to the stomata. At the same time stomatal apertures enlarge as guard cells expand in width. The entire leaf at this time becomes turgid. Actively functioning leaf stomata are considered to be the portals of entry for atmospheric smog, since the cells that line the substomatal chambers show protoplasmic injury first and are always the most severely damaged (*11*). Chloroplasts disintegrate and plasmolysis follows. Cell walls shrink slowly, maintaining plasmodesmatal connections with neighboring cells. Damage is usually limited to a few cells surrounding the affected substomatal chamber. There is no rupture of cell walls or dissolution of middle lamella. Intercellular air spaces enlarge as affected cells shrink. Since dehydration is slow, cellular "mummification" of affected tissue is not complete until 1 to 2 days following exposure (Fig. 1). The extent of tissue involvement is, as in any gas damage, in proportion to the concentration and duration of pollution.

In the grass, *Poa annua* (L), the sensitivity of the leaf tissue is a function of its maturity (*3*). Damage in the youngest leaf appears only at the tip; in a leaf somewhat older, close to midblade; and in a fully matured leaf, only at the base. This localization of damage has been shown to be related to the gradient of cellular differentiation from tip toward base in the maturing leaves and is probably true also in broad-leaved plants, such as spinach and tobacco, in which cellular maturity is likewise progressive from tip toward base (*12, 13*). Only the cells that have just completed maximum expansion are smog sensitive. Young leaves are not susceptible, probably by virtue of their compact cellular nature, absence of well-developed intercellular air spaces and substomatal chambers, and nonfunctional stomata. Old leaves are not sensitive by virtue of their comparatively heavily suberized cell walls (*14*).

Whatever the gross picture of smog damage, anatomical studies indicate that in all sensitive vegetation the microscopic picture is the same. It is evident from this work, that the damage produced by smog differs from that produced by any other phytotoxic agent studied, for example, frost, ozone (*15*), SO_2, HF, fungus, and insect. Smog-attacked cells are not disrupted as they shrink, resulting in a tissue "skeletonization" in the limited regions of the substomatal chambers; damage in response to other gases is usually unlimited, spreading throughout the lamina and, more often than not, resulting in complete necrosis (SO_2, O_3) or affecting vascular elements (HF) (*5*). The anatomy of stems and roots and the vascular elements of leaves are never affected by smog, indicating that the phytotoxic constituents of smog are not translocated within the plant.

Poa annua (L) is considered a very reliable biological indicator for atmospheric smog for several reasons: (i) the extreme sensitivity of its cells to minute quantities of phytotoxic materials; (ii) its method of cellular differentiation from tip toward base in the linear leaf with resulting marked transverse leaf banding; and (iii) its ubiquity as a weed in Los Angeles County, making a naturally occurring check available in many and scattered areas. Work is in progress in an attempt to calibrate this plant as a quantitative, as well as a qualitative, bioassay material. A detailed account of the anatomy of normal and smog damaged *Poa* has been completed.

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Contamination of Nuclear Fractions of Thymus Homogenates with Whole Cells

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A reexamination of methods for the isolation of nuclei of cells of the thymus gland of the rat and calf has led to the observation of atypical reactions that suggest a gross contamination with intact whole cells. In view of the recent use of nuclear fractions of thymus homogenates in studies on the localization of enzymes (*1, 2*), it is desirable to report a method (*3*) for demonstrating such contamination.

The isolation of a pure nuclear fraction from the thymus gland is complicated by the presence of large numbers of small thymocytes, that is, small cells with large nuclei surrounded by a very thin layer of cytoplasm. Histologically, these cells are indistinguishable from small lymphocytes (*4*). It is frequently very difficult, if not impossible, to detect contamination of fresh nuclear fractions with these cells by routine examination with the phase contrast microscope, since the layer of cytoplasm is so thin as to be indistinguishable. The difference in reaction of isolated nuclei and whole cells to changes in ionic composition or osmotic pressure of the suspending medium offers a ready means of determining the extent of contamination.

For this study, the nuclear fraction of calf thymus was isolated by the method of Stern and Mirsky (*1*), which employs a solution of 0.0018*M* $CaCl_2$ in ap-

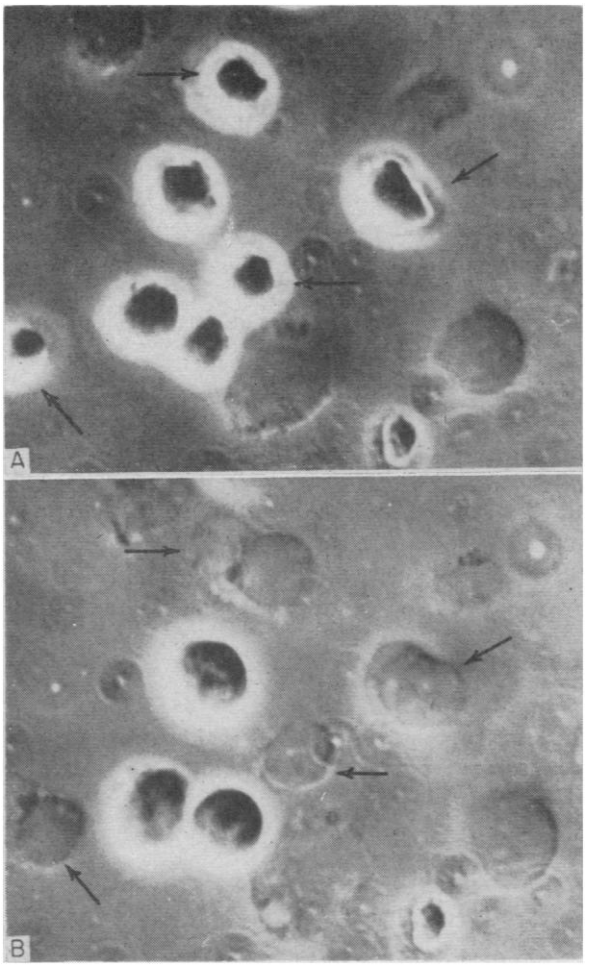

Fig. 1. (*A*) Photomicrograph of nuclear fraction of calf thymus homogenate 2 min after treatment with alkaline 0.25*M* sucross and 1 percent bovine serum albumin. (*B*) Photomicrograph of the same microscopic field as (*A*) taken 1 min after treatment with 0.001*M* $CaCl_2$.

proximately 0.25*M* sucrose as the isolation medium. Examination of the wet preparation with the phase contrast microscope revealed no evidence of contamination with whole cells. A solution of alkaline (*p*H 8.5) 0.25*M* sucrose and 1 percent bovine serum albumin was then added at the edge of the cover glass and drawn across the field by absorption of fluid in lens paper at the opposite edge of the cover glass. This solution causes the disintegration of isolated nuclei, as proved on other preparations. Figures 1*A* shows the result of this treatment on the nuclear preparation 2 min after adding the test solution. Only a few of the "nuclei" swelled and disintegrated, whereas the remainder were apparently unaffected by this medium. The remaining "nuclei" were then treated with 0.001*M* $CaCl_2$ added in the same manner. Figure 1*B*, which was taken 1 min after adding the 0.001*M* $CaCl_2$ solution, shows the same microscopic field as Fig. 1*A*. Note that some of the "nuclei" have ruptured, discharging their contents into the medium. The remainder show a distinct increase in size. On continued exposure to the hypotonic medium, the remainder of the "nuclei" also rupture.

These observations lead to the conclusion that the "nuclei" that swell in 0.001*M* $CaCl_2$ are in reality intact small thymocytes, for it has been shown (*5*) that, in isolated nuclei of other tissues, hypotonic $CaCl_2$ causes shinkage of the isolated nuclei, whereas whole cells swell in hypotonic solution.

The differential action of the alkaline sucrose medium probably is twofold. (i) It has been shown that isolated nuclei do not react osmotically to sucrose solutions but may swell and dissolve in 0.88*M* sucrose (*5*). (ii) Alkaline solutions cause swelling and disintegration of isolated nuclei (*6*). This contamination of the nuclear fraction with whole cells appears to be more pronounced in the preparations isolated in 0.25*M* sucrose and 0.0018*M* $CaCl_2$ than in those mediums containing citric acid, suggesting that *p*H and ion binding may be important factors in stability of the plasma membrane of small thymocytes. Preparations isolated at low *p*H, however, are not considered satisfactory for enzyme studies.

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Compared to the inner satisfaction over a problem successfully solved, any outside recognition becomes meaningless.—RÖNTGEN.