ME1609 POLYETHYLENE GLYCOL (PEG) AND ITS APPLICATION IN ELECTRON MICROSCOPY. John J. Wolosewick, Department of Anatomy, U. of Illinois at the Medical Center, Chicago, Il. and Keith R. Porter, M.C.D. Biology, University of Colorado, Boulder, Co.

Plastic embedding resins scatter electrons sufficiently to reduce the contrast of structures embedded in them. The absence of resins in whole-cultured cells observed with the HVEM yield images of increased contrast. This approach is limited to cells thin enough for electron-beam penetration. To observe cells in situ we have used PEG, a water miscible wax frequently employed for cytochemistry at the light and electron microscopical levels. PEG processed tissues may be examined also in the SEM. Tissues (e.g.,testis, brain) are fixed in glutaraldehyde/osmium,rinsed in buffer, infiltrated(60°C) in 50% PEG(MW 1500-6000) for 2 hrs, then in pure PEG for 2hrs and embedded in PEG in gelatin capsules. Blocks are cut(0.1-0.2μm) on a dry diamond knife, and the ribbon of sections transferred with a camel's hair to a well of a spot plate. 0.1% Triton-X 100 in dis. water is added to the well. The PEG dissolves while the sections maintain their integrity. The sections, while submerged, are placed on Formvar-polylysine-coated grids, dehydrated in ethyl alcohol and critical point dried. Cells of tissues so processed exhibit an ultrastructure similar to that in whole, critical-point dried cells. Cytoskeletal structures(microtubules, microfilaments, microtrabeculae) are identified and appear not to have been damaged during processing, though membranous components appear somewhat less distinct. This is an alternative method for cells too thick for whole-cell TEM or HVEM, and cells in situ. The gains in contrast and resolution in specimens devoid of electron-scattering resins is remarkable. Specimens for SEM are sectioned until the appropriate area is located. The block is placed in dis. water, dehydrated and critical-point dried. The block is mounted(sectioned side up) on metal stubs, coated with gold and viewed in the SEM.

ME1610 A FIBER-OPTIC PHASE-RANDOMIZER FOR MICROSCOPE ILLUMINATION BY LASER. Gordon W. Ellis. Department of Biology, University of Pennsylvania, Philadelphia, PA 19104.

Hard, et al (J. Cell Sci. 23, 335, 1977) have described the problems caused by the coherence of laser radiation when it is used for microscope illumination. The rotating-wedge/ground-glass system they describe for phase randomization serves effectively to render the illumination incoherent but requires rigid and exact placement of the laser and associated apparatus relative to the microscope. I have developed an alternative means of phase-randomization which eliminates these constraints. It utilizes a fiber-optic light guide vibrated by a piezoelectric transducer. In use the beam exiting the laser is focused on the end of a single-fiber step-index light guide. As the light is propagated down the fiber it is multiply reflected at the interface between the fiber core and its lower index cladding, thus converting the single-mode uniphase input into a polymodal multiphasic output. The complex wavefront emerging from the fiber illuminates the microscope condenser over a broad spatial frequency range. With the fiber stationary, the field of view shows at high contrast a complex interference (speckle) pattern because the phases of the superimposed components, though highly diverse, are time invariant. By vibrating the fiber with a piezoelectric transducer, the optical paths through the fiber are varied in time so that the contrast of the speckle pattern is time-averaged to zero. With this system, because the light is conveyed from the laser to the microscope through a flexible light guide, one has complete freedom in the placement of the laser and the microscope. A microscope may be adapted to the use of this system merely by placing the output end of the light fiber at the point normally occupied by the light source in the microscope's illuminator. However, improved performance can be obtained by replacing the illuminator collector lens with a suitable super-8 cine macro-zoom lens.
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ME1611 SEPARATION OF CELLULAR RIBONUCLEOTIDES, DEOXYRIBONUCLEOTIDES AND THEIR ANALOGS BY HPLC. William Plunkett. Department of Developmental Therapeutics, Univ. of Texas System Cancer Center M.D. Anderson Hospital and Tumor Institute, Houston, TX.

The proper balance of cellular nucleoside 5'-triphosphates is thought to be necessary for the faithful replication of nucleic acids, the integrity of cellular energy charge and the maintenance of their roles as cellular regulatory effectors. In addition, the biologically active and therapeutically useful forms of nucleoside analogs such as 3-deazauridine and ara-C are the respective 5'-triphosphates. Two procedures are presented, each employing high pressure liquid chromatography (HPLC) gradient elution of nucleotides from Partisil-10 SAX (Whatman, Inc.), that permit rapid resolution and quantitation of normal cellular 5'-triphosphates and those of the active nucleotide of each analog. The first procedure, carried out directly on acid-soluble extracts, separates deazaUTP, the active metabolite of 3-deazauridine, from other ribonucleotides including UTP, with which deazaUTP competes for the substrate site on CTP synthetase, and CTP, the affected nucleotide. The second procedure, conducted after removal of ribonucleotides by periodate oxidation, is suitable for the quantitation of cellular 2'-deoxyribonucleoside 5'-triphosphates. This method also separates araCTP from deoxyribonucleoside 5'-triphosphates, including dCTP with which araCTP competes for DNA polymerase. These separation procedures, requiring 30 and 45 min, respectively, are sensitive to 25 pmol of nucleotide and are readily adapted to the separation of the 5'-triphosphates of ara-A, xyl-A, 3'-dAdo and their derivatives. These HPLC assays for ribonucleotides and deoxyribonucleotides facilitate studies of the effects of external agents on the cellular concentration of these compounds and permit investigations of the biochemical pharmacology of nucleotide analogs. (Supported by NCI grants CA-11520, CA-14528 and American Cancer Society Grant CH-130.)