The Authors' Reply: Practical Advantages to Acridine Orange

To the Editor.—The acid phosphatase and nonspecific esterase reactions that Drs. Heaton, Howard, and Garrett mention are well-known supplementary techniques for demonstrating myenteric neurons but do not preclude the development of additional special stains that provide the pathologist with still other alternatives for confirming the presence of ganglion cells. In our article we proposed that acridine orange also may be used for this purpose and has a practical advantage of being a rapid and simple technique, applicable to either freshly frozen or formalin-fixed, paraffin-embedded sections of tissue, but we did not suggest that it is the only method or that it should replace all other special stains. We did point out that some techniques that have been used to demonstrate neurons in bowel wall, particularly those depending on immunocytochemistry, are more complex and require more time.

Regrettably, the quality of the color reproduction of our acridine-orange-stained sections was extremely poor in our AJCP article and does not convey the true brilliance of the fluorescent orange-red contrasting with the green background of smooth muscle and connective tissue. A much better illustration of the technique results is found in an earlier article of a more general nature on the acridine orange technique in relation to the central and peripheral nervous systems (Sarnat HB: Rev Neuril (Paris) 1985; 141:120–127).

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Peculiar Cytoplasmic Inclusions in Cells of Acute Lymphoblastic Leukemia

To the Editor.—Like Takemori and colleagues, we have been intrigued by the occurrence of "virus-like" particles (VLPs) in leukemic lymphoblasts. However, the light microscopic features of the inclusions they describe differ from those characteristic of VLP collections in acute lymphoblastic leukemia (ALL), i.e., orange-pink to red with Romanowsky, usually solid and single, not or faintly metachromatic with toluidine blue (Takemori and associates—blue, multiple, often as rings, strongly metachromatic). These differences suggest that the VLPs in their case differ from those in the generality of ALL, or that the phenotype of their case has additional features not characteristic of the generality of ALL.

The detection of what appear to be nuclear fragments in VLP-containing inclusions prompted us to undertake a more systematic ultrastructural examination of the occurrence of altered heterochromatin-like areas in the cytoplasm of leukemic lymphoblasts, with a view to determining their relation to VLPs. Ultrastructural identification of altered chromatin is supported by preliminary evidence of stainability with cis-dichloro-diammine platinum (II). We examined 51 cases of childhood ALL—35 non-T, 13 T, and 3 B-ALL. In brief, masses of altered chromatin were found in abnormally high frequency in ALL compared with control lymphocytes (normal child and fetal marrow, child and fetal thymus). In ALL, however, the increase was a feature of non-T rather than T- or B-types. A close physical and quantitative relation between altered chromatin and VLPs was demonstrated.

One interpretation of these findings is that VLPs, or a proportion of them, are a lysosomal-type response to damaged chromatin. The detection of acid phosphatase activity in inclusions of VLPs by light and electron microscopy would support this interpretation. However, acid phosphatase activity in these inclusions is inconsistent, from our own observations and those of others, and, when positive, may be a property of material other than VLPs in the inclusions. In view of this and the fact that some VLPs show such suggestively viral features as solid cores and budding an alternative explanation—that VLPs or a proportion of them are in fact viral, with chromatin damage as a morphologically recognizable cytopathic effect—may be the more likely. Evidence of transformation of normal cells by lethally irradiated VLP-containing cells, and preliminary evidence for the presence of nucleic acid in VLPs (S. Robbins, personal communication) are consistent with this interpretation. We are currently investigating the capacity of VLPs to induce chromatin damage in substrate lymphocytes.

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