Short Communication

The Effect of Light upon Development in Potato Tissue Slices

William F. Hanebuth and Robert M. Chasson
Department of Biological Sciences, Illinois State University, Normal, Illinois 61761

The dramatic changes in oxygen consumption which occur following slicing of dormant tuber tissue have been well documented (6, 7, 9). It has been difficult to interpret the results of various workers because the investigators have frequently employed different methods of slicing, aeration, and illumination, as well as various culture solutions, temperatures, and types of tissue. Lee and Chasson (10) suggested that these different techniques might contribute to the variety of interpretations assigned to the aging phenomenon. The relative contribution to the total respiratory rate by the glycolytic pathway, the pentose phosphate pathway, and the tricarboxylic acid cycle may be influenced by aeration, temperature, and light (11). Ap Rees and Beever (1), who suggested that pentose phosphate metabolism is largely responsible for the respiratory increase, allowed light from normal laboratory conditions to strike the aging slices. Romberger and Norton (12), on the other hand, indicated that culturing (aging) was carried out in the dark and have interpreted their results to indicate that the tricarboxylic acid cycle becomes enhanced with aging. The latter conclusions were also reached by Laties (8), although light conditions were not reported. It is not unreasonable to suspect that light might influence the activity of respiratory enzymes since it does affect other enzyme systems in potato (14). However, previous attempts in our laboratory have failed to demonstrate any relationship between tissue O2 consumption and either light quality or quantity. Neither was it possible for us to show any influence of light on the malonate or cyanide sensitivity of potato tissue slices following conventional aging treatments. The effects of these two inhibitors on the tricarboxylic acid cycle and terminal oxidation are well known, and their influence upon fresh and aged potato tissue is specific: cyanide greatly suppresses O2 uptake in fresh tissue but has little influence on aged tissue; malonic acid only inhibits the respiration of aged tissue (2, 9). In a further attempt to demonstrate a possible link between light and respiration in tuber slices, we have modified previously applied procedures such that the tissue is aged in the presence of either cyanide or malonate. The results reported here confirm one of the conclusions drawn from our previous experiments referred to above, namely, that red light and far red light yield essentially the same results. Therefore, only the data from the former will be presented here.

Cylinders of tissue having a diameter of 8.0 mm were prepared from potato tubers (Solanum tuberosum L., var. Russet) and cut into 1.0-mm slices with a hand microtome. Eight fresh slices weighed approximately 0.5 g. The dry weight of these slices ranged from 10 to 13% of the fresh weight. The slices were washed with sterile distilled water to remove starch freed from cut cells, then placed in Petri dishes containing 10.0 ml of test solution, which was just sufficient to cover the slices. Cutting and washing of the tissue was carried out in dim room light. Temperature was maintained at 23 ± 1°C. All solutions were prepared in 50 mM HEPES buffer. The slices were cultured in either 50 mM malonic acid, pH 5.6, or 5 mM KCN, pH 7.2. Dark controls were maintained in both HEPES buffer, pH 5.6, and HEPES buffer, pH 7.2. Light treatments were applied as 5 min of red light (0.72 mw/cm²) or far red light (5.7 mw/cm²) at the beginning of each experiment (Cinemoid Filters, refer to Fig. 1). Following removal of slices for oxygen

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2 NDEA Title IV Fellow. Present address: MSU/AEC Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48823.

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Fig. 1. Wavelength separation of light sources. ——— Red filter consisting of two S-506 primary red and one S-533 deep amber Cinemoid filters (Century Lighting, Inc., New York); ———: far red filter consisting of one primary red, one deep amber, and one S-520 primary blue filter. Red light source: 15-w "cool" white fluorescent; far red light source: 250-w incandescent bulb. Stippled and hatched areas indicate wavelength range and are not intended to show relative color intensities. Curves for the red source (cool white fluorescent lamp) and the far red source (tungsten incandescent lamp) were redrawn from curves furnished by the General Electric Company and Instrumentation Specialties Company, Inc., respectively. The percentage transmittance of the filters was determined spectrophotometrically.
uptake measurement, the remaining slices were given an additional 5 min of red or far red light treatment. Samples were removed at 3 hr and at 6 hr, rinsed in distilled water, and then placed in measuring vials containing HEPES buffer adjusted either to pH 5.6 or pH 7.2, depending on the prior incubation pH. Oxygen consumption was measured polarographically with a Clark oxygen electrode (Yellow Springs Instrument Co.) for 15 min at 30 C. Cyanide or malonate was then added (injection) to the vials to a concentration equal to that existing
in the aging medium, and oxygen consumption was measured for an additional 15 min. The data were calculated as μmoles O₂ consumed per hr per g dry weight of tissue. Fresh (unaged) tissue was measured in the same way. The data presented here are expressed as a factorial increase in the aged rates over this fresh rate. The graphs represent the average of at least four trials, with a maximal standard deviation of approximately 14%.

Respiration of the dark controls (aged in HEPES buffer alone), as shown in Figure 2, indicates the typical increase in oxygen consumption with time. Upon injection of malonate the respiration rate remained essentially the same, while injection of cyanide caused pronounced inhibition: fresh tissue was highly inhibited (25% of control rate) and there was a progressive reduction in the percentage of respiratory inhibition as the tissue aged (39% of control rate at 3 hr, 63% of control at 6 hr).

When tissue was aged in the presence of malonic acid and subjected to either continuous darkness or brief exposure to red light, marked differences in the respiratory development became apparent (Fig. 3). Tissue aged in darkness and malonate respired at essentially the same rate as the dark control, and subsequent addition (injection) of malonate had, if anything, a slight stimulatory effect. Tissue exposed to red light was severely inhibited by the malonate; injection of malonic acid caused a further reduction in the respiratory rate. Previous work in our laboratory has demonstrated that in the absence of inhibitors no statistical differences exist between dark, red, far red, and white light-treated tissues aged as long as 96 hr (3). We therefore assume that the dark control is sufficient to serve as a control for the other light conditions.

After 6 hr of aging in malonate, the respiratory rate of the red light-treated tissue was greatly reduced below the level of the tissue in continuous darkness. As shown in Figure 3, the inverse of this sequence occurred when the tissue was incubated in cyanide. Red light-treated tissue respired at a rate equal to that of the dark control (6 hr), indicating a complete loss of cyanide sensitivity. Dark tissue aged in cyanide retained its relative cyanide sensitivity over the 6-hr period, when compared with the dark control (Table I). Injection of cyanide caused all tissues to respire at still lower rates. However, after 6 hr the percentage reduction in the dark-treated tissue was approximately four times that observed in the red light-treated tissue.

These results clearly indicate that the tissue responded metabolically to the light it received. We had originally attempted to investigate the possibility that the developmental response was mediated by the phytochrome system. The available data do not support such a contention. While the tissue responds to red light, we have not yet been able to determine an action spectrum. We are aware that phytochrome has not been detected spectrophotometrically in fresh potato tissue (4), an observation which we had confirmed in both fresh and aged tissue (3). However, the possibility of functional phytochrome in nondetectable quantities still remains. No attempt was made to determine a dose-response curve. Results from other plant systems would suggest that saturating doses were provided (5, 13).

Fresh tuber tissue respiration is highly cyanide-sensitive, but essentially malonate-insensitive. As the tissue ages, its respiration becomes partially cyanide-insensitive and partially malonate-sensitive. In our studies these are the events which occur when the tissue is treated with either red or far red light. The tissue aged in either cyanide or malonate but receiving no light tends, however, to have respiratory characteristics similar to those of fresh inhibited tissue. This result appears to be due to the combination of inhibitor and darkness since the dark control (Fig. 2) shows at least one typical aging response, i.e., a decrease in cyanide sensitivity with time.

In these experiments tissue was obtained from the same variety of potato tuber and in most cases from the same tuber itself. All tissues were subjected to identical temperatures, culture times, culture solutions, and aeration procedures. The absence or presence of light, however, greatly influenced the development of respiratory characteristics typical of aged storage tissue slices.

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