Radiosensitivity and Rate of Cell Division: "Law of Bergonié and Tribondeau"

Abstract. *The rate of cell division decreases as tobacco leaves grow, and older leaves grow without any cell divisions. Disks were cut from leaves having differing degrees of cell division at various developmental stages. In the chlorenchyma, the induced susceptibility to photodestruction of chloroplasts was used to measure sensitivity to gamma radiation as a function of the rate of cell division. The same biological effect can thereby be studied both in dividing and in nondividing tissues of the same morphological and physiological cell types. The radiosensitivities were approximately the same, irrespective of the extent of cell divisions.*

The "Law of Bergonié and Tribondeau," perhaps more a point of view than a true scientific law, is almost universally accepted as a generalization concerning the greater effectiveness of ionizing radiation on dividing than on nondividing cells. It originated from observations of irradiated tissues having different degrees of mitotic activity (*1*). These tissues, however, differed in morphology and physiology as well as mitotic activity. Mitotic inhibition, chromosome breakage, and other irregularities of mitosis which are detected with ease in dividing cells but either cannot occur or cannot be detected in nondividing cells have also been studied. Radiosensitivity of dividing compared to nondividing cells of the same morphological types has not yet been investigated where the biological criterion of damage or death is equally applicable to dividing and nondividing cells. A useful test system for such a study is photodestruction of chloroplasts after γ-irradiation of tobacco leaf parenchyma. This system is favorable because (i) the chlorenchyma grows with many cell divisions in young leaves, few in intermediate, and no cell divisions in older leaves (*2*); and (ii) the photodestruction is an early reflection of general cell death rather than of a specific destruction of chloroplasts (*3, 4*), and is indicated by the decline in chlorophyll content in both dividing and nondividing cells. Even without ionizing irradiation, bleaching of green tissue resulting from chlorophyll destruction (in contrast to the occasional loss of capacity for plastid formation) in higher plants is a general characteristic of senescence (*5*). Our biological system, unlike those mentioned above, permits comparison of doses necessary to produce the same biological effect in cells of the same morphological and physiological cell types.

Tobacco (*Nicotiana tabacum*, Turkish variety) was grown as described by Haber and Foard (*6*). From the relation between cell number and leaf dimensions (*6*) and from measurements of leaf-growth rates, we calculated the relative rate of increase in the number of palisade cells as a function of leaf length. Relative rate of increase in cell number is defined as $(1/n)(dn/dt)$, where n is the cell number and t is time. The palisade layer was chosen because it contains most of the chloroplasts (*7*) and ceases dividing not long after the spongy parenchyma (*2, 6*), which contains the remainder of the chloroplasts (*7*). Feulgen-stained leaf squashes indicated the expected presence of many mitotic figures in "young" leaves (20 to 50 mm long); of fewer in "middle" leaves (80 to 110 mm long); and of none in the "old" leaves (130 to 155 mm long). Leaf disks (5 mm in diameter) were punched from "young," "middle," and "old" leaves of the same tobacco plants. The disks in water were irradiated in celluloid tubes immersed in ice water with ^{60}Co γ-rays (Gammacell 200 from Atomic Energy of Canada, Ltd.) at 5.9 krad/min. The disks, in groups of eight each, were floated on distilled water in covered petri dishes under a multiple water-cooled refluxing lamp unit (*8*) under equal intensities (4844 lu/m^2) from GE 150-watt floodlights. The temperature was 23°C. For other experiments, in which leaf disks were kept in darkness, the temperature was 20°C. Chlorophyll was analyzed by the method of Arnon (*9*).

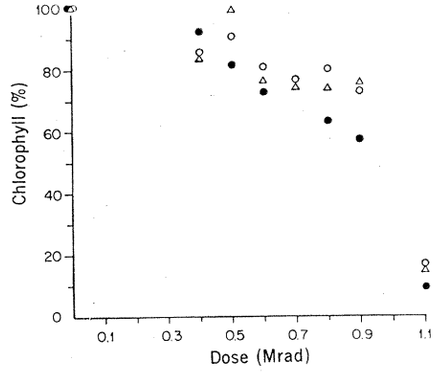

Fig. 1. Susceptibility to photodestruction of chlorophyll induced by γ-rays. For the three groups, disks were cut from growing leaves of the same plants. The disks were γ-irradiated and then illuminated for 24 hours with white, incandescent light of 4844 lu/m^2. Each point represents analysis of eight leaf disks. (●) "Young" leaves; 20 to 50 mm long; relative rate of increase in number of palisade cells was approximately 0.6 to 1.1 per day; 100 percent represents 6.7 μg per leaf disk. (○) "Middle" leaves; 80 to 110 mm long; relative rate of increase in number of palisade cells approximately 0 to 0.4 per day; 100 percent represents 9.7 μg per leaf disk. (△) "Old" leaves; 130 to 155 mm long; no cell divisions occur in the palisade or spongy parenchyma; 100 percent represents 8.1 μg per leaf disk.

The dose-response curves for γ-irradiation are similar, regardless of the relative rate of cell division in the chlorenchyma (Fig. 1). For each of the three groups, the slope becomes much steeper at a dose near 1 Mrad. The chlorophyll loss represents γ-ray-induced sensitivity to photodestruction, not direct destruction by γ-rays, because during 24-hour incubation in darkness there is negligible chlorophyll loss with or without γ-irradiation. After treatment with 1.1 Mrad, leaf disks slowly lost chlorophyll in darkness, but not faster than the unirradiated dark controls, which took about 9 days to reach half their initial chlorophyll content. Thus the effect of gamma radiation (Fig. 1) corresponds to one of the criteria of general lethality in wheat leaf chlorenchyma (*3*).

In our opinion, the law of Bergonié and Tribondeau is less a precisely stated scientific law subject to experimental test than it is a general point of view reflecting several aspects. (i) Radiation effects are more easily seen in dividing than in nondividing cells. (ii) After irradiation, cell divisions increase damage. (iii) Dividing cells have greater intrinsic radiosensitivity.

The first aspect is generally true but essentially trivial, because some of the most characteristic cytological radiation effects—mitotic inhibition, gross genic imbalance, and multipolar spindles—could not possibly occur in cells not undergoing mitosis. Moreover, other effects, such as chromosome breakage or somatic mutation, can occur in both dividing and nondividing cells but can be detected only in dividing cells.

The second aspect is not trivial; it is a generalization with which we agree (*3, 10*). Mitotic divisions produce gross chromosome imbalance, after chromosome breakage and rearrangements, and they also amplify somatic mutations. Thus, extensive cell division after irradiation will result in further damage.

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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References and Notes

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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*)

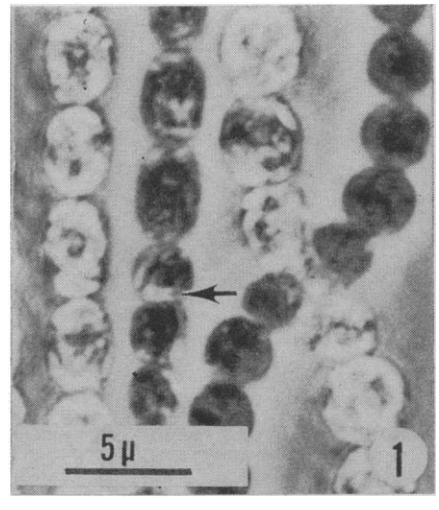

Fig. 1. *Nostoc* cells 72 hours after gas vacuole induction. Refractile areas (*arrow*) of cells contain gas vacuoles. Some cells fail to develop gas vacuoles and appear dark in phase-contrast microscope.

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.