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INTRACELLULAR LOCALIZATION OF WGA BINDING SITES IN DETERGENT-PERMEABILIZED PHOTORECEPTOR CELLS

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In the course of our study of glycosilation sites in photoreceptors, we employed post-embedding techniques for detection of N-Acetylglucosamine residues in the inner segments of rat photoreceptors. Thin sections of tissue embedded in Lowicryl were incubated with wheat germ agglutinin (WGA). These experiments did not yield satisfactory results since labeling densities were very low. This might be due to alterations of binding sites during tissue processing, or a result of the inherent low detection capabilities of the post-embedding procedures. As an alternative approach we explored the application of pre-embedding techniques with detergent permeabilized photoreceptors. Retinas were isolated from seven- to ten-day-old rats and fixed prior to treatment with detergents. Various fixation procedures were investigated. The two main alternatives were low concentration of glutaraldehyde or paraformaldehyde-lysine-periodate mixture. Best results were obtained with 0.2% glutaraldehyde + 2% formaldehyde in 0.1 M cacodylate buffer, pH 7.0, for 30 min at 4°C. For the permeabilization step, various concentrations of saponin and Triton X-100 were tried. Best results were obtained with 0.05% Triton X-100 for 5 min at room temperature. Incubation of permeabilized cells was carried out with 40 μg/ml of peroxidase-labeled WGA, for 2 hrs at room temperature. Bound peroxidase-WGA was visualized by a DAB-H2O2 reaction followed with osmium tetroxide. Under optimum combination of fixation and permeabilization sequences, WGA binding sites were clearly depicted in the Golgi cisternae and transport vesicles.

INTERFACE STRUCTURES INVESTIGATED BY CONVERGENT-BEAM ELECTRON DIFFRACTION

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Convergent-beam electron diffraction (CBED) has been applied in grain boundary structure determination, both for horizontal and vertical boundaries in thin specimens. First, the relation between the bicrystal symmetry and CBED pattern symmetry is developed for the two types of boundaries. These results are subsequently employed for a structure determination of twin boundaries in Au and Si. The CBED patterns from twinned bicrystals obtained experimentally are compared with computer-simulated patterns, for the case of a horizontal twin boundary in Au and Si. Finally, the symmetry of CBED patterns from vertical twin boundaries in Si is analysed and found to depend on whether a static incident beam or a rocking-beam geometry is employed.
applications in various fields, as well as data on the available systems and services, are discussed.

ENTERIC VIRUSES IN A COHORT OF RURAL COSTA RICAN CHILDREN*1

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Fifty-one rural children were recruited at birth and observed prospectively over a 20-month period in 1981-83. The presence of rotavirus, adenovirus, coronavirus-like particles, and small round viruses was investigated by transmission electron microscopy (TEM). Rotavirus was, in addition, studied by ELISA. A total of 2,516 weekly fecal specimens were collected and tested.

For TEM whole feces were made into 10%–20% suspensions in brain heart infusion broth and then clarified at 500 x g for 30 min at 4°C. Clarified supernatants were frozen at -70°C until ultracentrifuged at 200,000 x g in a Beckman SW50.1 rotor for 60 min at 4°C. Pellets were resuspended in 0.1 ml of distilled water and adsorbed onto Formvar-coated grids and negatively stained with 3% phosphotungstic acid at pH 6.5. Specimens were examined under code in a chronometered 10 min search of the grid's center and two corner areas using a Hitachi H300 instrument at 75 kV (40,000 magnification).

The incidence of diarrhea was 0.7 episodes per child-year. Rotavirus was the commonest virus (0.53 infections per child-year), followed by adenovirus (0.46 infections per child-year) and coronavirus-like particles (0.24 infections per child-year). Only 3 of 24 rotavirus infections and 2 of 21 adenovirus infections were associated with diarrhea. Children who excreted coronavirus-like particles and small round viruses were symptomless. Typical Norwalk, calicivirus, or astrovirus particles were not seen. Rural conditions, good hygiene and breast feeding may explain the low incidence and low pathogenicity of viral enteropathogens in rural Costa Rica.

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