Abstracts of Papers Presented at the Thirtieth Hokkaido Regional and Memorial Meeting of the Japanese Society of Electron Microscopy

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Special Lecture I: HIV structure and its morphogenesis
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The ultrastructural features of HIV and its related retroviruses in morphology and morphogenesis, comparing with SIV and the infection of HIV into the cells were presented. The virus particles were formed by budding process and all budding particles were doughnut form. The virus particles were extracellularly observed as various kinds of profiles which had conical electron-dense core, a central and an eccentric electron-dense round core, doughnut-like type and layered core. It seemed that the maturation of HIV occurs outside the cell from doughnut-like particles. In ultrathin-sections, HIV-2 was rarely found to be double-ring particles, but it was easily seen to have projections on the envelope. Consequently HIV-2 is quite in morphology to SIV. HIV-1 entered in MT-2 cell by two ways.

Special Lecture II: HVEM studies of advanced materials
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Electron channeling becomes prominent at high accelerating voltages by the simultaneous excitation of many waves. Rate of the electron channeling linearly increases with increasing voltage by the relativistic effect, and this fact results the unexpected advantage of HVEM. The present work is concerned with new applications of a 3MV ultra-HVEM to the following subjects: (a) Determination of necessary accelerating voltages for each individual research, such as in situ experiments of various phenomena, irradiation effect, and utility of environmental cells, as a function of sorts and conditions of materials, and (b) determination of the critical size of blocks necessary for formation of the non-equilibrium phases such as amorphous solids and super-saturated solid solution. Item (b) involves (1) electron irradiation induced solid amorphization, and (2) electron irradiation induced foreign atom implantation (ELI-PAI). Based on the experimental results, the general rules for solid amorphization and foreign atom implantation have been obtained. Furthermore, the formation mechanism of solute atom clusters and the relaxation mechanism of lattice distortion induced by solute atoms are verified by a combined method of HVEM and Auger valence electron spectroscopy.

1. Molecules, genes and chromosomes
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Electron microscopy has helped to a great extent in studying the ultrastructure and biological function of chromosomes, containing DNA molecules, namely genes which regulate genetic phenomena. It is presented that the results on the ultrastructure of the DNA molecules, chromatin fibers and chromosomes, as well as those of elemental analysis of Mg and Zn in the cells, especially in the sperm heads. Since 1961, Nakanishi and his coworkers have been engaged in the studies on the ultrastructure of the eukaryotic chromosomes based on a multicoiled model as a working model with the aid of conventional, scanning and high voltage electron microscopes. Ultrathin sections of mitotic cells at metaphase observed by conventional electron microscopy revealed no apparent spiral structure of chromosomes, showing thin fibers in irregular appearance. On the other hand, the spiral structure and three-dimensional configuration of the chromosomes are clearly visualized by scanning electron microscopy, and high voltage electron microscopy. It is suggested that the chromosome is constituted by spiralization of the chromonema which is formed by coiling of chromatin fibers consisting of the complex of DNA fibers and histone.

2. Electron microscopic studies on plant virus and viroid RNAs
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Electron micrographs of genome RNAs for plant viruses were first demonstrated on double-stranded (ds) RNA of wound tumor virus by Kleinschmidt et al. (1964), then dsRNA of replicative form for tobacco mosaic and turnip yellow mosaic viruses (Wolstenholme & Bockstahler, 1967). Murant et al. (1981) showed electron micrographs of single-stranded (ss) RNA for thirteen plant viruses. Results of these studies indicated that molecular weights (Mr) estimated by electron microscopy were in well agreement with those previously obtained by electrophoretic analysis. However, our estimation (Uyeda et al., personal communication) of Mr for segment 8–12
of rice dwarf virus dsRNA by electron microscopy was slightly higher than that of electrophoretic analysis. One of the striking figures for ssRNA was shown by Lebeurier et al. (1977) on tobacco mosaic virus assembly site, demonstrating clearly two ssRNA tails of both 5' and 3' ends at the same side of the partially assembled rods. The results lead to the conclusion that the initiation site of coat protein assembly was at about 800 nucleotides from 3' end of the genome. First electron micrographs of viroid RNA were demonstrated by Sogo et al. (1973). Electron microscopic studies revealed the rod, circular and linear forms of infectious molecules of potato spindle tuber viroid. Riesner et al. (1979) succeeded to demonstrate the dark field figures of circular forms of potato spindle tuber viroid, and investigated the conformation changes at different temperatures. We investigated the rod and circular forms of hop stunt and cucumber pale fruit viroids by electron microscopy, and showed that both viroid molecules were the similar size and shape, which were finally proved to be the same viroid species.

3. Role of electron microscope for basic study of freezing-preservation on biological materials

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Freezing-preservation technique is now widely used in a variety of fields, such as biology, medicine and food science. For successful freezing-preservation of biological materials, the basic studies to find the behaviour of cells under freezing are undoubtedly necessary. Electron microscopy, especially such as freeze-fracture technique and cryo-scanning electron microscopy which allow for observation of cellular ultrastructure under frozen state, have contributed greatly on this regard. By the use of electron microscopy, it has become clear that primary site for the freezing injury is plasma membrane and that a variety of plasma membrane ultrastructural changes, such as formation of intramembrane particle (IMP)-free patches, endoplasmic vesiculation, lamellar to hexagonal II phase transition and contact-induced IMP aggregation, caused by freezing have significant relation to occurrence of freezing injury. These basic knowledges obtained by electron microscopy provide useful information for the future development of more successful freezing-preservation.

4. Observation and X-ray microanalysis of cheese by the frozen thin sectioning method

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We have carried out structure observation and X-ray microanalysis of frozen thin sections of cheese. The frozen thin sections were prepared from a commercial processed cheese, and transferred into the transmission electron microscope (JEM-2000FX: JEOL/EDS: LINK) by using a cryotransfer holder (EM-CTH10: JEOL). The frozen thin sections were then observed and analyzed by X-ray microanalyzer. They were compared with Epon-embedded thin sections. In the frozen thin sections, profoundly interesting structures were clearly observed and many elements were detected. In the Epon-embedded thin sections, on the other hand, there were many movements and losses of constituent materials. Consequently the frozen sections were found much better than Epon-embedded sections because they preserve the native state of the cheese.

5. Taste buds as chemical receptor

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Gustatory (type-III) cells in the taste buds have afferent synaptic contacts with the nerve terminals, contain numerous microtubules, and are capable of taking up monoamine precursors (5-HTP and L-DOPA). Other type-I and -II cells have been supposed to be sustentacular cells. The origin of taste bud cells is still controversial, although both neural crest and epithelial origins have been suggested. Taste bud cells possess bundles of intermediate-sized filaments which terminate at desmosomes. The antibodies to keratins from the bovine muzzle and human stratum corneum stained taste bud cells as well as the surrounding epithelial cells in the mouse. This finding has led us to the supposition that all cell types comprising the taste buds—including gustatory cells—originate from the epithelial cells surrounding the taste buds. PKK3 monoclonal antibody against pig kidney epithelial cell line reacts with a 45-kD keratin that is present in the cytoskeleton of simple epithelia. We found that this monoclonal antibody reacted with mouse taste bud cells, but not with surrounding epithelial cells. Therefore, 45-kD keratin may be an excellent immunocytochemical marker for taste buds. PKK2 antibody against pig kidney epithelial cell line, in contrast to PKK3, reacted with surrounding epithelial cells, but not with taste bud cells. It is suggested that keratin subtype differs between taste bud and surrounding epithelial cells.

6. Filaments in the supporting and basal cells of mouse olfactory epithelia

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The filaments in the supporting and basal cells of the olfactory epithelium of mice were investigated by immunohistochemistry and electron microscopy. The antikeratin antibody from human hepatocellular carcinoma cells (MA 902) reacted with the basal cells just above
the basal lamina and with the supporting cells, which have their nuclei located in the upper third of the epithelium. The antikeratin antibody from human keratinocyte (KL-1) reacted only with the basal cells. Both of the supporting and basal cells contained many bundles of 10 nm filaments, and the basal cells possessed densely aggregated bundles of filaments than the supporting cells. The olfactory sensory cells, with their nuclei in the middle region of the epithelium, lacked 10 nm filaments and were negative with antikeratin antibodies. It is concluded that the 10 nm filaments in the supporting and basal cells of olfactory epithelia are composed of keratin proteins, although the keratin subtype differs between those cells. The basal cells of older mice (20–28 W) were flatter in shape, contained thicker bundles of filaments and showed stronger staining by KL-1 antibody than those of younger one (2 W).

7. Some electron microscopic studies in opthalmology
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1. Neutrophils in the peripheral blood of 6 patients with Behçet's disease at the exacerbation stage were examined by electron microscopy. The numbers of microtubules around the centrioles in a unit area of the electron micrographs were 4.96 ± 2.76 in the patients and 2.36 ± 1.88 in the control subjects; the difference was statistically significant (p<0.0005). The results suggest that microtubules may play a significant role in the pathophysiology of Behçet's disease. 2. Fibroblasts in the human conjunctiva possessed intracellular striated fibers which were variable in length and thickness. The length and thickness were 0.48 ± 0.38 μm and 62.3 ± 26.4 nm, respectively. The periodicity of the major crossbands was 69.7 ± 2.8 nm. At least six sub-bands were seen between the electron-dense major bands. 3. Ribosome-lamellae complexes were found in plasma cells in the normal palpebral conjunctiva. These structures were composed of hollow cylindric lamella-structures with or without interposed ribosome-like particles. 4. A mitochondrial inclusion body was demonstrated in mast cells of normal conjunctiva.

8. Morphological basis of the cochlear mechanics—A scanning electron microscopic study on the supportive tissues in the mouse cochlea
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Hearing mechanics of the cochlea are related to the regional differences in structures of the basilar membrane. In this study, we examined the supportive tissues in the mouse cochlea by scanning electron microscopy. The supportive tissues of the cochlear duct were exposed with H2BO3-OsO4 or NaOCl. The connective tissue on the lateral wall of the cochlear duct was perforated with holes for the external sulcus cell-cords. The holes varied in shape, size, and density along the cochlear duct, suggesting that the function of the sulcus cells is related to regional cochlear functions. The auditory teeth of the spiral limbus were clearly exposed; the size and density of the auditory teeth varied along the cochlear duct. These regional differences may be related to mechanics and production of the tectorial membrane. The edge of the tympanic lip of the spiral limbus consisted of bone in the basal turn and connective tissue in the apical half turn, suggesting that the displacement mode of the pillar cells differ between both regions. The width of the spiral fissure between the primary and secondary osseous spiral lamina and width of the secondary lamina suggests that the width of the basilar membrane increases in the hook and then is almost constant to the apex but its stiffness decreases.

9. Various aspects of dislocation behavior in the martensitic transformation
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The martensitic transformation is the phase transformation in which the crystal structure is changed through co-operative movements of atoms without accompanying any atomic diffusion process. In this transformation, a high stress field is necessarily produced inside and outside a transformed plate. The transformation process is therefore inter-related to various kinds of dislocation behavior through this stress field. Several possibilities of the inter-relations are enumerated as follows: (1) assisting action of pre-existing dislocations on the nucleation of martensitic plates, (2) hindering action of pre-existing dislocations on the growth of martensitic plates, and (3) production or multiplication of dislocations due to the stress field of martensitic plates. Although these possibilities have often inferred or imagined in literatures, they have not closely been investigated so far. In the present paper, recent research on these subjects performed with an electron microscope is presented, especially of the item (3) above. Emphasis is laid on the fact that with a skillful use of an electron microscope one is able to determine the Burgers vector of a dislocation, and that the Burgers vector is in turn a key factor to clarify the underlying mechanism of the dislocation behavior.

10. Electron microscopic study on the bainitic transformation in Cu-Zn-Al alloys
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The bainitic transformation is known to be a phase change associated with the martensitic transformation in addition to the diffusion-controlled process. However, less investigations have so far been performed to study the mutual relation between the co-operative movement of atoms in a group and the diffusion of individual atoms. In the present study, the crystallographic nature of the SiO film on SiO showed a constant value from 120 K to the temperature resistance of the film before the heat treatment was observed a multilayer structure of the constituent elements, however, the resistance of the film on SiO increased by 40 hr. This is caused by interdiffusion of the silicon into the surrounding matrix and the growth process follows autocatalytically.

11. Study of high-Tc Y-Ba-Cu-O superconducting multilayer thin film

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The Y-Ba-Cu-O thin films have been studied by a multilayer deposition and the following investigations were carried out in the present study. (1) a relationship between a room temperature electrical resistance and the heat treated time, (2) a cross-sectional observation of the multilayer interface using SEM, and (3) temperature-dependent resistances of the films in terms of the heat treated time. The metallic Y, Ba, and Cu were evaporated sequentially in order of Y-Ba-Cu-Cu-Ba-Y on ZrO/SiO/Si and SiO/Si substrates in the vacuum of 10^-10 Torr and they were heat treated at 900°C in air. The room temperature resistance of the film on SiO increased by increasing the heat treatment time from 10 hr to 40 hr. This is caused by interdiffusion of the silicon into the multilayer film during the treatment. However, the room temperature resistance of the film on ZrO was nearly constant value during the treatment and the ZrO buffer layer acts as a diffusion barrier to the silicon. The interface of the film before the heat treatment showed the multilayer structure of the constituent elements, however, the film which was annealed for 10 hr was observed a similar composition caused by the thermal diffusion and a chemical reaction of the elements. The resistance of the film on SiO showed a constant value from 120 K to the lower temperature, however, the resistance of the film on ZrO approached to zero at 83 K.

12. Analysis of diffraction contrast of small stacking fault tetrahedra

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In the study of damage structure evolution by energetic particles, the smallest stacking fault tetrahedra (SFT) whose size is around 0.5 nm are often observed in fcc metals by weak beam technique. The qualitative analysis and image calculation of diffraction contrast of such small defects are not performed yet. The conventional column approximation is not applicable to this problem. In the present study, the image calculation of small stacking fault tetrahedra under weak beam diffraction condition is performed by the multi-slice method. The visibility of these small SFT and correspondence between the structure size and the image size are examined. The atomic structure of SFT is determined from atomic relaxation of SFT-like structure surrounded by large perfect crystal. The core part of it is cut out and then it is sliced into few number of thin layers. Each of them is assumed to be the unit cell (about 2 nm x 2 nm x 0.25 nm). The whole crystal whose thickness is about 25 nm is constructed by the stack of these sliced SFT and slices of perfect crystal. The interaction of the transmitted beam and scattered beams is traced by the multi-slice method under the diffraction condition which corresponds to weak beam image. The image calculation is made for SFT whose size is 0.5 nm and 0.75 nm. Their image have strong intensity comparing to the background intensity. Moreover each image size shows the well correspondence with the structure size for both cases.

13. Light and electron microscopic study in the central nervous system of a new dystonic mouse, "Wriggle mouse sagami"

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"Wriggle Mouse Sagami (WMS)" is a new autosomal recessive mutant with dystonic movements such as remarkably extended and tonic hind limbs, difficulty in maintaining upright posture, twisting of the trunk and neck, etc. This abnormal behavior initially appears 10 days after birth and progresses until 12 weeks of age. In spite of the severe clinical symptoms, the cytoarchitecture and fiber connections of the motor system are normal. We intended to investigate the WMS cerebellum using P450-immunochemical staining, Golgi silver impregnation and electron microscopy. The conclusions were as follows: (1) Arborization pattern of the Purkinje cells gradually became coarser with aging and their dendritic spines were more irregularly arranged on the surface of the dendritic
trees. (2) The remarkable swellings of the Purkinje cell axons were present in the granule cell layer even at the onset stage. (3) Synaptic formation on the dendrites of Purkinje cells was so disturbed that some intrinsic factor might prevent accurate connection between the Purkinje cells and input terminals. This dysconnectivity might also be present in other parts of the brain.

14. Ultrastructural neuropathology
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1. Ultrastructural demonstration of paramyxovirus nucleocapsids in the SSPE brain-inclusion bodies suggests that SSPE is a persistent measles virus infection, which has been confirmed serologically. Similarly, presence of papovavirus-like particles in the enlarged nuclei of PML brain established that PML is a disease of polyomavirus infection. 2. Hypothesis that varicella-zoster virus infects neurons of dorsal root ganglia in childhood was first verified by ultrastructural detection of specific virus particles in neurons in a child who died of the acute varicella infection. 3. Negative staining with viral antibodies is useful tool to detect virus in a tissue homogenate. Corona virus was named after its “coronal” surface projections visualized by negative staining. 4. Primary demyelination and remyelination are also well demonstrated ultrastructurally. 5. Spongiform changes verified by ultrastructural detection of specific virus particles in neurons in a child who died of the acute varicella infection. 3. Negative staining with viral antibodies is useful tool to detect virus in a tissue homogenate. Corona virus was named after its “coronal” surface projections visualized by negative staining. 4. Primary demyelination and remyelination are also well demonstrated ultrastructurally. 5. Spongiform changes verified by ultrastructural detection of specific virus particles in neurons in a child who died of the acute varicella infection. 3. Negative staining with viral antibodies is useful tool to detect virus in a tissue homogenate. Corona virus was named after its “coronal” surface projections visualized by negative staining. 4. Primary demyelination and remyelination are also well demonstrated ultrastructurally. 5. Spongiform changes verified by ultrastructural detection of specific virus particles in neurons in a child who died of the acute varicella infection.

16. Ultrastructure of spleen innervation in the horse, cow, pig and chicken
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The innervation of the red pulp in the horse, cow, pig and chicken spleen was investigated by the electron microscopy and immunohistochemistry for S-100 protein. In the cow and pig, the red pulp consisted of a fine meshwork of reticular cells, reticular fibers and smooth muscle cells. Nerve terminals were observed around smooth muscle cells, but not reticular cells. In the horse and chicken, there were no smooth muscle cells in the red pulp, so nerve fibers terminated around reticular cells. Nerve fibers or terminals were found in the sheathed artery in the horse and chicken, but not in the cow and pig. All the terminals contained both dense and lucent core vesicles, therefore they were sympathetic nerves. As a result, it was thought that the structure providing the splenic movement greatly differed among these species.

17. Ultrastructural characteristics of carp blood cells
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The morphology of blood cells of carp was investigated by electron microscopy. Erythrocytes, thrombocytes, lymphocytes, granulocytes and monocytes were identified as the peripheral blood cells. Thrombocytes were round to long oval cells containing vesicular and microtubular structures in their cytoplasm, and had oval nuclei with abundant heterochromatin. Lymphocytes and monocytes were morphologically similar to those of mammals. Granulocytes were distinguished into three types (type I, type II and type III) according to the morphology of the nucleus and granules. Type I granulocytes possessed lobed nuclei and a large number of cytoplasmic granules. Some of the granules were oval in shape and contained electron-dense materials and a crystalloid, and the others were small and round in shape, and contained electron-lucent materials. Type II
granulocytes had eccentric nuclei and the cytoplasm were filled with large granules. Type II granulocytes were subdivided into IIa and IIb granulocytes by the ultrastructures of the granules. Granules of type IIa granulocytes were furnished with an electron-dense rim. Those of type IIb granulocytes contained electron-dense and electron-lucent materials distributed like a patchwork. Type III granulocytes possessed round nuclei and a few large granules. The granules were filled with regularly arranged fibriform materials and some needle-like structures.

18. Intraepithelial antibodies and immune cells in the digestive tract
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The immune substances given into the fetuses or neonates by the mother are associated with the prevention of microbial infection and the postnatal development of gut-associated lymphoid tissues (GALT). Although in placenta there are some species differences in morphology and in the permeability of transmissible antibodies from mother into fetuses, maternal antibodies transmitted across the placental tissue are concerned in the organization of intestinal local immune system of neonates together with those secreted via mammary gland after parturition. Maternal immunoglobulins (IgG, IgA) sucked with colostrum or milk were actively absorbed by the jejunal epithelial cells of rabbit neonates and found to be localized in the pinocytotic vesicles or absorptive granular materials in the apical cytoplasm. The immunoglobulins were then demonstrated in the larger granules transporting to the basal region to secrete into the blood capillaries. Although it was found only an inactive development of GALT within a couple of weeks in their postnatal life, a large number of IgG- or IgA-containing cells were demonstrated in the lamina propria of the digestive tract with advancing the postnatal development of the GALT which occurred from 3 or 5 weeks of age. These intramucosal localizations of immunoglobulins were also demonstrated in the wall of excretory system in liver and salivary glands as a first line of immunological barriers.

19. Ultrastructural study on lymphoproliferative disorder
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We conducted an electron microscopic study on buffy coat cells of peripheral blood from the patients with lymphoproliferative disorders. Acute lymphoblastic leukemia cells with B-markers (B-ALL) were noticed abundant rough-endoplasmic reticulum in cytoplasm, and had round, ovoid and indented nuclei. Acute lymphoblastic leukemia cells with T-markers (T-ALL) had convoluted nuclei, were observed clustered dense bodies and Gall bodies in cytoplasm, and had microspikes at the cytoplasmic projections. Chonic lymphatic leukemia with B-cell markers (B-CLL) were divided into three types from the shape of nucleus and surface. A relation between the ultrastructural findings and immunological differences and prognosis of the patients were recognized. Hairy cell leukemia (HCL) was rare disease in Japan. From the shape of the nuclei could be divided into three types; oval, irregular and indented. These shape related to prognosis of the patients. The cells of prolymphocytic leukemia (PLL) and lymphosarcoma cell leukemia (LSCL) closely resembled each other in ultramicroscopic findings. Therefore, in diagnosis of these disease clinical findings such as the presence of splenomegaly and lymphoadenoma should be taken into consideration. Adult T cell leukemia (ATL) cells had the nucleus with shape of flower leaf like, and HTLV-I virus had been found on the surface of cultured T cells from the patients with Adult T cell leukemia and lymphoma.

20. Structure and function of the cytoskeletal system in hepatocytes
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The cytoskeletal system is mainly composed of microtubules, microfilaments and intermediate filaments. Role of microtubules in hepatocytes is extensively investigated because administration of colchicine, a potent inhibitor for this element, specifically perturbs microtubule-related functions. It has been shown that hepatocyte microtubules are engaged in the secretion of plasma proteins and lipoproteins into the blood, translocation of organelles, and excretion of biliary constituents into the bile canaliculi. Electron microscopy revealed a close association of microtubules with the organelles related. On the other hand, microfilaments are shown to be involved in the contraction of bile canaliculi. Immunohistochemistry using anti-actin antibody demonstrated preferential localization of microfilaments in the pericanicular areas of hepatocytes. Disappearance of microfilaments was observed when cytochalasin B, an inhibitor for actin, was infused into the portal vein in parallel with marked decrease in the bile flow. Role of the intermediate filaments is not clearly shown due to the lack of specific inhibitors, but the element is believed to play a role in the establishment of cell architecture, because bundles of intermediate filaments course through the cell and interconnect the spot desmosomes at the plasma membrane.

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21. Actin in ooplasmic segregation
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It has been established that ooplasmic segregation in the Tubifex egg, which occurs after the second polar body formation, is a process dependent on actin-based cytoskeleton. Actin filaments are present not only in the cortex but also in the moving subcortical cytoplasm. They form networks in the cytoplasm. The cortex contains a sheet-like lattice of actin filaments. Shortly after the second meiosis it is thickest around the animal pole, and tapers toward the equator of the egg. Similar polarized distribution of cortical filaments is also observed in eggs at 50–60 min; at this stage, however, actin filaments near the equator of the egg are fewer than before. This polarized cortical organization becomes more pronounced at 90 min: a large part of filaments are localized near the animal pole, forming a polar dense lattice and a ring of filament bundles circumscribing it. In contrast to the increase in the polar zone, actin filaments in the remaining regions of the animal hemisphere become much fewer than before. These observations indicate that the cortical filament lattice in the Tubifex egg undergoes a polarized movement directed toward the pole during ooplasmic segregation. In view of the fact that the subcortical cytoplasm containing mitochondria and actin networks is physically connected to the cortex via actin filaments, it is strongly suggested that cortical contraction resulting in actin reorganization may cause movement of the underlying cytoplasm.

22. SEM observations on indented rings of tree
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The anatomy of indented rings in Sitka spruce (Picea sitchensis Carr.) was examined using SEM. Two SEM specimens, one having clean cut transverse and tangential longitudinal faces and the other having clean cut transverse and radial longitudinal faces, were prepared from small wood blocks. They were useful to understand correctly the complicated arrangement of tracheids and rays in the indented rings. In particular, SEM micrographs at lower magnification clearly revealed the gross anatomical features in these rings. The radial rows of tracheids were considerably disordered in the marginal zones between the middle of the indented rings and the normal zones. Most tracheids in each of two marginal zones were inclined in opposite senses, in the radial direction, to each other. The shape and size of the tracheids were also different from those in the normal zones and varied irregularly within an annual ring. Moreover, trabeculae were commonly found to occur in the tracheids of the indented rings, especially in the marginal zone. In the marginal zone the arrangement of the rays was not straight in the radial direction and biseriate rays lacking a radial resin canal were often found. The parenchyma cells of them were very variable in size and cross sectional shape. Some multiseriate rays with radial resin canals were also irregular in shape. Rays in which the epithelial cells of the resin canal directly contacted axial tracheids on one side were often found.

23. Three-dimensional structure of the osteoclast: Computerized representation
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Three-dimensional representation of tissue and cells by the reconstruction from the serial sections is almost difficult in the case of ultrastructural level not as the case of light microscopy. Because it is actually impossible to mark fiducial points in the specimen block. And incorrectly piling up leads to reconstruct impossible structures. Although the computer aided method for its purpose remarkably developed and practices became easy, the requirement for setting fiducial marker in each sections stay as usual. As one of the trials for it, after making the ultra thin section of the cell (A-plane), serial ultrathin sections of the same cell were made at right angle to the A-plane (B-plane). Then the structures in the B-planes were piled up on the base of the figure in the A-plane. Since structures in the A-plane appear as points or lines at the margin of the B-plane, if the B-planes were correctly piled up each other, these points and lines of B-plane will appear again the figures of the A-plane. This method was used for the reconstruction of the processes forming the ruffled border of the osteoclast. Three-dimensional reconstruction were examined by the personal computer using NIKON COSM0ZONE2SA. The reconstruction of an osteoclasts cultured on the dentine slab were also demonstrated.

24. Scanning electron microscopy of bone resorption and formation-traces of osteoclastic and osteoblastic activities
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To understand the activities of osteoclasts and osteoblasts the mouse parietal bones and femurs were observed by scanning electron microscopy after macerating the organic material by KOH and NaOCl treatment; the femurs were sagittally split into two pieces before the maceration to observe the interior. On the inner surfaces of the parietal bones were two types of bone resorptive rough areas: Type I areas with no remarkable patterns and Type II areas with feather or fire-frame patterns. In young mice before puberty, only Type I rough areas were seen and they changed parallel to the growth rate of the
skull. In adult mice after puberty, Type I and II rough areas were seen, and the total of these areas was larger in females than in males. In femurs, the bone resorptive rough areas were seen on the bone trabeculae and inner surface of the cortical bone. Their total also changed parallel to the growth rate of the bones and showed similar sex differences to the parietal bones. Bone formation areas showed the surfaces covered with minute granules. After administration of estrogen, calcitonin, and vitamin D, the amount of the bone trabeculae was increased. The sizes of the bone resorption and formation areas appeared to change according to the effects of these hormones and vitamin on bone resorption and formation. Scanning electron microscopy is of great benefit to study bone resorption and formation under various conditions.

25. Mineral-depositing mechanism in the calcification in fibroblast cultures

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We have examined the early mineral deposits within calcifying fibroblast cultures by bright field and dark field electron microscopy, and EDX microanalysis. A human gingival fibroblast cell line was cultured in α-MEM medium supplemented with fetal calf serum. The cells in culture showed both intracellular and extracellular ectopic calcification. The initial site of crystallization resided within vesicular structures (intracellular vesicles and extracellular matrix vesicles). Dark field imaging showed nascent sites of tiny crystals to be associated with the inner leaflet of the vesicle membrane. Such crystals sometimes were undetectable at these sites with conventional bright field image. These deposits gathered in clusters in extracellular spaces and extended to the contiguous collagen fibrils. Secondary collagen- and microfibril-associated calcification then occurred and continued. The collagen fibrils and microfibrils were not the initial mineralization sites, but they served to orient crystal precipitation. Coalescence of tiny calcific deposits and accumulation of crystals on collagen fibrils and microfibrils formed large calcified masses surrounded fibroblasts. EDX microanalysis of the mineralized regions revealed prominent peaks for calcium and phosphorus, and these crystals showed 100 planes (8.2 Å) and 002 planes (3.4 Å) indicating hydroxyapatite in nature. These results support the hypothesis that initiation of calcification requires a microenvironment delimited by a membranous (vesicular) structure derived from cells, and also suggest that collagen fibrils and microfibrils are important in determining the pattern of crystal deposits and orienting the growth.

26. Localization of calcium in the mucous lining of the rat duodenum

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Localization of calcium in a rapid frozen and freeze substituted duodenum of the rat (65–100 g) was demonstrated by means either of histochemical or electron microscopic method. In normally fed rats, the glyoxylic bis(2-hydroxyanil) (GBHA) staining of semi-thin Epon sections revealed many discrete granular Ca-GBHA reactions over a majority of absorptive cells of the duodenum, primarily along their lateral membranes. These cells did not show GBHA reactions in the cytoplasm. In unstained ultrathin sections, electron microscopy revealed calcium-loaded dense granules showing similar distribution as the GBHA granules. Such granules were lost after conventional staining for electron microscopy. Some of the absorptive cells at the tip region of each villus contained numerous GBHA-reactive tubulovesicular structures throughout the cytoplasm. In these cells, electron microscopy confirmed the presence of calcium-loaded dense-granules in mitochondria and its absence along lateral membranes. The number and intensity of all GBHA reactions increased in calcium-repleted rats and, in addition, diffuse but intense GBHA reactions appeared at regions of the lamina propria overlaid by the absorptive cells showing intracellular GBHA reactions. These results may suggest the presence of two distinct calcium absorption pathways (transcellular and paracellular) in the mucous lining of the rat duodenum.

27. Ultrastructure of the surface epithelial cells of the normal human rectum

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Surface epithelial cells excluding a few endocrine cells of the normal human rectum were investigated by electron microscopy. Some common features of the epithelial cells, except the Goblet cell, were: cylindrical shape, microvilli whose length and density had considerable variation, glycoalyceal bodies around the microvilli, thick surface coat and large lysosome-like bodies in the cytoplasm as seen especially in the Principal cell. Principal-1 cell was characterized by few tiny vesicles and Principal-2 cell had some tiny vesicles which seemed morphologically to belong to the absorptive cell group. Vesicle cells contained many tiny vesicles. Intermediate cells retained many tiny vesicles and some irregular mucous vacuoles. Columnar mucous cells accompanied by many tiny vesicles and some round mucous vacuoles seemed to be labeled as the secretory cell group apparently isolated from the Goblet cell because of cell shape, the characteristics of each mucous vacuole and the style of secretion. It is considered that the Principal-1 cell may become the Principal-2 cell.
and the Vesicle cell may transform into the Columnar mucous cell through the Intermediate cell owing to the similarity between them and that the reverse may also be true.

28. Electron microscopic histochemistry of glycoconjugates in the duodenal glands of the cow

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Secretion of the duodenal glands of the cow was examined using electron histochemical methods. A piece of the glandular tissues from eight adult Holstein cows was fixed in 3% glutaraldehyde at 4°C for 2 hours and embedded in Lowicryl K4M. Ultrathin sections were stained with periodic acid thiocarbohydrazide silver proteinate (PA-TCH-SP) and dialysis iron (DI). In the cow, two types of cell were present within lobules. One was chiefly distributed at central area of lobules. These cells contain mitochondria, endoplasmic reticulum, Golgi apparatus and few secretory granules (diameter of 300 nm–500 nm) which were electron lucent and weekly stained by PA-TCH-SP and DI. The cis-side of the Golgi apparatus was also strongly stained by PA-TCH-SP and DI. The other was distributed at outer area of lobules. Their cytoplasm contains mitochondria and many secretory granules (diameter of 300 nm–650 nm) which were electron lucent and stained by PA-TCH-SP and DI. Cis-side of the Golgi apparatus was stained by only PA-TCH-SP.

29. An immunocytochemical study of endocrine cells in the pancreas of Caiman latirostris (Alligatorinae)

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Pancreatic endocrine cells were investigated immunocytochemically in caiman (Caiman latirostris) using streptavidin-gold at electron microscopic level. Glucagon (A)-, insulin (B)-, somatostatin (D)-, pancreatic polypeptide (PP)- and motilin (M)-immunoreactive cells were identified in this study. The endocrine granules of each cell type were measured their maximum diameters and analyzed by computerized image analyzing system. The A-cell granules were round and largest (448 ± 103 nm in diameter) in this study and were characterized by the presence of dense-cored granules having electron lucent halo or moderately electron opaque zone around the dense core. The B-cell granules were round and medium size (369 ± 73 nm) containing polymorphous crystal. The D-cell granules were round and slightly smaller (401 ± 22 nm) than the A-cell granules and their granular contents were varied from low electron opaque to electron dense. The PP-cell granules were small (264 ± 27 nm), electron dense and most irregular in shape. The M-cell granules were smallest (218 ± 24 nm) in this study and slightly irregular in shape. It is suggested that in caiman pancreas, the motilin-immunoreactive cells are an additional type of the pancreatic endocrine cells.

30. A semiquantitative electron-microscopic study of 24-hr rhythms in the pineal gland of the mouse

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Semiquantitative electron-microscopic observations on the pineal gland of male dd-mice were made to examine whether pinealocytes, pericapillary spaces and capillary endothelial cells show 24-hr rhythms. Nuclear areas of pinealocytes, cytoplasmic areas of pinealocytes per nucleus and numbers of areas of various structures in pinealocytes per nucleus were determined over a 24-hr period. In addition, areas of pericapillary spaces per unit area and numbers of fenestrae per unit length of abluminal endothelial membranes were estimated. Nuclear and cytoplasmic areas and areas of pericapillary spaces were determined by a point-counting method. Areas of cell organellae and the length of endothelial membranes were measured using a semiautomatic picture analyzing system (Kontron MOP-AM03). Nuclear and cytoplasmic areas, areas of Golgi complexes, multivesicular bodies and pericapillary spaces, and numbers of granulated vesicles, vacuoles and endothelial fenestrae were increased during the light period, whereas areas of mitochondria and endoplasmic reticulum, and numbers of synaptic ribbons and myelin-like figures were increased during the dark period. Based on the above observations, possible 24-hr functional rhythms in pinealocytes of the mouse were discussed.

31. Observation of electron-irradiation processes in materials by HVEM

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When the crystallines are electron-irradiated with above a given threshold energy, vacancies and interstitials are produced in the crystal as a result of displacement of lattice atoms. These point defects aggregate and form the dislocation loops and/or voids. A part of these defects diffuse toward surface and grain boundary. In this process, solute atoms in material interact with the point defects and some solutes migrate with the defect the same or inverse direction of defect flow so that solute segregation occur at sink site, and some times produce the precipitates. On the other hand, when these precipitates are irradiated with high energy electrons, the precipitates dissolve again in matrix. Thus a series of damage process in alloying materials can continuously observe using HVEM. At the
same time HVEM can be applied to many material research such as simulation study of fusion research by connecting the ion accelerator.

32. Microstructure analysis for the fusion reactor materials development
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For the basic understanding of the mechanism of structure changes by cascade damages, the accumulation of data based on appropriate experimental techniques under the controlled irradiation conditions is required. In this paper a series of recently developed techniques of the observation and analysis relevant to variety of defect structures induced by energetic particles, mainly 14 MeV D-T neutrons are discussed. A consistency among specimen preparation, observation with TEM, data acquisition and analysis, which is inevitable for the understanding of microstructure evolution mechanism, is pursued. The selection of materials and the design of specimens are quite important to extract the elementary defect processes in the cascade damages. Irradiation of specimens with defined boundary condition for migrating point defects is expected to give the insight into defect processes. The comparison of defect structure evolution between thin foil specimens already thinned before neutron irradiation and bulk specimens thinned after irradiation enables us to estimate the return rate of interstitials to their own cascade and also cluterating rate of them. The irradiation of intentionally introduced defects such as dislocations, dislocation loops, voids and stacking fault tetrahedra brings the information of specified defect processes. The annihilation of pre-existing interstitial type dislocation loops in Al under neutron irradiation indicate the vacancy predominant atmosphere during the irradiation. The thin foil specimen is also used effectively to detect the subcascade structures in several metals such as Au, Ag, Cu and Ni, and cascade size, subcascade size, the energy divided into each subcascade are estimated.

33. Microstructural change on H+ implanted pure Al
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Modifications to the subsurface layer of pure Al on 30 KeV H+ ion implantation to fluences of 1-10 x 10^17 H+/cm^2 at room temperature were investigated by using transmission electron microscopy. At low fluence, dislocation loops and low number density bubbles were formed. And when electron beam was focused on a certain bubble, dislocation punching from the bubble was observed. It may be caused by high inner pressure of bubble raised by electron beam effect. By following implantation, the number density and mean size of bubble increased with fluence. When ion dose was greater than 5 x 10^17 H+/cm^2, the tunnel structure was observed and at the same time, surface blistering occurred. And the contrast changes of the tunnel structure near a blister were recognized when electron beam was focused around it. It may occur by the fluid motion of high pressure hydrogen.

34. Structure of anodic oxide films on aluminum—Composite oxide films
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Pure aluminum specimens were covered with hydroxide films by immersing in boiling distilled water and then anodized galvanostatically in a neutral borate solution at different temperatures to form composite oxide films. The formation behavior of the composite oxide film was examined by electron microscopy with an ultra thin sectioning technique. It was found that the composite oxide film consists of two layers; a crystalline oxide layer (outer layer) and an amorphous oxide layer (inner layer). During anodizing, a) the hydroxide film was dehydrated to form the crystalline oxide layer at the hydroxide/oxide interface, and b) the amorphous oxide layer was produced at the oxide/metal substrate interface. Also, c) the amorphous oxide converted to the crystalline oxide at the interface between the outer layer and inner layer. The rates of a) and b) were constant at all temperatures and the rate of c) increased with anodizing time and anodizing temperature. High concentrations of voids were observed in a region of the crystalline oxide layer close to the amorphous oxide layer. The distribution of the voids across the crystalline oxide layer suggested that the voids are formed by a rearrangement of oxide accompanied with the reaction 3).

35. Practical use of electron microscopy for the diagnosis of glomerular disease
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The development of a percutaneous technique to procure a small piece of tissue from a kidney and studies of renal disease using light microscopy (LM), electron microscopy (EM), immunofluorescence microscopy (IF) have produced an evolutionary change in the practice of renal medicine. Morphological studies of the kidneys using LM, EM and IF can provide us with an exacting anatomical diagnosis and aid the determination of the etiology and pathogenetic mechanism of disease process. EM and IF are mutually complementary in the characterization of immune glomerular deposits which are protein deposits resulting from immunologic reaction in glomeruli of patients with glomerulonephritis. EM findings are of value especially when interpreted in light of IF. EM can provide...
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Us with much more precise information about glomerular lesions and should be used whenever a renal biopsy specimen is to be critically. Ideally every biopsy specimen should be studied by LM, IF and EM. Objective evaluation of renal biopsy guides us in instituting appropriate therapy and in assessing the course and prognosis of the disease process. EM is now a routine technique for investigating renal parenchymal diseases such as glomerulonephritis and basement membrane nephropathy.

36. Application of X-ray microanalysis to the dermatology
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Superficial horny cells of the skin obtained from various regions of the body of a healthy man were analysed for elements by an X-ray microanalyzer in a scanning mode. Sulfur, potassium, chloride and calcium were constantly detected in all the specimens examined. In addition, there were peaks of aluminum, silicon, etc.; the source of these elements was suspected to be external substances such as air-borne dusts. Abnormal deposits of calcium in the dermis were confirmed by the X-ray microanalyzer in a scanning transmission mode in patients with pseudo-xanthoma elasticum, subepidermal calcified nodule, and dystrophic calcinosis cutis. Elemental analysis also contributed to a diagnosis of granulomas due to mercury and silica.

37. Ultrastructural difference of junctional communication between highly and weakly metastatic clones derived from rat mammary carcinoma
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We observed different metastatic abilities of highly and weakly metastatic clones of a rat mammary carcinoma cell line. The differences in metastatic ability of these clones are caused by different potentials to detach from the primary site and/or to invade into blood vessels. There are, however, no differences between highly and weakly metastatic clones with regard to their in vitro growth characteristics. Ultrastructural observation indicates that the numbers of desmosomes, tight and gap junctions of weakly metastatic clone cells were higher than those of highly metastatic clone cells. The gap junctional formation between weakly metastatic clone cells and normal fibroblasts was more frequently observed when compared with that between highly metastatic clone cells and fibroblasts. To examine the relationship between the metastatic ability of tumor cells and the tumor cell capability to make junctional communication with fibroblasts, we used a dye transfer method. The incidence of intercellular communication between weakly metastatic clone cells and fibroblasts was significantly higher than that between highly metastatic clone cells and fibroblasts. These results suggest that normal fibroblasts may regulate the metastatic ability of tumor cells by the intercellular communication.

38. Ultrastructural studies on exfoliated cells
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There have been several reports on the examination of exfoliated cells by scanning electron microscopy (SEM) or transmission electron microscopy (TEM). SEM provides valuable information on the surface ultrastructure of cells, such as the presence of microvilli or microridges, as well as on their morphology as a whole. TEM examination enables a determination of the origin and identification of the cells to be made. Consecutive application of the two methods would yield a more complete view of the external and internal ultrastructure of the cells. In our clinic, the same cytologic material was successively examined by light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The cytologic samples were smeared on a slide-glass-sized X-ray film, and stained with modification of the Papanicolaou method. The smears were examined by LM and after photography of the cells, selected area on the smears were marked with Indian ink from the opposite side of the film, then samples for SEM examination were prepared. After the SEM examination, the specimens were rehydrated to allow the penetration of Epon 812 into the cells. The TEM examination showed the cell organelles to be comparatively well preserved. These consecutively performed LM-SEM-TEM examinations provided useful information on cytologic subjects, especially concerning the origin of the cells.