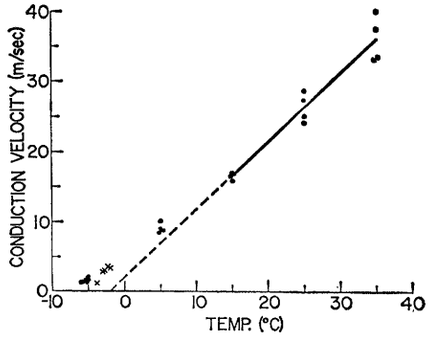

Fig. 3. Conduction velocity in red squirrel caudal nerves (fast fibers) plotted as a function of temperature. Filled circles are values obtained prior to spontaneous rewarming. Crosses are values during spontaneous rewarming.

showed that freezing of Locke's solution with concomitant warming of chamber air did not occur between 0° and −10°C without a nerve in the chamber.

That the temperature rise associated with spontaneous rewarming occurred within the nerve is shown by the large increases in the height of the action potential and in the conduction velocity that occurred at the onset of rewarming (Fig. 2, *B* and *C*). The studies of Mazur (*4*) on the thermodynamics of intracellular freezing predict that at low rates of cooling, such as those used in our studies, intracellular freezing would be unlikely at tissue temperatures warmer than −10°C. It is therefore probable that the spontaneous rewarming was associated with the freezing of extracellular fluid. The action potential usually disappeared soon after the temperature rise associated with spontaneous rewarming began its decline, lending support to the idea that the nerves were progressively freezing at this point. Nerves that were gently touched immediately after the disappearance of the action potential were stiff and opaque.

When nerves were cooled to the temperature where the action potential disappeared, or where spontaneous rewarming occurred, and then warmed again to 25°C, full recovery of function usually occurred. If artificial rewarming was not begun soon after disappearance of the action potential, the nerves either failed to recover or showed functional changes. In several instances cooling was continued after the disappearance of excitability, but none of the nerves cooled below −7°C recovered their excitability when rewarmed to 25°C.

The temperatures at which spontaneous freezing occurred are in the same range that Smith and her coworkers reported for spontaneous freezing in supercooled hamsters (*5*). Failure of excised nerves to recover function following freezing at −4° to −7°C occurred in the temperature range from which Smith (*5*) and Popovic (*6*) reported deaths from freezing of whole mammals. Our results with supercooled nerves also agree well with the recent findings of Luyet and Gonzalez (*7*) that whole muscle in rats can survive freezing for 15 to 20 minutes at −5°C, and not at all at −10°C. Evidence for recovery from freezing of peripheral nerves in man was provided recently by the observation of Mills (*8*) that frozen limbs may show a return of cutaneous sensitivity after being thawed by rapid rewarming techniques. Our results would corroborate such recoveries if nerve temperatures never fell below −4° to −7°C.

Figure 3 shows a plot of conduction velocity as a function of temperature in the caudal nerve of a red squirrel. Determinations of conduction velocity just prior to or during spontaneous rewarming are plotted in the lower left corner (temperatures below 0°C). Conduction velocity is commonly reported to be a linear function of temperature. Such a linear relationship appears to hold for our data between 15° and 35°C, but values obtained at 5°C and during supercooling are somewhat higher than might be expected.

It is not known if peripheral nerves ever must function in the supercooled state in an intact animal. The fact that caudal nerves of muskrats from a warm climate conduct in the supercooled state shows that exposure to severe cold is not, at least in every case, associated with conduction capability at supercooled temperatures. Perhaps the most important feature common to those species exhibiting subzero nerve function is the rather wide temperature range over which the body regions supplied by such nerves normally operate.

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Ultrastructure of Vegetative and Reproductive Apices of Chenopodium album

Abstract. *The apical meristem of the vegetative shoot of* Chenopodium album *(lamb's-quarters) exhibits alterations in cytoplasmic structure as early as 3 hours after the plant has been subjected to one photoinductive cycle which promotes flowering. The endoplasmic reticulum shows an altered distribution and there is evidence of an increase in acid phosphatase production. Dictyosomes increase in number per cell by the end of the second inductive cycle.*

The volume of literature relative to the process of flowering is considerable and there are recent reviews of the subject (*1*). Interest has centered mainly around the areas of response of the plant to its environment, the site of synthesis and movement of a flowering substance, and biochemistry of the receptor pigment system. There are very few studies in which attempts were made to correlate strictly physiological processes with early morphological changes which occur at the reactive sites—the shoot apical meristems.

In most of the early morphological studies of flowering, no attempts were made to control flowering experimentally, and in many instances such control would have been virtually impossible. In more recent studies, certain short-day plants in which flowering can be controlled conveniently have been used, for example, *Xanthium pennsylvanicum* (cocklebur), *Chenopodium album* (lamb's-quarters), and *Pharbitis nil* (Japanese morning glory). Several of these studies have yielded data relative to qualitative changes in such substances as nucleic acids and total proteins (*2–4*). It has been well established that only an active bud can be in-

duced—that is, one in which DNA multiplication is occurring (*5*). That RNA synthesis is an essential feature of induction has been well established (*2*, *3*, *6*, *7*). There is evidence that an increase in the concentration of sulfur-containing proteins occurs upon induction (*3*). Histone is considered normally to occur as nucleohistone, but histones (basic proteins) increase in concentration in the cytoplasm of cells of the reproductive apex (*3*). In one instance, the mitotic index of *Xanthium* was reported to double as early as 16 hours after the end of the inductive long night (*8*). Healy and Jensen (*4*) report a doubling of the number of ribosomes in the central zone of the apex 12 hours after the end of the long inductive night for *Pharbitis nil*. An increase in the histone concentration in the cytoplasm occurs 72 to 96 hours after the end of the inductive cycle in *Xanthium pennsylvanicum* and *Chenopodium album*, respectively (*3*).

If changes in distribution and concentration of substances can be detected as early as 12 hours after the end of the inductive cycle, one might assume that alterations may occur in the ultrastructure of the plant at this time. In this report we offer evidence of such changes.

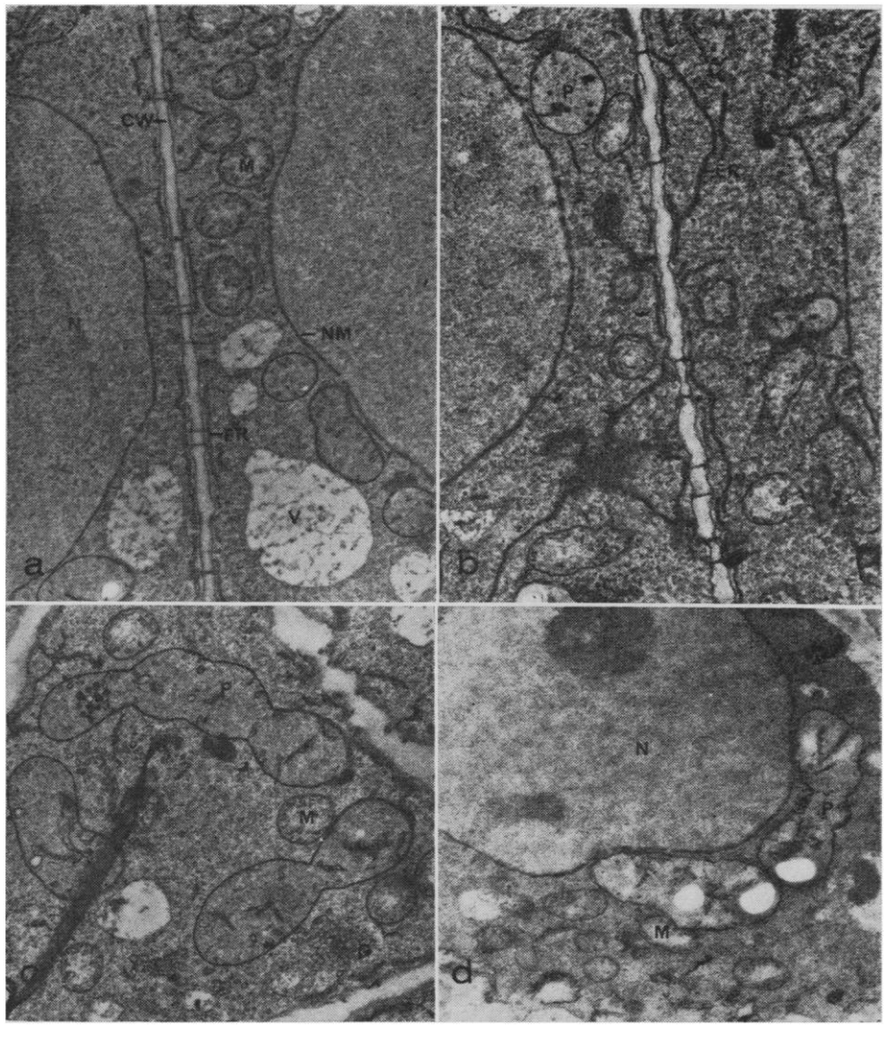

Fig. 1. Electron micrographs of cells from central zone of vegetative shoot apices of *Chenopodium album*. (*a*) Vegetative control showing restriction of endoplasmic reticulum (*ER*) to a position near the cell wall (*CW*). (*b*) After three photoinductive cycles there is a more extensive distribution of *ER* throughout the cytoplasm. (*c*) After two inductive cycles showing the commonly lobed appearance of plastids. [*a*, *b*, and *c*: apices fixed first in cold 3-percent glutaraldehyde, washed in cold water and then fixed in cold 2-percent $KMnO_4$ (× 9,000)]. (*d*) Cell from vegetative apex fixed in cold 10-percent formaldehyde, washed in water at room temperature, and then fixed with cold 2-percent $KMnO_4$. Note nearly continuous nuclear membrane and satisfactory preservation of mitochondria; compare with Fig. 2. (× 11,000). *CW*, Cell wall; *D*, dictyosome; *ER*, endoplasmic reticulum; *M*, mitochondrion; *N*, nucleus; *NM*, nuclear membrane; *P*, plastid; *V*, vacuole.

We used a "short-day" plant, *Chenopodium album*, for which there is considerable information on the morphological and cytochemical changes which occur at the shoot apex following induction (*2*). *Chenopodium album* can be kept vegetative on a regime of 16 hours of light and 8 hours of darkness. A reversal of the light regime will induce flowering. Normally, flowering will not occur uniformly in the variety we used unless the plants receive two or three inductive cycles. The plants (30 to 45 cm in height) for this experiment were subjected to a continuous inductive regime for three cycles. Apices of induced plants were collected 3 hours after the end of each long night and apices of vegetative plants were collected at various times within a 24-hour period. Collections were also made from plants given a short light period in the middle of the 16-hour dark period. The apices were fixed in a cold, 3-percent glutaraldehyde solution buffered with 0.1*M* phosphate buffer at *p*H 7.2. They were then washed in cold buffer, treated for 2 hours in a cold, 2-percent $KMnO_4$ solution, dehydrated, and embedded in Maraglas. The material was sectioned with a diamond knife on a Porter-Blum microtome.

The shoot apex of a vegetative plant grown under long days possesses two surface layers of cells (tunica) which enclose a more massive tissue region (corpus). A terminal axial zone, composed of the distal portions of the tunica and corpus, will henceforth be referred to as the central zone. Details of apical organization can be found elsewhere (*2*). The ultrastructure of the cells of the shoot apex is similar to that described for the meristematic cells of the root tip by Whaley *et al.* (*9*). Mitochondria, proplastids, vacuoles, dictyosomes, nuclei, and endoplasmic reticulum (ER) are present after fixation in $KMnO_4$. The ER is continuous with the nuclear membrane and also is probably continuous with the intercellular connections.

In cells at the tip of the vegetative apex the endoplasmic reticulum is most often seen as a single layer near the outer surface of the cell (Fig. 1*a*). After three inductive cycles the ER shows a more complex distribution in the cell and is seen throughout the cytoplasm (Fig. 1*b*). Although most pro-

nounced after three cycles, the change in ER distribution begins 3 hours after the end of the first long night. Lobed plastids (Fig. 1*c*) which may be in the process of dividing occur in cells near the tip of the apex of vegetative plants, but are much more commonly seen in cells on the flanks of the apex. After two and three inductive cycles lobed plastids are as common at the tip as along the flanks. The number of dictyosomes visible in sections of apical cells increased sharply after three inductive cycles. The average number of dictyosomes counted per cell in sections of the second tunica layer of the vegetative apex was 3.5 (based upon counts from apices fixed at various times during the 24-hour period). The average number of dictyosomes in cells from the same region in apices collected after one, two, and three long inductive nights were 4.7, 4.7, and 7, respectively. Observations of plastid behavior and ER distribution were made from several sections from each of six to ten apices of each stage. We took dictyosome counts from the axially located cells of the second tunica layer and from the next six cells of the same layer, moving down the flank of the apex. The counts were taken from four sections from each of three apices of each stage.

When sampled 3 hours after the end of the interrupted long night and on succeeding days, apices from plants given only one interrupted long night exhibited an ultrastructure similar to that of a plant on long days. If apices were sampled immediately after three successive interrupted long nights they exhibited the ultrastructural changes described for induced apices; such plants were in fact induced and reached anthesis a week to 10 days later than plants given uninterrupted long nights. The apices from plants given interrupted long nights were sampled in an effort to distinguish between the effects of induction and the increase of night length itself, but these effects are difficult to separate, especially with a plant that normally requires more than one long night for induction to occur.

The changes reported in plastid behavior, dictyosome number, and ER distribution may be associated with the induction process or they may be merely indicators of the increased rate of cell division that occurs upon induction.

When we began this study, the poor results we obtained with apices fixed in $KMnO_4$ alone led us to try fixing them first in cold 10-percent formaldehyde and then, after washing them for 1 hour in water, fixing them in cold $KMnO_4$. Although it is generally good practice to keep material being processed for electron microscopy at a low temperature, the 1-hour wash was carried out at room temperature to speed washing time. This procedure had no readily apparent ill effects upon the cells of the vegetative shoot apices (Fig. 1*d*), but samples taken 3 hours after the end of the first inductive night showed obvious signs of autolysis (Fig. 2, *a* and *b*). The mitochondria were swollen and the membranes of all organelles were thickened and dense. The nuclear membranes were broken into many short lengths. After two long nights the autolysis was more severe and after three long nights the contents of many cells were almost completely destroyed. It is possible, therefore, that upon induction there is an increase in concentration or activity of a hydrolytic enzyme. Acid phosphatase is present in the meristematic cells of plants; it attacks phospholipids (*10*) and is not greatly affected by dilute solutions of formaldehyde (*11*). Since the first effects of autolysis are upon the phospholipid-containing membranes, it is possible that the enzyme is a phosphatase. Other enzymes could, of course, be involved. Preliminary histochemical tests have shown acid phosphatase to have a general distribution in both induced and vegetative shoot apices of *C. album*, but since these tests are not quantitative, nothing can be said at this time concerning the relative concentration of the enzyme.

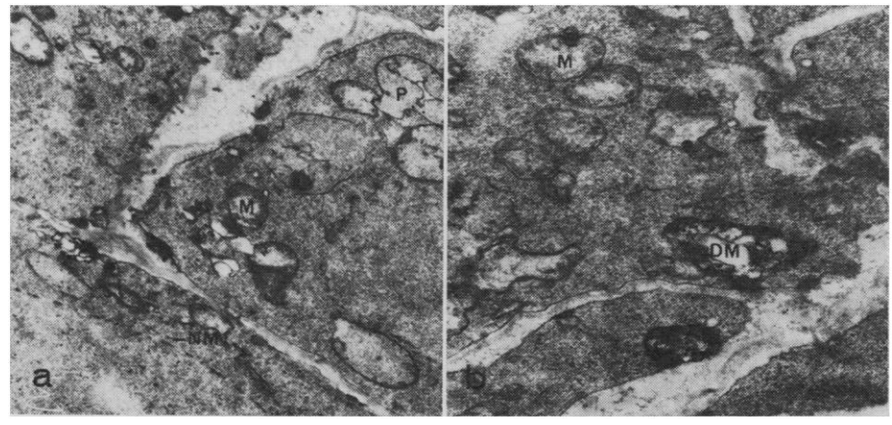

Fig. 2. Cells from central zone of induced apex 3 hours after the end of one inductive long night. Note high percentage of damaged mitochondria (*DM*) and interrupted nuclear membrane (*NM*) (both × 10,000). Method of fixation as in Fig. 1*d*.

Much research has been conducted relating the phytochrome system to flowering as well as to other photomorphogenetic responses (*5*). However, the nature of the flowering process differs from the noninductive responses of most other light-controlled biological systems (*6*). It is generally believed that flowering in day-sensitive plants is stimulated by the movement of a flowering hormone, "florigen," from the leaves to the shoot apex, and that young, fully expanded leaves are most effective in the production of florigen. The early flowering response noted for *Chenopodium album* hardly provides the necessary time for translocation to occur from mature leaves.

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