Chelate Staining(XXXVI) Histochemical Staining of Metals in the Mayfly Larvae (Baetis spp.) That Inhabited in a River Polluted with Heavy Metals

Yawara SUMI, Noriko H. MASUDA, Masayuki K. HARA, Takuro SUZUKI, *Shigehisa HATAKEYAMA and *Kazuo T. SUZUKI
Dept. of Anatomy, St. Marianna Univ. Sch. of Med., Kawasaki, *Natl. Inst. for Environ. Stud., Jpn., Ibaraki

The larva of the mayfly is known to be one of the species most tolerant to heavy metals. We histochemically examined the tissue distribution of metals taken up by the larvae that inhabited in the river Mazawa(Yamagata) contaminated with heavy metals such as copper(Cu), zinc(Zn), cadmium(Cd) and iron(Fe).

The concentrations of Cu, Zn, Cd and Fe in whole body homogenates of the larvae were 174, 1624, 17 and 348 μg/(g wet weight), respectively. The larvae of mayflies collected in the river were fixed in formalin or acetone. In the sections stained by dimethylaminobenzylidenerohdanin, high accumulation of Cu were observed in epithelial cells of the digestive tract. Unexpectedly, Zn was so lightly stained by dithizone with the formalin-fixed sections although Zn indicated maximum concentration in the homogenates among the four metals. In contrast, with acetone-fixed sections, dithizone stained with high optical density Zn in epithelial cells in the tract.

The results obtained suggest that Zn probably presents in the form of its salt or is weakly bound to some biological constituents in the larvae.

Immunocytochemical Studies on Association of Fibrinogen with Platelet during Aggregation

H. Suzuki[1), 3)], R.L. Kinlough-Rathbone[2)], M.A. Packham[3), 1)], J.F. Mustard[2)], K. Tanoue[1)] and H. Yamazaki[1)]

Dept.of Cardiovascular Res.,The Tokyo Metropolitan Inst. Med. Sci.[1)], Dept. of Pathol., McMaster Univ.[2)] and Dept. of Biochem., Toronto Univ.[3)]

Platelet aggregation is believed to be dependent upon the availability of fibrinogen (Fbg). The association of Fbg with washed human platelets was studied during ADP- and thrombin-induced aggregations. Platelets were fixed, embedded in Lowicryl K4M, sectioned, incubated with goat anti-human Fbg, washed, reacted with gold-labelled rabbit anti-goat IgG and prepared for electron microscopy. Resting platelets did not have gold particles on their surface although there was extensive gold labelling on the α-granules. In the presence of added Fbg, ADP caused extensive platelet aggregation and release reaction, and gold particles were evident between the adherent platelets and on the surface. Thus, we demonstrated that Fbg was essential for ADP-induced aggregation by the immunocytochemical method (Blood, in press). Thrombin caused similar aggregation and release reaction even in the absence of added Fbg. However, gold particles were seen between the adherent platelets only in a few regions. Therefore, it is concluded that thrombin aggregates platelets without extensive binding of released Fbg to the platelets.

**An Immunohistochemical Study of the Basement Membrane of the Rat Esophagus in Association with Malignancy**

**N. Suzuki, K. Nabeya, S. Oguchi, Y. Kobayashi, H. Kawakami & H. Hirano.**
Departments of Surgery and Anatomy, Kyorin University School of Medicine, Mitaka, Tokyo 181

Changes in the basement membrane (BM) of the rat esophageal epithelium during carcinogenesis were studied by light and electron microscopy using immunohistochemical staining techniques. Male Wistar rats were fed with carcinogens (0.25% N-methylbenzylamine, 0.16% $NaNO_2$) for 3 months. The middle portion of the esophagus was removed out and fixed with 4% formaldehyde. Frozen sections (7 and 40 μm thick) were made, incubated with the rabbit antiserum against laminin (mouse EHS sarcoma), then HRP-labeled goat anti-rabbit IgG.

In the normal esophagus, the binding of the anti-laminin antibody was observed linearly along the BM beneath the epithelium under the light microscope. By electron microscopy, reaction product representing anti-laminin antibody binding sites was detected continuously and diffusely along the BM.

In the epithelium of carcinogen-induced hyperplasia and cancer, the BM became wavy in profile. The anti-laminin antibody labeling appeared to increase in intensity along the BM both light and electron microscopically.

**Butyrylcholinesterase-containing Processes and Cell Bodies in Rat Brain as Visualized by a New Method**

Hisao TAGO, Yoshihiko NUMATA, Hisashi KUMASHIRO, Toshihiro MAEDA* and Hiroshi KIMURA*

Dept Neuropsychi, Fukushima Med Col, Fukushima 960
*Dept Anatomy, Shiga Univ Med Sci, Otsu 520-21

A new histochemical method for butyrylcholinesterase (BChE) was undertaken in rat brain. The method has enabled us to detect clearly BChE-containing fibers and cell bodies of neurons and glias in the CNS. Male Wistar rats weighing about 200 g were fixed by perfusion (2% paraformaldehyde + 2% glutaraldehyde), and then by immersion (4% paraformaldehyde). Cryostat sections (20 μm) of the brain were pretreated with 0.1 mM of BW284c51 or iso-OMPA to inhibit acetyl-ChE or BChE, respectively. After wash, the sections were incubated at 37 °C first for 30 min in Karnovsky and Roots medium (BCh as substrate) diluted 1:100 with 0.1 M maleate buffer and then for 5 min in a mixture of DAB and $H_2O_2$. No specific staining was observed in sections treated with iso-OMPA, or reacted in the first medium but omitting the substrate.

The technique is much more sensitive than previous methods, particularly for neural structures. In addition to positive glias, some vascular endothelia were also stained with a moderate intensity.