cosmus polymorphus[9], and the histoenzymological assays in toto have shown a chymotrpysin-like protease in the endostyle of *Ciona intestinalis* and *Phallusia mammillata*[16].
In the endostyle of the ammocoetes larvae of *Petromyzon marinus* a cathepsin-like protease, active at pH 4, was determined by biochemical assays[17].
On the basis of the above results the endostyle of *S. plicata* produces a trypsin-like enzyme, but does not show the presence of a chymotrypsin-like enzyme. This protease is likely to be mixed with the mucus secreted by the endostyle. As a result, the food particles entrapped by the mucous film begin to be digested in the pharyngeal tract, a process which appears to be rather common in the chordates.

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Cellular calcium binding state change during pentylenetetrazole-induced bursting activity in snail neurons

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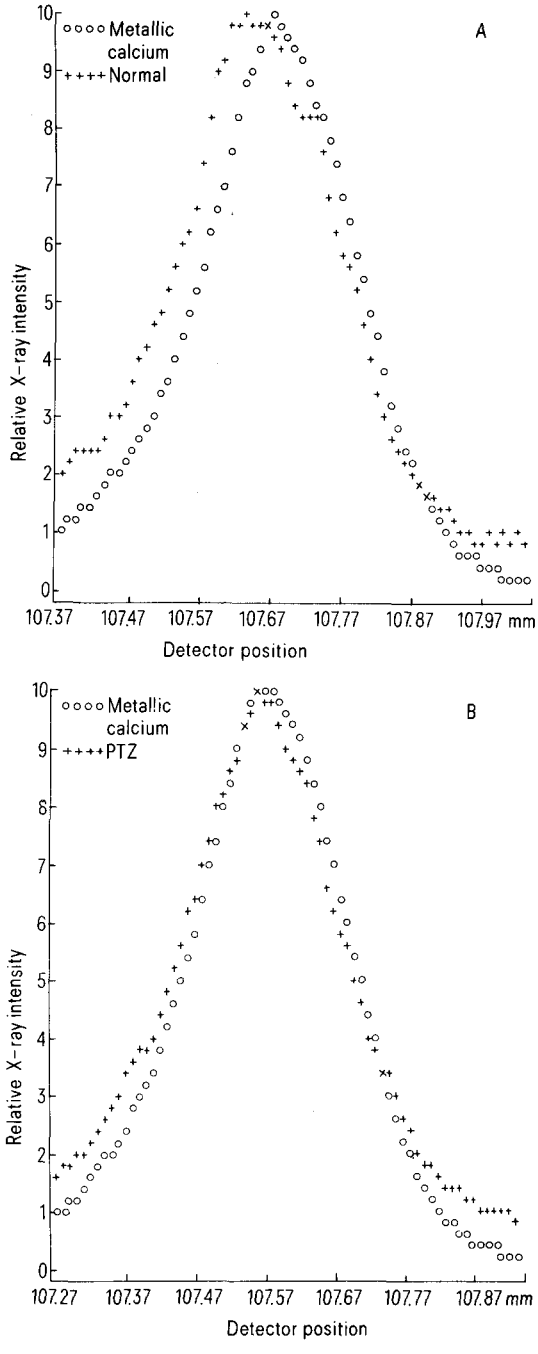
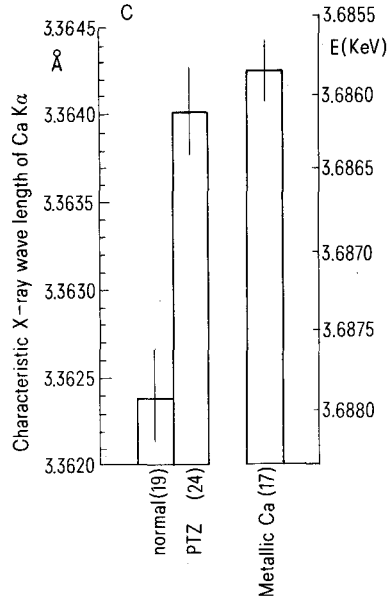
Summary. According to the examination by a computer controlled electron probe X-ray microanalyzer, the calcium binding state in the cell membrane during pentylenetetrazole-induced bursting activity was different from that of normal neuronal membrane.

Pentylenetetrazole (PTZ) induces bursting activity in the D-neurons of the Japanese land snail *Euhadra peliomphala*[1]. The intracellular scattered calcium bound to the subcellular organelles was found, by an examination with a computer controlled electron probe X-ray microanalyzer (CCEPXMA)[2], to move toward the cell membrane during bursting activity. If the movement of calcium toward the cell membrane has some relation to the bursting type neuronal discharges, the calcium should be bound to the cell membrane, and the binding state of calcium would become different from the normal membrane state.
The peak X-ray spectrum of elements shifts to some extent due to binding state change[3,4], and in the case of calcium, Chun[5] described a so-called chemical shift between calcium and calcium oxide. We tried to examine whether any kind of chemical shift exists in or near the cell membrane when the normal neuron and that in the state of bursting activity are compared.
D-neurons of the Japanese land snail *Euhadra peliomphala* were used, since the D-neurons of *Euhadra* are the group most sensitive to PTZ[1]. The D-neurons were divided into 2 groups, one of which was incubated in snail Ringer solution containing PTZ (5×10^{-2} M) and the other in snail Ringer solution alone. Each group was frozen rapidly and sectioned to about 10 µm thickness by a Lipshow cryomicrotome and freeze dried by the method described previously without passing through the liquid phase[2]. Both types of freeze dried specimens were placed on a hand-made carbon disk and the chemical shift was measured successively at the same accelerating voltage (15 kV) and absorbed current (10^{-8} A). The electron beam was about 1 µm in diameter and the beam spot was placed on the cell membrane area. For the standard X-ray peak spectrum position, metallic calcium was used. According to Bragg's law ($n\lambda=2d\sin\theta$), if θ is changed slightly for some unexpected reason, an X-ray wave length shift which should be distinquished from a true chemical shift occurs. The possible origins of the slight θ change are a) movement of the specimen in the z-direction (in our system, it was within $\pm3.12\times10^{-4}$ Å), b) movement of the analyzing crystal by a temperature change (in our system, within $\pm3.12\times10^{-4}$ Å), c) electron beam diameter change (in our system, undetectable) and d) movement of the beam in the X-Y direction (in our system, 0.94×10^{-4} Å). We measured these items first and if the chemical shift of samples exceeded this range, we decided that the results were positive for a chemical shift. The error range of metallic calcium after 8 h of repeated drawing of the X-ray spectrum curve was 3.36427 ± 0.000172 Å, and at this time the error range, including all the 4 items mentioned above, was considered as constant. Under such conditions, we started to measure the chemical shift of the specimens.
The steps for measuring the chemical shift were as follows. The analysing crystal was moved in 10-µm steps successively on Roland's circle at each measuring spot. The measuring time of each step (each crystal position) was 1 sec. We drew the curve of the relative X-ray strength when the maximum X-ray strength was 10. The curve was drawn after averaging of 5 arbitrary measuring spots on the neuronal membrane of one specimen.
The figure, A, shows an example of chemical shift of normal membrane. The peak of normal neuronal membrane showed a shift to a shorter wave length by about -16×10^{-4} Å. The peak of PTZ-treated membrane was almost the same as those of metallic calcium as shown in B. C shows the summary of chemical shift of normal and PTZ-treated neuronal membrane from metallic calcium, which demonstrates a clear difference between the 2 neuronal membrane states.
The above findings demonstrate that normal and PTZ-treated neuronal membrane or the cytoplasm near the cell membrane have different calcium binding states. Our previous study suggested that PTZ induced the release of calcium bound to subcellular organelles such as lysosome-





Chemical shift of calcium in normal and PTZ-treated freeze dried neurons. *A* An example of secondary X-ray intensity drawing by stepwise (10 μm) analysing crystal position movement (total of 67 steps) of metallic calcium (○) and normal neuron (+). The symbol × means the doubled position of the symbol ○ and +. Note the peak position shift of the normal neuron from the metallic calcium. *B* Same drawing but with metallic calcium (○) and PTZ-treated neuron (+). Note almost the same peak position between the two specimens. *C* Summary of chemical shift of the normal and PTZ-treated neurons. Values are mean ± SD. Numbers of experimental specimens are in parentheses.

like granules[6], and that membrane calcium density was remarkably increased during PTZ-induced bursting activity[7]. The above findings suggest that the increased calcium attached to the membrane area showed a different binding state. The significance of this change is completely obscure at this stage and requires further investigation.

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Intramembraneous particle change during pentylenetetrazole-induced bursting activity in snail neurons

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Summary. The freeze fracture study of snail neurons showed that during bursting activity induced by pentylenetetrazole, the intramembraneous particles demonstrated a different pattern from that of normal neurons.

When seizure discharge is produced in mammalian cerebral cortex by various methods such as pentylenetetrazole (PTZ) administration[1], penicillin application to the cerebral cortex[2] or repetitive cortical stimulation[3], the intracellular potential of the cerebral cortical neuron shows characteristic bursting activity (BA). Exactly the same pattern of BA can be seen when PTZ is applied to the specific neurons of the snail[4–6].