The observation of UEA-I binding sites of stomach by adjacent semithin and ultrathin sections.

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On the observation of lectin histochemistry, the different bindings sometimes observed between HRP labeled lectin at light microscopic level and colloidal gold labeled lectin at electron microscopic level. To overcome this problem, adjacent semithin and ultrathin sections were cut and stained with lectin gold conjugate. Rat stomach was fixed with half strength Karnovsky's fixative and embedded in Lowicryl K4M. Pairs of serial semithin and ultrathin adjacent sections were cut. Semithin section mounted on glass slide for light microscopy and ultrathin section mounted on nickel grid for electron microscopy were used. UEA-I lectin was labeled with colloidal gold by usual protein/gold conjugation method. Semithin section was stained with this conjugate and visualized by physical development and ultrathin section was stained with same conjugate. Excellent correspondence was obtained from light and electron microscopies. UEA-I bindings were found on covering mucous cell of superficial layer, mucous cell in foveolae and mucous neck cell. At electron microscopic level, mucous granules of covering mucous cell were strongly stained, and trans side of Golgi lamellae and mucous granules of foveolar mucous cell were also stained. Mucous granules of mucous neck cell were stained and heterogeneous stainability of these granules was found. At light microscopic level, this was also observed as mosaic staining. We conclude that this method is very useful for precise comparison if tissue shows the different results with HRP-labeled lectin at light microscopic level and gold-labeled lectin at electron microscopic level.

Biological characteristics of malignant and benign tumors of human salivary gland
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The neoplasms in the field of otolaryngology have often complicated morphological and biological characteristics, and the prognosis cannot be prospected on the simple basis of morphology. The aim of the present study was to search for more reliable cancer markers in order to know the changed biological behaviors during the course of tumor progression in the salivary gland neoplasms. For this purpose, increased DNA instability common to cancer cell (Fukuda et al., 1986, 1992) was used as the specific marker of malignancy. PCNA, AgNORs or FlnORs (Imamura et al., 1992) were also used for the supporting markers of malignancy. Lectin histochemistry was applied for the same tumors to detect the abnormal gene expressions for the glycoproteins during tumor progression. Ordinary paraffin sections taken from 51 malignant and benign human salivary tumors were hydrolyzed with 2N HCl at 30°C for 20 min for the denaturation of unstable DNA of cancer cell, and successively stained with anti-single-stranded DNA antiserum immunohistochemically. As the result, all cancer cells were positively stained, in sharp contrast to the negative stainability of non-malignant cells. But in poorly differentiated and invasive areas, the stainability of cancer cells was much stronger than that of well differentiated cancer cells, reflecting the increased DNA instability by tumor progression. Even in benign pleomorphic adenoma, some neoplastic cells were positively stained sporadically, indicating the presence of malignant clones, indicating the their presence in early progression phase, although the prognostic value is not clear. PCNA index also tended to be higher in invasive lesion. The number of AgNORs or FlnORs was slightly increased in cancer with marked increase of the volume and bizarre shape.

A dual staining method for neutral carbohydrates and DNA (deoxyribonucleic acid) using periodic acid-thiocarbohydrazide-silver protein-physical development (PA-TCH-SP-PD) and Feulgen (hot HCl-Schiff) techniques.

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A dual staining method was established for the histochemical detection of neutral carbohydrates and DNA in light microscopy. The method consisted of combined periodic acid-thiocarbohydrazide-silver protein-physical development (PA-TCH-SP-PD) and hot hydrochloric acid (hot-HCl)-Schiff (Feulgen) procedures, which produced reaction products of blackish and magenta shades respectively. The results obtained by a series of the control procedures for the present dual staining method could be taken to substantiate its superior properties such as the precision, specificity, reliability and reproducibility. The present dual staining method is believed to be unusually useful for the light microscopic histochemical studies on neutral carbohydrate-containing cells, since it exhibits a high contrast between blackish and magenta shades and reveals distinctly the localization of cytoplasmic neutral carbohydrates and nuclear DNA in one and the same tissue section.

Study of Epstein-Barr virus infected lymphocytes in infectious mononucleosis
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Epstein-Barr virus (EBV) has been known as a B-cell lymphotropic virus and to cause infectious mononucleosis (IM). In this study, four lymph node specimens of IM were examined to determine the phenotypic expression of EBV infected lymphocytes.

The 3µm thick paraffin sections were first stained by immunohistochemistry using alkaline phosphatase (ALP)-labeled avidin method employing primary antibodies of L26 (DACO) as a B-cell marker, CD3 and UCHL-1 (DACO) as T-cell markers. They were colored bright red by the ALP reaction with naphthol-AS-BI phosphoric acid as a substrate and hexazotized new fuchsin as a coupler. Then in situ hybridization was performed using digoxigenin (Dig)-labeled oligonucleotide antisense probe for EBV encoded small RNA (EBER-1). The sections were treated by anti Dig-ALP-conjugated antibody and visualized by 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitroblue tetrazolium salt (NTB) to produce purple-black signals. The EBV positive lymphocytes with phenotypic expression were calculated on microphotographs.

The number of EBV positive lymphocytes of these IM cases was more than 90 per 10³ lymphocytes. The distribution of B/T cells among EBV positive cells were 65/19, 24/66, 17/26 and 12/3 (%). In two cases, EBV positive cells were predominantly B-cells and the others were T-cell predominant. These EBV positive cells ranged small lymphocytes to transformed large ones. In addition to the EBV positive small T-cells, EBV positive large transformed T-cells were also observed. We concluded that EBV infected T-cells may play an important role in the pathogenesis of infectious mononucleosis, and this double staining method is useful for these study.

The demonstration of carbonic anhydrase activities in the hypodermis of molting crayfish

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As the main component of crayfish outerskeleton is calcium carbonate, the carbonic anhydrase activities were supposed to work some part of their metabolism of outerskeleton. The crayfish of each molting stages were obtained by molting hormone injection. The hypodermis was dissected from carapace portion and separated from calcified outerskeleton. After fixation by 0.25 % glutaraldehyde and 2% paraformaldehyde, the 18 µm thick sections were made by cryotome. The histochemical demonstration was done by Hansson's method. To obtain constant reaction products we picked up sections on membrane filter slip (pore size 0.45 µm) which was attached on slide glass, and each process were proceeded on this membrane filter. After reaction, the observations were made by light microscope and electron microscope. Results: The reaction products were observed restrictedly in the aftermolting stage. New outerskeleton and apical part of the hypodermis had strong reaction products. Apical reaction were observed transversely through the epithelium. These portion was defined as intercellular space which work as open vascular system by EM observation. The results suggest that the intercellular enzyme may synthesize the carbonic acid which transported through the hypodermal cell. The enzyme in the outerskeleton may analyze the carbonic acid which secrete from the hypodermis.

Expression of CA19-9, SLX, and STN antigens in glandular dysplasia and related lesions.

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Forty cases of glandular abnormalities of the uterine cervix were identified histologically by our department from January, 1984 to April, 1992. These included 17 cases of endocervical glandular dysplasia (EGD), 6 of adenocarcinoma in situ (AIS), 5 of microinvasive adenocarcinoma (MI), and 12 of invasive adenocarcinoma (AD). Immunohistochemical localizations of CA19-9, SLX, and STN antigens were examined in these specimens by the streptavidin-biotin method.

CA19-9 staining was often observed on the apical portion of AD. SLX staining was characteristically found on the perinuclear portion in EGD but not in other lesions. The localizations of STN were often seen on the cytoplasmic portion of each lesion.

The CA19-9, SLX, and STN antigens were found in EGD. However, they were present in AIS, MI, and AD with increasing frequency, with AD having the highest level.

We noted that, initially, glycosynthesis was just beginning and that aberrant glycosylations were accumulating in EGD.

EGD, therefore, may involve pre-cancerous lesions.

Immunohistochemical and Biochemical Characterization of Radical and Lipid Peroxides Scavenging Systems in LEC Rat Livers.

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Long-Evans Cinnamon (LEC) Rats are prone to develop fatty liver, hereditary hepatitis and hepatocellular carcinoma, and it is explained that abnormal intercellular retention of copper in the hepatocytes could be the pathogenesis of these changes. Like as other metal ions, e.g. ferric ion, copper ion (Cu⁺⁺) stimulates to generate oxygen radicals and lipid peroxidation in cells.

The present study was thus designed to investigate the significance of those radicals and lipid peroxidation (LPO) as the more direct pathogenesis of the hepatocytic abnormalities mentioned above. For this purpose, radicals and LPO scavenging enzymes, such as Mn- and Cu-Zn-superoxide dismutase (SOD) and glutathione peroxidase (GSH-PO) in the livers of LEC rats (9, 19, 35, 75 weeks of age) were biochemically and immunohistochemically examined, and they were compared with those of their control rats (LEA, Long-Evans Agouti).

As results, GSH-PO (effective LPO scavenger) was gradually but consistently decreased in LEC rats livers up to 19th week. Cu-Zn-SOD was also gradually decreased, but no significant changes were recognized between LEA and LEC. It is of interest that Mn-SOD was meaningfully increased in the hepatocytes of LEC rats. Since Mn-SOD is solely localized in mitochondria, H₂O₂ (the reaction products of Mn-SOD), may be accumulated in them, because GSH-PO, the specific scavenger for the mitochondrial peroxides, is markedly diminished in those LEC rats of 19th week. The intramitochondrially accumulated H₂O₂ may generate oxygen radicals and lipid peroxides which would cause in mitochondrial dysfunction and subsequent serious cell injuries.

Detection of nRNA for GFAP gene in mouse liver, and purification of RNA-dependent RNA polymerase for the nRNA

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Fukuda et al. (1992) recently discovered a novel nuclear RNA which plays a fundamental role as a gene repressor in the process of ontogeny and cytodifferentiation. This type of nuclear RNA (nRNA) is localized in the nucleus in hybridization with nuclear DNA having a life span as long as that of the cell, duplicated in the late phase of DNA replication localized in the G-bands of metaphase chromosomes. The synthesis of new nRNA should be accomplished by using the pre-existing nRNA as the template, and RNA-dependent RNA polymerase should be necessary for all proliferating cells to make inheritance of irreversible gene repression possible. In the present study, nRNA suppressing the transcription of GFAP gene in mouse liver was detected by Northern blot hybridization, and RNA-dependent RNA polymerase was purified from the fetal mouse. The total nucleic acids were extracted from mouse liver. After digestion with DNase I, Northern blot hybridization was performed using the cDNA labeled with P-32 for mouse GFAP (Glial fibrillary acidic protein) messenger RNA. Specific autoradiographic signal was detected showing the presence of nRNA for suppressing the transcription of GFAP gene in mouse liver, because the gene is only active in glial cells in the brain. RNA-dependent RNA polymerase was extracted and purified by columns and the activity was assayed by the system containing NTP, DTT, 3H-UTP, RNase inhibitor, primer, and rabbit globin mRNA as the template. The activity of the RNA-dependent RNA polymerase was demonstrated by scintillation counter determination, showing the existence of new RNA polymerase in the proliferating fetal tissue.

Heterogeneous Distribution of Glucose Transporter in Mouse Brain Microvessels

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We have previously investigated the glucose transport in mouse brain by using a fluorescent glucose analogue, NBD-glucosamine (NBDG) and a confocal laser scanning microscope (CLSM). At 0.5 min after i.v. injection of NBDG, the fluorescent intensity was exclusively and heterogeneously distributed in the brain microvessels.

In this report, the distribution of glucose transporter in the mouse brain was examined with an immunogold method using polyclonal anti-glucose transporter antibody. The cryosections (30 μ m thick) were prepared from the mouse brain perfused with 4% paraformaldehyde. The sections were incubated with primary antibody and secondary gold-conjugated antibody. After enhancement with silver, and staining with neutral red, the sections were observed by the CLSM. The results show that glucose transporter is heterogeneously localized in the microvessels.

The previous and present data indicate that the transport of glucose from the microvessels to brain parenchyma occurs through glucose transporter heterogeneously distributed on the microvessels.

The effects of 12-O-tetradecanoylphorbol-13-acetate (TPA) on the bladder epithelium. The duration of TPA-treatment and the proliferation of basal cells.

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By injecting the TPA into a rat urinary bladder, the superficial cells of the epithelium were changed their morphology and were desquamated from the epithelium selectively and completely⁽¹⁾. Following this desquamation, the epithelium was regenerated by proliferation of basal cells. In this study, to confirm the relation between the duration of TPA treatment and basal cell proliferation, injected TPA was excreted artificially from the bladder at 10, 30 and 60 min after TPA injection, and then sterile saline was instilled into the bladder to wash the remaining TPA. Each Rat was injected BrdU (i.p.) 18 hours after TPA treatment and fixed at 24 hours after TPA treatment.

The BrdU positive cells were detected in all rats tested and were specific for the basal cell layer. By electron microscopy, the luminal surface of the superficial cells at 10 min after TPA injection was irregularly foliated and few large spherical vacuoles were observed in the cytoplasm of the superficial cells. These morphological responses to TPA were principally the same as that observed in the previous studies. These results indicate that the TPA affects on bladder epithelium in a very short time periods.

(1) Watanabe, S and Sasaki, J: Cell Tiss. Res. (1992) 268 239-245.

Effect of salt-loading on tyrosine hydroxylase- and peptide-immunoreactivities in the hypothalamic supraoptic and paraventricular nuclei

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Salt-loading induces profound metabolic changes in magnocellular vasopressin containing neurons in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus. Previously, it was found that salt-loading induces the increase of messenger RNAs encoding vasopressin (AVP) and their coexisted bioactive substances such as galanin (GAL) and tyrosine hydroxylase (TH) in these neurons by in situ hybridization histochemistry. In the present study, we investigated the salt-loading-induced change of immunoreactivities of AVP, GAL, and TH by immunocytochemistry using specific antisera against these substances in the SON and PVN without any drug pretreatment. Adult male Wistar rats were given food and water ad libitum. Salt-loading was performed for 2, 4 or 7 days in 2% NaCl to drink. For control, rats were allowed to freely be accessed to water ad libitum. The TH-immunoreactive neurons appeared at 2nd day of salt-loading, and gradually increased its number or immunoreactive intensity at 4th and 7th day. On the contrary, AVP- or GAL-immunoreactivity decreased at 2nd day salt-loading, more severely being depleted at 4th and 7th days. The immunoreactivities of these substances in PVN showed similar changes as those found in SON. The result suggests that TH and its coexisted peptides change in similar time course in magnocellular neurons in the salt-loaded condition.

Immunohistochemical study on steroid-synthesizing enzymes in mammalian brain

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To elucidate the steroid-synthesis and -metabolism in the mammalian brain, cytochrome P450 enzymes were immunohistochemically studied using the brain of male adult Wistar rats. The steroid-synthesizing P450 enzymes examined in this study, were cholesterol side-chain cleavage (SCC), 17 α -hydroxylase/lyase (17 α), C21-hydroxylase (C21), 11 β -hydroxylase (11 β) and aromatase (AROM). The primary antibodies to SCC, 17 α , C21 and 11 β (J Endocrinol, 119:191, 1988) were provided from Dr. S. Kominami and Dr. S. Takemori, Hiroshima University; AROM-antibody (Endocrinology, 124:1417, 1989) was supplied from Dr. J. Kitawaki, Kyoto Pref. University of Medicine. Vectastain ABC-peroxidase system was applied to the histochemical detection. The immunoreactivities were observed in the neurons of various region of the rat brain. The existence of "neurosteroids" were strongly suggested.

Attempt to detect cancer cells in head and neck tumors by immunohistological staining using antikeratin antibody and flow cytometry (FCM)

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Recently, quantification of DNA of the cell has been simplified and accelerated with the use of FCM, a technique with hold strong potential for the diagnosis of the degree of cancer malignancy. In head and neck tumors, cells other than cancer cells are often contain in the tissue, thus making the analysis of the DNA histogram difficult. In the present study, epithelial cells were stained selectively with use of antikeratin antibody and attempt was made to obtain a selective DNA histogram of epithelial cells from tumor tissue. **[Methods]** KB cells and A single-cell suspension prepared from freshly resected specimen was reacted with antikeratin antibody and labeled with FITC.

This suspension was then double stained with propidium iodide and quantified with FACS tar. **[Results]** ① With the use of antikeratin antibody, KB cells and epithelial cells (cancer cells) were stained selectively. ② FCM allowed selective gating of keratin-positive cells, and a DNA histogram with increased accuracy was obtained. **[Summary]** The method discussed in the present study was capable of detecting epithelial cells (cancer cells) selectively and was useful in exploring cell kinetics.

Importance of acidic mucin secretions by foveolar and mucous neck cells of rat fundic mucosa as the defence mechanisms against HCl as revealed by fasting

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The localization of neutral mucin and acidic mucins in both control and fasted rat gastric fundic mucosa were examined by microscopic and electron microscopic histochemical methods. By Carnoy's fixation, the surface mucous coat of the control rat gastric mucosa was found to be composed of alternating layers of acidic mucins and neutral mucin, indicating the synchronous and cyclic secretions of them. In many gastric pits, the acidic mucins were found to spring out from the deep foveolar regions like volcanoes. This phenomenon may suggest that the acidic mucins play a fundamental role in protecting the pit cells against HCl during its passage, and the layers of neutral mucin and acidic mucins in the surface coat is the safeguard against the HCl and digestive enzymes in the gastric lumen.

In the fasting rat gastric mucosa, the acidity and the amount of the gastric juice were markedly decreased, indicating the suppressed secretions of mucins and HCl. The decreased production of sulfomucin was directly demonstrated by ³⁵S₃₅-auto-radiography. Many mucous neck cells were ballooned due to accumulation of alcian blue-positive but high iron-diamine-negative sialomucin, which was not demonstrable in the control.

In the control rat fundic mucosa, acidic mucins were demonstrable microscopically and ultra-structurally only in the deep foveolar cells, but because of the retarded mucin secretion, they became visible even in the middle and surface foveolar cells in the fasting rat.

It was shown that the acidic mucins are an important barrier for the pit cells against HCl.

Immunocytochemical localization of PTHLP in breast cancers
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The presence of PTHLP (Parathyroid Hormone-Like Protein) in breast cancers was assessed by immunohistochemistry using polyclonal antiserum (Oncogene Science) and indirect immunoperoxidase technique.

The primary tumors from 33 patients were 8 papillo-tubular carcinomas, 10 solid tubular carcinomas, 9 scirrhous carcinomas, 2 invasive lobular carcinomas, 3 medullary carcinomas and 1 mucinous carcinoma.

All 33 tumors exhibited positive staining in the tumor cytoplasm and stromal fibroblasts were also stained with anti-PTHLP antiserum.

Immuno-electron microscopic localization of PTHLP in breast carcinoma cells was examined using pre-embedding method and PTHLP was localized in the dilated cisterna of endoplasmic reticulum.

Detection of IL-6 and IL-6r in MRL Mice by ISH at EM Level

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We developed *in situ* hybridization (ISH) system at electron microscopical (EM) level, using bromodeoxyuridine (BrdU)-labeled DNA probes. This method was used to detect quantity and location of interleukine-6 (IL-6) and its receptor (IL-6r) expression in organs of autoimmune disease MRL/lpr mice. Eight µg endotoxin (ET) was injected to 20 weeks age male mice and killed them after 15 minutes and 60 minutes. Other same age and sex MRL/lpr and ddY mice were used as control groups. After Zamboni fixation, OCT compound frozen section of the kidneys, lungs, liver, spleen and lymph nodes were obtained. BrdU labeled IL-6 and IL-6r cDNA probes (the gift from Dr. Hirano, Osaka University) were used for ISH post-embedded immunogold colloids staining and EM observation was performed. At 15 min., after ET injection, IL-6 mRNA signals were found mainly in nucleus of mesangial cells. At 60 min., signals were found both in nucleus and cytoplasm. IL-6r mRNA signals, at both 15 and 60 min., were found in nucleus and cytoplasm. Laser Confocal Imaging System at only 60 min. specimens were detected positive staining in glomerulus, liver, lung, spleen, and lymph nodes. Northern Blotting, also gave IL-6 mRNA band only in ET-injected 60 min. specimens. Anti-mouse IL-6 monoclonal antibody was used for ABC staining, but all of the specimens were not be found any IL-6 protein. Our study is showed that ISH at EM method was highly sensitive. IL-6 is a inflammation related cytokine, it only appears by stimulation factor.

Localization of gamma-glutamyl transpeptidase in the Paneth cell in small intestine

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Distribution of gamma-glutamyl transpeptidase (GGT) in the crypt of mucous epithelium of small intestine was examined by an immunohistochemical technique using a monoclonal antibody. Small blocks of human duodenum and jejunum, obtained from the surgical operations, were fixed with PLP fixative. Paraffin sections were prepared and immunostained for GGT.

The immunoreaction was observed along the brush border of small intestine. The reaction decreased toward the crypts of intestinal glands. Nevertheless, intense reaction was observed on the luminal surface of Paneth cells as well as in the secretory granules located at the apical portion of the cells.

GGT was demonstrated in kidney, liver, prostate, epididymis, pancreas, sublingual gland and in apocrine as well as eccrine sweat glands, by immunohistochemical method. Among the organs which undergo secretion, the reaction was recognized in the luminal surface of the cells and in the secretory granules in the apical cytoplasm of secretory cells of apocrine sweat gland. The reaction pattern of Paneth cell is quite similar to that of the cells of apocrine sweat gland, though the Paneth cell is not known as apocrine-type secreting cells. GGT probably take part in the secretion of the product of Paneth cell, but, the elucidation of the function of GGT in Paneth cell requires further investigation.

3-D Observation of Bile Canaliculi "Varicoid Changes" in Rat Livers with Ischemia-reperfusion Injury

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By using a confocal laser scanning microscope, we found out characteristic changes of bile canaliculi, named "varicoid changes" in the rat livers with ischemia-reperfusion injury. In a reperfusion group, left hepatic lobes were clamped temporarily and sampled 12 hours after declamping. Liver tissues were also taken after 12 hours-clamping in a ischemia group and after sham operations in a control group. Each liver section obtained was stained with FITC-phalloidin after fixation. Serial confocal images and stereo images showed that the bile canaliculi had rather uniform diameters in the control. In the reperfusion group, multiple diverticula appeared in the pericanalicular regions throughout the livers with the actin filaments increased in intensity, similar to phalloidin-treated livers; the deformed canaliculi were dilated according to the periods of ischemia. On the contrary, no "varicoid changes" occurred in the ischemia group. It is concluded that the "varicoid change" of the bile canaliculi is one of the initial signs of the ischemia-reperfusion injury in the liver and that unbalanced actin polymerization-depolymerization cycle may result in such a characteristic change.

Detection of BGP and collagen type I gene expression in bone tissue of hypophysectomized rat - Application of in situ hybridization technique with non-radiolabeled probes -

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In this study, we have developed an in situ hybridization method utilizing non-radiolabeled RNA probe. Using this technique, we attempted to demonstrate bone Gla protein (BGP) and type I collagen m-RNA expression in femurs from both normal and hypophysectomized rats.

The operation of hypophysectomy was performed at 4 weeks of age, and 8-week-old operated and age-matched normal rats were used. The femurs from both experimental groups were fixed with 4% paraformaldehyde in 0.2 M phosphate buffer for 6 hours. They were then decalcified with 0.2 M EDTA for 3 weeks at 4 °C. The tissue blocks were washed with PBS and then dehydrated through a graded series of ethanol, and infiltrated in paraffin wax. In situ hybridization was performed with DIG labeled probes.

In normal rats, BGP and type I collagen m-RNA were expressed mainly in osteoblasts lining along the cortical and trabecular bone surface. In hypophysectomized rats, on the other hand, the intensity of the m-RNA expression in the osteoblasts of trabecular bone was dramatically decreased, but not in cortical bone.

It was suggested that matrix producing property of osteoblasts of the trabecular bone were depressed by hypophysectomy. The results indicated that in situ hybridization technique, using non-radiolabeled probes, is feasible for application into bone tissue research.

Double staining by combination of enzyme cytochemical staining with immunocytochemical labeling at electron microscopic level

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Protein A-gold technique is widely used for detection of multiple antigens in the same thin sections. However, no all antibodies against antigens we want are always available. It would be very convenient, if we could cytochemically stain the antigen in such case. In this paper, we investigated the application of protein A-gold technique to the thin sections stained previously by enzyme cytochemical methods. Rat liver was fixed by perfusion with 4% paraformaldehyde + 0.25% glutaraldehyde + 0.15M cacodylate buffer (pH 7.4) for 5 min. Sections (80 µm or 200 µm thick) were cut with a Vibratome and divided into two groups. The first group (200 µm) was dehydrated and directly embedded in LRWhite. The second group (80 µm) was incubated in cerium medium for acid phosphatase (ACPase) activity for 1 h at 37°C and were dehydrated and embedded in LRWhite. Polymerization of the resin was performed at -20°C under UV light for 24 h. Thin sections were stained by the protein A-gold technique using polyclonal antibodies against cathepsins B, D, and H. ACPase activity was found in lysosomes in hepatocytes and sinus-lining cells. Gold particles showing cathepsins were present exclusively in lysosomal compartments in the hepatocytes and the sinus-lining cells with quite varied labeling intensity. When the protein A-gold technique was applied to the thin sections from ACPase-stained tissue, three kinds of lysosomes were noted; 1) positive for both ACPase and cathepsins, 2) positive for ACPase only, and 3) positive for cathepsins only. The results show that the protein A-gold technique is able to be applied to the sections stained previously by cerium method and the distinguishable heterogeneity exists among the lysosomal compartments of the liver cells. This work is supported in part by grand-in-aid (No. 03833013) from the Japanese Ministry of Education and Culture.

Proliferation of myocardial peroxisomes in experimental rat diabetes: a biochemical and immunocytochemical study

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In a previous study, we have shown that catalase and acyl-CoA oxidase activities are increased in the diabetic rat heart. Since these enzymes are contained in peroxisomes, it would be expected that the increased enzyme activities would be associated with proliferation of myocardial peroxisomes. The purpose of this study was to examine this suggestion by analyzing normal and diabetic rat myocardium using biochemical and immunocytochemical methods. Sprague Dawley rats aged 6 weeks were administered with 65 mg/kg of streptozotocin to induce experimental diabetes and were divided into two groups. The hearts of the first group were processed to the biochemical analyses including enzyme assay and immuno-blotting and those of the second group were used for the morphological analyses including alkaline DAB and immunocytochemical staining. Catalase and acyl-CoA oxidase activities were increased in the diabetic rat heart and immunoblot analysis showed that both enzyme proteins were markedly enhanced in diabetic heart homogenates. After immunoenzyme staining, catalase and acyl-CoA oxidase were localized in fine granules in the myocardium which were increased in number in diabetic rats. The numerical density of the granules stained for catalase was increased 1.7 times and that for acyl-CoA oxidase was 1.8 times, compared with controls. Protein A-gold labeling for catalase and acyl-CoA oxidase was present in myocardial peroxisomes. The labeling density for both enzymes was increased in diabetic rats by 1.6 times for catalase and 1.5 times for acyl-CoA oxidase, compared with controls. The results indicate that myocardial peroxisomes are increased in the diabetic rat and that this proliferation is accompanied by an increase in catalase and acyl-CoA oxidase activities.

Protection of mitochondria by Rikkunshi-to (TJ-43) from adriamycin cytotoxicity in rat parietal cells

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An anticancer drug, adriamycin (ADM) is known to have cytotoxic effects in several tissues caused by formation of hydrogen peroxide or hydroxyl radicals which cause peroxidation of the mitochondrial membrane lipids. We tried to reveal the toxicity of ADM on mitochondria by cytochemical means in rat parietal cells and examined the prophylactic effect of the traditional Chinese drug, Rikkunshi-to (T-43, Tsumura) against ADM-induced toxicity in rat gastric mucosa.

By single i.p. injection of ADM (0.5-1.0 mg/100 mg) to the rats, the parietal cells showed disintegration of the tubulo-vesicular system 20-22 hrs after the injection, but no apparent ultrastructural changes were observed in mitochondria except for high density of the outer membrane of some mitochondria. These morphological changes were not obvious in the rats pretreated by T-43 (100 mg/100 g, orally administered daily for 6 days). From the histochemical activities of a mitochondrial enzyme, cytochrome C oxidase, which was mainly observed in parietal cells in gastric mucosa, the lowest enzyme activity was observed in rats treated only with 1.0 mg/100 g of ADM and the highest in the normal rats. The activity of the rats pretreated by TJ-43 was apparently higher than the untreated group and the rat with injection of ADM (0.5 mg/100 g) showed almost the same intensity as the normal rats. This decrease of activity was recognized in EM observation as an absence or decrease of the reaction products in most mitochondria of parietal cells (especially in intermembrane space) of ADM-treated group without pretreatment by TJ-43.

From these results, we conclude that TJ-43 pretreatment prevents ADM-induced cytotoxicity in rat parietal cells.

Immunocytochemical Localization of bFGF (basic Fibroblast Growth Factor) and Cell Proliferation in Immature Rat Ovary Treated with Gonadotropins
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In the ovary, cell proliferation and neovascularization are correlated with follicular development, ovulation, and formation of corpora lutea. It is well known that bFGF is related to the vascular proliferation. In the present study, immunolocalization of bFGF and its relation to the cell proliferation are studied in immature (3-week-old) rat ovaries treated with pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG). Rats were sacrificed 12, 24, 36, 48 hours after PMSG treatment, and 3, 6, 9, 12, 24 hours after the injection of hCG. Immature rats were given the injection of hCG 48 hours after the PMSG treatment. Ovulation could occur about 12 hours after the injection of hCG. BrdU (Bromodeoxyuridin) (200mg/kg) were injected about 30 minutes before fixation.

(1) Some flattened cells near the capillary around the growing follicles were positively stained with anti-bFGF antibody. Some granulosa cells of the growing follicles and endothelial cells of the capillary of these follicles were positive against anti-BrdU antibody.

(2) On the follicle growing, some restricted preovulatory Graafian follicles were surrounded by flattened cells positively stained for bFGF, but some follicles were not.

(3) Around the ovulation, some cells which were positively stained with anti-bFGF antibody were intermingled with the granulosa cells in the large preovulatory follicles.

(4) After the ovulation, few cells were positively reacted against bFGF in the newly formed corpora lutea.

(5) Basic FGF may correlate with the proliferation of endothelial cells of capillary with follicular development and ovulation.

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2. Tokuyasu, K. T.: Immuno-cryoultramicrotomy. In "Immunolabelling for Electron Microscopy", ed. by J. M. Polak and I. M. Varndell, Elsevier Science Publishers B. V., Amsterdam, 1985, pp. 71-82.
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