procedure. First, the exponentially growing cells were washed with an inorganic salt solution SMB II (Miyake & Beyer, 1973). These cells were, then, seeded onto a PY agar plate medium (0.9% agar) containing antibiotics and were incubated at 23C. At 7 days after the start of incubation, pure colonies of the ciliate were yielded on the agar plate. Finally, each of these colonies was transferred to different containers that held 2ml of PY liquid medium containing antibiotics. However, almost of cells broke down at the late stationary phase of growth in the axenic culture. In order to determine whether the cause of this phenomenon was due to the unsuitable concentration of the PY medium, we examined the growth of the ciliate in various concentrations of media. However, the cells also broke down in any PY concentration at the late stationary phase of growth. These phenomena, therefore, imply that such a cytolysis might be induced by some accumulated substances in the medium during cultivation.

EXISTENCE OF SIALOMUCIN IN THE EXTRACELLULAR CYST IN THE THYMUS OF JUVENILE MICE

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The extracellular cyst which exists at the cortico-medullar region in the thymus of many vertebrates might be a graveyard for T cells undergoing death.With a lectin, we observed whether or not mucous cells in the epithelium of the extracellular cyst secreted the sialomucin.Animals used were, 2 days of age, male mice of the IVCS strain.The thymus was fixed with glutaraldehyde, embedded in LRwhite, stained with 10nm gold colloid conjugated lectin (LPA), re-stained with uranyl acetate and lead citrate and viewed with an electron microscopy.Sialic acid existed in the secretory granules in the mucous cells and also in the content of the cyst. These findings indicated that the mucin secreted by the mucous cells had a high viscocity and might play a role for catching negative selected T cells which might move ino the cyst.

SUBCELLUAR LOCALIZATION OF NEUROTROPHIN RECEPTOR IN CULTURED SLICES OF MOUSE CEREBELLUM

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It has been well demonstrated that the binding of neurotrophins to their relevant Trk receptors plays a siginificant role in regulation of survival, proliferation and differentiation of neurons.To date, however, there is little information on the subcelluar localization of these neurotrophin receptors in the brain. The expression and localization of p75, TrkA, TrkB in cultured slices of mouse cerebellum were examined by Western blot and immunohistochemistry. In the organotypic slices, the immunoreactivity of p75 was found mainly in Purkinje cells, and the immunostainig of TrkA and TrkB was intense in Purkinje cells and granule cells. At the electron microscopic level, we found that TrkB localized in the plasma membrane, small vesicles in the cytoplasm and dendritic processes of Pukinje cells.

THE MUCOUS CELLS OF THE ADHESIVE ORGAN OF THE TERRESTRIAL PLANARIAN *BIPALIUM SP.*

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Histological observations were examined on the adhesive organ of the terrestrial planarian, *Bipalium sp.* We already reported that the epidermal mucous cells of this animal were classified into six types with electron microscopy. In present study, we investigated the mucous cells with scanning and transmission electron microscopy. The organ was composed of the tile shaped papillae and observed comb like arrangement on the margin of the head. The component cells of the papillae-surface were observed eosinophilic. Small pore, pit which was covered with cilia, existed among papillae. Two types of mucous cells were observed on the surface of the papillae. One was lattice granular cells and the other was electron dens granular cells. The result suggests that the two types of mucous granules play an important role of taking up the live baits.

CHANGE OF BROWN ADIPOSE CELLS OF LAND LEECHES, *HAEMADIPSA ZEYLANICA JAPONICA* WITH TEMPERATURE IN SUMMER

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Brown adipose cells were swollen eggs shaped in the winter and summer. They included large oil droplets and mitochondria. We studied again how its form appears in summer during 1999 to 2003 a University Forest in Chiba. The monthly average temperature in late May was 18.1, at which time the form was spindle shaped. The temperature in late June was 21.3, at which time the form showed the shape of swollen eggs. Also, in late July it averaged 23.9, at which time the shape of swollen eggs spread throughout the internal area. The results indicate that these cells adapt to change in temperature.

FIBROMUSCULAR LAYER IN THE RESPIRATORY TRACT OF RED-BELLIED NEWT (*CYNOPS PYRRHOGASTER*)

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Serial sections of the respiratory tract of red-bellied newt (*Cynops pyrrhogaster*) were made, and the relationship between the density (cells/$10^6\mu m^3$) of serotonin-immunoreactive neuroepithelial endocrine (NEE) cells and fibromuscular layer was studied. The respiratory tract was divided into five laryngotracheal portions (LT1-LT5) and one pulmonary portion (P). Then, each portion was further subdivided into ventral (V), dorsal (D) and lateral (L) surfaces. The density of serotonin-immunoreactive NEE cells was remarkably high in LT4-L and LT3-L subdivisions. In those portions, smooth muscle and many capillaries were observed in fibromuscular layer between the lateral cartilages and epithelium.

CULTURE OF LANCELET CELLS DERIVED FROM GONADS

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To establish lancelet (amphioxus) assay systems for analyzing gene function and control of gene activation under background of this animal, we are culturing cells from lancelet branchial epithelium, gut epithelium, nerve cord, and gonads. Of these, cells from gonads spread well and proliferated in a medium consisting of Millipore filtered seawater 77%, HEPES 10 mM, D-MEM 16%, calf serum 5%, and L-glutamine supplemented with 4% (v/v) B27. GFP-expressing vector was successfully introduced into cells from gonads with an electroporator changing cell density or DNA concentration, and/or voltage/duration of pulse, but at very low rate.

EFFECTS OF LYSENIN ON CULTURED CHROMATOPHORES OF TELEOST FISH

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Lysenin binds sphingomyelin (SM) specifically. It has lethal effects on various cultured cells of mammals excepting mouse melanoma cells (B16F10). In the present study, we examined the effects of lysenin on normal pigment cells cultured from swordtail, Nile tilapia, zebrafish, sumatra and topmouth minnow. Several min after the beginning of the treatment with lysenin (100 ng/ml), saltatory movements of pigment granules began at the tips of dendritic processes and the peripheral regions of the cell. The saltatory movements then spresded all over the cytoplasm of pigment cells. Tilapia melanophores, and melanophores, xanthophores and erythrophores of swordtail changed to a sphere in form or were cut into some small spheres, although the outlines of cultured pigment cells of other species were almost maintained. Immunofluorescence study using swordtail melanophores showed that radial arrays of microtubules within cells disappeared by the treatment with lysenin. Any lysenin-treated cells did not respond to norepinephrine, suggesting lethal effects of lysenin. Lysenin (100 ng/ml) that had been incubated beforehand with SM-liposomes (0.03 mM) for 10 min had no effects on pigment cells.

SUBCELLULAR LOCALIZATION OF INNEXIN, GAP JUNCTION RELATED PROTEIN IN INSECT CELLS

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Innexin, gap junction related protein in insect, is thought that it isconcerned with intercellular direct communication. But, the cell biologicalanalysis has not been performed so far. To date, we have showed that gapjunction is formed and innexin is expressed in cultured insect cells, usingby microinjection of Lucifer yellow and immunocytochemistry of anti-innexinantibody. In this study, we will report localization of innexin in detail byimmunoelectron microscopy, as well as the changes of amount of innexinexpression under the various culture conditions by western blotting.