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with 10 nm Au and examined at 3-20 kV. Fractured human enamel surfaces were ion-beam-sputtered with Au in layer thickness varying from 1-10 nm and examined at 5-25 kV. All samples were investigated with an ISI SEM, type DS-130.

In approximately 50% of all investigated sound erythrocytes one or more pores were found of size ranging from 40-100 nm. At a given Au layer (10 nm) the pores and biconcave shape of the erythrocytes were optimally visualized at 3 kV, with inferior image quality at higher kV. At 5 kV and a Au layer of only 1 nm, sharp edges of the enamel fracture surface were very well imaged; at 10 kV the same sample showed an inferior quality due to charging. At 25 kV and a Au layer of 10 nm some masking of the sharp edges occurred.

**STUDY OF CELL ATTACHMENT AND CELL SURFACE ON ISOLATED OSTEOCLASTS AND OSTEOCLAST PRECURSOR CELLS USING AN INSTRUMENT PERMITTING COMBINED LIGHT AND SCANNING ELECTRON MICROSCOPY-LM/SEM**

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Techniques for the isolation of osteoclasts from mammalian (rat, cat, rabbit) long bone marrow cavities have recently become available and permitted in vitro study of osteoclast surface structures. It has become clear that osteoclasts are highly variable in morphology, depending on their metabolic state. Murine osteoclasts have thus far not been studied extensively. In our work on osteopetrosis, an inherited bone disease caused by osteoclastic dysfunction (lack of resorptive capacity), we studied differences in cell surface between osteoclasts from normal and osteopetrotic mice (mi/mi stock) during fetal development. The fact that osteoclasts in fetal mi/mi mice are mainly mononuclear, however, posed the problem of identifying mononuclear osteoclasts in mixed mononuclear cell populations in the SEM. The LM/SEM (Prof. J.S. Ploem, Department of Cytochemistry and Cytochemistry, Leiden University) enabled us to solve this problem. We isolated osteoclast containing cell suspensions from long bones of fetal mice, purified the cells on Percoll and allowed the cells to adhere to glass cover-slips. After formalin fixation they were stained for the osteoclast marker enzyme tartrate-resistant acid phosphatase (TRAP), postfixed and prepared for SEM by routine procedures. We screened for TRAP positive cells by LM mode. The surface of the same cell was then studied in the SEM mode.

Preliminary results suggest no major differences in cell surface between mi/mi and normal osteoclasts. This, however, may be due to selection of adherent osteoclasts only. It is also possible to identify (normal) preosteoclasts (mononuclear, TRAP positive) and their adherence to stromal cells in vitro with the LM/SEM. First results are presented.

**IMMUNOCYTOCHEMICAL INVESTIGATION AND BIOCHEMICAL FUNCTION OF NATIVE MATRIX GRANULES IN THE HEART MITOCHONDRIAL MATRIX**

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The existence of matrix granules was first demonstrated by Palade (1953) and Sjöstrand and Rhodin (1953) in liver mitochondria. Since then they are detected in the mitochondria of almost every tissue or organ. They are 20-30 nm in diameter, strongly osmiophilic and not surrounded by a membrane. They can be distinguished from electron-dense particles (EDP) visible in the matrix in pathologic situations such as ischemia or Ca-overload. These EDPs are formed by hydroxyapatite precipitation. In the native matrix granules (NMGs), which are believed to be precursors of the inner mitochondrial membrane, a proteinaceous component was detected (Suter and Staßiibli, 1970) by pronase digestion. Because these NMGs are closely related with the inner mitochondrial membrane, we sought to find components in the NMGs which also make up the inner membrane. We have chosen cytochrome c oxidase because this complex is partly synthesized in the matrix. An antiserum was prepared in rabbits against cytochrome c oxidase isolated from beef heart mitochondria. By an indirect method with labeled goat anti-rabbit IgG (5 nm colloidal gold) as
secondary antisera, specific labeling was obtained in the mitochondrial profiles and in the NMGs. Based on these and earlier results about the composition and behaviour of these granules, in function of the energy state of the mitochondrion, we propose the following hypothesis (Herstens, 1986). In heart mitochondria the NMGs are a pool of components which can be incorporated in the inner mitochondrial membrane. Consequently fusion between the outer and inner mitochondrial membrane (inverted micelle formation) is induced (Jacob and Hertsens, 1984). These phenomena allow the mitochondria to cope with a sudden energy demand.

EM DIAGNOSTIC DETERMINATION OF FAECAL VIRUSES: COMPARISON OF THE DIRECT STAINING METHOD AND THE ULTRACENTRIFUGATION CONCENTRATION METHOD

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Faecal samples from patients suffering from gastroenteritis were processed according to the direct staining method and according to the ultracentrifugation concentration method. All samples were suspended in distilled water (20% v/v) and centrifuged at 2000 rev/min for 10 min at room temperature. For the direct staining method a drop of supernatant was placed on a pioloform-coated grid coated with carbon, touched with filter paper to remove excess fluid, dried, and negatively stained with 2% PTA, pH 6.0. For ultracentrifugation the above mentioned supernatant was centrifuged for 90 min at 164,000g at 4 C. The pellet was resuspended in 2 to 3 drops of distilled water and negatively stained with PTA as described above. The best results were obtained with the direct staining method. With the ultracentrifugation method no increase in concentration of any of the viruses (rotavirus, adenovirus, coronavirus, small round viruses) could be found. Besides, rotavirus could hardly be detected with this method as most of the virus had disintegrated. Furthermore, it was observed that the cohesion of the small round viruses had diminished considerably when this method was employed, making identification more difficult.

PULSED LOW-ENERGY INFRARED LASER IRRADIATION OF HUMAN DENTIN, A SEM STUDY

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Polymer bonding to dentin is mostly performed after chemical treatment of the dentin. Laser treatment of dentin, as a pretreatment to produce a clean and rough surface, may be an alternative. The dentin of 12 teeth were individually subjected to one of the following wavelengths: 9.32 μm, 9.57 μm, 10.27 μm and 10.59 μm, carried out with a Lumonics TEA 103-l multimode line tunable CO2 gas laser producing 100-200 nsec pulses; 20 pulses were given with a pulse energy density of 10-50 J/cm2 at a spot size of 2-5 mm.

The dentin (appr. 1 cm²) of another 5 teeth was subjected to a 9.21 μm line treatment, while another 5 teeth were not lased. All 10 dentinal surfaces were rinsed with 3% hypochlorite and water resp. for 30 sec each and air-dried; they then received two layers of Scotchbond adhesive™ and 0.45 cm diameter cylinders of P-10R composite resin to these surfaces. Specimens were stored in water at 25°C for 36 hr and fractured to visualize composite resin-dentin interface. Finally all samples were sputter-coated with Au (10-12 nm) and examined at 25 kV.

All four lines produced a quite rough surface melt after 20 pulses, contrary to the result obtained at enamel. The melt pattern is almost identical for all four lines: a fungi-like pattern of caps on top of a melted mass of dentin with deep invaginations is observable. The roughening of the surface is not related to the dentinal tubules. At shear experiments of the lased dentin-polymer bonding, fracture occurred in the dentin. Laser pretreatment of dentin increased bond strength of composite resin to dentin by 300%.