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PLASTID FUNCTION IN PLANT TISSUE CULTURES. I. PORPHYRIN SYNTHESIS BY DARK-GROWN HAPLOID AND DIPLOID ALBINO CULTURES[1]

WALTER TULECKE

Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y.

ABSTRACT

Tissue cultures lacking chlorophyll formed porphyrins when fed δ-aminolevulinic acid, a precursor of tetrapyrroles. When grown in the dark tissues from *Ginkgo biloba* L., *Taxus*, and *Rosa* formed protoporphyrin and several unidentified compounds. When grown in the light cultures did not form these pigments. The protoporphyrin was detected in the tissues after 3–6 hours incubation with δ-aminolevulinic acid; it was localized in the plastids by ultraviolet light microscopy and was identified by extraction procedures, chromatography, and absorption spectroscopy. No magnesium protoporphyrins were found, suggesting that chlorophyll synthesis was blocked at this point. Both male and female haploid albino tissues from *Ginkgo* formed protoporphyrin. The female albino tissue was derived from a chlorophyll-containing tissue culture from the female gametophyte by serially subculturing the green tissue in the dark. Upon exposing the female albino tissue to light, no greening occurred. The treatments used thus far have not caused chloroplasts to develop in the haploid albino tissues, even though the tissues contain many amyloplasts. Concurrent with the loss of chloroplasts, the female tissue loses all capacity to differentiate specialized cells, such as tracheids, resin cells, and chlorenchyma.

MANY TISSUE cultures of higher plants lack chlorophyll and functional chloroplasts, even though the cultures may be derived from green photosynthetic plant parts. Some cultures retain their functional chloroplasts, but the chlorophyll content is often less than that in the intact plant (Vasil and Hildebrandt, 1966). Other tissues deficient in chlorophyll may also be derived from the male gametophyte of gymnosperms; these tissues are undifferentiated, haploid, albino, and form only amyloplasts (Tulecke, 1960, 1964). The restricted function of plastids from the male gametophyte is consistent with studies on maternal inheritance, i.e., that the plastids from the male gametophyte do not function as chloroplasts and do not contribute to the chloroplast population of the next generation. Only the plastids from the egg cell pass to the zygote; the plastids from the male gametophyte either degenerate or are excluded soon after fertilization. Within the male gametophyte, however, and in tissue cultures derived from them the plastids may function as amyloplasts; rarely (usually never) do these plastids develop into chloroplasts.

Thus the lack of chlorophyll synthesis in certain tissue cultures may be the result of inherent biochemical deficiencies of the cells or it might be the result of nutritional or environmental conditions provided. In the present study several haploid and diploid albino tissue cultures of higher plants were fed δ-aminolevulinic acid (ALA), a precursor of tetrapyrroles such as the porphyrins and chlorophyll. Some of the porphyrins formed from ALA were determined and the conditions for their synthesis defined.

The experimental approach of using precursors to study blocks in biosynthetic pathways has been (Bogorad and Jacobson, 1964; Marschner, 1965), the study of *Chlorella* mutants (Granick, 1955), photosynthetic bacteria (Lascelles, 1964), and animal systems (Granick, 1966). In some cases, such as the work of Carell and Kahn (1964) with *Euglena*, the studies have been carried out on isolated chloroplasts.

The common biosynthetic pathway for tetrapyrrole synthesis is via glycine and succinic acid condensation in the presence of pyridoxal phosphate and δ-aminolevulinic acid synthetase to form δ-aminolevulinic acid (ALA). ALA in turn forms porphobilinogen (PBG), uroporphyrinogen, coproporphyrinogen, and protoporphyrinogen. The latter three compounds form uroporphyrin (URO), coproporphyrin (COPRO), and protoporphyrin (PROTO), respectively. Magnesium is thought to be inserted into protoporphyrinogen to yield magnesium protoporphyrin (MgP) followed by magnesium protoporphyrin monomethylester, magnesium vinyl protoporphyrin, chlorophyllide, and, finally, chlorophylls *a* and *b* (Granick, 1963b; Lascelles, 1964).

MATERIALS AND METHODS—*Tissues*—The tissue cultures used in these studies were the

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following: male albino and female green and albino tissues from the gametophytes of *Ginkgo*, diploid green tissues from the leaf and stem of *Ginkgo*, a pollen-derived haploid tissue from *Taxus* sp. (Tulecke, 1959), diploid tissues from the stems of *Rosa* sp. (Tulecke, Taggart, and Colavito, 1965), leaves of *Agave toumeyana*, leaves of *Lactuca sativa*, petioles of *Rheum rhaponticum*, tubers of *Solanum tuberosum*, and the endosperm of *Zea mays* (Straus, 1960). The tissues of *Agave* and *Rheum* occasionally showed a small amount of green pigmentation when grown on defined media.

The defined medium used for *Ginkgo*, *Taxus*, *Rosa*, *Lactuca*, *Rheum*, and *Agave* tissues was the same as that cited for the female gametophyte of *Ginkgo* (Tulecke, 1964); the medium for *Zea* was that of Straus (1960); and for *Solanum*, White's basal plus 6.0 ppm 2,4-dichlorophenoxyacetic acid and 18% coconut water. The tissues were maintained routinely on these media in test tubes in the light in a 12-hr-day–12-hr-night photoperiod. The tissues used for studies on porphyrin synthesis from ALA were grown in the dark, since light was inhibitory to porphyrin formation.

Haploid tissue cultures derived from the male and female gametophytes of *Ginkgo biloba* L. are very different in their growth, pigment production, and their capacity to differentiate (Tulecke, 1965). The male tissue is an albino which forms amyloplasts but no chloroplasts; the tissue grows well on a defined medium and does not differentiate. In contrast, the tissue from the female gametophyte is green, forms chloroplasts, and differentiates resin, tracheid, and chlorenchyma cells. Chromosome counts on both tissues show the majority of cells to be haploid ($n = 12$). Tissue cultures of leaf and stem from *Ginkgo* are green and show the same cell types as the green female haploid tissue cultures.

The albino strain of the green female tissue was derived by repeated subculture of the original green tissue in the dark over a 3- to 5-month period. The bleaching occurred gradually from green to brown to white. The tissue lost its capacity to differentiate specialized cell types, lost its chlorophyll, and became brown; the brown tissue consisted almost entirely of elongated parenchyma cells. At this stage the tissue would regreen when returned to light. If continued in the dark beyond this time, white outgrowths of tissue were formed; these cells were spherical, parenchymatous, and usually formed starch in the light or dark. This white tissue was subcultured as an albino strain in the dark. When grown in the light, the albino tissue did not become green and growth was somewhat inhibited. In most aspects of growth and differentiation, the female albino tissue appeared identical with the male albino pollen-derived tissue. Even a sweet aromatic odor characteristic of the male tissue was also detected from the female albino. The derivation of the albino strain from the green tissue could be obtained repeatedly.

Extraction and analysis—Tissue cultures were harvested, weighed, and ground in cold acetone in a mortar and pestle. After centrifuging, the tissue macerate was re-extracted with acetone until no fluorescence remained in the pellet. The *p*H of the combined supernatants was adjusted to 10.0 with 1 N NaOH, and the aqueous acetone partitioned with petroleum ether in a separatory funnel. This removed carotenoids and lipids. The *p*H was brought to 5.3 with acetic acid and the porphyrins were extracted by partitioning with ethyl ether. The porphyrin content of tissue extracts was estimated by absorption spectroscopy; the peak of the Soret band of extracts was compared to a standard curve for protoporphyrin IX.

The criteria for the identification of porphyrins (URO, COPRO, and PROTO) were (1) the extraction procedure, (2) the absorption spectrum of extracts, and (3) co-chromatography in at least three solvents.

The solvent systems used for paper or thin layer chromatography were *n*-decane–chloroform, 1:9 (DC); kerosene–2,4-pentanedione–methylbenzoate, 12:7:1 (KPM); 2,6-lutidine–1 N NH_4OH–1 M EDTA, 10:4:3 (2,6 L); and kerosene–*n*-propanol, 5:1 (KP). The first two solvents were those of Chu and Chu (1966); other solvents were from Falk (1964).

Chlorophyll, phaeophytins, and carotenoids from the petroleum ether extraction were separated on cellulose columns by stepwise elution with acetone (Duranton, Galmiche, and Roux, 1958). Absorption spectra were used to confirm the pigment class; quantitation of pigments was made using the appropriate formula of Röbbelen (1957).

Experiments on feeding ALA were set up as follows: liquid medium was prepared in Erlenmeyer flasks and 0.5 mM ALA was added by sterile filtration; weighed amounts of tissue were transferred to the medium and the cultures incubated in the dark. The tissues were recovered at different times, but usually after 3–10 days, and extracted for porphyrins. The 0.5 mM concentration of ALA was selected as an optimum level for porphyrin synthesis, based upon preliminary experiments on the dose response of several tissues. Similar extractions of albino tissues grown on media minus ALA were also run; they gave no detectable porphyrins.

The light sources for action spectra were those described by Klein (1964). The peak emission and ranges (mμ) were: far-red, 760 (695–785); red, 660 (620–685); green, 550 (510–585); blue, 420 (385–490).

RESULTS—The first experiments on porphyrin synthesis were carried out on the pollen-derived

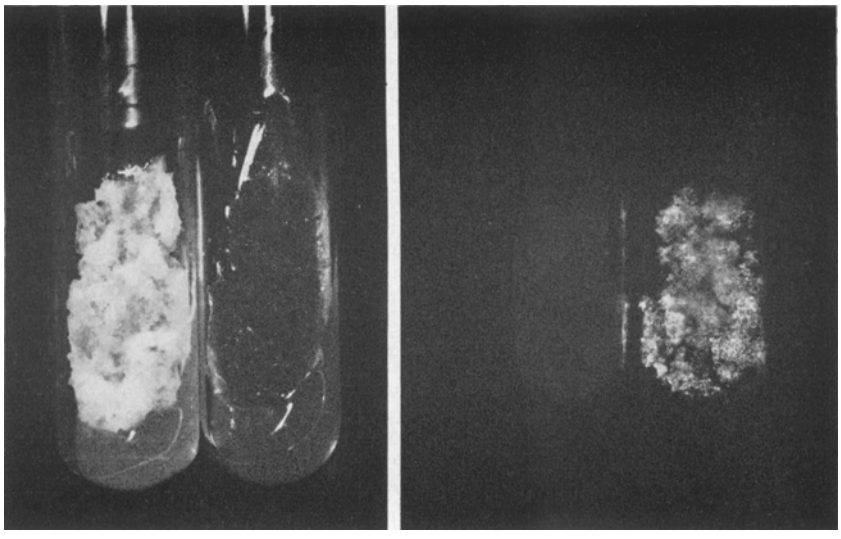

Fig. 1. Pollen-derived tissue of *Ginkgo* grown in the light (on the left in each case) and in the dark (on the right) on media containing δ-aminolevulinic acid. A. Photographed in white light; B. photographed in 366 mμ ultraviolet light.

tissue culture from *Ginkgo biloba*. When grown in the dark on a medium containing 0.5 mM of ALA the tissue turned tan to brown in color and gave a pink to red fluorescence when illuminated with 366 mμ ultraviolet light. No pigment formation was observed when the cultures were grown in the light. This is shown in Fig. 1 A, B. No pigment of this type had been observed previously in this tissue. Exposure of other tissues to similar treatment indicated a variable amount of fluorescence, as shown in Table 1; albino tissues on media without ALA showed no fluorescence.

Ultraviolet microscopy—The examination of tissue cultures by ultraviolet light microscopy was made before and after feeding ALA. It was important for the observer to dark-adapt his eyes for these observations. Cultures not fed ALA showed a small amount of fluorescent material in organelles of the male and female albino tissue cultures of *Ginkgo* and *Taxus* (male); cultures of *Rosa*, *Agave*, *Solanum*, *Zea*, and *Lactuca* gave no fluorescence before feeding ALA. The green tissue cultures of *Ginkgo* (female gametophyte, leaf and stem) and *Rheum* showed strong fluorescence. Tissues fed ALA were also observed and all of the albino tissues mentioned above showed strong localized fluorescence. Some of this fluorescence faded during microscopic examination with strong ultraviolet light. However, the amount of fluorescence in the cells varied with the age of the tissue; younger tissue was more active and showed more fluorescent organelles than older tissue. Similarly, not all organelles in a cell fluoresced; the number that did varied from 1 to 20. This variability from cell to cell can be seen in Fig. 2, which shows clusters of cells from the rose tissue, viewed under UV and bright-field illumination. These factors may also account for some of the variability in estimates of fluorescence of tissues shown in Table 1.

TABLE 1. *Relative porphyrin production by plant tissue cultures grown in the dark on media containing ALA*

Tissue	Estimation of fluorescence	Protoporphyrin, μg/g fresh weight
Ginkgo biloba L.		
Female albino	+++++	21.0
Male albino	+++++	40.0
Taxus sp.	+++	4.0
Rosa sp.	++++	5.3
Agave toumeyana Trel.	+++	—
Lactuca sativa L.	+	—
Rheum rhaponticum L.	+	—
Solanum tuberosum L.	+++	—
Zea mays L.	+	—

The nature of the fluorescing material in the control non-green tissues is not known. Extracts of the controls gave spectra with very low absorption around 400 mμ ,but there was insufficient material for chromatographing. The fluorescence in the control may be attributed to other tetrapyrrole compounds, such as cytochromes, catalase, or peroxidase; however, these compounds were not identified in the present study.

Extractions—Extracts of most tissues treated with ALA showed the typical absorption spectrum for protoporphyrin IX (Fig. 3). The Soret band at about 405 mμ is diagnostic for PROTO and was obtained from the male albino, female albino, and rose tissues. The *Taxus* tissue extract, however, gave a different absorption spectrum; this is shown in Fig. 3 and Table 2. Chromatographic results confirmed these findings and also showed that

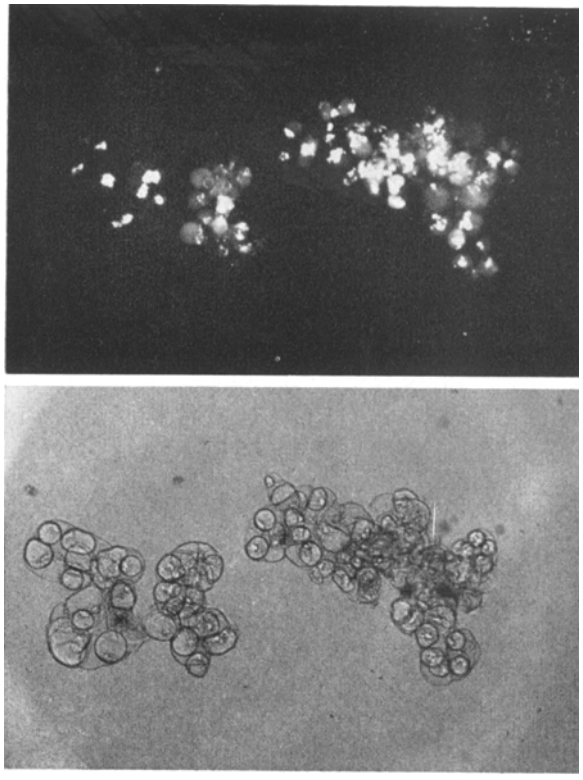

Fig. 2. Absorption spectra of ether extracts of plant tissue cultures grown in the dark on media containing δ-aminolevulinic acid; P, protoporphyrin standard; M, male gametophyte tissue from *Ginkgo*; F, female gametophyte tissue from *Ginkgo*, albino strain; R, 'Paul's Scarlet' rose stem tissue; T, *Taxus* sp. pollen derived tissue.

additional unidentified compounds were present in some extracts.

Chromatography—Ascending paper chromatography of tissue culture extracts in four solvent systems (Fig. 4) indicated the presence of protoporphyrin in the male and female albino tissues of *Ginkgo* and in the rose tissue treated with ALA. Traces of unidentified fluorescent compounds were also detected in some solvents. The fluorescent compounds from *Taxus* were markedly different from those obtained from the other tissues. In three solvents the major spot was coordinate with COPRO. However, in the 2,6-lutidine solvent the major spots were the lowest (#1) and the middle (#2); the highest spot (#3) was faint (see Fig. 4). Absorption spectroscopy of these fluorescing compounds after elution from chromatograms gave Soret peaks for spots 1, 2, and 3 of 395, 394, and 403 mμ respectively. The latter reading for spot #3 substantiates the presence of PROTO in trace amounts. Co-chromatography of spots #1 and #2 with COPRO in three solvents (DC, KP, and KPM) and the absorption spectrum were considered tentative evidence for the presence of COPRO in the *Taxus* tissue culture. The

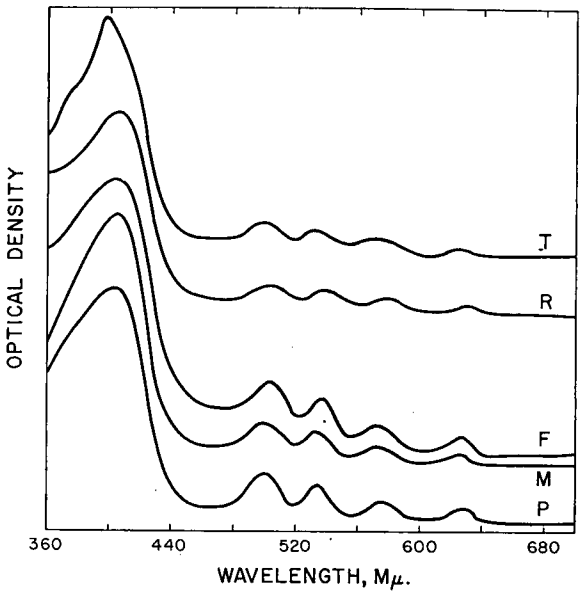

Fig. 3. Chromatography of ether extracts from four plant tissue cultures grown in the dark on media containing ALA, compared to porphyrin standards. The spots indicated are those fluorescing under 366 mμ ultraviolet light; standards: U, uroporphyrin, C, coproporphyrin, P, protoporphyrin; extracts of tissues: M, *Ginkgo* male albino, F, *Ginkgo* female albino, R, rose, T, *Taxus*; solvents: 2,6 L, 2,6 lutidine—1 N NH_4OH–1.5 mM EDTA (10:4:3); DC, *n*-decane–chloroform (1:9); KPM, kerosene–2,4 pentanedione–methylbenzoate (12:7:1); KP, kerosene–propanol (5:1).

results from 2,6-lutidine did not confirm this, hence the major products formed from ALA (spots #1, #2) are unknowns which are not coordinate with the standards URO, COPRO, or PROTO.

The identity of PROTO extracted from the female albino tissue culture from *Ginkgo* (grown on media containing ALA) was confirmed by one additional procedure, i.e., the conversion of the extracted protoporphyrin acid to the ester by esterification in methanol-sulfuric acid (Falk,

TABLE 2. *Absorption spectra of porphyrins extracted from plant tissue cultures fed δ-aminolevulinic acid*

Tissue	λ max. of tissue extracts in ether				
	Soret	IV	III	II	I
Ginkgo (n)					
Female albino	404	502	535	576	628
Male albino	404	503	535	576	630
Taxus (n)	397	500	533	572	624
Rosa (2n)	406	504	538	578	630
Coproporphyrin std.[a]	397	498	527	568	623
Protoporphyrin std.[a]	404	503	536	576	633

[a] Values from Falk (1964).

1964). A similar reaction was run on the PROTO standard and both esterified samples were co-chromatographed in three solvents; the R_F was identical in both samples.

The chlorophyll and carotenoid content of the female green gametophyte tissue culture of *Ginkgo* grown in the light (approximately 250 ft-c) was compared to the content of the same pigments in the female gametophyte from seeds. Acetone extracts were made of the tissue culture (serially transferred once a month for over two years) and of the female gametophyte (from 3-month-old seeds, post-fertilization). The pigments were separated on powdered cellulose columns and quantitated. The content of chlorophyll *a*, chlorophyll *b*, and carotenoids, in μg/g fresh weight, were 11.1, 4.1, and 9.5 for the female tissue culture and 6.6, 1.3, and 3.2 for the female gametophyte from the seed. In appearance the tissue culture derived from the gametophyte was obviously greener than the original tissue of the gametophyte. A similar extraction procedure applied to the female and male albino tissues detected no chlorophyll, but carotenoids were present. The extraction procedure for porphyrins was used for the female (green) tissue culture; the absorption spectrum indicated little or no porphyrins.

Environmental and nutritional conditions—In

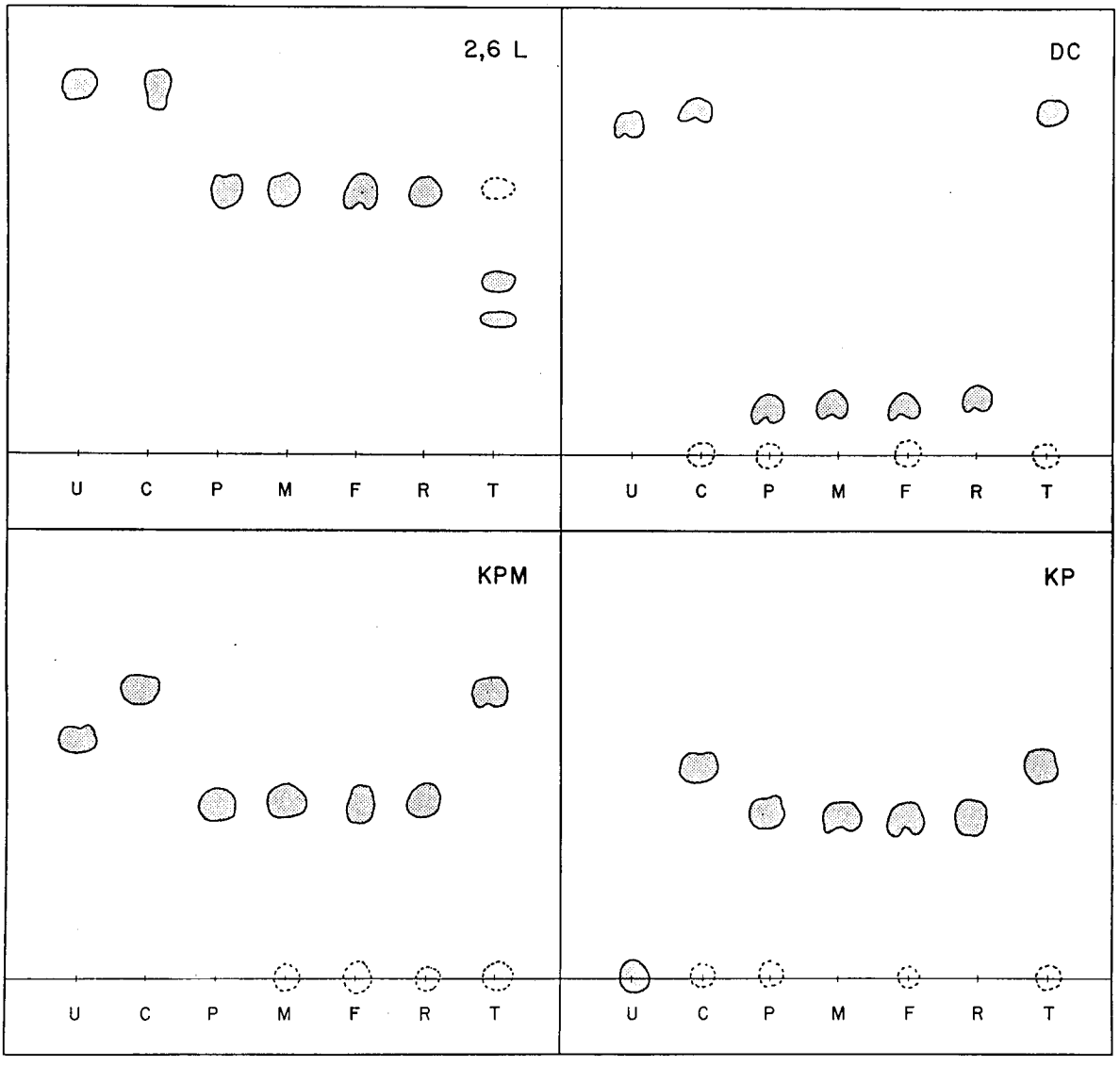

Fig. 4. Cells of 'Paul's Scarlet' rose tissue grown on a defined medium containing δ-aminolevulinic acid: *top*, photograph taken with ultraviolet light; *bottom*, bright field of the same cells. Since some fluorescence fades on exposure to light, the ultraviolet light photograph was taken first; the cells in bright field show considerable plasmolysis after exposure to UV.

the early experiments on porphyrin formation from ALA, it was noted that synthesis took place only in the dark; white light (250 ft-c) inhibited the conversion. Various regions of the visible spectrum were tested for activity and it was found that green and blue light was inhibitory. A qualitative evaluation of fluorescence for the male *Ginkgo* tissue showed a maximum of fluorescence in the dark, a slight reduction in far-red light, but only trace amounts in red, cool white, and green light; no fluorescence was observed in tissues grown in blue light.

Excretion of porphyrins into the media was not detected in any of the experiments. Examination under 366 mμ UV light showed an absence of fluorescence material in the media.

Media supplemented with glycine and succinic acid at millimolar equivalents to promote the production of ALA were not successful in inducing protoporphyrin synthesis in any of the albino tissues; the pyridoxine requirement was presumed to be met by its presence in the medium. In other experiments, decreasing the sucrose concentration from 2.0% to 0.2, 0.02, or 0.0% in the medium containing ALA was found to have no effect on porphyrin synthesis by the male albino tissue culture.

Granick (1963b) reported that *a*, *a*-dipyrridyl increased the formation of magnesium protoporphyrin in *Chlorella* by facilitating magnesium incorporation into tetrapyrrole. When tissues were grown in media supplemented with ALA alone (0.5 mM), or ALA (0.5 mM) and *a*, *a*-dipyrridyl (0.1 mM), and extracted, the *Ginkgo* male albino tissue yielded 5.8 and 6.2 μg of protoporphyrin per gram of fresh tissue and the rose tissue yielded 3.4 and 10.5 μg, respectively. The absorption spectra of these extracts gave no indication of magnesium protoporphyrin. The values for the *Ginkgo* tissue were not significantly different, but the amount of PROTO from the rose tissue on *a*, *a*-dipyrridyl and ALA was increased about threefold over the ALA alone controls.

The *Ginkgo* male albino and rose tissues were placed on media containing ALA and incubated in an anaerobic jar which was flushed daily with 5% CO_2 in nitrogen. These conditions were reported by Lascelles (1964) to increase PROTO formation in *Rhodopseudomonas sphaeroides*. Compared to control tissues of the same age grown aerobically, the rose tissue gave more fluorescence under anaerobic conditions than in the controls, the *Ginkgo* tissue less; this occurred only in the dark. Absorption spectra of the rose extract gave maxima at 398, 498, 530, 570, and 623 mμ suggesting that compounds other than PROTO were formed; further identification was not made.

The loss of chlorophyll from female albino tissue appears to be irreversible, since it has been impossible to restore the green color. Prolonged exposure to white light, repeated transfer in white light, and grafting experiments with the male, female, or green diploid strains of tissue have all failed to induce greening. Thus prolonged exclusion of light appears to have changed the green female tissue to one which resembles the male in all respects thus far tested.

Discussion—The mechanisms for the control of tetrapyrrole and chlorophyll synthesis in higher plants are not well known. Probable mechanisms may be inferred from work on algae (Granick, 1955), animal cells (Granick, 1966), and photosynthetic bacteria (Lascelles, 1964). Some information is also available from studies on etiolated plants (Sisler and Klein, 1963; Bogorad and Jacobson, 1964). Other aspects of chlorophyll formation and chloroplast development have been reviewed by Bogorad (1965), Gibor and Granick (1962, 1964), Granick (1963a, b), Marks (1966), Schiff and Epstein (1965), Smillie, Evans, and Lyman (1964), and others.

In the present experiments with tissue cultures the block in chlorophyll synthesis appears to be at the point of magnesium insertion into protoporphyrinogen; no magnesium protoporphyrin or other intermediates beyond this point were identified; protoporphyrin accumulated in most of the tissues fed ALA. In *Taxus*, *Solanum*, and *Agave* small amounts of PROTO and larger amounts of other unidentified porphyrins were formed. Early in this work it was thought that the various tissue cultures would exhibit different blocks in porphyrin synthesis and yield intermediates similar to those obtained in *Chlorella* mutants (Granick, 1963a, b) or etiolated plants (Bogorad, 1965). In the latter systems, various magnesium-containing porphyrins were detected; however, in the work with tissue cultures, no magnesium-containing compounds were identified. Of course, it is possible that a block exists prior to ALA synthesis, since no protoporphyrin was formed when the tissues were fed glycine and succinic acid. However, this question was not pursued in the present studies.

Comparatively little is known about the enzymes or the control mechanisms by which magnesium is combined with tetrapyrroles (Lascelles, 1964). However, a control mechanism for tetrapyrrole synthesis has been suggested by Granick (1966) for animal cells, by Lascelles (1964) for photosynthetic bacteria, and by Bogorad (1965) for higher plants. In photosynthetic bacteria, for example, iron is needed for heme biosynthesis and the heme acts by negative feedback inhibition to inhibit ALA synthetase, thus decreasing ALA and tetrapyrrole production. Feeding ALA circumvents this control mechanism and tetrapyrroles may accumulate. In the plant tissue cultures, it is quite probable that a similar mechanism is operative, but this remains to be demonstrated. The accumulation of PROTO in the tissues could be explained by the fact that feeding

ALA bypasses the normal controls on ALA synthetase. What limits the biosynthesis to PROTO is not known.

Another mechanism for the control of tetrapyrrole synthesis has been advanced by Granick (1966). Using the induction of ALA synthetase in chick embryo liver cells as a test system, Granick has suggested a mechanism to explain the action of a wide variety of compounds which cause increased porphyrin synthesis. The theoretical scheme would have ALA synthetase production governed by a structural gene and an adjacent operator segment. When heme is formed, it combines as a corepressor with a protein aporepressor formed by a repressor gene. The heme-protein repressor suppresses the operator segment and therefore the structural gene, thus limiting the production of ALA synthetase. A number of chemicals essentially replace heme as the corepressor, rendering the repressor complex ineffective; the result is that ALA synthetase production continues. This entire regulatory mechanism is circumvented by feeding ALA and it is not affected by PROTO formation.

Marsh, Evans, and Matrone (1963) have shown that iron-deficient cowpeas fed ^{14}C-ALA in the dark form PROTO but no Mg PROTO; however, in the light the plants make chlorophyll. This implies a light-dependent reaction for Mg PROTO formation, but there is no other evidence for this at the present time. In the present studies the exposure to light of plant tissue cultures containing protoporphyrin did not yield any Mg PROTO.

Isolated chloroplasts of *Euglena* were shown by Carell and Kahn (1964) to be capable of forming PBG, URO, and COPRO, but not PROTO, from ALA. Incubating the chloroplasts with PBG showed no porphyrin synthesis since the plastids are impermeable to PBG. The actual site of ALA synthesis, whether in the chloroplasts or the mitochondria, remains to be determined. In other experiments, Carell and Price (1965) have shown that iron is required for the conversion of COPRO to PROTO in *Euglena*; under extreme iron deficiency, COPRO is accumulated and the rate of PROTO formation is decreased.

Albino and pigmented strains of tobacco tissue have been studied by Venketeswaran and Mahlberg (1962). They found that rapid growth and friability were correlated with the albino strain. Similar results were obtained in the haploid albino tissues of *Ginkgo*. However, Venketeswaran and Mahlberg reported no anatomical differences, other than the absence of chloroplasts, between green and albino tissue cultures. This contrasts markedly with results from *Ginkgo* where the albino tissues are undifferentiated.

The chlorophyll content of plant tissue cultures has been studied by Vasil and Hildebrandt (1966), and Dobberstein and Staba (1966); Stetler and Laetsch (1965) and Laetsch and Stetler (1965) have shown that tobacco tissue cultures require cytokinins for chloroplast differentiation; and work by Jaspars (1965) and Oh-hama, Shihira-Ishikawa, and Hase (1965) suggests that carbohydrate content of media may influence plastid differentiation and function. Likewise, the repression of chloroplast development by high concentrations of certain carbon sources, such as glucose or acetate, has been cited by App and Jagendorf (1963). This repression was observed in dark-grown *Euglena* cells placed in the light. In the present studies on plant tissue cultures there was no difference in chlorophyll or porphyrin production between tissues grown on varying amounts of sucrose.

The albino and green tissues from the female gametophyte of *Ginkgo* provide an interesting experimental system for studying the relationship between chloroplast function and metabolic and cellular differentiation. The albino tissue lacked the specialized cell types present in the green tissue and also failed to regreen in the light.

The change in plastid function from green to colorless, which is induced by growth in the dark, was interpreted by Granick (1963a) as not the result of a loss of proplastids, but to the loss of ability of plastids to differentiate. The results from the present experiments support this interpretation. The proplastids of the tissue cultures replicate, but they differentiate as amyloplasts rather than chloroplasts.

Looking beyond the present results, it seems reasonable to suggest that products of plant-tissue cultures (other than porphyrins) could be synthesized in increased amounts by using appropriate precursors and metabolic blocks, i.e., either chemically induced blocks or selected strains of tissue.

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