amount ,than the intact plant, the level of potassium in the

culture medium strongly influenced the amounts of free amino acids in the tissue cultures (Bove, Bove and Raveux 1957). The protein of actively dividing cells in tissue culture of Solanum tuberosum was found richer in hydroxyproline and basic amino acids than the protein of mature tuber cells (Steward, Thompson and Pollard 1958). The bulk protein of Agave also showed a greater amount of hydroxyproline in the tissue culture (Weinstein, Nickell, Laurencot, jr., and Tulecke 1959). Hydroxyproline is the predominant amino acid in the cell wall of the tissue cultures. This suggests that probably hydroxyproline is a structural component of cell wall protein. Evidence in support of this view came from a report by Lamport & Northcote (1960) who found hydroxyproline in the protein of cell walls from tissue cultures of Acer pseudoplanus and Phaseolus vulgaris.

The amino acid arginine occurs in large amount in the winter storage tubers of the Jerusalem artichoke Dauranton (1958) has studied the metabolism of arginine and found that the degradation pattern known to occur in microorganisms, i.e. from arginine to urea and ornithine and thence through intermediates to proline. Nevertheless, the use of cultures established from normal plant tissue to synthesize the more unusual amino acids of plants has been, as yet, unrewarding.

Gamborg and Finlayson (1969) examined cultures derived from 12 plant species and found that total protein content was 27—35 per cent. Soluble proteins constituted 1.7—8.7 per cent of the total dry weight of the cells. The amounts of essential amino acids particularly the basic ones and methionine, were proportionately higher in the cultured cell proteins than those reported for the seed proteins.

Khanna et al. (1974) have extracted insulin from static and suspension culture of *Momardica charantia* Linn and predicted the feasibility of insulin production on a commercial basis from these cultures.

Enzymes

In view of the increasing importance of enzymes in medicine and commerce (Beckhorn 1962), attempts have been made to use plant tissue cultures as a possible future source of economically or biochemically useful enzymes. Normally enzymes are secreted in nutrient media by tissue cultures The first demonstration of extracellular enzymes in higher plants was the demonstration that Rumex virus tumour tissue secretes r–amylase into the culture medium (Brakke & Nickell 1951). Among the enzymes known to be present in tissue cultures are: proteolytic enzymes (Gainor & Crisley 1962, Krikorian 1965), invertase (Straus 1962), X and B-amylase (Brakke & Nickell 1951, 1955, Nickell & Brakke 1954, Karstens & De Meester-Manger Cats 1960, Jaspars & Veldstra 1965), ascorbic acid oxidase, phenolase, and peroxidase (Witham & Gentile 1961, Straus & Campbell 1963), trytophan synthetase (Klein, Caputo and Witterholt 1962) and chlorogenic oxidase (Spurr. Holcomb, Hilderbrandt and Riker 1962). Scott, Daly and Smith (1964) found that a combination of IAA and kinetin caused a decrease in the activity of hexase monophosphate enzymes of nicotiana tissue cultures. Gentile (1965) has suggested that enzymes released by plant tissue cultures are bound to the cell wall or outer membrane surface and are released under certain physiological conditions. Nickell (1962) speculated that the only predictable worthwhile use for plant enzymes would be for use in specialized circumstances such as transformation of alkaloids, steriods, antibiotics and other physiologically active molecules.

Miscellaneous Products

Becker, Hui and Albersheim (1964) reported that Acer pseudoplatanus grown in suspension secretes into the medium poylsaccharides which are similar to the non-cellulosic wall polysaccharides of the cells and which differ slightly from those of cambial cells in the intact tree.

Routien & Nickell (1956) reported the production of oxalic acid by tumour tissue cultures of Rumex acetosa and of ascorbic acid from Nicotiana crown gall tissue cultures.

TABLE 1 Primary plant products in plant tissue culture

Cell Mass	(a) as a food source	Byrne (1962) Mandels et al (1968) Tulecke (1963,1965)
	(b) production of carbohydrates and organic acids	Routien and Nickell (1956) Scott et al (1955) Alleweldt and Radler (1962) Matthern (1962)
	(c) accumulation of starch grains	Norstog (1956) Steward et al (1961)
Amino Acids	(a) Synthesis of hydroxyproline in Rosa sp., Solanum tuberosum Agave, Acer pseudoplatanus and Phaseolus vulgaris	Weinstein et al (1962) Steward et al (1958) Weinstein et al (1959) Lamport and Northcote 1960
	(b) r-aminobutyric acid, proline, citric acid in Citrus limonum	Bove et al (1957)
	(c) arginine in Jerusalem artichoke	Duranton (1958)
	(d) insulin in Momordica charantia L.	Khanna et al. (1974)
Enzymes	(a) proteolytic enzymes	Gainor and Crisley (1961, 1962) Krikorian (1965)
	(b) invertase enzymes	Straus (1962)
	(c) & and B–amylase	Brakke and Nickell (1951, 1955) Nickell and Brakke (1954) Jaspars and Veldstra (1965)
Enzymes	(d) ascorbic acid oxidase, phenolase and peroxidase	Witham and Gentile (1961) Straus and Campbell (1963)
	(e) tryptophan synthetase	Klein et al (1962)
	(f) chlorogenic oxidase	Spurr et al (1962)
Miscellaneous Products	(a) polyscacharides in Acer pseudoplatanus	Becker et al (1964)
	(b) oxalic acid in Rumex acetosa tumour tissue	Routien and Nickell (1956)
	(c) ascorbic acid in Nicotiana crown gall tissue cultures	Routien and Nickell (1956)
	(d) ethylene production in several cultures	Gamborg and La Rue (1968)

Gamborg and La Rue (1968) found that several plant ell cultures produced ethylene. They suggested that thylene formation depended not only on the kind of lant from which the cultures originated but also on the hysiological state of cells.

ɔummary

The possibilities of using cell mass as a food source is ɔeing seriously considered after the feasibility of large-ɔcale production of plant cells in suspension. The table 1 ists the usefulness of plant tissue culture in the production ɔf various primary plant products such as amino acids, ɛnzymes, carbohydrates etc.

Secondary Plant Products:

Nickell (1962) has defined secondary plant products as compounds not produced by all plants, whose functions are not known and which are not essential metabolites, but which generally have considerable biological activity.

Mitra (1966) reviewed the work concerning synthesis of constituents in the plant and related it to their synthesis in cell cultures. He concluded that there exist biochemical differences between the intact plant part and the cell culture derived from it. Although their genetic constitution is the same, their physiology of growth becomes different in vitro under the new environmental conditions.

Alkaloids

The greatest interest shown in secondary plant products of medical importance has been in alkaloids.

West & Mika (1957) were the first to have obtained callus cultures of *Atropa belladonna* root. They demonstrated that the root callus possessed the capacity for unlimited growth in vitro and for development of an entire plant in that roots, stems and leaves were formed. They noted that only isolated roots and root callus could synthesize atropine, stem and leaf cultures could not unless macroscopic adventitious roots had been formed. The amount of alkaloid found was 0.047–0.053% of the dry weight in the roots callus cultures. Excised roots grown in organ culture made slightly less alkaloid (0.042% of the dry weight), roots of intact plants, however, are known to accumulate many times more atropine. Atropa belladonna alkaloids are synthesized by excised root organ cultures (French & Gibson 1957, Reinouts Van Haga 1957, West & Mika 1957). The site of synthesis of hyoscyamine has been studied in Atropa and Datura (Evans & Partridge 1953, James 1946, Peacock, Leyerle & Dawson 1944, Van Haga 1954). Petiard and Demarly (1972) and Petiard et al. (1972) established tissue cultures of *Atropa belladonna L.* and *Vinca minor L.* and found the same compounds present in cultures as originally present in parent plant. Telle & Gautheret (1947) grew root tissues of *Hyoscyamus niger* and crown gall tissue of *Datura stramonium* has been cultured by Nickell (Gautheret 1959). Chan & Staba (1965) studied alkaloid production by *Datura stramonium* stem callus culture. The highest total alkaloid yield occurred at the end of third week in Murashige-Skoog medium supplemented with coconut water and precursors. The highest percentage, on a dry weight basis (0.161 per cent) was obtained from cells grown in the above medium containing 0.2 per cent L–phenylalanine. Alkaloids were present in the lower concentration in the medium than in the cells. Elze and Teuscher (1967) have shown that cells of Datura tatula and D. metel are able to absorb added atropine from the medium and to accumulate it. Cell free enzyme preparations from Datura innoxia are able to reduce hyoscine to hyoscyamine (Babcock & Plotkin 1967). Datura species have been used to study the incorporation of various compounds into the principal alkaloids (Gibson and Danquist 1965). The growth of *Hyoscyamus niger* ovaries in culture has been described by Singh Bajaj (1966).

Metz and Lang /1966) grew *Hyoscyamus niger* root cultures and found the dried roots to contain 0.2–0.3 percent scopolamine. Stohs (1969) reported on the production of scopolamine and hyoscyamine in callus suspension culture of *Datura stramonium*. Dhoot and Henshaw (1974) studied the synthesis of alkaloids in callus and suspension cultures of Hyoscyamus niger and found the levels of alkaloids varying between 0.04% of the dry weight during the phase of rapid growth and 0.08% during the stationary and lag phases.

Solt (1957) reported that the alkaloid nicotine is biosynthesized by tobacco root cultures only when the root tips are present. Alkaloids have been found in the excized root organ cultures of *Nicotiana* species (Dawson 1942) Dawson (1942) stated that the nicotine produced is not retained within the root tissues but is liberated into the external medium. Dawson (1960) also reported that the qualities of nicotine alkaloid produced by cultured tobacco (N. glutinosa) decreased rapidly during the initial stages of tissue culture so that none was detectable when a typical callus state was achieved. On the contrary, speake et al (1964) could still identify nicotine in free cell and callus cultures of tobacco root, stem or leaf (N. tabacum var. 'Virginica') after 23 transfers. The general level of nicotine produced by cell and callus cultures is 0.1–1.0mg/mg dry weight of tissue whereas nicotine produced by the intact plant root is 29mg/mg dry weight. Dawson (1960) stated that excised tobacco root cultures supplied with various labelled nicotinic acid compounds (0.01–0.03mg/ml) did not increase nicotine production but the cells did incorporate them into nicotine. Furuya et al (1966, 1967) have identified small amounts of nicotine and antabine in tobacco (N. tabacum var. 'Bright Yellow') callus tissue. Cultured tissue of embryo origin from N. *rustica* showed 0.85% of its dry weight as total tobacco alkaloids (Krikorian 1965).

Neumann and Muller (1971) reported that callus cultures of *Nicotiana tabacum L.* do not synthesize alkaloids in different growth media but they start to produce alkaloids (nicotine and two other alkaloids, not identified and not occuring in Nicotiana plants under normal conditions) after addition of cycloheximide. However, suspension cultures started with tissue from the callus cultures did produce alkaloid (nicotine and other two alkaloids) and the alkaloid content depended on the growing conditions.

White (1945) appears to be the first to have obtained tissue cultures of *Catharanthus roseus* from crown galls rendered bacteria free by pasteurization. These showed a very rapid growth, and when grafted back into the host plant they produced typical tumours which regularly increased in size. A total of 66 alkaloids have been found in *Catharanthus roseus* (Vinca rosea). Four of the alkaloids, leurosine, vincaleukoblastine, leurosidine, and leurocristine have demonstrated varying degrees of antitumour activity (Neuss, Johnson, Armstrong and Jan-

sen 1964). Carew and Krueger (1974) have studied the factors influencing growth and alkaloid formation in Catharanthus tissue culture. Pecursors added to influence alkaloid formation included geraniol, tryptamine, trytophan, catharnathine and vindoline. Trytamine exerted the strongest influence on alkaloid formation.

Hadwiger and Weller (1964) demonstrated the biosynthesis of ricinine in excised roots of *Ricinus communis*. Staba and Laursen (1966) have examined tissue cultures of three varieties of *Ipomoea violacea* (morning glory) and *Rivea corymbosa*. Only trace amounts of alkaloids were dectected in some calluses, no alkaloids were detected in others.

Steinhart (1962) has reported the synthesis of candicine in the stem cultures of the cactus *Trichocereus spachianus*. Sander (1956) has reported the formation of tomatine in tomato cultures. Callus of *Papaver somniferum* (opium poppy) has been cultured by Koblitz et al (1967) and Rangnathan et al (1963). Alkaloid free suspension systems which incubated with thebaine are known to metabolize this alkaloid (Griitzmann and Schroter 1966). Skythanthine has been isolated from callus cultures derived from shoot cultures of *Solanum xanthocarpum* (Heble, Narayanswami, Chadha 1968). Reinhard et al (1968) have identified harmine in tissue cultures derived from *Peganum harmala*.

Furuya et al (1972) reported the formation of a number of alkaloids in callus tissue derived from root, stalk and capsule of *Papaver somniferum* (opium poppy). Alkaloids detected were Sanguinarine, dihydrosanguinarine, oxysanguinarine, protapine, cryptopine, magnaflorine and choline. Along with these a new alkaloid norsanguinarine was also isolated.

Resperine alkaloids, useful as tranquilizers and hypotensive drugs, were produced by *Rauwolfia* tissue culture (Koichi, Suzuki and Maruoka 1974). Callus tissue from the budded plant was planted on a basal medium. The differentiated young plant was cultured on a solid or liquid medium. The young plant was found to contain resperine alkaloids in the same amount as in the natural root i.e. (0.025%). Shaking culture further increased the alkaloid content. Resperine has been isolated from cultures of *Alstonia constricta* (Carew 1971) and Rauwolfia (Mitra and Kaul 1964) in small amounts.

Benjamin and Mulchandani (1973) investigated the secondary metabolic products of callus tissue from *Tylophora indica* and found the tissue lacked the ability to synthesise phenanthroindolizidine alkaloids, the known constituents of the intact plant. The incorporation of phenylalanine, the known precursor of Tylophora alkaloids, into the basal medium did not induce alkaloid synthesis.

Veliky (1972) reported on the synthesis of carboline alkaloids, by cell suspension culture of Phaseolus vulgaris.

Cultures were established from the roots of the plant that is not known to produce alkaloid but were shown to produce *B*-carboline (non harman) and -l-methyl-*B*-carboline (harman when grown in the presence of tryptophan. Probably these cells possessed a biosynthetic potential for transforming tryptophan into indole alkaloids. Similarly, trans-Cinnamic acid-3-C^{14} has been shown to be coverted to various ameryllidoceous alkaloids when supplied to excised promorial tissue from buds of *Hippeastrum vittatum* (Suhadolnik, 1964) Alkaloid content of the callus tissue is markedly different from that of the seeds. Hippeastrine and lycorine are not present in the callus tissue, whereas these two alkalods are present in the seeds.

Summary

The following table (2) lists the alkaloid production by plants in culture.

TABLE 2 Alkaloids in Plant tissue culture

Source and type of Culture	Nature of alkaloid	Yield	References
Atropa belladonna root	atropine	0.047-0.058 %dry wt.	West and Mika (1957) French and Gibson (1957)
Atropa and Vinca minor L.	as in parent plant		Patiard and Demerly (1972) Patiard et al (1972)
Datura stramonium stem callus	tropane alkaloids (Scopalamine and hyoscyamine)	0.161% dry wt.	Chan and Staba (1965)
Hyoscyamus niger root callus	Scopalamine	0.2 0.3% dry wt.	Metz and Lang (1966)
Hyoscyamus nigher callus and suspension	alkaloids	0.04-0.08% dry wt.	Dhoot and Henshaw (1974)
Nicotiana sp.	nicotine	0.1-1.0 mg/mg dry wt.	Speake et al (1964)
N. rustica cultures of embryo origin	tobacco alkaloids	0.85% dry wt.	Krikorian (1966)
Catharanthus roseus crown gails	indole alkaloids	0.01% dry wt.	Neuss et al (1964)
Ricinus communis roots	ricinine		Hadwiger and Waller (1964)
Cactus tricho-cereus sphachianus stem	candicine		Steinhart (1962)
Ipomoea violacea, Rivea corymbosa	alkaloids		Staba and Laursen
Tomato	tomatine		Sander (1956)
Papaver somniferum callus	thebaine		Koblitz et al (1967)
Papaver somniferum root, stalk and capsule callus cultures	sanguinarine, dihydrosanguinarine, protopine, cryptopine, magnoflorine, choline and norsanguinarine		Furuya et al (1972)
Solanum xanthocarpum callus	skythanthine		Heble et al (1968)
Peganum harmels	harmine		Reinhard et al (1968)
Rauwolfia	resperine	0.025% dry wt.	Koichi et al (1974)
Alstorica constricta	resperine		Carew (1965)
Phaseolus vulgaris suspension	carboline alkaloids		Veliky (1972)
Claviceps purpurea Rye callus	Ergot alkaloids	1.3 mg/ml	

ilycosides

When a sugar is combined through its glycosidic ydroxyl with an alcohol, a glycoside is formed. The non ugar portion of the molecule is known as the aglycone. ilycosides are often responsible for the taste and aroma f some foods and are also often used medicinally.

Sargent and Skoog (1960, 1961) isolated and charac-erized scopolin and three other glycosides of scopoletin om tobacco. Scopoletin was released by tobacco ith cultures into the agar medium on which they were rown. The amount of free scopoletin in the fresh growing allus tissue was relatively low, 5 mg/g. Most of the copoletin in the tissue was present in a bound form nd was retained within the cells. Skoog and Montaldi 1961) found that the amount of scopoletin released aries with the concentration of auxin in the medium nd increases markedly at high, toxic levels of auxin either IAA or NAA). Phenylalanine was found to be ighly active in releasing scopoletin into the medium. ritig et al (1966) have shown C^{14} labelled phenylalanine nd tyrosine to be incorporated into scopolin and copoletin. Tyron (1956) claimed the capacities of arious strains of tobacco callus to form buds and diffe-ntiate is very much related with the content of their copoletin.

Benveniste et al. (1964, 1966, 1966) have identified everal phytosterols in cultures of N. tabacum. Cultures f *Agave toumeyana, Dioscorea composita, Rubus uticosus, Chichorium endivia* and Carrot incubated vith acetate—1—C^{14} do not incorporate label into anosterol, lanostadienone, and lanostadiene; however, ycloartenol was so labelled (Ehrhardt, Hirth and Ouri-son 1967). Benveniste (1968) also reported on isolation f diosgenin and related steroids from tissue cultures f N. tabacum.

Hecognenin, which is a steroidal sapogenin is found n Agave. Agave toumeyana were grown in semisolid ulture as well as in submerged growth (Nickell 1962, ulecke and Nickell 1960) but it did not contain hecogenin Weinstein, Nickell, Laurencot, Jr., and Tulecke 1959). imilarly, *Dioscorea composita*, which contains dios-enin, a steroidal sapogenin could be grown in culture Nickell 1962, Tulecke and Nickell 1960). Kaul taba (1968) reported the production and isolation f a compound identified as diosgenin from root callus uspension cultures of *Dioscorea deltoidea*. Kaul t al. (1969) studied the influence of various factors n diosgenin production by Dioscorea deltoidea callus nd suspension cultures. Mehta and Staba (1970) eported on the production of diosgenin in callus and uspension cultures of a number of Dioscorea species: . composita, D. deltoidea, D. floribunda and D. spicu-flora. Of these, maximum diosgenin content was resent in tissue cultures of D. deltoidea and then in ecreasing order of diosgenin production in tissue cultures n D floribunda, D. spiculiflora, and D. composita.

Sarkisova (1974) reported on the production of iosgenin and Steroidal saponins in D. deltoidea wall ssue culture and found the water soluble saponins roduced in these cultures possessed pharmacological ctivity.

Callus derived from seedlings of *Dioscorea tokoro* tains the ability to synthesise diosgenin, yonogenin nd tokorogenin, though isodiotigenin and kogagenin, formed in the intact plant, are not produced in tissue cultures (Tomita, Uomori and Minato 1970). Tomita and Uomori (1971) have established the general path-way of sapogenin biosynthesis in tissue cultures of D. tokoro.

Diosgenin was isolated from *Solanum laciniatum* callus cultures; its quantity was identical with that of field plants. However, the main steroid of the solanum species, solasodine was not detectable in the callus (Vagujfalvi, Maroti and Tetenyi 1971). Isolation of dios-genin was also reported from *Solanum xanthocarpum* tissue cultures (Heble, Narayanswami and Chadha 1968). Later, Heble et al. (1971) reported that analysis of tissue cultures of *Solanum xanthocarpum* subjected to the influence of different plant hormones singly and in synergistic combinations showed changes in the steroidal content indicating chemical regulation by auxins.

Khanna and Mohan (1971, 1973) reported on the production of diosgenin, *B*-sitosterol and stigmasterol by tissue cultures of *Momordica charantia L.*

Cultures of **Digitalis lanata** and D. purpurea have been initiated by Staba (1962) with the intent of study-ing the glycosidic components of these cultures. Although Kedde-positive substances were detected (Buchner and Staba 1963, 1964) there were no chemical data to prove that cardiac glycosides were in fact synthesized. On the contrary, Stohs and Staba (1965) reported that root suspension cultures of D. lanata and callus cultures of D. purpurea and D. mertonensis were unable to convert digitoxigenin or a number of other precursors to glycosides. After 16 days, traces of conversion products were detected. The work by Medore, Tsao and Albert (1967) on D. mertonensis revealed Baljet-positive spots when various steroidal precursors like sterol A, a multisterol surfactant, cholesterol, and polyethylene glycol 1000 were added to the culture medium. Medore et al. (1967) also reported the biological activity of extracts of callus of guinea pigs. There were in all these instances considerable variation in the biological development of the cultures, for it is known that Digitalis callus formed organized structures (Staba and Lamba 1963, Staba, Laursen and Buchner 1963). Preliminary examination for the formation of cardenolides by Digi-talis tissue cultures were carried out (Buchner and Staba 1964). It may well be that it is these organized 'plantlets' which, in fact, synthesize the small amounts of substances that were detected. Furuya et al. (1973) examined the *Digitalis purpurea* tissue culture and found that it maintained the same biosynthetic potential regarding the conversion of digitioxin into purpurea glycosides A and B and into gitoxin as the intact plant, but did not convert progesterone into the cardenolides.

Cultured tissues of *Iberis sempervirens* were found to be devoid of mustard oil glucosides. It sppears that cultures of I. sempervirens do not accumulate these com-pounds whereas they are formed during normal deve-lopment of this plant. Cultures of **Taxus cuspidata** of leaf origin did not contain any cyanogenetic glycoside taxphyllin (Bleichert 1964). The same was found for tissues of *Trifolium repens* which although genetically competent to produce cyanogenetic glucosides are unable to effect their synthesis in culture (Hughes 1968).

Visnagin, a furanochrome, has been isolated from and identified in suspension cultures of **Ammi visnaga.** (Kaul and Staba 1965, 1968). A large amount of 1-maackiain and a small amount of 1-pterocarpin have been isolated from callus tissue of **Sophora angustifolia** (Furuya and Ikuta 1968). Coumarin (1,2—benzopyrone) has been detected in cultures of Melilotus (Routien and Nickell 1956), although Trifolium cultures do not synthesize it. Steck et al. (1971) reported that cultured cells of *Ruta graveolens L.* produced the coumarins umbelliferone, scopoletin, psoralen, xanthotoxin, isopimpinellin, rutamarin and rutacultin (6, 7-dimethoxy-3-(1-dimethylallyl) coumarin), a new natural product.

Goris (1965) found that carrot root cultures were capable of converting vanillin to glucovanillin. Smirnov and Kuzovkina (1974) studied the formation of the secondary compounds in *Ruta graveolens* root tissue and found that the tissue retained the ability to synthesise the main lipophilic contents typical of the whole plant root. The essential oils, coumarins, furocoumarins, furoquinoline and acridone alkaloids typical of the intact plant were identified in the tissue.

Brain et al. (1973) have studied the steroidal constituents of suspension cultures of *Trigonella foenum graecum* (Fenugreek) under different environmental conditions and found that all the steroidal fractions present in the seed were observed in at least some of the cultures but the appearance of particular components was dependent on the medium composition and culture age. Stevens and Hardman (1974) initiated the tissue cultures from cotyledons of *Trigonella foenumgraecum* and identified diosgenin, yanofenin, stigmasterol and *B*—sitosterol.

Chemical analysis of *Apocynum cannabinum* tissue cultures revealed the presence of various compounds, of which only two *B*—sitosterol and stigmast-4-en-3-one could be identified. However, none of the known glycosides or genins commonly associated with A. Cannabium was detected (Carew 1971). Further work showed that these tissue cultures contained substances which were chromatographically very close to but not identical with the reference compounds K-strophanthin, cymarin and straphanthidin (Lee, Carew and Rosazza 1972).

Chemical components of callus and other tissues of pumpkin were investigated. Squalene and 3 sterols were isolated and identified. Stigmastadienol was not present in the callus (Yanagawa, Kato and Kitahara 1971)

Summary

The table (3) lists the glycoside production by plants in culture.

Tomita et al. (1969) reported on the production of sesquiterpenes in tissue cultures of **Lindera strychnifolia.** Lindenenol, lindenenol acetate, linderane, linderalacton, lindesterene and caryophyllene were detected. Butcher (1971) reported on the biosynthesis of abnormal terpenoids by tissue cultures of **Andrographis paniculata.** Cultures did produce detecable quantities of andrographolide and two related compounds but produced relatively large amounts of a new group of sesquiterpene lactones.

TABLE 3 Glycosides in plant tissue culture

Source and type of Culture	Nature of glycoside	Yield	References
Tobacco pith cultures	scopolin and related glycosides	5Mg/g dry wt.	Sargent and Skoog (1960, 1961)
N. tabacum	diosgenin and related steroids		Benveniste (1968
Dioscorea deltoidea wall tissue culture	diosgenin and steroidal saponins		Sarkisova (1974)
D. deltoidea root callus suspension	diosgenin	1.00% dry wt	Kaul and Staba (1968)
D. composita, D. deltoidea, D. floribunda and D. spiculiflora callus and suspension	diosgenin		Mehta and Staba (1970)
Solanum liciniatum	diosgenin, solasodine		Vagujfalvi et al (1971)
Solanum xantho-carpum	diosgenin, -sitosterol	0.04% dry wt.	Heble et al (1968
Momordica charentia	diosgenin, -sitosterol and stigmast-erol		Khanna and Mohan (1971, 1973)
Digitalis lanata and D purpurea callus	cardenolides	0.002% dry wt.	Buchner and Staba (1964)
Digitalis mertonensis	cardenolides		Medore et al (1967)
Ammi visnega suspension	visnagin (furanochrome)		Kaul and Staba (1965, 1968)
Sophora angustifolia	1-mackiain and 1-pte-nocarpin		Furuya and Ikuta (1968)
Melilotus officinalis	coumarins dry wt.	0.05% dry wt.	Routien and Nickell (1956)
Ruta graveolens	coumarins and furocoumarin		Steck et al (1971), Smirnov and Kuzovkina (1974)
			Brain et al (1973)
Trigonella foenum-graecum suspension	steroidal saponins		
T. foenumgraecum cotyledons	steroidal saponins		Stevens and Hardman (1974)
Apocyanum cann bium	-sitosterol, stigmasterol		Carew (1971)
Pumpkin callus	squalene and related sterols		Yanagawa et al (1971)
Cassia senna callus	anthracene derivatives	1.2% dry wt.	Rai et al (1974)
Rheum Palmatum callus	anthracene derivatives		Rai and Turner (1974)

Miscellaneous compounds: Anthocyanins

Anthocyanins are the substances which give the varied and brilliant colours to plants and their products —flowers and fruits, etc. Anthocyanins give red, mauve, violet and blue colours. All higher plant species have the potentiality of producing anthocyanins. In vivo anthocyanin synthesis requires certain environmental and physiological conditions such as light of sufficient energy, favourable temperature and an ample source of carbohydrate. However, in vitro anthocyanin synthesis is uncommon, the amounts found are usually very minute, and the anthocyanin—synthesizing strains may lose their ability for pigment formation after repeated culture.

There are a number of reports of anthocyanin formation in callus and cell suspension cultures (Ardenne 1965, Blakely and Steward 1961, 1962 DeCapite 1955, Harborne 1963, Slabecka-Szweyknowska 1952) as well

as in organ cultured in vitro (Klein & Morgan 1961, Mishra 1966, Thimann and Edmondson 1949). The cultures from which anthocyanin pigments have been identified with certainty are those of Happlopappus graciles (Ardenne 1965), *Parthenocissus tricuspidata* (Stanko and Bradinskaya 1962), Corn endosperm (Straus 1959), a red lycopene-containing carrot (Sugano and Hayashi 1967) and *Dimorphotheca auriculata* (Harborne, Arditti and Ball 1970). Ibrahim et al. (1971) have reported on the formation and nature of anthocyanins in callus cultures of *Jerusalem artichoke* tuber, flax cotyledon, carrot root, rose cytyledon and McIntosh apple cotyledon. Goldstein et al. (1962) have found leucoanthocyanins in the sycamore maple (Acer pseudo-platanus) cell suspensions derived from cambium. Constabel (1971) have studied the effect of hormones on anthocyanin accumulation of the Happlopappus graciles.

Alfermann (1974) attempted to isolate different cell lines from callus cultures of *Daunus carota* producing cyanidin derivatives.

Carotenoids

Carrot accumulates carotenoids in the storage root. Carotenoids of various intensities of orange and red are reported present in carrot callus cultures (Caplin and Steward 1949) and gall tumours (Nickell and Tulecke 1961). Carotenoid synthesis in tissue culture does occur but not in quantity and kind, as in the root of plant. Some callus cultures, however, do synthesize carotene as in the mature plant (Naef and Turian 1963).

Chlorophyl

Chlorophyl or green pigments are present in certin tissue culture strains of fruit embryo (Tukey 1938), Cuscuta (Loo 1946), Rumex virus tumour tissue (Buck-holder and Nickell 1949, Nickell and Tulecke 1961), Sequoia stem callus (Ball 1950), Carrot (Audus 1959), Cocklebur callus (Fox and Miller 1959), Taxus, Ginkgo and Pseudolarix pollen (Tulecke 1961), Nicotiana crown gall (Van Lith-Vroom, Gottenbos and Karstens 1960), Haplopappus (Blakely and Steward 1961), Citrus ovules (Sabharwal 1961). Eight different tissue cultures containing green pigments were established from various plants used for food (Hilderbrandt, Wilmar, Chen, Rajagopal and Riker 1962). Mahlberg and Venketesh-waran (1966, 1962) have shown chlorophyllous tobacco cultures to possess pigmentation ratios similar to those of the normal leaves, although the total amounts (micro-grams per gram fresh weight) were very much less. Hilderbrandt et al. (1963) reported that the greatest concentrations of chlorophyl, in several different callus cultures, occurred in the culture medium devoid of sugars or those which contained high sugar (8%) concentrations. (Fukami and Hilderbrandt 1967). Vasil et al (1964) studied the nutrition and growth of chloro-phyllous callus tissues and Kumar (1974) reported on the Chlorophyll development in *Dolichas lablab* and *Archis hypogaea*.

Flavanoids

Kordan (1959, 1962), Rangaswamy (1958, 1961), and Sabharwal (1962, 1963) cultured mature juice vesicles from lemon and other citrus fruits and observed that the callus masses lacked the ability to synthesize the necessary pigments. Later, Kordan and Morgenstern (1962) reported that the vesicle stalks from mature lemon fruits proliferating in culture release flavinoid. However, hisperidin, the well known flavanone glycoside which occurs in lemon fruits was absent in the cultures.

Antibiotics

Many higher plants are known to contain antimicrobial agents (Skinner 1955, Nickell 1958, 1959, 1962). Antibiotics are produced by tissue cultures of avocado (Nickell 1962), lettuce and cauliflower (Campbell, Chan and Barker 1965), *Ipomoea violaceae* var. Pearly Gates, and a number of other plant species (Mathes 1967, Anonymous). Khanna and Staba (1968) have studied the antimicrobial activity of 24 plant species as static and suspension cultures against *Escherichia coli* (Gram negative), *Staphylococcus aureus* (Gram positive), mycobacterium Smegmatis (Acid fast) and *Candida albicans* (fungus). Aspen (populus sp.) tissue cultures of cambial origin yield antimicrobial, substances which produce inhibitory zones when culture plates are innoculated with a number of bacteria and fungi (Mathes 1963). Sharma and Khanna (1974) have reported the isolation and identification of insecticidal substances from *Tephrosia* sps.

Tannins

Tannin deposition occurs in crown gall tissue (Braun and Stonier 1958) and in Sequoia callus tissues (Ball 1950), probably as secondary metabolic products. Plants suspension cultures may modify tannins in a similar way to that reported for some microorganisms (Gray 1959). Chafe and Durzan (1973) have detected tannin in cell suspensions cultures of white spruce (Picea glauca (Moench) Voss).

Lignins

Gautheret (1966) and White (1963) have elaborated on the histogenesis and organized development in plant tissue cultures. The range of structural differentia-tion in tissue culture is different from what was observed in the intact plant. Gautheret (1966) stated that the lignin synthesized in cultured tissue is different from the lignins in organ from which the cultures were derived. (Barnoud 1965, Koblitz and Koblitz 1962). It has been reported that kinetin may increase lignification (Koblitz 1962), and so can gibberellic acid (GA) Kob-litz 1966). Bergmann (1964) showed that a suitable supply of kinetin increased a hundredfold the number of tracheids in colonies of tobacco, and that this was acco-mpanied by synthesis of lignin. Wetmore et al. (1963) found that sugar levels greatly influence the formation of phloem and xylem and the degree of lignification. Lippard et al. (1971) have studied the external surface of cell walls of plant cells grown in culture and reported the formation of lignin on the outside surface of suspension-cultured cells of Daucus, Ipomoea, and Phaseolus, and suggested that natural fibrous lignin may be a much commoner component of plant tissue than suspected hitherto.

Essential oils and Isoprenoids

The terpenes are a group of unsaturated hydrocarbons, found in the essential oils and oleo-resins secreted by a number of plants.

Peppermint (Mentha piperita) has been as economically important crop plant, due to its volatile oil content. In 1951 How we recorded the attempted culture of peppermint. Some slow-growing tissue cultures were obtained using a basal medium supplemented with 2,4–D and coconut water. Cultures were examined to detect their volatile oils. All attempts to detect volatile oils in the tissue cultures of Mentha were unsuccessful (Krikorian 1965). Lin and Staba (1961) reported the culture of peppermint (M. piperita) and spearmint (M. spicata). It appeared that a high-salt medium was necessary as well as an extraordinary amount of inositol (5000 mg/ litre). Peppermint and spearmint tissue cultures were unable to synthesize oils even when fed appropriate precursors. However, Rosa cultures are known to be odiferous. It is presumed that the metabolism which leads to the essential oils is linked to the environment in situ and to the development of oil secreting glands which do not form in cultures. (Krikorian and Steward 1969).

Callus cultures of *Ruta graveolens* are so far the only tissue cultures capable of synthesizing volatile oil. Comparison of the components of the volatile oil of the organs of the differentiated plants (Kubeczka 1967) of *Ruta graveolens* with those of the tissue cultures (Reinhard, Corduan and Volk 1968) showed that a leaf oil is most similar to the volatile oil of tissue cultures gorwn in the light. In both cases the main components are undecanone, nonanone and their acetates. Corduan and Reinhard (1972) investigated the influence of several factors on volatile oil composition in tissue cultures of *Ruta graveolens*. Autotrophic or heterotrophic conditions did not affect composition. However, drastic differences were found between light- and dark-grown callus cultures. The compsosition of light-grown cultures corresponded to that of leaf tissue.

TABLE 4

Miscellaneous secondary products in plant tissue culture

Source and type of Culture	Nature of Constituents	References
Cuscuta	Chlorophyll	Loo (1946)
Rumex virus tumour tissue	Chlorophyll	Nickell and Tulecke (1961)
Sequoia stem callus	Chlorophyll	Ball (1950)
Carrot	Chlorophyll	Audus (1959)
Nicotiana Crown gall	Chlorophyll	Van Lith-Vroom et al (1960)
Haplopappus	Chlorophyll	Blakey and Steward (1961)
Dolichos lablab	Chlorophyll	Kumar (1974)
Taxus, Ginkgo and Pseudolarix	Chlorophyll	Tulecke (1961)
Rutagraveolens leaf callus	Essential oils and terpenoids	Kubeczka (1967), Reinhard et al (1968)
Lindera strychnifolia	sesquiterpenes	Tomita et al (1969)
Andrographis paniculata	abnormal terpenoids	Butcher (1971)
Happlopapapus gracilis	anthocyanins	Andenne (1965)
Parthenocissus tricuspidata	anthocyanins	Stanko and Bredinskaya (1962)
Corn endosperm	anthocyanins	Straus (1959)
Carrot	anthocyanins	Sugano and Hayashi (1967)
Dimorphotheca auriculata		Harborne et al (1970)
Jerusalem artichoke tuber callus	anthocyanins	Ibrahim et al (1971)
Acer pseudoplatanus suspension	leucoanthocyanins	Goldstein et al (1962)
Carrot callus cultures	carotenoids	Caplin and Steward (1949)
Carrot gall tumours	carotenoids	Nickell and Tulecke (1961)
Lemon fruit cultures	flavonoids	Korden and Morgenstern (1962)
Avocado	antibiotics	Nickell et al (1962)
Lettuce and cauliflower	antibiotics	Campbell et al (1965)
Ipomoea violaceae	antiobitics	Mathes (1967)
Tephrosia sp.	antibiotics	Sharma and Khanna (1974)
Sequoia callus	tannins	Ball (1950)
White spruce suspension	tannins	Chafe and Durzan (1973)
Daucus, Ipomoea and Phaseolus suspensions	lignins	Leppard et al (1971)
Guayule stem culture	rubber	Arreguin and Bonner (1950)
Tea plant stem callus	caffeine and theobromine	Ogutuga and Northcote
Theobroma cacao callus and suspension	polyphenol, caffeine, theobromine and theophylline	Hall et al 1974)

Rubber

Guayule stem tissue cultures, grown on solid media, produced rubber equal to the normal percentage (0.1%) present in the tree stem, or could be increased 1–3 fold in the presence of acetone, sodium acetate, *B*–methyl crotonic acid, or their combinations (Arreguin and Bonner 1950).

Polyphenol, Caffeine

Ogutuga and Northcote (1970) established callus tissue from segments of the stems of the tea plant and found that they produced Caffeine (1,3,7-trimethylxanthine). The Caffeine was found in the tissue and was also present in the growth medium. Theobromine was also produced by the tea callus tissue. Hall et al. (1974) investigated the production of polyphenol, caffeine, theobromine and theophylline in the callus and suspension cultures of theobroma Cacao.

Summary

Table 4 lists the secondary products, mainly essential oils and terpenoids, anthocyanins, chlorophyll, carotenoids, flavinoids, antibiotics, tannins, lignins, rubber and polyphenol biosynthesized in cultures of higher plants.

CONCLUSION

During the last quarter of a century, fermentation techniques have developed extensively, and numerous useful substances such as antibiotics, amino acids, enzymes etc. have been produced on an industrial scales. On the other hand, the techniques of plant tissue culture have also progressed and advances in this field during the past decade have raised the possibility of plant cell cultures for a similar production of metabolites.

As emphasized in excellent reviews by Puhan and Martin (1971), Mitra (1966) and others, industrial application of plant tissue culture to the production of useful substances seems to be difficult because of the slow growth of plant cells, the low content of many secondary compounds accumulated and many other problems. For solution of these problems studies should be carried out on the problems of isolation of appropriate biochemical mutants, production of high cost compounds such as drugs, improvement of suspension culture techniques on a large scale and so on.

In spite of many problems, it is believed that application of plant cell cultures to industrial production of therapeutic compounds and physiologically active substances will before long be realized.

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BIBLIOGRAPHY

Alfermann, A. W., 1974; 3rd International Congress of Plant Tissue and Cell Culture, Leicester, U.K. (Abstracts), 152.
Alleweldt, G. and Radler, F., 1962; Plant Physiology, 37: 376–379.
Anonymous, 1964; Drug Trade News, Nov. 9: 45–69.
Ardenne, R., 1965; Z. Naturforschg. 20: 186.
Arreguin, B and Bonner, J., 1950; Archives of Biochemistry and Biophysics, 26: 178–186.
Adus, L. J., 1959; "Plant Growth Substances", Leonard Hill (Books) Ltd., London.
Babcock, P. A. and Plotkin, G. R., 1967; Lloydia 30: 285.
Bajaj, Y. P. S. 1966; Phyton 23: 57–62.
Ball. E., 1950; Growth 14: 295–325.
Barker, H.A., 1956; "Bacterial Farmentation", Wiley, New York.
Barnoud, F., 1965; Proceedings of the International Conference on Plant Tissue Culture, Pennsylvania State, University:
Becker, G.E., Hui, P.A. and Albersheim, P., 1964; Plant Physiology 39 913 - 920.
Beckhorn, E.J., 1962; Drug Cosmetic Industry 90 : 680 - 682.
Benveniste, P., 1968; Phytochemistry 7 : 951 - 953.
Benveniste, P., Hirth, L. and Ourisson, G., 1964; Comptes Rendues des Sciences de l'Academic de Sciences 258 : 5515 - 5518.
Benveniste, P., Hirth, P. and Ourisson, G. 1966; Phytochemistry 5 : 31 - 44.
Benveniste, P., Hirth, L. and Ourisson, G., 1966; Phytochemistry 5 : 44 - 58.
Benzamin, B.D. and Mulchandani, N.B, 1973; Planta Medica 23 : 394-397
Bergmann, L., 1964; Planta 62 : 221-254.
Blakely, L.M. and Steward, F.C., 1962; American Journal of Botany; 49 : 653.
Blakely, L.M. and Steward, F.C., 1961; American Journal of Botany 48 : 351 - 3.58
Bleichert, E.F., 1964. Unpublished results.
Bove, J., Bove, C. and Raveux, G., 1957; Revue General de Botanique 64 : 572 - 592.
Brain, K.R., Lockwood, G.B. and Turner T.D., 1973; Pharm. Aikak (Abstracts), 5.
Brakke M.K. and Nickell L.G., 1955; Annals of Biology 31: 215-226
Brakke, M.K. and Nickell, L.G., 1951; Archives of Biochemistry and Biophysics 32 : 28—41.
Braun, A.C. and Stonier, T, 1958; Protoplasmatologia 10 (5a) : 1—93, Wien, Springel—Verlag.
Buchner, S.A. and Staba, E.J., 1963; Lloydia 6 : 208.
Buchner, S.A. and Staba, E.J., 1964; Journal of Pharmacy and Pharmacology 16 : 733 - 757.
Buckholder, P.R. & Nickell, L.G., 1949; Botanical Gazette 110 : 426—431
Butcher, D.N., 1971; International Symposium on Morphogenesis in plant. cell, tissue and organ culture, New Delhi, India.
Byrne, A.F., 1962; Activities Report 14 : 177 - 180.
Byrne, A.F. and Koch, R.B., 1961; Science 35 : 215 - 216.
Campbell, G., Chan, E.C.S. and Barker, W.G., 1965' Canadian Journal of Microbiology 11 : 785 - 788.
Caplin, S.M. and Steward, F.C., 1949; Nature 163 : 920 - 921.
Carew, D,P., 1971; Internaional Symposium on morphogenesis in plant cell tissue and organ cultures, Delhi.
Carew, D.P. and Krueger, R.J., 1974, 3rd International Congress of Plant tissue and Cell culture, Leicester, (Abstracts) 178.
Carew, D.P. and Staba, E.J., 1965; Lloydia 28 : 1 - 26.
Chafe, S.C. and Durzan, D.J., 1973; Planta; Berl. 113 : 251 - 262.
Chan, W.N. and Staba, E.J., 1965; Lloydia 28 : 55 - 62.
Constabel, F., 1971; Planta 96 : 306 - 316.
Constabel, F., Gamborg, O. L., Kurz, W.G.W. and Steck, W., 1974; Planta Medica 25 : 158 -165.
Corduan, G. and Reinhard, E., 1972; Planta Medica 11:917-922. Dauranton, H. 1958; Comptes Rendues de Seances de l'Academie de Science 246 : 3095 - 3098.
Dawson, R.F., 1942; American Journal of Botany 48 : 813 - 815.
Dawson, R.F., 1960; American Scientist 48 : 321 - 340.
De Capite, L., 1955; American Journal of Bo any 42 : 869.
Dhoot, G. K. and Henshaw, G. G., 1974; 3rd International Congress of Plantt issue and Cell culture, Leicester, (Abstracts) 258
Ehrhardt J. D., Hirth L. and Ourisson, G., 1967, Phytochemistry 6 : 815 - 821.
Elze, H. and Teuscher, E., 1967; Flora (Jena) N.S. 158 : 127 -132
Evans, W.C. and Partridge, M.W., 1953; Journal of Pharmacy & Pharmacology 5 : 293 - 300.
Fox, J.E. and Miller, C., 1959; Plant Physiology 34 : 577 - 579
French, D. I. and Gibson, M.P., 1957; American Pharmaceutical Association Journal 46 : 151 - 155.
Fritz, B., Hirth, L. and Ourisson, G., 1966: Comptes Rendues de Seances de l'Academie de Science 263 : 860 - 863.
Fukami, T. and Hilderbrandt, A.C., 1967; Botanical Magazine (Tokyo) 80 : 199 - 212.
Furuya, T. and A, Ikuta, 1968; Chemical & Pharmaceutical Bulletin (Tokyo) 16 : 771.
Furuya, T., Ikuta, A. and Syono K. 1972; Phytochemistry 11, 3041 -3044.
Furuya, T., Kojima. H. and Syono, K. 1967; Chemical & Pharmaceutical Bulletin (Tokyo) 15 : 901 - 903.
Furuya, T. Kawaguchi, K. and Hirotani, M. 1973: Phytochemistry 12 : 1621 - 1626
Furuya T., Shibata, S. and Iizuka, H. 1966; Journal of Chromatography 21: 116 - 118
Gainor, C. and Crisley, F. D., 1962; Nature 193: 1076–1077.
Gamborg, O. L. and Finlayson, A. J., 1969; Canadian Journal of Botany 47: 1857–1863
Gamborg, O. L. and La Rue, T. A. G. 1968; Nature 220: 604–605.
Gautheret, R. J., 1959: "La Culture des tissues vegetaux techniques et realisations", Masson, Paris.
Gautheret, R. J., 1966; "Cell Differentiation and Morphogenesis- International Lecture Course, Wageningen, The Netherlands" pages 55–95. North-Holland Publ., Amsterdam.
Gentile, A. C., 1965; "Tissue Culture" (ed. C. V. Ramkrishanan), W. Zunk, The Hague.
Gibson, M. R. and Danquist, G. A., 1965; Journal Pharmaceutical Science 54 1526–1528.
Goldstein, J. L., Swain, T. and Tjhio, K. H., 1962; Archives of Biochemistry and Biophysics 98: 176–178.
Goris, A., 1965, Annales Pharmaceutiques Francaises 23: 275–279.
Gray, W. D., 1959; "The relation of fungi to human affairs"–Henri Holt & Co. Inc. New York.
Griitzmann, K. D., and Schroter, H. B., 1966; "Biochemie und Physiologie der Alkaloide" (ed K. Mothes) 3, page 347.
Hadwiger, L. A. and Waller, G. R., 1964; Plant Physiology 39: 244–246.
Hall, T.R.H., Foiziel, Jelal, M.M.A. and Collin, H.A., 1974; 3rd International Congress of Plant tissue and Cell culture, Leicester, (Abstracts) 220.
Harbone, J. B., 1963; "Chemical Plant Taxonomy" (T. Swain, ed.) Academic Press, New York, page 618.
Harbone, J. B., Arditte, J. and Ball, E. A., 1970; American Journal of Botany 57: 754.
Heble, M. R., Narayanswami, S. and Chadha, M. S., 1971; Phytochemistry 10: 2393–2394.
Heble, M. R. Narayanswami, S. and Chadha, M. S., 1968., Science 161: 1145.
Heble, M. R., Narayanswami, S. and Chadha, M. J., 1968, Naturwissenschaften 55:350-351.
Hilderbrandt, A.C., Wilmar, J. C., Chan, P.K., Rajgopal, M. V. and Riker, A. J., 1962; Plant Physiology 37: 854.
Hilderbrandt, A.C., Wilmar, J. C., Hohns, H. and Riker, A. J., 1963; American Journal of Botany 50:248–254.
Howe, K. J., 1951; M. Sc. Thesis, University of Rochester "The structure and development of the mint plant Mentha piperita L., with speical reference to the secretion of essential oils".
Hughes, M. A., 1968; Journal of Experimental Botany, 19: 52–63.
Ibrahim, R. K., Thakur, M. L. and Premanand, B., 1971; Lloydia 34: 175–182.
James, W.O. 1946; Nature 158: 377-378
Jaspers, E. M. J. and Veldstra, H., 1965; Physiologiae Plantarum 18 933–940
Karsten, W.K.H. and V. De Meester-Mangercats, 1960; Acta Botanica Neerlandica 9:263–274.
Kaul, B. and Staba, E. J., 1968; Lloydia 31:171–179.
Kaul, B. and Staba, E. J., 1965; Science 150: 1731–1732.
Kaul, B., Stohs, S. J. and Staba, E.J., 1969; Lloydia 32: 347–359.
Kent, A. E., 1966; Ph. D. Thesis, Cornell University "The totipotency of cultured plant cells: Its control during development and morphogenesis."
Khanna, P. and Staba, E. J., 1968; Lloydia 31:180–189.
Khanna, P. and Mohan, S., 1973; Indian Journal of Experimental Biology 11: 58–60.
Khanna, P. and Mohan, S., 1971; International Symposium on Morphogenesis in Plant cell. tissue and organ cultures, Delhi.
Khanna, P., Nag, T. N., Jain, S. C. and Mohan, S., 1974; 3rd International Congress of Plant tissue and Cell culture Leicester, (Abstracts), 257
Klein, A. O., and Morgan, C. W., Jr., 1961; Plant Physiology 36:1.
Klein, R. M., Caputo, E. M. and Witterholt, B. A., 1962; American Journal of Botany 49: 323.327.
Koblitz, H., 1962; Faserforschung und Textiltechnik 13: 270–275
Koblitz, H, 1966; Papier 20: 622–627.
Koblitz, H, and Koblitz, D., 1962; Faserforschung und Textiltechnik 13: 571–574.
Koblitz, H., Grutzmann, K. and Hagen, I, 1967; Z. Pflanzenphysiol. 56 27–32.
Koichi, Y.M. Suzuki and M. Maruoka, 1974; Chemical Abstracts 80: 743249:.
Kordan, H. A., 1962; Bulletin of the Torrey Botanical Club 89: 49–52.
Kordan, H. A., 1959; Science 129: 779–780.
Kordan, H. A., and Morganstern, L. 1962; Nature 195:63.
Krikorian, A. D., 1965; Ph. D. Thesis, Cornell University "The synthetic potentials of cultured plant cells and tissues".
Krikorian, A. D. and Steward, F. C., 1969; "Plant Physiology" (Ed. F. C. Steward), Academic Press, New York, pages 227–326.

Kubeczka, .K. K., 1967; Flora Abstracts A. 158: 519.
Kumar, A., 1974; 3rd International Congress on Plant tissue and Cell culture, Leicester, (Abstracts), 289.
Lampont, D.T.A. and Northcote, D.H., 1960; Nature 188: 665–666.
Lee, P.K., Carew, D. P., and Rosazza, J., 1972; Lloydia 35: 150–156.
Lin, M. and Staba, E.J., 1965; Lloydia 24:139–145.
Lippard, G. G., Ross Colvin, J. Dyson Rose and Marin, S.M., 1971; Journal of Cell Biology 50:63–80.
Loo, S., 1946; American Journal of Botany 33:295–299.
Mahlberg, P. G. and Venketeswara, S, 1962; American Journal of Botany 49: 655.
Mahlberg, P.G., and Venketeswaran, S. 1966; Botanical Gazette 127: 114–199.
Mandels M., Jeffers, J. and El-Bisis, H.M., 1968; Technical Report 69–36–7L. U.S. Army Natick Laboratories.
Mathes M. C., 1967; Lloydia 30: 177–181
Mathes, M. C., 1963; Science 140: 1101–1102
Matthern, R. O., 1962; Activities Report 14:170–176.
Medore, R., Taso, D.P.N. and Albert, L.S., 1967; Journal of Pharmaceutical Science 56:67–72.
Mehta, A. R. and Staba, E.J. 1970; Journal of Pharmaceutical Science 59:864–865.
Metz, H. and Lang, L., 1966; Auslegeschrift, B. D., Patentant No. 1,216,009
Mishra. D., 1966; Naturwissenschaften 53:307.
Mitra, G. C., 1966; Bulletin of National Botanical Gardens 119:1–36.
Mitra, G. C. and Kaul. K. N. 1964; Indian Journal of Experimental Biology 2:49–51.
Moham Ram. H. Y. and Steward, F. C., 1964; Canadian Journal of Botany 42:564–565.
Naef, J. Turian, G., 1963; Phytochemistry 2:173–178.
Neumann, von D. and Muller, E., 1971; Biochemie und Physiologie der. Pflanzen 162: 503–513.
Neuss, N., Johnson, I. S., Armstrong, J. G. and Jansen, C. J., 1964; Advan Chemotherapy 1:133–174.
Nickell, L. G., 1962; "Advances in Applied Microbiology" vol. 4: Academic Press, New York, pages 213–236.
Nickell L.G., 1958; Phyton 11: 93–96.
Nickell, L. G., 1959; Economic Botany 13: 281–318.
Nickell, L. G. and Brakke, M. K., 1954; American Journal of Botany 41:390–394.
Nickell, L. G. and Tulecke, W. R., 1961; "Plant Growth Regulation" pages 675–685, 4th International Conference Iowa State University Press Iowa, U.S.A.
Norstog, K. J., 1956; Botanical Gazette 117: 253–259.
Ogutuga, D.B.A. and Northocote, D.H., 1970; Journal of Exp. Botany 21:258–273.
Peacock, S.M., Leyerle, D.B. and Dawson, R.F. 1944; American Journal of Botany 31:463–466.
Petiard, V. and Demarly, Y., 1972; Ann. Amelior Plantes 22: 361–374.
Petiard, V., Demarly, Y. and Paris, R R. 1972; Plantes medicinales et phytotheripie 1:41–49.
Puhan, Z. and Martin, S.M., 1971; "Progress in Industrial Microbiology", 9:13–39.
Rai, P. P. and Turner, T. D., 1974; 3rd International Congress of Plant tissue and Cell cutlure, Leicester (Abstracts) 155.
Rai, P. P., Turner, T. D., and Greensmith, S. L., 1974; Journal of Pharmacy and Pharmacology 26:722–726.
Rangaswamy, N. S., 1958; Experientia 14: 111–112.
Rangaswamy, N. S., 1961; Pytomorphology 11:109–127.
Rangnathan, B., Mascarenhas, A. F., Sayagaver, B. M. and Jagannathan V., 1963 "Symposium of plant tissue organ culture, Delhi", pages 108–110.
Rau, W., 1967; Planta 74: 263–277.
Reinhard, E., Corduan, E. G. and Volk, O. H., 1968; Phytochemistry 7:503:504.
Reinouts van Haga, P, 1957; Abhandl. deut. Akad, Wiss. Berlin Kl. Chem. Geol. Biol. 7: 102–105.
Routien, J. B. and Nickell, L. G., 1956; U. S. Pattern(s) 2,747,334.
Sabharwal, P. S., 1962; Plant Embryology Symposium. Council of Scientific and Industrial Research, New Belhi, India, Pages 239–243.
Sabharwal, P. S., 1963; Plant tissue organ culture Symposium, University of Delhi, pages 265–274.
Sabharwal, P.S., 1961; Symposium on plant tissue and organ culture, University of Delhi and UNESCO South Asia Science Conference Office. New Delhi.
Sander, H., 1956; Planta 47: 374–400.
Sargent, J. A. and Skoog, F., 1960; Plant Physiology 35: 934–941.
Sargent, J. A. and Skoog, F., 1961; Physiologiae Plantarum 14: 504–519.
Sarkisova, M. A., 1974; 3rd International Congress of plant tissue and cell culture, Leicester (Abstracts) 259.
Scott, K. J., Daly, J. and Smith, H. H. 1964; Plant Physioology 39: 709–711.
Scott, R. W., Burris, R. H., and Riker, A. J., 1955; Plant Physiology 30:355–360.
Sharma, R. and Khanna, P., 1974; 3rd International Congress on Plant tissue and Cell culture, Leicester (Abstracts) 77.
Skinner, F. A., 1955. "Antibiotics" (Eds. K. Peach and M. V. Tracey) vol. 3. Springer, Verlag. pages 626–724.
Skoog, F. and Montaldi, E., 1961; Proceedings of the U.S. Academy of Science 47: 36–49.
Slabecka-Szweykowska, A., 1952; Acta Botanica Sec. Poloniae 21:537.
Smirnov, A. M. and Kuzovkina, I. N., 1974; 3rd International Congress of Plant tissue and Cell culture, Leicester (Abstracts) 180.
Solt, M. L., 1957; Plant Physiology 32:480–484.
Speake, T., McCloskey, P., Smith, W. K. Scott, T. A., and Hussey, H., 1964; Nature 201:614–615.
Spurr, H.W. Jr., Holcomb, G. E. Hilderbrandt, A. C. and Riker, A. J., 1962; Plant Physiology 37: Suppl. 731.
Staba, E. J., 1963; Development in Industrial Microbiology 4: 193–198.
Staba, E. J., 1962: Journal of Pharmaceutical Science 51:249–254.
Staba, E. J. and Laursen P., 1966; Journal of Pharmaceutical Science 55: 1099–1101.
Staba, E. J., Laursen P. and Buchner, S. A., 1963; Proceedings of International Conference on plant tissue culture, Pennsylvania State University, pages 191–210.
Staba, E. J., and Lamba, S. S., 1963; Lloydia 26: 29–35.
Stanko, S. A. and Bradinskaya, M. S., 1962; Proceedings of Academic Science of USSR 146:1152.
Steck. W., Bailey, B. K., Shyluk, J. P. and Gamborg, O. L., 1971; Phytochemistry 10:191–194.
Steinhart, C. E., 1962, Science 137:545–546.
Stevens, P. G. and Hardman, R, 1974; 3rd International Congress on plant tissue and cell culture, Leicester (Abstracts) 75.
Steward, F. C., Mapes, M. O. and Mears, K., 1958; American Journal of Botany 45:705–708.
Steward F. C. Mapes M. O., Kent, A. E. and Holsten, R. D., 1964; Science 143:20–27.
Steward, F. C., Kent, A. E. and Mapes. M. O., 1966; Current Topics in Developmental Biology 1:113–154.
Steward, F. C., Shantz, E. M., Pollard, J. K., Mapes, M. O. and Miltra, J., 1961; "Molecular and cellular Structure" Ronald Press Co., New York, pages 193–246.
Steward, F. C., Thompson, J. F. and Pollard, J. K. 1958; Journal of Experimental Botany 9:1–10.
Stohs, S. J. 1969; Journal of Pharmaceutical Science 58: 703–705.
Stohs, S. J. and Staba, E. J., 1965; Journal of Pharmaceutical Science 54: 56–58.
Straus, J., 1959; Plant Physiology 34:536.
Straus, J., 1962; Plant Physiology 37:342–348.
Straus, J. and Campbell, W. A., 1963; Life Sciences 1:50–62.
Street, H. E., Henshaw, G. E. and Bulatti, M. C., 1965; Chemistry and Industry (London) 27–33.
Sugano, N. and Hayashi, K., 1967; Botanical Magazine (Tokyo) 80:440.
Suhadolnik, R. J., 1964; Lloydia 27:315–321.
Telle, J. and Gautheret, R. J., 1947; Comptes Rendues des Seances de l'Academie de Science 224: 1653–1654.
Thimann, K. V. and Edmondson, Y. H. 1949; Archives of Biochemistry 22:33.
Tomita, Y., and Uomori, A., 1971; Chemical Communications: 284.
Tomita, Y., Uomori, A. and Minato, H., 1970; Phytochemistry 9:111.
Tomita Y., Uomori A. and Minato, H., 1969; Phytochemistry 8: 2249–2252.
Torrey, J. G. 1957: Proceedings of the U.S. National Academy of Science 43:887–891.
Tukey, H. B., 1938; Botanical Gazette 99: 630–665.
Tulecke, W., 1961; Bulletin of the Torrey Botanical Club 88: 350–360.
Tulecke, W., 1963; Tech. Documentary Report AMRL–TDR–124, New York.
Tulecke, W., 1963; REPT RM RL-TR–65–101. Aerospace Medical Researh Lab. Aerospace Medical Division, Airforce Systems Command, Wright Patterson Airforce Base, Oiho USA.
Tulecke, W., and Nickell, L. G., 1960; Trans. N. Y. Acad. Sci. 22: 196–206.
Tulecke, W., Weinstein, L. H., Rutner, A. and Laurencot. H. J., Jr., 1962; Contributions to the Boyce Thompson Institute 21:291–302.
Tyron, K., 1956; Science 123:590.
Vagujfalvi, D., Maroti, M. and Tetenyi, P., 1971; Phytochemistry 10: 1389–1390.
Vanlith-Vroom, M.L., Gottenbos, J. J. and Karstens, W. K. H., 1960; Acta Botanica Neerlandica 9:275–285.
Van Haga, P. R., 1954; Pharmaceutisch Weekblad 89: 71–72.
Vasil, I. K. and Hilderbrandt, A.C., 1966; Planta, Berl. 68:69–82.
Vasil, . K. Hilderbrandt, A.C. and Riker, A. J., 1964; American Journal of Botany 51:677.
Veliky, I. A., 1972; Phytochemistry 11: 1405–1406.
Warwick, R. P. and Hilderbrandt, A. C., 1966; Plant Physiology 41: 573–585;
Weinstein, L. H., Nickell, L. G. Laurenoct, H. J., Jr., and Tulecke, W., 1959; Contributions to the Boyce Thompson Institute 20: 239–260.
Weinstein, L. H., Tulecke, W., Nickell, L. G., and Laurencot, H. J., Jr., 1962; Contributions to the Boyce Thompson Institute 21:371–386.
West, F. R. Jr., and Mika, E. S., 1957; Botanical Gazette 119: 50–54.
Wetmore, R. H., and Rier J. P. 1963; American Journal of Botany 50:418–430.
White, P. R., 1963; "The Cultivation of Animal and Plant Cells" 2nd Ed. Ronald Press, New York.
White, P. R., 1945; American Journal of Botany 32: 237–241.
Wickremasinghe, R. L. and Swain, T., 1965; Phytochemistry e: 687–691.
Witham, F. H. and Gentile, A.C., 1961; Journal of Experimental Botany 12:188–198.
Yanagawa, H., Kato T. and Kitahara, Y., 1971; Phytochemistry 10: 2775–2780