cytopathic capacity for more than 5 months' storage at −40°C.

Plaques induced by lysate material are formed on cultures of chick embryo cells under hard agar, attaining diameters of up to 1 cm in 3 weeks. Material recovered from the plaques initiates cytopathic responses on chick cells in liquid medium similar to those initiated by the original amoeba lysates.

The presence of new infectious material in the chick cells was determined by making end-point dilutions of the supernatant and cellular fractions of inoculated cultures sampled at daily intervals. The cells were inoculated with material that was infectious at a 10^{-2} dilution for 1 hour at 37°C, thoroughly washed, and placed in medium (2 percent calf serum in Gibco 199). The cells remained indistinguishable from the uninoculated control cells for 4 days following inoculation, and during this time they were noninfectious in both the supernatant and the cellular fractions. On the fifth day after inoculation about 25 percent of the cells were visually cytopathic in areas that spread to include the entire plate by the next day. In direct correlation with the appearance of the cytopathic cells, the supernatant and cellular fractions of the cultures were infectious at dilutions of 10^{-3} and 10^{-4}, respectively, on the fifth day. The infectivity of the supernatant fraction increased somewhat to equal that of the cellular fraction at a 10^{-4} dilution by the sixth day.

It is concluded that the EG amoebas contain a material capable of infecting chick embryo cells and causing them to undergo cytopathic changes with the release of more of the infectious material. The material is present in two lines of the amoeba which were separated shortly after the initial isolation of the EG strain and maintained in separate laboratories in media with different growth supplements.

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12 July 1971

Potassium Flux: a Common Feature of Albizzia Leaflet Movement Controlled by Phytochrome or Endogenous Rhythm

Abstract. *Leaflets close when potassium moves into dorsal and out of ventral pulvinule motor cells and open when the flux direction is reversed. This is true whether leaflet movement is controlled by an endogenous rhythm or by phytochrome.*

Various leguminous plants, including *Albizzia julibrissin*, have doubly compound leaves with paired leaflets that regularly move toward and away from each other in response to a variety of endogenous and exogenous stimuli. Leaflets usually open in the light and close when darkened, but their movement will persist with a circadian rhythm if plants are kept in constant intensity light or uninterrupted darkness (*1*). During certain parts of the diurnal cycle, nyctinastic closure is controlled by the regulatory pigment phytochrome (*2*, *3*), which also controls many aspects of plant development such as seed germination, photomorphogenesis, and floral initiation (*4*).

Leaflet orientation depends on the relative turgor of motor cells on dorsal and ventral sides of the pulvinule, a thickened organ at the base of the leaflet (*5*). Potassium (K) flux is involved in turgor changes in *Mimosa pudica* motor cells (*6*) and in stomatal guard cells (*7*). The K concentration of the *Albizzia* pulvinule is sufficiently high (about 0.47*N*) that K flux can exert significant osmotic effect. We have reported (*8*) that *Albizzia* leaflets close in the dark when K moves into dorsal motor cells and out of ventral motor cells, and that preirradiation with red light is required for K efflux from ventral cells and for optimum movement of K into dorsal cells. Thus K flux in key pulvinule cells is the basis for phytochrome-controlled nyctinastic leaflet closure. Our study was undertaken to determine whether it is also the basis for endogenously rhythmic leaflet movement.

Albizzia plants were grown from seed in the greenhouse and transferred to controlled growth chambers 3 days or more before experiments, to allow time for plants to adjust to new environmental conditions. Growing conditions, experimental procedures, and light sources were as described (*8*).

A typical *Albizzia* leaf contains hundreds of pinnule pairs with similar K content and physiological behavior. We obtained comparative kinetic data on leaflet movement and K flux by measuring the angle between paired leaflets at designated time intervals, and then excising, freezing, and sectioning

Table 1. Leaflet closure in the light and correlated changes in K, the ratio of K to Ca, and the ratio of K to P in dorsal and ventral motor cells. The experimental procedure was the same as that described for Fig. 1, except that leaflet angles were measured and pulvinules were excised during the 12th to 16th hour of the photoperiod only. Data include standard deviations.

Time (hr)	Angle between leaflets	Ventral			Dorsal		
		K	K/Ca	K/P	K	K/Ca	K/P
12	180°	302 ± 98	1.15 ± .38	0.35 ± .11	215 ± 31	0.64 ± .23	0.23 ± .05
13.5	110°	300 ± 45	0.96 ± .14	0.30 ± .07	268 ± 63	0.87 ± .17	0.30 ± .07
14.5	60°	214 ± 40	0.87 ± .13	0.25 ± .06	297 ± 45	0.99 ± .18	0.35 ± .11
16.5	10°	163 ± 40	0.48 ± .11	0.14 ± .04	344 ± 44	0.98 ± .26	0.42 ± .20

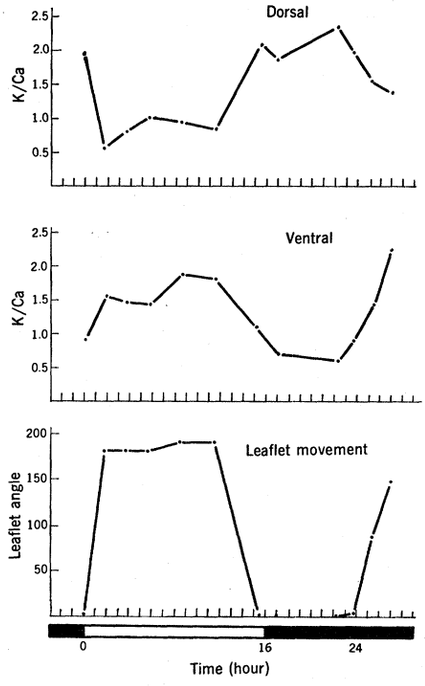

Fig. 1. Diurnal variations in leaflet angle and in the ratio of K to Ca in motor cells on the dorsal and ventral sides of the pulvinule. A plant was grown under its usual light conditions [about 1000 ft-c mixed (10 : 1) cool white fluorescent and incandescent], temperature (23°), and 16-hour photoperiod except that the final dark period was extended for 3 hours. At stated intervals the average angle between the leaflets on a mature leaf was measured and six pairs of pulvinules were excised, trimmed, and frozen in modified Tissue Tek at −30° in a cryostat. Longitudinal dorsiventral sections were cut at 24 μm, freeze-dried, and mounted on graphite rods for Acton electron microprobe analysis of K and Ca in dorsal and ventral motor cells. Measured values are scintillations during 25 seconds. Each datum is an average of 36 measurements (six sections, three areas per section, two measurements per area). Further details, including microprobe operating conditions, are described in (*8*).

the pulvinules in a cryostate in preparation for elemental analysis with an Acton electron microprobe (*8*). This instrument permits the simultaneous determination of the distribution of several different elements within a microstructure; we routinely determined K, Ca, and P in dorsal and ventral motor cells. Data are expressed either as K scintillations, or, to correct for changes in cellular volume during leaflet movement, as the ratio of K to one of the other relatively immobile elements (*8*).

With plants grown under a 16-hour photoperiod, leaflets open rapidly on transfer from dark to light, begin to close after 12 to 14 hours, and usually close completely before darkness. They remain closed during the 8-hour dark period, but will open without illumination if the usual dark period is extended 2 or 3 hours. Diurnal variations in leaflet angle and in the ratio of K to Ca in dorsal and ventral motor cells are shown in Fig. 1. The kinetic curves of leaflet movement and of K flux in ventral cells are almost parallel, while the curve for K flux in dorsal cells is almost their mirror image. The K content of both dorsal and ventral motor cells changes threefold during the diurnal cycle.

We studied K flux during the closure period (Table 1) to see whether kinetic data were consistent with a shuttle of K from contracting cells on one side of the pulvinule to expanding cells on the other side. K movement started at approximately the same time in ventral and dorsal motor cells, but K movement into dorsal cells was completed before K efflux from ventral cells. These data suggest that not all the K leaving contracting ventral cells enters expanding dorsal cells.

When *Albizzia* plants are maintained in constant intensity light or uninterrupted darkness for a prolonged period, leaflets alternately open and close, with each phase persisting for about 12 hours. Microprobe analysis of pulvinar tissue excised from a darkened plant during alternate periods of opening and closure revealed rhythmic variation in the K content of the motor cells, particularly those on the ventral side of the pulvinule (Fig. 2). Endogenously rhythmic leaflet movement may thus depend on an endogenous rhythm in K flux.

Studies of rat and cat brain have demonstrated diurnal rhythms in the concentration of the neurohumors acetylcholine and norepinephrine (*9*) and in the *N*-acetyltransferase activity controlled by norepinephrine (*10*). Our data on rhythmic K movement provide additional evidence for oscillatory changes in membrane permeability which may play a central role in the operation of the biological clock (*11*).

Comparative study of several plants with moving leaves or leaflets reveals both anatomical (*12*) and physiological similarities with *Albizzia julibrissin*. Both phytochrome and an endogenous rhythm control movement in *Mimosa pudica* (*13*), *Samanea saman* (*14*), and

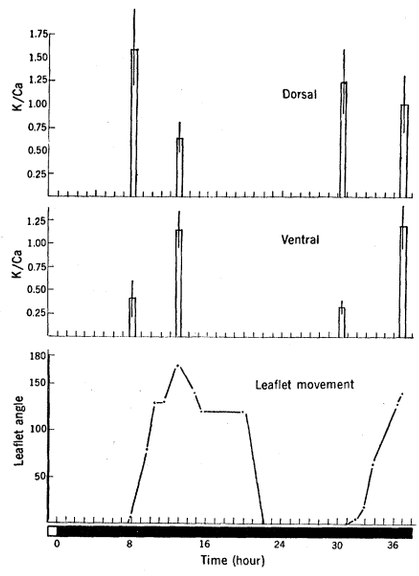

Fig. 2. Endogenously rhythmic variations in leaflet angle and in the ratio of K to Ca in dorsal and ventral motor cells. A plant that had been grown with a 16-hour daily photoperiod was transferred to continuous darkness. Pulvinules were excised and analyzed as in Fig. 1. Thin vertical lines indicate standard deviations.

Albizzia lophanta (*1, 2*). Phytochrome-controlled nyctinastic leaflet closure in *Samanea* and *Albizzia* both require aerobic metabolism and phytochrome that absorbs in the far red during the entire closure period (*8, 14*). The opening of both *Mimosa* and *Albizzia* leaflets is promoted by blue light, although continuous irradiation with far red light also opens the leaflets of *Mimosa* (*15*) but not those of *Albizzia*. K moves out of contracting cells of the primary pulvinus when *Mimosa* responds to a mechanical or a dark stimulus (*6*). These facts raise the possibility that K flux is a general prerequisite for turgor change in all species with moving leaves or leaflets.

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Prolonged Color Blindness Induced by Intense Spectral Lights in Rhesus Monkeys

Abstract. *Prolonged exposure of rhesus monkeys to intense blue light produces long-term changes which are consistent with loss of response of those cones that contain a photopigment with peak absorption at 445 nanometers. The 90 percent reduction of spectral sensitivity in the blue region has lasted more than 5 months. Reduced sensitivity to long wavelengths is produced by adaptation to light of 520 nanometers. This reduced sensitivity, which lasts no more than 30 days, is attributed to a temporary loss of response of the cones containing a photopigment with peak absorption at 535 nanometers.*

Studies on the effects of intense spectral light on the eye have been few, and have not dealt with recovery time of the eye after exposure. In an early study, Burch (*1*) created effects similar to hereditary color blindness in human subjects with "intense color adaptation." These effects lasted up to 1 hour. Burch exposed his subjects briefly to sunlight of unknown intensity filtered through broad-band filters. Auerbach and Wald (*2*) produced sizable but brief changes in photopic spectral sensitivity with 5-minute exposures to white light and to red, orange-red, orange, yellow, green, and blue light produced by broad-band filters. The retinal illuminances of these lights were up to 10^7 trolands. The subjects required 5 to 10 minutes to return to the initial state of adaptation to dark.

Brindley (*3*) exposed his own eye to retinal illuminances of 25,000 to 300,000 trolands for repeated 30-second periods. Using color matching as a measure, he found that the effects he produced were similar to hereditary dichromacy and in some instances to monochromacy. These effects lasted only 40 seconds, but Brindley also reported intense afterimages that lasted for 6 to 10 hours and a faint afterimage that lasted for 8 months. In other reports of duration of effects after exposure to intense light, subjects were exposed to intense but brief flashes of white light or broad-band light of an unspecified wavelength-energy distribution (*4*). In most studies (*4*), recovery of visual function was measured. In no instance, except Brindley's report of afterimages, has evidence been presented for long-term changes in visual thresholds in humans after brief exposures to radiant intensities below those which create gross pathology.

When rats have been exposed to intense white light for a long period, the rods of the eyes show microscopic degeneration of the membranes of the outer segments. This degeneration is not accompanied by gross retinal lesions (*5*). Recently, Kuwabara (*6*) found similar outer segment degeneration in cones of monkeys exposed to a high retinal irradiance, only 10 percent below that which produces gross thermal lesions.

These examples of rod and cone damage by white light led us to look for selective degeneration of different classes of cones caused by long exposure to intense light of different wavelengths. We exposed rhesus monkeys to intense blue and green lights for long periods, and measured spectral sensitivity before and after exposure.

Intense blue light caused a loss of short wavelength sensitivity that has persisted for more than 5 months. In contrast, we found a recovery time of 18 to 30 days for the changes in sensitivity to long wavelengths that followed exposure to green light. Using our model of spectral sensitivity (*7*), we relate the change effected by blue light to the cones that contain a photopigment with peak absorption at 445 nm; and the change caused by green light to the cones that contain a photopigment with peak absorption at 535 nm. Our results give us the hope of distinguishing between classes of color receptors by associating morphological changes of the type seen by Kuwabara and others (*5*, *6*) with the functional loss.

The optical system for both the spectral sensitivity measurements and for the spectral retinal irradiation consisted of three Maxwellian-view channels. (i) The white background channel produced a white background path of equal energy, which subtended a visual angle of 18 deg and produced retinal illuminance of 3400 trolands. All of the data are from increment-thresholds taken against this background. (ii) The test channel produced a 50-msec flash of 2-deg diameter, which was superimposed on the center of the white background. This channel used a 1600-watt compact xenon arc lamp, double monochromator, and counter-rotating neutral density wedges to reduce the flash to threshold. (iii) The spectral background channel used collimated light from the xenon arc lamp to produce a high intensity 18-deg spectral exposure path through narrow-band interference filters. The maximum radiant intensity, at the cornea, of the spectral exposure path was 10^{-4} watt/steradian, (*8*) which equals retinal power density of 10^{-3} watt/cm^2 (neglecting preretinal absorption). This is one-thousandth of the retinal power density for threshold retinal burns reported by Ham *et al.* (*9*) or for pigment epithelial damage reported by Friedman and Kuwabara (*10*).

Subjects for the experiment were adolescent rhesus monkeys (*Macaca mulatta*) (*11*). The operant behavior procedure for increment-threshold measurements has been described (*12*, *13*). Threshold measurements were made at consecutive 10-nm intervals from 410 to 690 nm and then from 690 to 410 nm. The average of the two values was the threshold for that day.

The same operant behavior proce-