Ultracytochemical Study of Glucose-6-Phosphate Dehydrogenase Activity
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The ultrastructural localization of glucose-6-phosphate dehydrogenase (G6PD) activity has been investigated using a copper ferrocyanide method in the rat liver cells by immersion fixation and freeze substitution fixation by using glutaraldehyde.

After immersion in 2% glutaraldehyde fixative for 30min at 4°C, the site of reaction products, copper ferrocyanide, were on the cytoplasm close to the r-ER in hepatic parenchymal cells of rat. These findings were ascertained by several control experiments and X-ray microanalysis.

After the metal contact quenching at -196°C, freeze substitution fixation, 2% glutaraldehyde in acetone for 3 days at -80°C, was performed. The reaction products were not observed on the cytoplasm close to the r-ER, but also on the cytoplasm near the r-ER. It is suggested that freeze substitution fixation were useful to elucidate the ultrastructural sites of soluble enzyme.

Immunohistochemical study of basic FGF related to the development of capillaries in the bovine retina.

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The purpose of this study is to establish the clearer relationship between the localization of basic FGF and the morphological capillary development. The adult and fetal bovine eyes were immunohistochemically and morphologically examined for the presence of basic FGF and anatomical development of retinal vessels.

Immunohistochemically, basic FGF was clearly detected in the retina around 150 days of gestation. Morphologically, primary capillaries were already observed at 90 days of gestation, and development of secondary capillaries were detected at 150 days, when development of inner & outer segments of photoreceptors were seen in the subretinal space.

From our results, basic FGF is thought not to act as an angiogenesis factor for the development of primary capillaries in the bovine retina. Our data suggest the possibility of biological function of basic FGF in the development of secondary capillaries as an angiogenic stimulus, and it also reasonable to think that basic FGF may act in the development and differentiation of photoreceptors.

Analysis of Ki-1 antigen(CD30)mRNA expression in rat tissues, cell lines and malignant lymphoma

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Ki-1 antigen(CD30) is a 105/120KD glycoprotein, selectively expressed on human anaplastic large cell lymphoma(ANL) and/or Hodgkin's(HD) disease. We have previously reported the characterization of Ki-1 antigen by immunohistochemical methods, suggested that this molecule may play a role in the regulation of cell growth and transformation. To help clarify the intercellular signaling system involved ANL, HD and its pathogenesis. We have examined the expression of CD30 by northern blot, Flow cytometry, Immunohistochemical methods. **Materials and Methods** Biopsy specimens of malignant lymphoma, rat tissues(lung, liver, kidney, spleen, brain, spinal cord) and cell lines(T cell:CEM, Jurkat, HSB2, MT1, MT2, HUT102. B cell: Namaruwa, Ball., Neuron: A172, IMR32, PC12) used in this study. The gene probe for the northern hybridization was prepared by TA cloning system. **Results and Conclusion** Expression of CD30 mRNA is found in CD30 antigen positive tissues and cell lines, especially, over expression are seen in large cell anaplastic lymphoma case. At T cell line, The amount of CD30 mRNA was highest in CEM, HSB2 and HUT102, followed by other Jurkat, MT1, MT2 and activated peripheral blood lymphocyte with ConA and PHA. Almost of normal rat tissues, IMR32, A172 and PC12 cell lines were no expression its mRNA but only the spinal cord was detected. These results suggest that the CD30 is responsible for cytokine directed growth regulation of lymphocyte and NGFRs sensory neuron.

CA19-9 in Mature Cystic Teratoma

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We investigated 250 patients with mature cystic teratoma (MCT), using an enzyme immunoassay to determine the tissue source of their high serum levels of CA19-9. The mean preoperative serum CA19-9 level was 79.4 U/ml, and the mean postoperative level was far lower (27.4 U/ml). There were 41 patients positive for CA19-9. In the 31 with serum CA19-9 levels over 101 U/ml, CA19-9 was immunohistochemically demonstrated in the bronchial glands (14 cases) and bronchial mucosa (6 cases) of the MCTs. CA19-9 levels were higher in the cyst fluid of MCTs than in the serum. These findings suggested two routes for the transfer of tumor-produced CA19-9 into the bloodstream: 1) direct secretion from MCT bronchial tissue, and 2) leakage from MCT cysts into the surrounding capillaries through the interstitium.

Histological Evaluation of Yolk Sac Tumor

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Morphological analysis of imprint cytology and histology was performed in 7 patients with YST. These seven cases of YST were morphologically evaluated. Endodermal sinus pattern was most frequently identified, followed by polyvesicular vitelline pattern. On the whole, as with imprint cytology findings of YST, we could classify 4 patterns. Mostly cuboidal cells were frequently observed; cuboidal cells and nuclei were both cuboidal in shape and 25 - 40 μ and 20 - 25 μ in diameters respectively. Next to cuboidal cells, spindle-shaped cells were most frequently noticed; 20 - 40 μ in size with spindle-shaped nuclei 10 - 20 μ in diameter. Naked cells and bizarre cells were also noticed. We postulated cuboidal cells and spindle-shaped cells were originated from the endodermal sinus pattern and the loose reticular pattern of YST, respectively.

Histochemical Analysis of Blood Group Antigens in Human Sublingual Glands. An Application of High Performance Liquid Chromatography (HPLC) to Estimate the Quantity of Galactose Liberated from Tissue Sections by α -Galactosidase Digestion

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Using immunostaining with monoclonal antibodies (MAbs) from three different sources as well as anti-B lectin GSAI-B₄ staining and α -galactosidase digestion, localization and analysis of blood group B antigens were carried out in tissue sections of sublingual glands. Quantitative analysis of galactose was simultaneously carried out on the supernatant enzyme solution used for treating tissue sections by a method of HPLC. MAb-B(H079) and GSAI-B₄ reacted uniformly with the mucous cells from blood group B and AB secretor individuals. Among reagents used, only MAb-B(H079) can recognize the B antigens in mucous cells from nonsecretor individuals. Digestion with α -galactosidase resulted in the consistent appearance of H and Le^b antigens in mucous cells of all the secretor individuals examined. HPLC analysis of galactose revealed that α -galactosidase can specifically liberate the terminal galactose residues of B antigens and no marked difference was present in the contents of liberated galactose from mucous cells of the glands among individuals investigated (8.5-11.7 nmoles/cm²). No galactose was detected in samples from the glands of nonsecretor individuals although the enzyme digestion resulted in the appearance of Le^b and Le^x antigens. These results suggest that observed difference in the reactivity of different reagents is ascribed to both quantitative and qualitative heterogeneity of B antigens.

Experimental Studies of Toxicological Aspect of Methylene Chloride on Changes in Kidneys

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The effect of methylene chloride on the kidneys of rats has been studied. Methylene chloride, at a dose of 0.5ml/ B.W., was administered intraperitoneally into the subject rats.

After a certain period, the injection rats were killed at various intervals, and postmortem hematological and histological examinations were performed.

After 30mins, hematuria, an increase in urinary NAG activity, urinary creatinine concentration, and urinary Al-p activity were found. An increase in the red blood cells and large deposits of hemosiderin in the spleen were observed by berlin-blue staining. Microscopic examination of the distal renal tubules revealed edema, decudation and degeneration of epithelial cell, and hemoglobin cast in several tubules increased. The degeneration was also the same in proximal renal tubules. Under electromicroscopy, vacuolization of renal tubular cells, an increase in the lysosome, and removal of Micro Villi and exfoliation from basement membrane in the epithelial cells were observed.

The above results suggest that nephrotic syndrome occurred as a result of the toxic effect of methylene chloride on the red blood cells.

Histochemical Visualization of Subcellular Organelles by Confocal Laser Scanning Microscope (CLSM) without Using Fluorescent Probes. J Itoh, H Utsunomiya, N Komatsu, R Y Osamura and K Watanabe. Cell Biol Res Lab and Pathol. Tokai Univ Sch of Med Isehara Kanagawa Japan 259-11

Recently, the application of confocal laser scanning microscopy (CLSM) in the fields of biology and medicine was enormously expanded.

This CLSM was found to be extremely useful to observe not only very fine intracellular or extracellular localizations of various biologically active substances but also three dimensionally reconstructed features of those in immunohistochemistry or in situ hybridization utilizing fluorescent probes as their visualization markers. Since the degradation of the fluorescence of these probes is rather quick and serious, the CLSM with fluorescent signals often prohibits precise and elaborate observations. Repeated observation on permanently preservable specimens are certainly impossible. In the present study, therefore, the peroxidase (histochemically stainable) was applied to CLSM to replace fluorescent probes and to avoid those disadvantages.

[MATERIALS AND METHODS] ① Endogenous peroxidase (azure granules and rER) in immature monocytes in mouse liver typhoid nodules and ② IgG (well developed rER) in plasma cells in rat lymphnodes were enzyme- and immuno-histochemically stained. Their final reaction products, oxidized DAB, were reacted with OsO₄ to form "osmium black" which can be detected by the reflectance mode of CLSM. ③ Rat hepatocyte were overfixed for 48 hours with OsO₄ to observe their Golgi apparatus which are known to be specifically stained by the osmium. In the above experiments, ② and ③, 40 μ m thick "micro-chopper" sections were employed. For CLSM observation, LSM-10 with IBAS (an image analysis system, CARL ZEISS) was used. [RESULTS AND COMMENTS] Osmium, which was deposited on azure granules (monocytes), rER (monocytes and plasma cells) and Golgi apparatus (hepatocytes) as the fine reaction products of peroxidase histochemical staining or by itself, was successfully demonstrated by the reflection mode observation of CLSM. The finer suborganelle localizations were obtained from 40 μ m thick non-frozen sections.

Light and electron microscopic localization of peptidyl-glycine alpha-amidating monooxygenase (PAM) in the rat pituitary and mouse pituitary tumor cells (Att20). Y. Itoh, R. Y. Osamura, K. Watanabe, Dept. of Pathol. Tokai Univ. Sch. of Med., Isehara, Kanagawa JAPAN 259-11

Some peptide hormones are known to be amidated as a post-translational processing to obtain bioactivities. PAM is known to be involved in alpha-amidation. This study is aimed at to elucidate the immunohistochemical localization of PAM in the normal rat pituitary and mouse cultured Att20 cells. Att20 cells are the cultured mouse pituitary tumor cells. (Materials and Methods) For light microscopic observation, mature Wistar male rats were perfused with low picric acid pH4 Bouin's fixative and the removed pituitaries were further fixed in the same fixative for overnight. Colocalization of PAM and pituitary hormones or subunits were observed in mirror section. For immunoelectron microscopy (pre-embedding method), the pituitary tumor cells (Att20) were fixed for 1 hour with Zamboni solution. We used L.R. White resin for post-embedding method, on the rat pituitary. These methods were performed in conjunction with indirect immunoperoxidase method using Ab26 (Dr. B. A. Eipper, Dr. Takeuchi) polyclonal antibody. (Results and Comments) This study elucidated the immunohistochemical localization of PAM, in the secretory granules which were thought to be the major sites of amidation. And the paraffin mirror sections showed colocalization of PAM with peptide hormones, such as GH, TSH β and LB. The function of PAM in these cells remained to be further investigated.

Investigation of Albumin (Alb) Synthesis in Regenerating Liver after Partial Hepatectomy (PH)

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We investigated Alb synthesis in regenerating liver, using immunocytochemistry, in situ hybridization and liquid scintillation (LS). **Materials and methods** Male Wistar rats were partially hepatectomized by method of Higgins and Anderson. They were sacrificed at days 2, 5 and 10 after PH and some of them were injected ^3H -leucine (20 μCi) before sacrifice. The liver was fixed by PLP or PFA, frozen and sectioned into 6 μm slices by cryostat. They were incubated by rabbit anti-rat Alb, followed with HRP conj. anti-rabbit IgG and stained with DAB solution. They were osmicated, dehydrated by ethanol and embedded in Epon for ultrathin sections. Some slices were hybridized by ^{35}S -labelled oligo DNA complementary for Alb m-RNA, and covered by emulsion, developed and fixed. The homogenized tissue was fractionated into microsome and cytosol after centrifuge, and supernatant of the cytosol was incubated with excessive amount of anti-rat Alb, and tritiated activity in the immunoprecipitate was counted by LS counter. **Results** Immunoreaction of Alb was seen in all hepatocytes as fine granules after PH and their granules were larger and more numerous at days 5 than those at days 2 or 10. Subcellular location of Alb was in r-ER and Golgi apparatus, and Alb-positive organelles were well preserved and developed at days 5 or 10. In situ hybridization study showed presence of Alb m-RNA in all hepatocytes and grains of Alb m-RNA were more numerous at days 5 than those at days 2 or 10. LS counter revealed significantly higher activity of ^3H -leucine incorporated into Alb synthesis at days 5 than that at days 2 or 10. **Conclusion** Our study suggested that activity of Alb synthesis was varied during liver regeneration and that Alb synthesis was most active at days 5 after PH.

Ultrastructural Investigation of Nucleolar DNA in Blasts from 40 Patients With Acute Leukemia
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The nucleolus, which consists mainly of RNA, has a small amount of DNA in it, the localization of which is known to differ by cell type. We examined the distribution of DNA in the nucleolus of blasts from 40 patients with acute leukemia at the ultrastructural level.

Materials and Methods: Heparinized bone marrow specimens from 20 patients with acute lymphocytic leukemia (ALL), and 20 patients with acute myeloid leukemia (AML) were fixed with 2.5% glutaraldehyde, dehydrated and embedded in L-R white resin.

Osmium-amine-B was prepared according to the method of Olins (J Histochem Cytochem 37:395, 1989). A mixture of 0.5g of disodium hexachloro osmate and 5ml of concentrated ammonium hydroxide was heated in a heavy-walled sealed bottle at 100 $^{\circ}\text{C}$ for 12 hours in an oil bath. After the solution was filtered, 2.5ml of concentrated HCl was added to the filtrate, and the deposit (osmium-amine-B) was obtained. It was then dried and stored until use.

Ultra thin sections mounted on gold grids were floated on a drop of 5N HCl at room temperature for 25 minutes. The grids were placed on drops of 0.2% osmium-amine-B solution bubbled with SO_2 . The samples were observed under an electron microscope without counter staining.

Results: The DNA in ALL blasts was clumped in the nuclei, while in AML blasts, it tended to be dispersed. The DNA distribution in the nucleoli could be classified into 3 types, i.e., granular structure, linear structure and a combination of these two structures. The granular structure of DNA was predominant in ALL blasts and the linear structure of DNA was predominant in AML blasts. A combination of the two structures was observed both in ALL and AML blasts. The granular DNA in the nucleoli was often connected with a nucleolar wall by linear DNA.

Mapping of the enzyme activities in thylakoid membrane of a cyanobacterium, *Nostoc muscorum*.

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Localization of NADH-dehydrogenase, cytochrome oxidase, and photosystem II and I were mapped with BSPT-, and DAB-histochemical reaction (the 30th annual meeting of this society, 1989).

Following three criteria were considered for the mapping procedure.

1. Bulk area possessing the activity of an enzyme should be equivalent to the reaction-positive area. Reaction-positive areas of the above four enzymes were calculated by stereology (the 32th annual meeting, 1991). PSI activity spread whole area of the membrane. The other three activities distributed as patch-like pattern and shared with PSI. The area of dehydrogenase and that of cytochrome did not overlapped each other.

2. Individual size of each reaction-positive area was estimated from the analysis of the reaction-positive length along the membranes on cross-sections. The smallest size of the area was 4,000 nm^2 .

3. Comparison of dark-grown cells and light-grown cells suggests a close relationship between the reaction-positive area and the particles on freeze-fracture faces. Activity of dehydrogenase showed 23.8% increase in the dark-grown cells compared with that of the light-grown cells. Increase of the cytochrome oxidase was 27.5%. Number of the particles, presumed to be related to respiration, increased on both PF and EF. Increase of area filled with the particles was 5.7% and 5.3% respectively.

Localization of Collagen Type X mRNA in Embryonic Chick Endochondral Bone and Heterotopic Bone Induced by Chick Periosteal Allograft

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Type X collagen is synthesized by hypertrophic chondrocytes of the growth plate and deposited in the calcifying region, while type II collagen is synthesized by resting and proliferating chondrocytes. We examined the spatio-temporal localization of collagen type I, II and X mRNAs in embryonic chick vertebrae undergoing endochondral ossification and in the heterotopic endochondral bone induced by chick periosteal allografts. In the embryonic vertebra at stage 45, a reversed pattern of distribution between collagen type II and X mRNAs was noted in maturing chondrocyte zones. High signals of collagen type I mRNA were restricted in osteoblasts. On the other hand, different expressions of these collagen mRNAs were observed in the subcutaneously grafted heterotopic bone. Five days after transplantation, developing non-hypertrophic chondrocytes with rapid expression of collagen type X mRNA were identified in the mass of atypical cartilage. At day 9 after transplantation, sparsely distributed chondrocytes having high signals of collagen type X mRNA were demonstrated in osteoid and/or woven bone.

The phenotype of chondrocytes showing rapid expression of collagen type X gene derived from the grafted periosteum seems to participate in the important role of endochondral bone formation in the early stage of fracture repair.

Production and Characterization of a New Anti-rat Macrophage Monoclonal Antibody, RM-4

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Utilizing a homogenate of Silica-induced lung granuloma of rat as immunogen, a new monoclonal antibody designated RM-4 was produced. By the immunohistochemical evaluation, RM-4 was found to be specific for most macrophage populations in many different tissues and body cavities, such as Kupffer cells in the liver, tingible body macrophages in the lymphatic follicles, sinus macrophages in the lymph nodes, and red pulp and marginal zone macrophages in the spleen. RM-4 was also reactive with interdigitating cells in the lymph node and Langerhans cells in the skin. On the other hand, RM-4 failed to recognize peripheral blood monocytes, granulocytes, lymphocytes, any epithelial cells, or any cells other than macrophages. Immunoelectron microscopically, reaction to RM-4 was observed in phagosomal membrane and a part of cell membrane relate to phagocytosis. In addition, under the various stimulation, RM-4 positive rates became higher. In double staining of the spleen, RM-4 revealed distinct reactivity in contrast to the previously reported several anti-rat macrophage monoclonal antibodies. RM-4 recognized an antigen with 46kD in Western blot analysis. Accordingly, RM-4 was considered to be a very useful tool not to specifically recognize most macrophage populations as an anti-pan-macrophage monoclonal antibody, but also to evaluate macrophage phagocytotic activities.

Relationship between Staining Quality of GFAP and Cell Cycle Determined by FCM in Glioma

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Glial fibrillary acidic protein (GFAP) is often used in histologic diagnosis of glioma, but its staining quality differs by the cells examined even processed with a specific method. A flowcytometric analysis was made to clarify the relation of the staining quality of GFAP to the cell cycle, assuming that the latter might contribute to induction of the difference.

TC430 cells (glioblastoma strain established by us) were cultivated in a chamber slide, fixed with 70% ethanol following 3 to 4 days' incubation, and stained with anti-GFAP monoclonal antibody by the PAP method. By employing double staining of propidium iodide and FITC-labeled GFAP, GFAP-positive cell rates were calculated on the DNA histograms obtained from gated cells at the G₀/G₁, S and G₂M phase in established cell lines (Vero cells, TC430 and KMU100). The results are: 1) Vero cells were GFAP-negative, and 2) TC430 and KMU100 were positive for immunohistologic staining, and the GFAP-positive rate of the gated cells was highest for the G₂M cells, compared with those for G₀/G₁ and S phase cells.

It is suggested that GFAP staining quality may differ according to the cell cycle.

Characterization of acidic glycoconjugates in rat gastric surface epithelia during development by postembedding staining using cationic colloidal gold
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The postembedding staining procedures using cationic colloidal gold (CCG) at both light and electron microscopic levels were applied to evaluate changes of acidic glycoconjugates in rat gastric epithelia during development. Semithin and ultrathin sections from the Lowicryl K4M embedded tissues from embryonic (14-16day), fetal (18-22day), suckling (1-20day) and weaning (21-28 day) rats were incubated with CCG at pH 2.5 [CCG (2.5)] and CCG at pH 1.0 [CCG (1.0)] to characterize sialylated and sulfated acidic glycoconjugates. At the LM level, the sections were further processed for silver amplification. In adult rat stomach, intense labelings of both CCG (2.5) and CCG (1.0) were found on the mucous cells around gastric pits. At the EM level, gastric pit cells contain monophasic granules which react with both CCG (2.5) and CCG (1.0) and foveolar cells contain biphasic granules without CCG (2.5) nor CCG (1.0) labelings. Mucous cells with CCG (2.5) labeling appeared on the 21st day of gestation and after birth they mainly located around gastric pits. Besides, during suckling phase, CCG (2.5) labeling cells appeared on the upper surface. At the EM level, mucous cells contain biphasic granules with larger dense cores and CCG (2.5) labeled peripheral portions. On the other hand, CCG (1.0) labeling mucous cells located around the gastric pits after birth. Present results showed the characteristic staining patterns of sialylated acid glycoconjugates in surface mucous cells during suckling phase and after weaning staining pattern became that of adult type.

Secretagogue-induced Calcium Signaling in Mast Cell as Revealed by Confocal Laser Scanning Microscope

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By using confocal laser scanning microscope (CLSM), we analyzed calcium signaling in single mast cell induced by secretagogue, compound 48/80. Rat peritoneal mast cells obtained by a peritoneal lavage were degranulated with compound 48/80 after loading fluorescent calcium indicator, fluo-3/AM. Confocal fluorescence images showed the elevation of free intracellular calcium concentration $[Ca^{2+}]$, transferred in both the cytoplasm and the nucleus immediately after compound 48/80 stimulation. Local application of the secretagogue to the mast cell through micropipette evoked heterogeneous increase of $[Ca^{2+}]$. Line scanning mode of CLSM revealed that $[Ca^{2+}]$ waves began at the side of a cell near the micropipette, then spread to another side at average speeds of 35 μ m/sec.

Expression of the inositol 1,4,5-triphosphate receptor in the cultured human malignant cell lines
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One of the authors(KM) had found the mouse inositol triphosphate receptor (IP₃-R) protein and raised rat monoclonal antibodies (moAbs) reacting with the protein at three different epitopes. We investigated in the present report whether these moAbs reacted with the antigen in human malignant cells. HPL-ESC-M, -S and -K were human esophageal squamous cell carcinoma cell lines established from a single primary culture. They expressed different phenotypes regarding with squamous cell differentiation. U266, P3HR-1, HPL-Hod-1 and ARH77 were of lympho-hematopoietic origin; myeloma, Burkitt's lymphoma, Hodgkin's disease and IgE myeloma, respectively. Frozen sections of these cells were prepared from the cell pellet of in vitro cultured cells and fixed with 10% buffered-formalin for 5 minutes. For immunocytochemistry they were sequentially reacted with moAb, biotin-F(ab')₂ anti-rat IgG and avidin-alkaline phosphatase. Of 3 moAbs only one (10A6) reacted with the human antigen. Its reactivity with the cell lines was ubiquitous but varied among cell lines. The antigen was located either diffusely or localized in the cytoplasm of each cells, but its distribution on the plasma membrane was not apparent. No particular relationship between its distribution and cell differentiation or cell types was observed. Thus this moAb would be a new and useful tool for the investigation of human IP₃-R. It is urgently required to confirm directly that the antigen in the human cells is the IP₃-R protein.

Dynamics of chromogranin A granules in adrenal medullar cells after treatment with nicotine
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Effects of nicotine on the secretion and the formation of granules were studied by electron microscopic immunocytochemistry of chromogranin A as a marker of chromaffine cells.

The adrenal medulla of Wistar-Imamichi male rats were fixed chemically 5, 30, and 60 minutes after a single subcutaneous injection of nicotine (5mg/kg). The materials were embedded in Lowicryl K4M and the immunostaining of antichromogranin A of bovine adrenal medulla was performed by post-embedding techniques using colloidal gold conjugated second antibodies.

Five minutes after the injection of the drug, the number of granules in adrenaline cells decreased rapidly and the vacuolation of the rough endoplasmic reticulum occurred. Colloidal gold was observed only on granules. The Golgi apparatus was developed well and the formation of the granules began 30 minutes after the treatment with the drug. Only mature granules, however, were stained with antichromogranin A. After 60 minutes of the injection of nicotine, adrenaline cells increased the granules as many as normal controls, while the well developed Golgi apparatus still remained. Colloidal golds were observed almost on all granules except immature granules around the Golgi apparatus. The rough endoplasmic reticulum and the lamellae of the Golgi apparatus were devoid of colloidal gold.

From these results, nicotine induces the rapid secretion of granules but the cells begin immediately to make granules. However, the immunoreactivity appears after the maturation of granules.

Immunohistochemical Study of Plasminogen Activator Expression in Human Colorectal Carcinoma

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Fifty cases of colorectal carcinomas were immunohistochemically examined for the expression of urokinase (u) and tissue (t) type of plasminogen activators (PA) in relation to their differentiated characteristics. U-PA-A was mainly observed at the apical portion of cancer cells with higher degree of differentiation. And then it changed its localization predominantly into cytoplasm and its staining pattern from heterogeneous to homogeneous with progressive dedifferentiation. The number of u-PA-A positive cancer cells increased with these changes. But u-PA-B t-PA-A and t-PA-B usually tended to distribute at the apical portion of cancer cells and not to change its localization with the tumor differentiation. And their positive cancer cells were observed mostly less than 50% of tumor cells. The present findings suggest that the mode of u-PA-A localization and its positive rate in carcinoma tissue and the degree of tumor differentiation reflected by the pattern of CEA distribution are closely related.

Preparation of a protein-specific monoclonal antibody to β 1 $\rightarrow$ 4 galactosyltransferase, and its application to immunohistochemistry.

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β 1 $\rightarrow$ 4Galactosyltransferase (GalTase) has various kinds of sugar chains including blood group substances. Polyclonal antibodies to GalTase, that have been used in previous immunohistochemical studies of GalTase, may recognize such non-specific sugar chains as well as specific epitopes including polypeptide sequences (Child et al., 1986). The previous immunohistochemical studies with the polyclonal antibodies, therefore, should be reexamined using protein-specific monoclonal antibodies (mAb).

In the present study, we have prepared a protein-specific mAb GTL2 (mouse IgG2b) to GalTase purified from rat liver (J. Biochem. 111, 568-572, 1992). The protein-specificity of GTL2 was confirmed by immunoblot analysis, in which GTL2 specifically recognized non-glycosylated human GalTase expressed in *E. coli* (provided by Dr. Narimatsu, Soka Univ.) as well as rat GalTase deglycosylated with glycosidases (N-Glycanase, sialidase, and O-Glycanase).

By the indirect immunoperoxidase method with GTL2, we studied the immunohistochemical localization of GalTase. Intense staining was observed only in *trans*-Golgi stacks within serous cells of submandibular gland, epithelial cells of epididymis, and hepatocytes.

The study for histological heterogeneity about DNA ploidy pattern and cell kinetics by PCNA stain

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Mucin-producing colon cancer sometimes shows heterogeneity in the tumor. In order to analyze the relationship between cell kinetics and histological difference of mucinous adenocarcinoma (including signet-ring cell carcinoma), it was divided into two kind of parts: cancer cells floating in mucin (M) and signet-ring cells or adenocarcinoma showing solid part without mucin (S). We used 18 cases of mucinous adenocarcinoma, and 6 cases of signet-ring cell carcinoma, all of which were advanced colon cancer. DNA ploidy pattern of the cancer cells was determined by DNA cytofluorometry, detecting intracytoplasmic mucin by PAS and DAPI double stain. Though cancer cells in the M portion had almost diploid pattern (96%), cancer cells in the S portion of 72% cases had diploid pattern, and those in that of 28% cases had aneuploid pattern. Though all signet-ring cell carcinomas showed diploid pattern, in the portion composed of solid structure, they showed a tendency to have aneuploid cells and polyploid cells. PCNA immunostain revealed that the growth fraction of cancer cells in S portion was larger than that in M portion significantly.

A Comparison of Histochemical Characteristics Between Hydroquinone-tetrazolium System and Alloxan-tetrazolium System

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In the 97th meeting of the Japanese Association of Anatomists, I reported that reducing reagents in tissues were histochemically demonstrable with alloxan and tetrazolium salts. It is known that quinones also react with tissue reducing reagents such as SH compounds. In this study, some histochemical characteristics of the hydroquinone-tetrazolium system and the alloxan-tetrazolium system were compared.

The composition of reaction medium was 0.05 mM Na-phosphate buffer solution containing 10 mM substrate, 2 mM nitroblue tetrazolium (NBT), and 5% sucrose. Hydroquinone (HQ) and alloxan (ALX) were used as substrates. Unfixed fresh frozen-sections of the pancreas and the liver were incubated in the reaction medium for 30 minutes at room temperature. Blotting papers bearing well known substances were also incubated in the reaction medium as simulation reactions.

The reaction products by both methods were pinkish violet and mainly distributed in the cytoplasm of cells observed. In exocrine pancreatic cells, the cytoplasm below the nucleus was stained stronger than the supranuclear cytoplasm. In the endocrine pancreas, the staining characteristics were common in all cells. The distribution pattern in the pancreatic tissue resembled between both methods. In the liver, however, granular formations appeared when the hydroquinone method was used. In the simulation reactions, alloxan as well as hydroquinone reacted well with SH compound. Hydroquinone could react with oxidized coenzyme Q, but not alloxan. With NAD(P)H as substrates, the results were reversed. Thus histochemical characteristics of both methods were a little different each other. The difference observed in the liver may be related to the fact that hydroquinone can react with oxidized coenzyme Q.

EXPRESSION OF PARATHYROID HORMONE-RELATED PROTEIN IN HUMAN PARATHYROID TISSUES

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We have described the expression and localization of parathyroid hormone-related protein (PTHrP), a major factor responsible for hormonal hypercalcemia of malignancy, in the parathyroid glands from normal and primary hyperparathyroidism (adenoma) subjects and the hyperplastic parathyroid glands from patients with chronic renal failure by light and electron microscopic immunohistochemistries using an anti-hPTHrP monoclonal antibody 4B3 and *in situ* hybridization (ISH) using BrdU-labelled cDNA probe. Twelve of 14 normal glands, 13 of 19 adenomas, 16 of 18 cases of secondary parathyroidism represented positive reactivities for 4B3. Immunostaining of 4B3 was completely extinguished by preabsorption with the excess of hPTHrP. In normal glands, the ratio of positive cells tended to increase in relation to ages of the subjects. PTHrP staining was mainly observed in typical oxophilic cells and some transitional cells, but scarcely seen in chief and water-clear cells. With immunoelectron microscopy, secretory granules sized 150 nm in diameter were seen in the cytoplasm abundant of mitochondria. Transcripts of PTHrP were clearly demonstrated in consistency with PTHrP-immunoreactivity by an ISH in semiserial sections. Conclusively, PTHrP and PTHrPmRNA were proved to be located characteristically in oxiphilic cells regardless of the pathogenesis of parathyroid glands. The presence of PTHrP might connect with the phenotypic differentiation of oxiphilic cells, though the pathophysiological role of PTHrP in the parathyroid gland still remain unclear.

Immunohistochemical examination of epidermal tumors with amyloid deposition
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We were studied secondary localized cutaneous amyloidosis and its precursor protein. 54 of cases of Bowen disease, 350 seborrheic keratosis, 67 basal cell carcinoma, 70 solar keratosis from our files (1986-92) were investigated. Biopsy and surgical specimens were fixed in 10% formalin, embedded in paraffin, sectioned and stained with H&E, Congo red and Dylon. Finally, 14 Bowen disease, 12 basal cell carcinoma, 50 seborrheic keratosis and 3 solar keratosis, all positive for Congo red and Dylon stain were examined in detail. Biotin Streptavidin technique was used to deparaffinize the sections. As primary antibodies, anti-human AL kappa, AL lambda, amyloid A, amyloid P and cytokeratin (#1, 5, 6, 7, 8, 10, 12, 14, 17, 18, 19) were used. AL kappa, AL lambda and AA antibodies were negative, whereas AP was positive, in all cases. Cytokeratins #10, 17, 18 were positive for amyloid deposition (34/79). As were #1, 5, 10, 14 (50/79) and #6, 18 (24/79). Furthermore, #7, 8, 12, 19 were negative in all cases. Normal epithelium, basal layer to prickle cell layer showed the same findings. The precursor protein of secondary localized cutaneous amyloidosis appears to be related to cytokeratin.

Localization of acid phosphatase activity in *Trypanosoma cruzi* in infected mouse cardiac tissues T. KOBAYASHI, H. SEGUCHI and J. FIGUEROA DA SILVA* Dept. Anat. Cell Biol., Kochi Med. Sch., Kochi and LIKA, Perunambuco Fed. Univ., Brazil

Chagas' disease initiates with the entry of infectable trypomastigote forms of *Trypanosoma cruzi* (T. cruzi) into the vertebrate host. These parasites penetrate into phagocytic and nonphagocytic cells, where they multiply as amastigotes and again differentiate into trypomastigotes. Biochemically, amastigotes have been reported to exhibit stronger acid phosphatase (ACPase) activity than trypomastigotes. In the present study, the localization of ACPase activity in T. cruzi was investigated by the cerium-based ACPase cytochemistry. T. cruzi exhibited ACPase activity in lysosomes and Golgi complex throughout the whole life cycle. Two weeks after infection, the enzyme activity was localized on the surface of the amastigote in the pre-proliferation stage in the cardiac muscle cells. Proliferating amastigotes, exhibited weak ACPase activity on their surface as compared with those before proliferation. In the late stage of proliferation, the enzyme activity was restricted to the plasma membrane of flagellar pockets. Three weeks after infection, the trypomastigotes that left the infected cardiocytes were found in the intercellular space. In these protozoa, the enzyme activity was also found on the plasma membrane of the flagellar pockets. T. cruzi phagocytosed by macrophages and not digested in the phagolysosomes exhibited ACPase activity on the amastigote surface. The present results demonstrated that the localization of ACPase activity on the surface of T. cruzi changes during its life cycle. The ACPase activity may be involved in maintaining the environment for the proliferation of T. cruzi in host cells.

PCNA immunohistochemistry on formalin-fixed specimens - Antigen retrieval by microwave irradiation -

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Although PCNA is the only method to be detected in the proliferating cells on conventional formalin-fixed and paraffin-embedded tissues, the staining intensity decreases with fixation time. Up to date, no attempt to enhance PCNA staining had succeeded. Recently retrieval of various kinds of antigenicity had reported employing microwave irradiation of tissue sections in heavy metal solutions. In order to examine suitable conditions for antigen retrieval of PCNA, we compared various kinds of solution with different irradiation time. Microwave irradiation of tissue sections in 0.001N NaOH solution for 15-30 minutes was the most effective. This effect seemed to be dependent on heat, pH and sort of buffer or solution used. These methods enabled PCNA immunostaining on specimens which were fixed with formalin for long time.

Furthermore, quantitative evaluation of PCNA staining intensity seems to be important for analysis of cell kinetics.

Ultrastructural comparison of perinatal mouse retinal cells by radioautography and image analysis

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For the purpose of comparing ultrastructural changes during embryogenesis and postnatal development, we studied mouse retinal cells by radioautography and image analysis. The ddY strain mice from embryonic day 9.5 to postnatal day 2 weeks were used in this experiment. Ocular tissues were incubated in the medium consisting of Eagle MEM, 10% FBS and ³H-thymidine (specific activity 185MBq/mM, 3.7MBq/ml), in a CO₂ incubator at 37°C for 1 hour. The tissues were doubly fixed with glutaraldehyde and osmium tetroxide and then embedded in Luveak 812. Thin sections (0.1µm) were cut and coated with Konica NR-H2 emulsion. After 13 months exposure they were developed with GL-phenidone developer, stained with lead citrate solution and observed with a Hitachi H-700 electron microscope. About 100 cells of middle portion of retina in each group (50 cells of labeled and unlabeled, respectively), totally about 200 electron microscopic radioautograms, were observed and analyzed by means of an image analyzer (Macintosh Quadra 900) to calculate the average area of nuclei, cytoplasm, mitochondria and ER, the number of mitochondria per labeled and unlabeled retinal cells. The results showed that the labeled cells were located mainly in the inner nuclear layers of retina from late embryonic stages to postnatal day 7. The area and number of cell organelles showed a declined tendency during aging. However, no significant difference was found between labeled and unlabeled retinal cells in the same age group. It should be concluded that the labeled mouse retinal cells were located in the inner nuclear layers of retina until postnatal day 7. The area of cytoplasm, nuclei and organelles between labeled and unlabeled retinal cells did not show significant difference.

Effect of Formalin Fixation on DNA

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The effect of formalin fixation on DNA was investigated. Aldehyde fixatives resulted in degradation of lambda phage DNA at room temperature but not at 4°C. The degradation also occurred in formalin when the pH or the salt concentration was low, or the formic acid level was high. Restriction endonuclease digestion of lambda phage DNA was incomplete after formalin fixation and this was also temperature dependent, but not affected by salt concentration of the fixative. Thus, relatively intact DNA was obtained from the tissues fixed in buffered formalin at 4°C.

Lambda phage DNA fixed in unbuffered formalin showed poor PCR amplification due to degradation of DNA during fixation. Lambda phage DNA fixed in buffered formalin evaded degradation and suited for template of amplification. Integrity of the PCR-amplification was confirmed by the sequencing of the subcloned PCR products.

Feasibility of formalin-fixed tissues as sources for PCR amplification was investigated with primers producing 128 bp fragment of c-Ki-ras exon 2. Although DNA from tissues fixed for 3 months showed amplification, there was no amplification from tissues kept in unbuffered formalin for longer than 6 months.

Comparison of cell kinetics of head and neck
tumor by flow cytometry
and immunohistological methods.Hirobumi Kumazawa, Shinnichi Sai, Jun Kita,
Yasuhiro Wada, Noriko Yamasaki, Yoshirou Hori,
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It is important to analyze the cell kinetics of head and neck tumor cells after treatment of anticancer agents. To analyze the cell kinetics, double staining with BrdU and PI by flow cytometry method is useful. However, when we look at the localization of positive tumor cells in a solid tumor, it is necessary to apply the immunohistological method, which can detect positive tumor cells in sections. In this study, we compared the positive ratio of oral epidermid tumor cells (Cisplatin sensitive cells:KB cells, Cisplatin resistant cells:KBrc cells) between flow cytometry method and immunohistological method in various condition. In terms of pulse labelling time of BrdU and concentration of BrdU used, no significant difference of positive tumor ratio was observed between two methods.

Immunohistochemical Detection of Chromo-
granin in Rat Submandibular Gland

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Chromogranins (CGs) are a family of closely related acidic proteins first detected in secretory granules of adrenal medullary cells. They are co-stored and co-released with catecholamines from storage vesicles, and sympathetic nervous system, but the function of CGs are largely unknown. The autonomic innervation of submandibular gland (SMG) has tempted to study an immunohistochemical detectable CGs and their possible function in the rat. The present study was evaluated for immunohistochemical detection of CGs in SMGs of the rats including postnatal development (1-98 day), anti CGs antibody injected, isoproterenol (IPR) treated and duct ligated rats. From the first to 14th day of the postnatal stage, proacinar cells and/or acinar cells in the SMG indicated a strong positive reaction for CGs, with a highest on 14 day. Subsequently, the stainability for CGs gradually decreased, and the staining was observed with negative reaction on 28th day specimens. The injection of antibody against CGs to the rats, from 7 till 21 day, showed a change in the cytomorphology of SMG cells. In these circumstances, CGs might be correlated with the cell proliferation of acinar cells in rat SMG. Chronic administration of IPR leads to several-fold enlargements of the acinar cell in the rat. It showed positive reaction for CG. The duct ligated SMG, 21st day specimens showed a slight regenerative proliferation of acinar cells, it took place with CG positively. The present study has clearly demonstrated that, CGs may play a vital role for mechanism in acinar cell differentiation.

Reactivity of the monoclonal antibody MRG-1 in the transitional epithelium and transitional cell carcinoma of the human bladder
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The monoclonal antibody MRG-1 (immunogen: human ovarian mucinous adenocarcinoma cell line, RMUG-L) reacts selectively with the type 3 chain of blood group A sphingolipid. As for the squamous cell and squamous cell carcinoma of the uterine cervix, its expression normally seen at the plasma membrane was lost in the cancer cells. We attempted here to examine changes in localization of MRG-1 antigen in the transitional cell and transitional cell carcinoma (TCC) of the bladder. Light microscopic study was performed using paraffin-embedded specimens. The positive staining was observed at the plasma membrane and in the cytoplasm in the normal epithelium. In the superficial TCC, the positive staining of the supranuclear region was observed, while no staining was seen at the plasma membrane. The invasive TCC showed negative staining. Ultracytochemical localization of MRG-1 antigen was revealed by the HRP-labeling method. In the superficial TCC the Golgi apparatus and the plasma membrane was positively stained. The differences of the staining at the plasma membrane between the light microscopic and the electron microscopic studies may be due to the treatment of specimens with organic solvents, since the positive staining at the plasma membrane was lost by the treatment with ethanol or xylene of the semithin frozen sections. This antibody could be useful for the early detection of the invasive tumor in the transitional neoplasms.

Expression of Sialyl-Tn Antigen in Gastric Cancers

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The expression of sialyl-Tn antigen in gastric cancers was examined immunohistochemically. In the non-neoplastic mucosa, STN was detected in almost all cells of the intestinalized tubules. Firstly, using early cancers, we examined the correlation between STN-expression and mucin-histochemical findings of cancers. In the intramucosal carcinomas, STN was expressed in 22 of 33 intestinal-type adenocarcinomas (63.6%), 8 of 23 gastric-type adenocarcinomas (34.8%) and 9 of 19 undifferentiated-type carcinomas (47.4%). In the carcinomas with submucosal invasion, it was respectively 6 of 7 (86.7%), 4 of 5 (80%) and 13 of 13 (100%) carcinomas. Secondly, using early and advanced cancers, we examined the correlation between STN-expression and clinicopathological findings of cancers. The incidence of positive staining for STN was higher as the depth of invasion or clinical stage progressed.

These results indicate that the expression of STN may reflect a kind of intestinalization of tumor cells accompanied with tumor progression in gastric cancers.

Demonstration of ATPase Activities in the Rat Synovial Membrane

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To know the function of the synovial membrane, the localization of ATPase activities were studied ultracytochemically on the rat knee joints. Mg^{2+} -ATPase activity was examined with Wachstein-Meisel's method(1957), Ca^{2+} -ATPase was with Ando's (1981) and Na^{+},K^{+} -ATPase was with Mayahara's(1979). Mg^{2+} -ATPase and Ca^{2+} -ATPase activities were found on the plasma membrane of both A and B cells. Particularly the filopodia of type A cells and the pinocytotic vesicles of type B cells were active portions. Na^{+},K^{+} -ATPase activity was noticed on the filopodia of type A cells, but the Golgi apparatus was positive on the type B cells. Both enzymes were negative on capillaries. These findings may raise the possibility that both type A and B cells might have mechanisms governing the electrolytic metabolism of the synovial fluid, since ATPase is the enzyme which supplies energy needed at active transport or cytosynthesis.

Developmental Change of the Bile Canaliculi in the Fetal and Postnatal Rat Liver:

With Special Reference to Pericanalicular Actin Filaments and Mg^{2+},Ca^{2+} -ATPase Activity

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Developmental change of the bile canaliculi in the Wistar rat liver, fetal 13th~21th days, postnatal and adult rat, was histochemically examined by light and electron microscopy. Pericanalicular actin filaments concerning with the contractile motility of the bile canaliculi and the microvilli formation, were observed by TEM and NBD-phalloidin fluorescence microscopy. Mg^{2+},Ca^{2+} -ATPase activity that is presumed to be involved in the bile acid transport of the bile canalicular membrane was detected by the modified Wachstein and Meisel method (Mg^{2+} 10mM, Ca^{2+} 0.2mM) under light and electron microscopy.

Pericanalicular actin filaments appeared from the 13th day of gestation, sparsely. Since then, pericanalicular actin filaments increased gradually with the formation of bile canalicular network that seemed to be completed by the 7th day after birth.

Mg^{2+},Ca^{2+} -ATPase activity appeared from the fetal 19th day in less than 10% of the bile canaliculi.

Mg^{2+},Ca^{2+} -ATPase-positive bile canaliculi rapidly increased around the birth (fetal 21th day and postnatal 1st day). About half (40-80%) of the bile canaliculi in that period showed positive ATPase activity.

By the 5th day after birth, almost all the bile canaliculi became ATPase positive.

Bile canalicular development seemed to have different course between the morphological maturation and the functional ability such as Mg^{2+},Ca^{2+} -ATPase activity.

Enzyme cytochemical Study on the Trophoblast of the Human Chorion Laeve

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The fetal membrane, which separates the amniotic cavity from the maternal uterine cavity, consists of the following three layers: the amnion, chorion laeve and the decidua. Trophoblast exists in this chorion laeve, but data is very little concerning the function of this cell. Here, the question is provoked whether this cell is a remnant of the placental development and merely serves as a covering of the amniotic cavity, or whether it has an active role in the physiology of the fetal membrane. In this study, we elucidated the subcellular localizations of some enzymes in the human chorion laeve.

Pieces of chorion laeve were fixed in a cold 0.25-2.0% glutaraldehyde. Cryostat sections of 40 μ m thickness were incubated in the following reaction media in order to detect the enzyme activity; lead citrate medium for ALP, Ca^{++} -ATPase and adenylate cyclase (AC), and cerium medium for 5'-N. Strong activity of ALP was demonstrated on the plasma membrane of the trophoblast. Heating at 65°C did not affect the activity, but 10 mM levanisole abolished the activity. As for 5'-N, Ca^{++} -ATPase and AC, the activities were also confined to the plasma membrane of the trophoblast. Prolactin, at a concentration of 10-1,000 ng/ml, activated the AC activity.

These observations strongly suggested that the trophoblast in the chorion laeve was not merely a remnant or a covering, but it was metabolically active and had an important role in the function of the fetal membrane.