The third aspect is a statement contradicted by our experiments. Because we examine our material only 1 day after γ-irradiation, the contribution of postirradiation cell division to lethality is reduced or eliminated. Consequently, our experiments pertain to radiation sensitivity of cells only in relation to mitotic activity of the tissue at the time of irradiation. In a complex system, where the magnitude of a given biological effect is not proportional to radiation dose, relative radiation sensitivity can be quantitatively defined only in terms of the radiation doses necessary to produce the same biological effect in tissues of the same morphological cell types. In our system, comparable effects are caused by equal γ-ray doses, without regard to the relative rate of cell division. Thus, there is no difference in intrinsic radiosensitivity conferred by the occurrence of cell division.

We do not think our findings contradict any of the observations pertinent to the law of Bergonié and Tribondeau, because other correlations of apparent radiation sensitivity with mitotic activity have entailed observations of different morphological cell types involving different effects or have used criteria of radiation effects inapplicable to nondividing cells. Within the same type of tissue, there undoubtedly are physiological differences associated with the presence or absence of cell divisions. Our results suggest, however, that such physiological differences do not significantly alter the intrinsic radiosensitivity of the whole cell.

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7 October 1968

Gas Vacuole Development in a Blue-Green Alga

Abstract. De novo *production of gas vacuoles can be induced in the blue-green alga* Nostoc muscorum *by transferring the cells from a defined medium to distilled water. The unusual ultrastructure of the gas vacuole membranes permits their easy recognition when specimens are prepared for electron microscopy by freeze-etching. The youngest gas vacuoles are biconical organelles; 48 hours after induction the gas vacuoles reach their maximum observed length when they are cylinders (1.5 by 0.1 μ) with cornical ends.*

Gas vacuoles are gas-filled organelles (Fig. 1) found in certain prokaryotic organisms (*1*, *2*). In some organisms gas vacuoles are present at all times (*2–4*); in others they occur sporadically (*2*, *5*, *6*). When present in sufficient quantity they cause the cells to float, but other details of their role have not been explored (*7*). Gas vacuole membranes have an unusual composition: they contain no lipid and are largely protein (*8*). They likewise have an unusual structure: they are composed of bands which may be subunits arranged in rows (*9*). This unique structure is readily observable in electron micrographs of specimens prepared by freeze-etching (*10*). Freeze-etching exposes the inside, gas interface of the vacuole membrane which is easy to identify and distinct from other cellular membranes.

There are three possible ways in which membranes can originate: (i) as outgrowths or products of other membranes or organelles, (ii) by division of existing organelles, and (iii) by assembly of the membrane from cytoplasmic components unconnected with any existing membranes or organelles (*de novo*). Smith and Peat (*3*) speculated that gas vacuoles might be produced by the photosynthetic membranes of the cell. In their electron micrographs of *Anabaena flos-aquae*, the gas vacuoles are in intimate contact with the photosynthetic membranes. Pringsheim (*11*) reported gas vacuoles as arising by fission of preexisting gas vacuoles, based on his light-microscope observations of *Oscillatoria agardhii* in which gas vacuoles are present at all times. Neither of these studies (*3*, *11*) was primarily intended to study gas vacuole origin, and the techniques employed were not designed to provide such information.

Gas vacuoles originate *de novo* in the isolate of *Nostoc muscorum* (*12*)

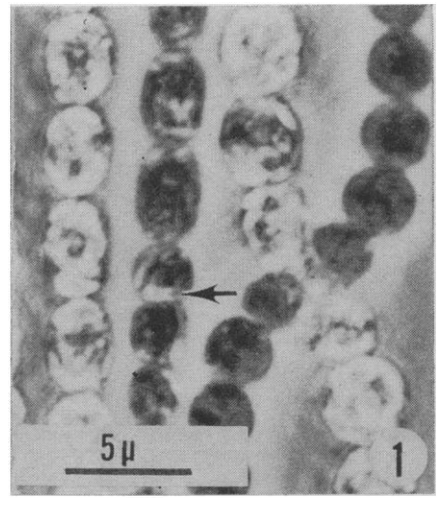
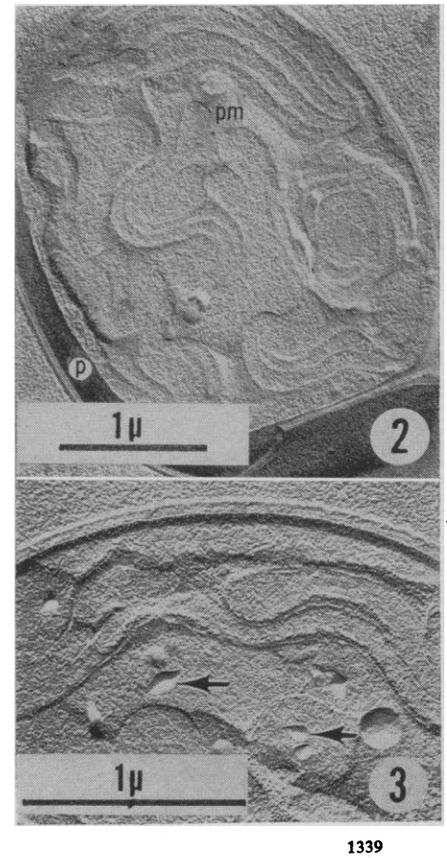


Fig. 1. *Nostoc* cells 72 hours after gas vacuole induction. Refractile areas (*arrow*) of cells contain gas vacuoles. Some cells fail to develop gas vacuoles and appear dark in phase-contrast microscope.

Fig. 2. At start of induction, *Nostoc* cell prepared by freeze-etching has photosynthetic membranes (*pm*) and plasmalemma (*p*) but no gas vacuoles.

Fig. 3. Seven hours after induction, new gas vacuoles (*arrows*) are visible in *Nostoc* cell.



which we are studying. When grown in aerated, liquid culture [25°C, continuous warm white fluorescent illumination (3850 lu/m^2)] in M. M. Allen's medium (*13*), this strain of *Nostoc* does not produce gas vacuoles. When gas vacuole formation is induced in these cells, the gas vacuoles develop without any evident connection with other membranes of the cell, and there are no preexisting gas vacuoles which could produce them by division.

Gas vacuole induction is accomplished by a treatment based on the work of Canabaeus (*5*). Cells from cultures about 14 days old are harvested by centrifugation, and approximately 2 ml of cells and medium are diluted with 100 ml of double-distilled water in a flask maintained in the light and temperature regime described above. At intervals after this induction treatment, cells are sampled and prepared for freeze-etching by adding glycerol to a concentration of 20 percent by volume, waiting for 30 minutes, and harvesting by Millipore filtration followed by rapid freezing in liquid Freon-22; freeze-etching is done as described by Moor *et al.* (*10*).

As gas vacuoles develop, certain changes in the culture are apparent to the unaided eye. The flasks initially have a layer of clear greenish cells at the bottom; after 24 hours a layer of milky green cells is seen floating at the surface of the water. Cells lacking gas vacuoles appear dark when examined with phase-contrast optics (Fig. 1); as gas vacuoles develop, bright, highly refractile regions appear in the cells. These areas contain the gas vacuoles, and the clear areas can be made to disappear by applying hydrostatic pressure.

At the start of the induction treatment the cells contain no gas vacuole membranes but other membranes are evident (Fig. 2) and are clearly distinguishable from the gas vacuole membranes (Figs. 3 and 4). No gas vacuole membranes were seen in the cells examined 4 hours after the start of induction. Seven hours after induction the first gas vacuoles become evident as small, inflated, biconical organelles (Figs. 3 and 4a). In most cases all cells in a filament develop gas vacuoles simultaneously. In *Nostoc* growth occurs by division of cells in a filament, but dry-weight measurements indicated that no new growth occurred during gas vacuole formation. Even in complete medium where no gas vacuoles were formed, the doubling time was 110 hours. The gas vacuoles must therefore originate in preexisting nonvacuolate cells. Nine hours after induction the gas vacuoles are longer but of the same diameter, and they have conical ends (Fig. 4b). Growth in length continues (Fig. 4, c and d), reaching a maximum

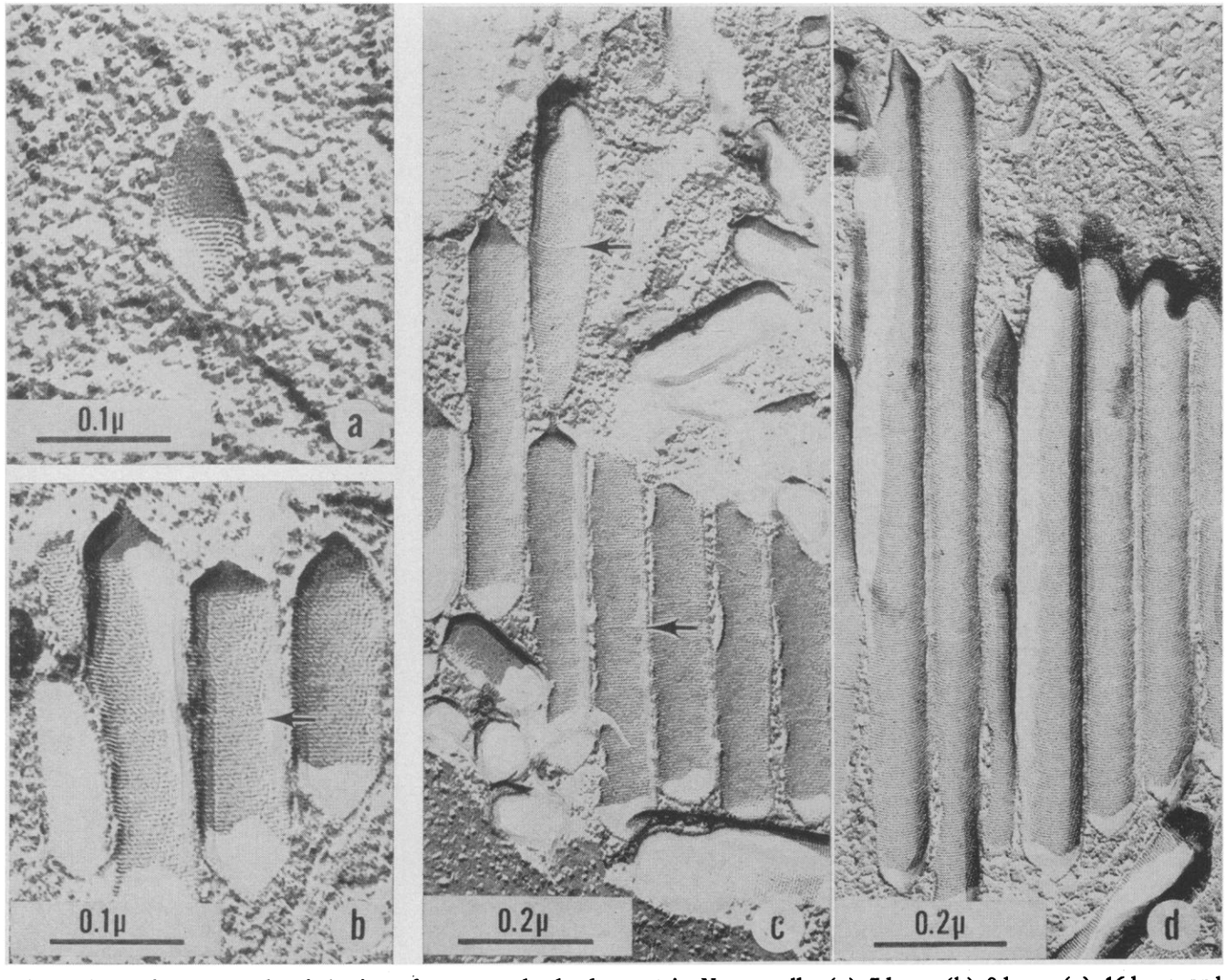


Fig. 4. Successive stages after induction of gas vacuole development in *Nostoc* cells; (a) 7-hour, (b) 9-hour, (c) 16-hour, and (d) 48-hour. Arrows indicate possible growing point.

of 1.5 μ 48 hours after induction. Although the gas vacuoles do develop in the chromoplasm, there is never any evident connection with the photosynthetic membranes or other cellular membranes.

Because the youngest gas vacuoles are short cylinders with conical ends, and the older gas vacuoles have similar ends but are longer in the middle, the new components of the membrane may be added at the middle of the cylinder as it increases in overall length. In some micrographs there is one band which stands out slightly from the others (Fig. 4, b and c); this could be the growing point of the gas vacuole.

It is unlikely that existing gas vacuoles were overlooked in the hundreds of uninduced and 4-hour-induced cells we examined. The unique banding pattern of gas vacuole membranes is readily identifiable in both the inflated and collapsed states. Whether unassembled gas vacuole membrane components existing in the cell prior to induction assemble, or whether synthesis of these components is initiated by induction is not known. In any case, as there are no parent gas vacuoles present at the time of induction, we conclude that the appearance of the assembled gas vacuole membranes in preexisting, nonvacuolate cells demonstrates their *de novo* origin.

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18 November 1968

Uracil Photoproducts from Uracil Irradiated in Ice

Abstract. *Two new products were isolated from uracil irradiated with ultraviolet light in frozen aqueous solution. As judged by mass, nuclear magnetic resonance, and ultraviolet and infrared spectra, one is a photopolymer, U_3, and the structure of the other is probably 6-4′-[pyrimidin-2′-one]-uracil. Formations of these products between pyrimidine bases are apparently common photoreactions, and may be important to the study of the photochemistry and photobiology of nucleic acids.*

Dimerization occurs when uracil and a number of its derivatives are irradiated (254 nm) in ice or as solid films (*1*, *2*). The major product, cyclobutyl type homodimer (U = U), has *cis-syn* configuration (*2*, *3*). The formation of *cis-syn* U = U as the major product is explicable from the crystalline structure of uracil (*2*, *4*). We now report photoproducts other than cyclobutyl dimers.

An aqueous solution of chromatographically pure uracil (2 mmole/liter) was irradiated in a frozen state for 2 hours at a distance of 7 cm from the G.E. germicidal lamps of an irradiator (*1*). This caused a decrease of 65 percent of the absorbancy at 260 nm. The solution was evaporated to dryness, and the residue was washed with portions of warm water until the washings showed no absorption above 300 nm. The washings were filtered and concentrated until precipitation started. A trace of precipitate was removed by filtration, and the filtrate was applied to a column of 100 to 200 mesh Dowex 50W X-12 (2.5 by 60 cm).

The residue was dissolved in boiling water for recrystallization. This

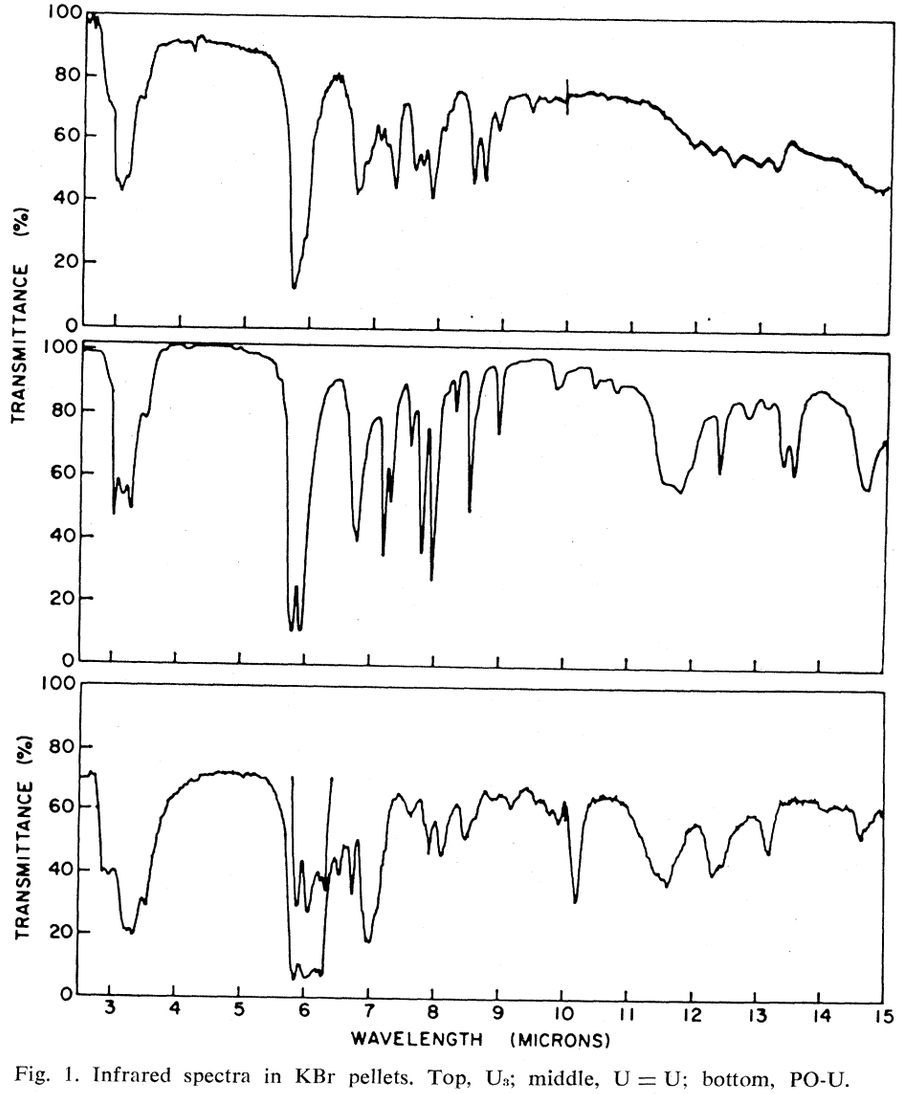

Fig. 1. Infrared spectra in KBr pellets. Top, U_3; middle, U = U; bottom, PO-U.

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