The Influence of Light on Synthesis of Protein and of Chlorogenic Acid in Potato Tuber Tissue [1, 2]

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Disks of tuber tissue maintained in a moistened environment increase substantially in both protein content (18, 19, 21) and phenolic content (28). The 2 processes have not previously been considered as related. However, the stimulation by light of phenolic synthesis in tuber slices (11, 16) has been found to depend largely on the synthesis of proteins. Data which relate the production of phenolic substances to a stimulation of protein synthesis by light are presented in this paper. It is proposed that much of the phenolic synthesis in tuber slices exposed to light results from the initiation of chloroplast development in the tissue.

The changes in respiration which develop in aerated tuber disks (4, 10, 15, 24) were linked to protein synthesis by Steward and Preston (18, 19) as early as 1940. Subsequently, chloramphenicol, a substance used to inhibit protein synthesis, was shown to inhibit the development of an altered respiration in aerated disks (2), an observation which suggested a direct causative relationship between protein synthesis and the alteration of respiratory activity in the disks. An analogous relationship between the synthesis of proteins and of phenolic substances is described in the present study.

Materials and Methods

Treatment of tissue. The experimental conditions used to study the accumulation of chlorogenic acid in disks of pulp tissue from potato tubers (var. Kennebec) were similar to those described previously (28). Tubers stored less than 5 months at 4° were used for most experiments. Uniform samples of disks were obtained by slicing pulp tissue 1 mm thick and then cutting disks 1.5 cm in diameter from the slices. Treatments were run in duplicate using 10 disks per sample (fr wt 2.5–3 g), and duplicate samples placed in a single petri dish were moistened with 2 ml of culture solution. The entire procedure for preparation of disks exposed the tissue to approximately 50 ft-c of incident light for about 30 minutes. In some experiments where the light of the laboratory was excluded during preparation of tissue, disks were cut from tubers in a dim green light providing less than 1 ft-c at the surface of the tissue. This source was a fluorescent tube wrapped with several layers of green and yellow cellophane transmitting maximally at 525 mμ. Disks cultured in the light were placed under a bank of fluorescent and incandescent bulbs providing 500 foot-candles (measured with a Weston light meter) at the surface of the disks. Except where noted, experiments were of 24 hours duration, and data are usually expressed as the net increase in chlorogenic acid per gram original fresh weight of tissue.

A marked quantitative difference in capacity for chlorogenic acid synthesis was found in tissue from tubers of different sizes. Large tubers, weighing 700 grams or more, yielded disks which responded more slowly than those from smaller tubers weighing 200 to 300 grams. In experiments where more than one tuber was used as a source of tissue, care was taken to choose tubers of similar size and to include in every sample approximately equal numbers of disks from each tuber used. Results from duplicate samples were normally within 5 to 10 % of the mean. Individual experiments were repeated several times with good qualitative agreement although exact quantitative relations were difficult to reproduce.

Assays. Chlorogenic acid was assayed by a modified Hoepfner procedure (27). Small columns (1 × 3 cm) containing alumina were used to absorb the chlorogenic acid from extracts before treating it with nitrous acid and then alkali. Recent work (5) has shown that at least 7 different conjugates of caffeic and quinic acids exist in the tuber, chlorogenic acid being the major one. All of these phenolic esters behave like chlorogenic acid in the assay used and are regarded as equivalent for the purpose of this study. Disks cultured on L-phenylalanine accumulate large quantities of free *p*-coumaric and caffeic acids and *p*-coumaroyl conjugates (5). Although these phenolic substances contributed to the absorption of ultraviolet light by crude alcoholic extracts, they did not interfere with the chlorogenic acid assay at the concentrations encountered.

Protein was determined by Kjeldahl digestion and Nesslerization of material insoluble in cold alcohol. Absorption spectra were determined in a Cary 11 spectrophotometer. Relative chlorophyll values were estimated from the absorption maximum at 665 mμ of alcoholic extracts of green tissue. The source of

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[2] A preliminary report has been published (6).

chloramphenicol was a sample of Park-Davis Chloromycetin-succinate. The *n*-butyl alcohol-ammonia solvent described previously (5) was used for paper chromatography.

Results

Light Effects. The effect of light on the synthesis of phenolic substances in disks of tuber tissue maintained on water is compared quantitatively in table I with the stimulation obtained by culturing disks on phenylalanine. The production of chlorogenic acid is increased by continuous illumination. Within 24 hours the illuminated water controls accumulate as much of the compound as disks supplied with phenylalanine. However, light does not induce the accumulation of large quantities of *p*-coumaroyl conjugates. The presence of these phenolic esters, whose absorption maxima are near 310 mμ, produces a shift of the absorption peak from 323 mμ (the absorption peak of caffeoyl conjugates) to about 317 mμ in extracts of tissue cultured on phenylalanine. No change in absorption maximum is observed in extracts of disks cultured either in darkness or in light on water alone (table I). Use of paper chromatography confirmed the absence of *p*-coumaroyl esters in the water control samples, although such substances do eventually appear in the tissue. Phenylalanine stimulates synthesis of both chlorogenic acid and coumaroyl esters in darkness, and its effect is further increased in light. The accumulation of total phenolic material in phenylalanine cultures was doubled in the light.

Table I

Effect of Light on Chlorogenic Acid Synthesis in Disks of Potato Tuber

Initial chlorogenic acid concentrations were 0.09 μmole/g fr wt.

Condition of culture (24 hour duration)	Net chlorogenic acid synthesis μmoles/g fr wt	Total phenolic substance*	
		λmax	μmoles/g fr wt
Water-Darkness	0.03	323 mμ	0.08
Light (500 ft-c)	0.30	323 mμ	0.48
L-phenylalanine, 0.05 M			
Darkness	0.19	317 mμ	1.02
Light (500 ft-c)	0.25	317 mμ	2.02

* Calculated from the absorption at λmax of alcoholic extracts, assuming an average extinction coefficient of 20,000.

Kinetic studies of chlorogenic acid synthesis indicate that an autocatalytic process is involved. A time course of chlorogenic acid production in disks maintained on water under continuous illumination, in darkness, or in light followed by darkness is shown in figure 1. The rate of accumulation of chlorogenic acid increases continuously in the light. A slow linear rate of accumulation is characteristic of tissue in the dark.

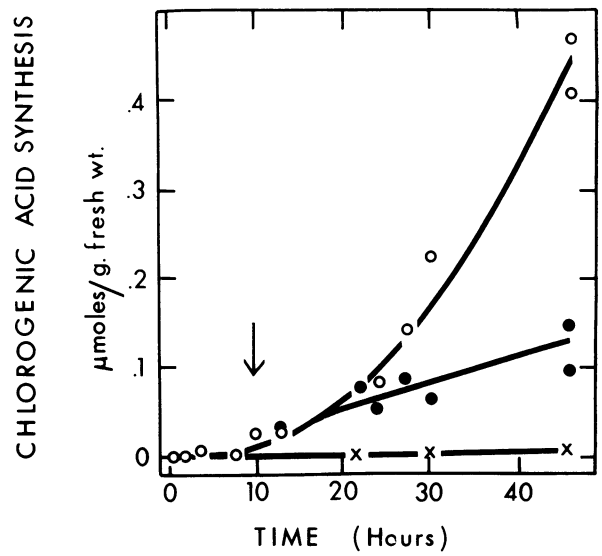

FIG. 1. Time course of chlorogenic acid accumulation. Data show the net increase in chlorogenic acid content expressed as μmoles/g original fr wt. ○—disks maintained on water in the light (500 ft-c.); ●—disks maintained in the light for 10 hours and then transferred to darkness, the point of transfer being indicated by the arrow; ×—disks maintained in the dark throughout, except for an initial exposure to daylight of 30 minutes during their preparation. Initial chlorogenic acid concentration; 0.06 μmoles/g fr wt.

Disks which were exposed to light for 10 hours and then placed in the dark at the point indicated by the arrow accumulated chlorogenic acid during the ensuing 36 hours at a rate much greater than that of disks maintained in darkness throughout. The increased rate of synthesis was roughly that attained by the disks before they were transferred from light to darkness. A longer initial exposure to light correspondingly increased the subsequent rate of chlorogenic acid synthesis in the dark. If light were acting merely to increase the availability of phenolic precursors, continued synthesis of chlorogenic acid in the dark at a greatly accelerated rate would not be expected. These results as well as the increasing rate of chlorogenic acid formation in the light suggest that production of phenolic substances could involve the synthesis or activation of enzymes by the tissue slices.

The curves in figure 1 suggest the participation of a light reaction involving high intensities and prolonged exposures. A light effect involving only low intensities and short exposures was also demonstrated by eliminating the 30 minute exposure to the light of the laboratory which all disks normally received during their preparation. When disks were prepared under a dim green safety light, the subsequent syn-

Table II

Protein and Chlorogenic Acid Synthesis in Disks Receiving No Light Exposure or Exposures of Different Durations

Initial protein concentration 11.6 mg/g fr wt. Initial chlorogenic acid concentration 0.15 μmole/g fr wt.

Treatment	Net chlorogenic acid synthesis μmoles/g fr wt	Net protein synthesis mg/g fr wt
No light exposure (except safety-light)	+ 0.05	— 0.4
Brief light exposure (30 min; 50 ft-c)	+ 0.12	+ 0.5
Continuous illumination (500 ft-c)	+ 0.59	+ 2.2

thesis of chlorogenic acid in the dark decreased. Table II shows that the exclusion of all light, except that from the dim green source, can reduce the amount of chlorogenic acid formed to one-half of that in disks prepared under normal conditions of room lighting. The data show further that the net synthesis of protein also requires a brief exposure to light. When light is excluded, a small but consistently observed decrease in protein content of the disks occurs. Continuous illumination produced a large net increase in both protein and phenolic substances.

After 24 hours of light, no visible greening of the disks was detected with the variety of potato used. If the slices were allowed to remain in the light another 24 to 48 hours, they developed a green cast. No algae could be detected microscopically on the surface of the disks. The absorption spectra of alcoholic extracts clearly indicated that chlorophyll was being synthesized in the tissue. If disks were maintained on water for several weeks in room light, they became noticeably green. The color was confined within the disks and was not produced by contamination of the disks or of the surrounding fluid. Microscopic examination of these tissues showed that small, irregularly shaped chloroplasts had developed in cells throughout the disks. Bright green chloroplast pellets have been prepared from tissue under continuous illumination for 5 days.

Effect of Protein Inhibitors. A direct indication that the synthesis of enzymes is necessary for the production of phenolic substances was obtained by the use of inhibitors of protein synthesis. Figure 2 shows that L-ethionine can reduce the accumulation of chlorogenic acid by 80 %. This inhibition occurs regardless of whether disks are maintained in light or darkness. Figure 3 shows that the inhibition of chlorogenic acid synthesis by ethionine can be reversed by methionine. Since L-methionine itself can be inhibitory, conditions for demonstrating a reversal must be chosen with care. If concentrations of ethionine greater than 0.01 M are used, methionine itself becomes inhibitory in amounts required to reverse the inhibition. The rates of chlorogenic acid synthesis in the water control samples of figure 2 and 3 are much greater than those of comparable

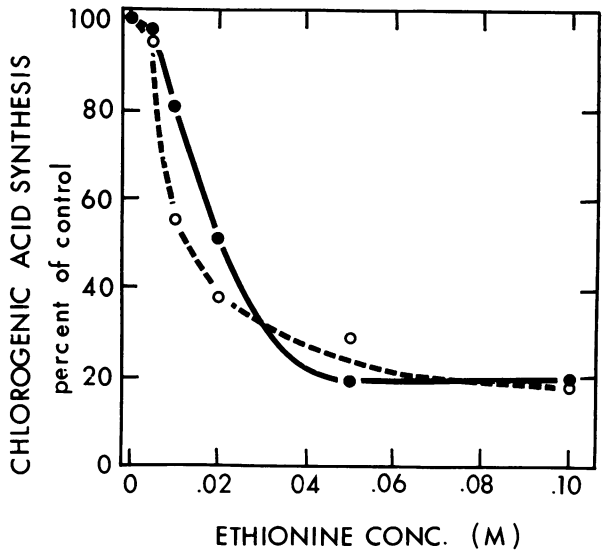

FIG. 2. Inhibition of chlorogenic acid synthesis by L-ethionine. ○—disks cultured in the light. Net synthesis of chlorogenic acid in water controls, 1.55 μmoles/g fr wt.; ●—disks cultured in the dark. Net synthesis of water controls, 0.98 μmoles/g fr wt. The initial concentration of chlorogenic acid was 0.19 μmoles/g fr wt. of tissue.

samples in the previous tables and figure. Small tubers were used as a source of tissue in the experiments with ethionine whereas large tubers which have a reduced synthetic capacity were used in experiments where low rates of synthesis were observed. The qualitative response of the tissue to the experimental treatments was not influenced by tuber size.

Direct evidence that ethionine inhibits protein synthesis in tuber slices is provided in table III. A net increase in protein content of 25 to 30 % occurred in illuminated slices maintained 24 hours on water. No external source of nitrogen was required because tubers contained exceptionally large quantities of free amino acids which furnish the nitrogen for protein synthesis (18, 19). In the presence of ethionine, at concentrations high enough to produce a 70 to 80 % inhibition of chlorogenic acid production, protein synthesis was inhibited by 40 %. The general appearance of such disks was quite normal. Although

Table III

Inhibition of Protein Synthesis by Ethionine

Initial protein concentration: Exp I 14 mg/g fr wt; Exp II 8.6 mg/g fr wt. Cultured 24 hours at 500 ft-c.

Exp	Culture solution	Net protein synthesis mg/g fr wt	Inhibition %
I	Water	5.1	
	L-ethionine, 0.05 M	3.1	39
II	Water	2.1	
	L-ethionine, 0.10 M	1.4	36

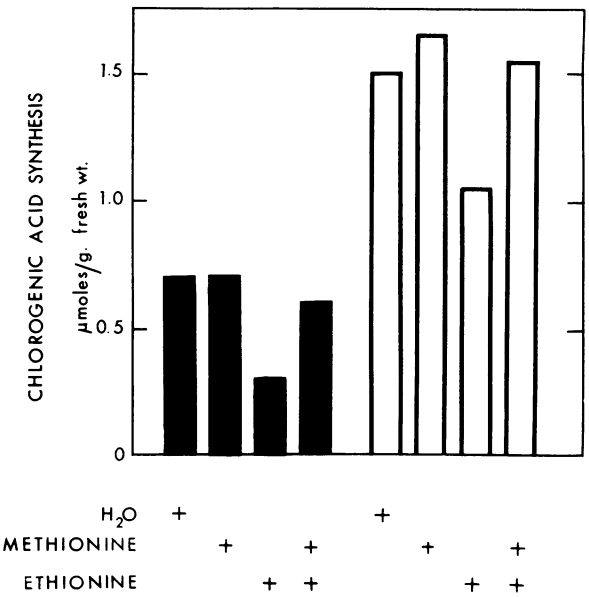

FIG. 3. Reversal of ethionine inhibition of chlorogenic acid synthesis by methionine. Solid bars represent net synthesis of chlorogenic acid by disks maintained in darkness 24 hours except for a brief exposure to daylight during preparation. Open bars represent synthesis in disks under continuous illumination. L-ethionine was employed at a final concentration of 0.01 M in culture solutions and DL-methionine was used at 0.001 M. Initial concentration of chlorogenic acid, 0.20 μmoles/g fr wt.

water uptake was reduced somewhat, ethionine-treated disks remained turgid. Chlorophyll synthesis in disks illuminated for 48 hours was also reduced in the presence of 0.1 M ethionine.

Evidence that ethionine does not interfere greatly with the enzymic reactions involved in the conversion of phenylalanine to chlorogenic acid is given in table IV. At concentrations which markedly inhibit the synthesis of chlorogenic acid from endogenous substrates, ethionine had little effect on the stimulation of chlorogenic acid synthesis produced by culturing disks on phenylalanine. Nor was the total phenolic production, determined from the absorption spectra of extracts, decreased in cultured disks.

These results suggest that sufficient enzymic activity exists in the tissue to metabolize the added phenylalanine without requiring the synthesis of new protein. The experiment also indicates that ethionine does not stimulate the oxidative removal of chlorogenic acid from the tissue. The formation of unreactive polymers during phenolic oxidation could yield low chlorogenic acid values without a concomitant decrease in synthesis.

Table IV

Effect of Ethionine on the Stimulation of Chlorogenic Acid Synthesis by Phenylalanine

Initial chlorogenic acid concentration 0.19 μmoles/g fr wt. Disks were cultured in darkness for 24 hours.

Culture solution	Net chlorogenic acid synthesis μmoles/g fr wt	Inhibition %
Water	0.46	
L-ethionine, 0.05 M	0.14	70
L-phenylalanine, 0.05 M	0.61	
L-phenylalanine + L-ethionine, 0.05 M each	0.50	18

Chloramphenicol also reduces the formation of chlorogenic acid in tuber slices. Concentrations as high as those required for ethionine inhibition are necessary to demonstrate an inhibitory effect on phenolic production. The synthesis of chlorogenic acid in both light and darkness is decreased 60 % by 5 mg/ml of chloramphenicol. When only 0.05 mg/ml of inhibitor is employed, some stimulation of synthesis is obtained in the light. Disks treated with chloramphenicol were completely turgid and viable. At the highest concentration employed, the only visible effect was a slight reduction of browning on the surface of the disks. Thiouracil at 5×10^{-3} M inhibits chlorogenic acid production by 50 %. Under these conditions no browning of the tissue occurs.

Discussion

Although synthesis of chlorogenic acid will occur in almost complete darkness, the accumulation of this phenolic ester can be doubled by exposing the disks briefly to light of relatively low intensity. If much longer light exposures at higher intensities are given, chlorogenic acid synthesis is further stimulated; the longer the exposure, the greater the subsequent rate of synthesis in darkness. Maximal endogenous rates are obtained under continuous illumination. An action spectrum of chlorogenic acid synthesis in potato disks indicates a broad maximum toward the blue end of the spectrum, but conclusive evidence for the participation of the phytochrome system has not been obtained (16).

Kinetic studies of the light effects have suggested that the activation or actual synthesis of enzymes is involved in the process. The inhibition of chlorogenic acid production by ethionine and chloramphenicol provides a direct indication that protein synthesis is required. In fact, light appears to stimulate chlorogenic acid formation by virtue of its effects on protein synthesis. A brief exposure of tissue slices to light is necessary for a net increase in protein content. Continued exposure of tuber slices to light results in a much larger increase in synthesis of both protein and chlorogenic acid.

Chlorophyll formation can be detected in tuber disks maintained in the light, and chloroplasts develop in the tissue. Greening is a response of whole tubers exposed to sunlight during growth, and less than 20 ft-c of continuous illumination during storage will induce chloroplast development in the outer cell layers of harvested tubers (9). The growth of chloroplasts in tuber slices and in etiolated tissue, following illumination, provides a logical explanation of light effects on protein synthesis. Develop-

ing plastids of tissue transferred from darkness to light increase markedly in protein content (3). The synthesis of proteins in developing chloroplasts is not necessarily linked to chlorophyll synthesis. Mego and Jagendorf (13) have observed that brief illumination of etiolated bean seedlings is sufficient to initiate chloroplast development. Subsequently, extensive synthesis of chloroplast proteins occurs in the dark although almost no chlorophyll is formed. The formation of proteins and phenolic substances in tuber slices does not depend upon the development of photosynthetic activity. Disks cultured in the dark can have no such activity, nor is enough chlorophyll formed during the first 24 hours of illumination to produce much photosynthesis. Whole tubers require 12 to 24 hours of illumination before chlorophyll synthesis can be detected (26).

In previous studies of protein synthesis in potato disks (18, 19, 21), no rigorous attempts to exclude light are reported. Consequently, the results may reflect initial stages in the growth of plastids, even though no greening was obvious.

This does not detract from Steward's hypothesis of a salt-induced protein synthesis, for chloroplasts are known to accumulate large quantities of salts (20). The observed stimulation of chlorogenic acid synthesis in disks cultured on various salts (28) probably results from a similar stimulation of protein formation.

The increase in respiration of aged potato disks is another metabolic alteration involving protein synthesis (2), and increases in the activity of several enzymes of electron transport (4) and of the pentose phosphate cycle (1) have been demonstrated. Newly activated pathways could increase the supply of substrates such as phenylalanine whose availability appears to be a factor limiting phenolic synthesis in tuber slices (5). However, the fact that light can double the production of phenolic substances in the presence of an excess of phenylalanine argues against indirect effects of this nature.

The behavior of polyphenoloxidase, an enzyme system capable of hydroxylating *p*-coumaroylquinic acid to chlorogenic acid in vitro (5), suggests a more direct relation between enzyme synthesis and phenolic production. Polyphenoloxidase activity declines markedly in the tuber after it reaches maturity and is harvested (25). Although the potato is a classical source of this enzyme, its activity in the mature tuber is very low compared with that at an early stage of growth. Preliminary experiments indicate that polyphenoloxidase activity increases 3- to 4-fold in tuber slices illuminated 16 hours. Although changes in the level of this enzyme alone could not produce the results obtained above, other enzymes responsible for phenolic synthesis may behave in a similar manner. A low level of such enzymes in the tuber would account for a virtual lack of phenolic synthesis in this tissue, while their reappearance in the tuber slice would restore the high capacity of the tissue for phenolic production. Amino acid activating enzymes, whose presence is required for renewed enzyme synthesis, can be extracted from tuber tissue immediately after its removal from dark storage (unpublished observations of Dr. K. R. Hanson).

Phenolic synthesis is not usually associated with chloroplast development. Enzyme formation in structures other than chloroplasts could occur. Hackett et al. (4) have shown that mitochondrial and microsomal fractions increase in nitrogen content as a result of aging tuber disks. However, the possibility exists that their preparations were contaminated with fragments of developing plastids.

The initiation of phenolic synthesis in leaf tissue also appears to involve the synthesis of proteins. Thimann and Radner (22) found that anthocyanin formation in Spirodella leaves is particularly sensitive to ethionine. Recently they obtained evidence for participation of a polynucleotide and suggest that protein synthesis is involved in anthocyanin pigmentation (23). Anthocyanin synthesis in expanding flower petals is also inhibited by ethionine (8).

Chloramphenicol inhibits anthocyanin synthesis at high concentrations and is stimulatory at low concentrations (12, 16, 17). The chlorogenic acid system responds similarly. Marked inhibition of synthesis can be obtained at concentrations 100 times greater than those producing stimulation. High levels of chloramphenicol are required to inhibit incorporation of amino acids into proteins of whole plant tissues (7) and of particulate preparations from plants (14).

The survival of plant tissues treated with such unusually high concentrations of inhibitors is evidence for a specificity of action. The fact that ethionine inhibits chlorogenic acid synthesis more than general protein synthesis in tuber disks may also reflect a differential sensitivity to ethionine of specific sites of protein synthesis in the cell.

Summary

1. Light stimulates the synthesis both of chlorogenic acid and of proteins in disks of pulp tissue from potato tubers. A brief exposure of disks to light of relatively low intensity doubles the synthesis of chlorogenic acid in the dark. Much longer exposures to light of relatively high intensity further stimulate the synthesis of chlorogenic acid. In disks cultured under continuous illumination, the rate of chlorogenic acid accumulation increased continuously for at least 40 hours, attaining the highest levels observed.

2. An initial brief exposure to light is also required for a net increase in protein content of tuber disks placed in the dark for 24 hours. Continuous illumination greatly increases the synthesis of proteins and leads to greening and to chloroplast development in the disks.

3. Chlorogenic acid synthesis is inhibited by high concentrations of ethionine and chloramphenicol. Ethionine inhibition can be reversed by methionine. Ethionine was shown to inhibit protein synthesis in the disks, but did not interfere with the conversion of phenylalanine to chlorogenic acid.

4. Formation of new proteins appears to be required for maximal rates of phenolic synthesis in tuber disks. Light is thought to be stimulatory by virtue of its effects on protein synthesis. The similarity in response to light of tuber slices and etiolated tissue suggests that the high capacity for phenolic synthesis observed results from a light induced development of chloroplasts in the slices.

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Literature Cited

1. Ap Rees, T. and H. Beevers. 1960. Pentose phosphate pathway as a major component of induced respiration of carrot and potato slices. Plant Physiol. 35: 839–47.
2. Calo, N., J. D. Marks, and J. E. Varner. 1957. Respiratory metabolism of aerated potato disks. Nature 180: 1142.
3. De Deken-Grenson, M. 1954. Grana formation and synthesis of chloroplastic proteins induced by light in portions of etiolated leaves. Biochim. Biophys. Acta 14: 203–11.
4. Hackett, D., D. W. Haas, S. K. Griffiths, and D. J. Niederpruem. 1960. Studies on development of cyanide-resistant respiration in potato tuber slices. Plant Physiol. 35: 8–19.
5. Hanson, K. R. and M. Zucker. 1963. The biosynthesis of chlorogenic acid and related conjugates of the hydroxycinnamic acids. Chromatographic separation and characterization. J. Biol. Chem. 238: 1105–15.
6. Hanson, K. R. and M. Zucker. 1963. Biosynthesis of cinnamic and hydroxycinnamic acid conjugates of quinic and shikimic acids. Federation Proc. 22: 650.
7. Jacoby, B. and J. F. Sutcliffe. 1962. Effects of chloramphenicol on the uptake and incorporation of amino acids by carrot root tissue. J. Exptl. Botany 13: 335–47.
8. Klein, A. O. and C. W. Hagen, Jr. 1961. Anthocyanin production in detached petals of *Impatiens balsamina*. Plant Physiol. 36: 1–9.
9. Larsen, E. C. 1950. Chlorophyll formation in potato tubers as affected by temperature and time. Science 111: 206–07.
10. Laties, G. G. 1962. Controlling influence of thickness on development and type of respiratory activity in potato slices. Plant Physiol. 37: 679–90.
11. Levy, C. C. and M. Zucker. 1960. Cinnamyl and *p*-coumaryl esters as intermediates in the biosynthesis of chlorogenic acid. J. Biol. Chem. 235: 2418–25.
12. Margulies, M. M. 1962. Effect of chloramphenicol on light dependent development of seedlings of *Phaseolus vulgaris*, var. 'Black Valentine', with particular reference to development of photosynthetic activity. Plant Physiol. 37: 473–80.
13. Mego, J. L. and A. T. Jagendorf. 1961. Effect of light on growth of black valentine bean plastids. Biochim. Biophys. Acta 53: 237–54.
14. Rabson, R. and G. D. Novelli. 1960. The incorporation of leucine-C^{14} into protein by a cell-free preparation from maize kernels. Proc. Natl. Acad. Sci. 46: 484–88.
15. Schade, A. and H. Levy. 1949. Studies on the respiration of the white potato. III. Changes in the terminal oxidase pattern of potato tissue associated with time of suspension in water. Arch. Biochem. 20: 211–19.
16. Siegelman, H. W. 1963. Physiological studies on phenolic biosynthesis. In: Biochemistry of Phenolic Compounds, J. B. Harborne, ed. Academic Press. London. (in press).
17. Siegelman, H. W. and S. B. Hendricks. 1957. Photocontrol of anthocyanin formation in turnip and red cabbage seedlings. Plant Physiol. 32: 393–98.
18. Steward, F. C. and C. Preston. 1940. Metabolic processes of potato disks under conditions conducive to salt accumulation. Plant Physiol. 15: 23–61.
19. Steward, F. C. and C. Preston. 1941. The effect of salt concentration upon the metabolism of potato discs and the contrasted effect of potassium and calcium salts which have a common ion. Plant Physiol. 16: 85–116.
20. Stocking, C. R. and A. Ongun. 1962. The intracellular distribution of some metallic elements in leaves. Am. J. Botany 49: 284–89.
21. Thimann, K. V. and G. M. Loos. 1957. Protein synthesis during water uptake by tuber tissue. Plant Physiol. 32: 274–79.
22. Thimann, K. V. and B. S. Radner. 1958. The biogenisis of anthocyanin. VI. The role of riboflavine. Arch. Biochem. Biophys. 74: 209–23.
23. Thimann, K. V. and B. S. Radner. 1962. The biogenisis of anthocyanin. VII. Requirement for both purines and pyrimidines. Arch. Biochem. Biophys. 96: 270–79.
24. Thimann, K. V., C. S. Yokum, and D. P. Hackett. 1954. Terminal oxidases and growth in plant tissues. III. Terminal oxidation in potato tuber tissue. Arch. Biochem. Biophys. 53: 239–57.
25. Todd, G. W. 1953. Enzyme studies on dormant and active potato tubers. Physiol. Plantarum 6: 169–86.
26. Yamaguchi, M., D. L. Hughes, and F. D. Howard. 1960. Effect of color and intensity of fluorescent lights and application of chemicals and waxes on chlorophyll development of white rose potatoes. Am. Potato J. 37: 229–36.
27. Zucker, M. and J. F. Ahrens. 1958. Quantitative assay of chlorogenic acid and its pattern of distribution within tobacco leaves. Plant Physiol. 33: 246–49.
28. Zucker, M. and C. C. Levy. 1959. Some factors which affect the synthesis of chlorogenic acid in disks of potato tuber. Plant Physiol. 34: 108–12.