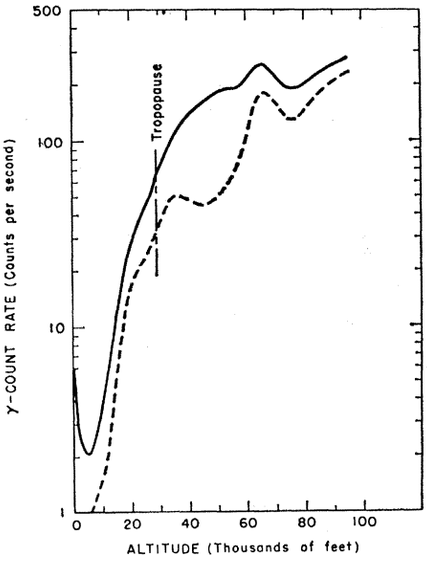

Fig. 1. Gamma-ray count rate as a function of altitude. Solid line, gross count rate; dashed line, net count rate, cosmic ray component removed.

natural sources has been assumed to occur at Thule, and has been subtracted, yielding the net γ count rate curve also indicated in Fig. 1.

Three peaks in the net γ distribution are apparent; one slightly above the tropopause (tropopause height, 29,200 ft on this date), a second located in the vicinity of 65,000 ft, and a third rise in count rate above 80,000 ft. This particular flight terminated with balloon burst at 97,000 ft. Similar distribution patterns were observed on the other three flights to altitudes above 80,000 ft, with the highest counting rate occurring at the highest altitude attained. In three flights a sharp transitory rise in count rate was observed between 1500 and 2000 ft, at which height a strong temperature inversion was noted in the radiosonde flights. Because of the decreasing density of the atmosphere with altitude, the net count rate data per se may be misleading, as the mean free path, and hence the effective physical volume from which γ-radiation is being detected, varies inversely as the air density. Suffice it to say at this time that fission product radioactivity was present at the time of these flights at altitudes up to 100,000 ft.

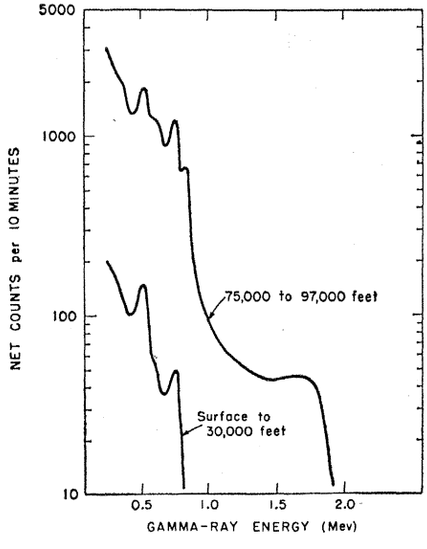

Fig. 2. Net γ-ray spectra observed *in situ*.

Gamma spectra accumulated directly on a multichannel analyzer as well as from magnetic tape support the contention that the net γ count rate arises from fission product radioactivity. Figure 2 shows net γ spectra observed between the surface and 30,000 ft, and 75,000 to 97,000 ft during flight No. 2. The photon spectrum due to cosmic ray interactions in the atmosphere has been removed by assuming that the spectral shape of this component as seen by the phoswich is a continuum and may be represented by a simple mathematical function. The magnitude of this component in a specific case was determined in the region 2.0 to 4.0 Mev, and its spectral contribution then subtracted from the spectrum in the region 0.25 to 2.0 Mev. Both spectra shown in Fig. 2 indicate γ-ray lines at 0.5 and 0.75 Mev. The former is attributable to positron annihilation and Ru^{106}, with a lesser contribution from Ru^{103} and possibly naturally occurring Be^{7}. The line at 0.75 Mev is presumably due to Zr^{95}–Nb^{95}. The spectrum observed above 75,000 ft is more complex, with indications of a line near 0.6 Mev, one at 0.85 Mev, and a low intensity line in the region of 1.6 to 1.7 Mev. The presence of Zr^{95}–Nb^{95} in relative abundance signifies a fairly recent origin, and it was presumably produced in the Soviet tests conducted during the autumn of 1961. The line at 0.85 Mev is attributed to Mn^{54} on the basis of energy and of a half-life sufficiently long to survive until April 1962; in similar fashion the lines at 0.6 and 1.6 to 1.7 Mev are attributed to Sb^{124} (*3*).

These investigations have shown the presence of readily detectable quantities of fission radioactivity in the atmosphere up to 100,000 ft over northern Greenland in early April 1962. The bulk of this activity was contained well within the stratosphere and may reflect the initial injection of debris to high altitude in the two largest shots of the series. Furthermore, it was observed that the remnants of the circumpolar vortex, a band of strong winds from the west at high altitudes in the arctic winter, were still present at the time that these measurements were made (*4*). The presence of a portion of the debris from the Soviet 1961 series at high altitude ($>$ 80,000 ft) within the polar stratosphere as late as April 1962 may explain, in part at least, the fact that observed fallout levels at temperate latitudes have been somewhat lower than anticipated thus far this year.

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References and Notes

1. This work was performed under the auspices of the U.S. Atomic Energy Commission in cooperation with the U.S. Weather Bureau. The invaluable assistance of personnel of Detachment 48, 12th Weather Squadron, U.S. Air Force, in making these flights, and the construction of the balloon-borne electronic equipment by the Health and Safety Laboratory, New York Operations Office, AEC, is hereby acknowledged.
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29 August 1962

Chloroplasts and Mitochondria in Living Plant Cells: Cinephotomicrographic Studies

Abstract. Chloroplasts *in situ* are surrounded by jackets of a material which does not contain chlorophyll. The chlorophyll-bearing inner structure lacks motion while the jackets constantly change shape. Long protuberances may extend from the jackets into the cytoplasm and may segment into particles that cannot be distinguished from mitochondria. Segmentation and other dynamic characteristics of living plant cells have been recorded on cine film.

We have photographed (*1*) a structural feature of higher plant chloroplasts which may be a factor in understanding the metabolism of chloroplasts. In the living condition, as viewed by phase contrast microscopy, chloroplasts are surrounded by jackets of material not containing chlorophyll. The chlorophyll-bearing inner structure appears to lack motion, whereas the jackets constantly change their shapes. Frequently, long

protuberances extend from the jackets into the surrounding cytoplasm. The protuberances may then segment into structures that cannot be distinguished from mitochondria. Furthermore, individual mitochondria can also be observed to unite with the chloroplast jackets and lose their identity completely. Figure 1 illustrates the segmentation of a protuberance and also shows the extent of the jackets surrounding the chloroplasts in a living spinach palisade cell.

After separation from the chloroplasts, the mitochondrial-like organelles join in the procession of mitochondria and sphaerosomes which stream within the cell. Usually, a view normal to a spinach mesophyll cell face shows that the mitochondria and sphaerosomes follow paths around the chloroplasts but that they occasionally seem to pass directly across the chloroplast face on either the vacuolar or the cell wall side. When viewed from the side, the spinach mesophyll cell appears stratified as follows from outside to inside: cell wall; the nonstreaming cortical gel in which the chloroplasts are partially embedded; two layers of kinoplasm [the streaming cytoplasm; see Kamiya (*2*)]; the layer adjacent to the cortical gel containing the bulk of mitochondria and the inner layer containing the bulk of the sphaerosomes; and the main vacuole. Mixing occurs between the two layers of kinoplasm and mitochondria, and sphaerosomes can be seen passing from layer to layer. In general, the innermost layer near the vacuole streams most rapidly. Strands of kinoplasm also enclose small vesicles or vacuoles to form a pattern, the cytoplasmic network. Portions of the network may be a visible extension of the endoplasmic reticulum (see Rose and Pomerat, *3*).

We have recorded on cine film (*4*) novel features of the protoplasmic organization in living plant cells, such as the cytoplasmic network, the sphaerosomes which outnumber all of the other visible organelles, and the dynamic behavior of mitochondria and chloroplasts. The photographs in Fig. 1 can in no way convey the sense of dynamic motion observed in living plant cells. Cinephotomicrography more closely depicts a realistic view of this activity than time-lapse exposures. Plant cytoplasmic streaming is so vigorous that slow-motion exposure may better resolve some of the motion.

For recording observations on cine film, free-hand sections of fully expanded spinach leaves provided excellent material for observation. The sections were cut so that virtually a single layer of palisade and spongy parenchyma cells was exposed. In most sections nearly all of the cells remained alive and were observed at the highest magnification of the phase contrast microscope. That the cells were alive was ascertained by the cytoplasmic streaming within them. Exposure of leaf cells as a single layer did not obviously impair the activity of the cells, which remained in a streaming condition for several days after sectioning when kept in water. When the cells were illuminated or supplied with sucrose in the dark, starch grains appeared within the chloroplasts. Buffer solutions such as phosphate or tris arrested the streaming activity in the sectioned material.

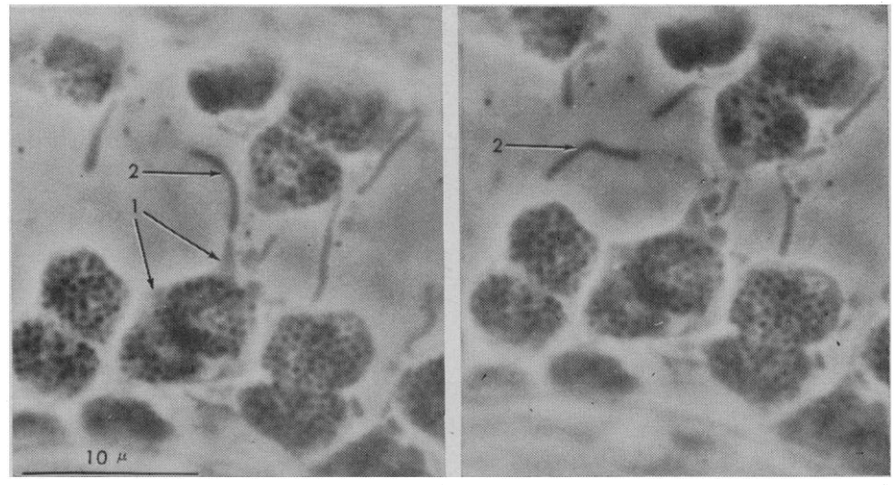

Fig. 1. Photomicrographs of a portion of a living spinach palisade cell. Arrow 1 points to the non-chlorophyll containing jackets which surround the chloroplasts (note conspicuous grana). Arrow 2 points to a mitochondria-like organelle in the process of segmentation from the chloroplast jacket. In the left photograph the organelle is still attached by a thread to the jacket. In the right photograph the organelle has joined in the streaming activities of the cell. The pictures were taken by flash photograph about 30 seconds apart on Kodak Plus X film. (Phase contrast with Neofluar 100X, oil immersion objective lens, Komplan 12.5X ocular lens, Zeiss 35-mm attachment camera.)

Phase contrast observations were recorded at 16 frames per second on Ektachrome ER film with a Bolex H 16 camera fitted with a 50-mm Switar lens and with a Wild focusing attachment for observation during exposure. A Zeiss research model microscope equipped with Neofluar phase contrast objective lenses, Komplan ocular lenses, and V Z phase contrast condenser was used. Filtered illumination was provided by an Osram XBO high-pressure xenon lamp.

The cine film also records some features of pressure effects as viewed in hair cells from tobacco leaves. When pressure was placed on cells, individual mitochondria became joined with the cytoplasmic network and lost their identity. Release of the pressure resulted in the reappearance of individual mitochondria and diminution in the extent of the network. Although pictures were not obtained, we observed mitochondria separating from the cytoplasmic network as well as coalescing with the network when the cells were not subjected to pressure. Since the cytoplasmic network may be a visible extension of the endoplasmic reticulum, our observations appear to support the interpretations of electron micrographs by Robertson (*5*) regarding the development of mitochondria from the endoplasmic reticulum.

The cine film records the streaming behavior of organelles in living cells of mature potato tubers. It shows the lobed and furrowed appearance of nuclei in different kinds of cells and the heterogeneity in structure of nucleoli. It also shows various kinds of inclusion bodies that arise in living cells as the result of tobacco mosaic virus infection, including a sequence showing the movement of the cytoplasmic network over the surface of a hexagonal tobacco mosaic virus-inclusion body.

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6 June 1962

Pollen Tube Growth in vivo as a Measure of Pollen Viability

Abstract. Observation by fluorescence of tomato pollen tubes in fixed styles clearly showed that all pollen samples grew regardless of age and fruit or seed setting potential. Methods commonly used to evaluate pollen viability, such as staining and germination, may predict growth in the style but do not always predict seed setting ability.

In the course of a study of the physiological and cytogenetic consequences of aging tomato pollen, it was discovered that older pollen lots often induce fruit setting without seed formation. Similarly induced parthenocarpy has been observed by McGuire (*1*) and others. Since this response suggested that fruit setting was induced as a result of pollen tube activity even without fertilization, a study was undertaken of tube growth in styles to which aged pollen had been applied. The dependable and rapid method of Martin (*2*) for staining pollen tubes in the style has greatly facilitated the study.

Although in vitro pollen germination has served as a useful indicator of fruit set in apples and pears under controlled conditions (*3*), a dependable measure of pollen viability (seed-setting ability) has been sought for a long time in other plants. Research on pollen physiology and programs of plant hybridization would be expedited if there were available a method for estimating seed-setting potentials which would not require waiting until ripe fruits could be sectioned. The frequent failure of such methods as staining the pollen itself or germination of pollen in vitro to predict seed set or even fruit set has been repeatedly demonstrated (*4*); in fact, Visser cites a negative relationship between germination in vitro and fruit set in a number of crops (*3*).

Several series of experiments with stored and fresh tomato pollen from the various varieties in the field and greenhouse were undertaken in 1959 (*5*) and repeated in 1960. The pollen samples were collected in gelatin capsules and stored immediately at 0°C over $CaCl_2$. These conditions were found to be the best for tomato pollen storage by McGuire (*1*). The oldest pollen was collected in 1956 and 50 other samples were collected at various later times. Three genetically different male-sterile lines (*6*) were pollinated in the greenhouse with both stored pollen and control pollen which had been freshly gathered from genetically identical sources. All pollen lots were judged to be 90 to 100 percent "viable" after testing by acetocarmine staining and germination in 20 percent sucrose. Twenty or more hours after pollination, two to five styles from each group of pollinations were fixed in formalin-aceto-alcohol, stained with aniline blue, and observed under ultraviolet light according to Martin's technique (*2*). Since pollen tubes penetrate to the ovule of the tomato in 12 to 15 hours, styles may be removed from the ovary after that time and the tubes observed without jeopardizing potential fruit or seed set. Thus information on tube growth, fruit set, and seed set can be obtained from each pollination.

Table 1. Pollination results from tomato pollen samples stored at 0°C over $CaCl_2$.

Parents		Pollen age (mo)	Pollen tubes per style*	Flowers pollinated	Total fruits	Total seeds
Female	Male					
ms_{17}, a_1, c, d_1, l	Early Pak	50	20–50	5	0	0
Pearson (ms_2)	Early Pak	38	10–20	17	0	0
Pearson (ms_2)	Pearson	17	50–100	12	0	0
Pearson (ms_2)	XL	14	150–200	27	6	4
Pearson (ms_2)	Pearson	14	150–200	7	5	0
ms_2, a_1, hl	VF36	6	75–100	5	1	4
Pearson (ms_2)	Pearson	4	150–200	11	9	100
ms_2, a_1, hl	VF36	3	100–150	5	2	40
ms_2, a_1, hl	VF36	Fresh	150–200	5	3	60
Pearson (ms_2)	Pearson	Fresh	150–200	5	5	250
ms_{17}, a_1, c, d_1, l	Pearson	Fresh	150–200	5	4	220

* Range observed in sample of two to five styles.

Table 1 gives representative findings from 8 of the 50 stored samples. The number of pollen tubes in column 4 represents the range found in the two- to five-style sample. The majority of the pollen tubes had grown the full length of the styles. Pollen lots older than 6 months produced some seedless fruits, and those older than 14 months failed to produce any fruits; yet pollen tube growth was observed in all styles regardless of pollen age. While staining and germination may predict growth in the style, such growth certainly does not measure fruit and seed setting capacity. It is clear from these simple tests that observing pollen tube growth in vivo also fails to serve as a reliable index of fruit or seed setting ability.

Comparable results have recently been reported by Nishiyama and Tsukuda (*7*) for x-irradiated tomato pollen, although responses in styles were not investigated. In their experiments, doses up to 10^5 r did not reduce germination in vitro but did eliminate fruit and seed set. At lower levels of irradiation, seedless fruits were reported. Fruits with viable seeds were produced only at the lowest levels of irradiation.

The seedless fruits produced in our experiments characteristically contained aborted ovules of varying sizes. Those seeds which appeared normal in size germinated in soil and were 60 to 80 percent viable. However, the seedlings from the older samples of stored pollen produced a high proportion (40 to 50 percent) of mutant plants abnormal in appearance or in pollen production (*8*).

The ability of stored and irradiated pollen to produce pollen tubes which elongate both in the style and in sugar solutions but which do not produce seed adds to the evidence of Johri and Vasil (*4*) and Visser (*3*) that "pollen viability" tests, other than seed production itself, may be quite unreliable. The large proportion of mutant seed produced by aged pollen suggests damage to the nucleus. However, this does not necessarily mean that the nuclei are functionless in directing pollen tube growth or even incapable of entering into fertilization. A useful measure of seed setting ability of pollen will have to be sought with these possibilities in mind. Meanwhile, further studies of the pollen tube mitosis, fertilization, and embryo development with aged or irradiated pollen should add important knowledge to these phenomena.

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