disc and their processes extend from there toward the centre. As a whole, their arrangement in the frog's gustatory disc shows a characteristic pattern resembling the spokes of a wheel.

Discussion. The present study has revealed a characteristic cytoarchitecture of MC cells in the frog's gustatory epithelium under the condition that the cells are probably filled with the fluorophore derived from 5,6-DHT. Recently, the ultrastructural study suggested the presence of Merkel cells in the frog's taste organ[6]. MC cells do not have apical processes extending toward the free surface. Serotonin-like monoamine-containing cells had been also observed in the taste organ of other species of vertebrates. In the rabbit's taste bud, the MC cell[7] was confirmed to be identical with the type III cell[8] which had been regarded as a gustatory receptor[9]. It extends from the basal lamina to the free surface and makes afferent synaptic contacts with intragemmal nerves. In the taste bud of the sheat-fish (Amiurus nebulosus), the basal cell contains an aminergic transmitter, serotonin[10]. Reutter[10] suggested that the basal cell modifies the impulse which is transmitted from sensory cells to afferent nerves. Further study is needed to clarify the role of MC cells in the frog's taste organ.

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The calcium-magnesium-deficient rat: A study on the distribution of calcium in the spinal cord using the electron probe microanalyser[1]

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Summary. Using the scanning electron probe X-ray microanalysis technique, calcium distribution in the spinal cord of the calcium-magnesium-deficient rat was studied. Calcium accumulations were observed within and around the perikaryon of certain unspecified motoneurons in the spinal cord.

In experimental rats fed a calcium-magnesium (Ca-Mg)-deficient diet for a period of several weeks, the concentration of spinal cord calcium showed an occasional rise despite a reduced blood calcium and magnesium level[3]. Furthermore, histochemical studies on the neuronal perikaryon of the spinal motoneuron showed a decrease in succinate dehydrogenase (SDH) activity with the perikaryon appearing swollen, and an atrophy and decrease in SDH type 2 muscle fiber was noted in gastrocnemius muscle tissue specimens in these Ca-Mg-deficient animals[3,4].

It became of interest to know the location of the calcium deposit within the spinal cord. The purpose of this study is to report the distribution of calcium in the spinal cord of the Ca-Mg-deficient rat by use of the electron probe microanalyzer, and to discuss a possible relationship between the calcium accumulations in the spinal cord and the appearance of a neuromuscular disorder in the experimental animals.

A Ca-Mg-deficit was induced in juvenile male albino rats by a synthetic diet containing 0.01% Ca and 0.003% Mg. The control rats were fed a normal diet containing 2.0% Ca and 0.34% Mg. Both groups were sacrificed after 6 weeks of this diet schedule, and the spinal cords were obtained for the experimental material. Atomic absorption spectrophotometry was used to determine the concentration of calcium in entire spinal cords with the following readings[3]:

	μg/g wet weight
Ca-Mg-deficient	286.6 ± 90.2
Controls	91.0 ± 10.7

Each value is the mean ± SE of the mean of 14.

The specimens were immersed in 4% formaldehyde containing 1% sodium oxalate, dehydrated gradually with alcohol, then cleared in xylene and embedded in paraplast using the method of Iwata et al.[5]. 10 micron sections were picked up from the solution onto a quartz disc and dried at 45 °C. The paraplast was removed and sections were coated with a thin layer of vacuum-evaporated carbon. The scanning electron probe microanalyzer (JXA-500A) was operated at 25 KV accelerating voltage and at about $1–10 \times 10^{-11}$ A absorbed current. The calcium LαX-ray integration time was 250 sec. Secondary electron images were recorded before recording the X-ray images. Polaroid photographs were then made of the display on the cathode ray tube.

The characteristic calcium LαX-ray images of the spinal cord specimens of the Ca-Mg-deficient and control animals and the secondary electron images appear in the figure. The image generated by the LαX-ray of calcium in the Ca-Mg-deficient animal is seen in figure B. Observation of the secondary electron image in figure D showed

Ratios of calcium-accumulated motoneurons in the anterior horn of the spinal cord of rats fed Ca-Mg-deficient or control diets

	Total motoneurons	Ca-accumulated motoneurons	Ca-accumulated motoneurons/total motoneurons
Control[6]	52	3	5.8%
Ca-Mg-deficient[6]	53	19	35.8%*

Values in parenthesis refer to number of animals studied significant level; *$p < 0.001$.

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the localization of calcium in the anterior gray column. Occasionally the accumulation of calcium occurred at the region of the spinal motoneurons. The posterior and lateral gray column and the white matter of the spinal cord appeared unaffected. In the control animal, the calcium LαX-ray and electron images only showed a diffuse distribution of the element. In the spinal motoneurons, the intensity of calcium did not exceed the background (figures A, C). Ratios of calcium-accumulated motoneurons in the anterior horn of the spinal cord of rats fed Ca-Mg-deficient or control diets are summarized in the table.

Thus, when the calcium LαX-ray are compared with the secondary electron images, it is seen that the accumulation of calcium occurs at the anterior gray column in the spinal cord of the Ca-Mg-deficient rats and tends to located in and around the region of the perikaryon of the spinal motoneuron. This finding is in agreement with the description of calcium accumulation reported in the previous study using the atomic absorption spectrophotometry.

The reason for the calcium accumulation at the site of the anterior gray column is not presently known, but suggests a relationship to a calcium dysmetabolism and/or other metabolic consequence, such as a secondary hyperparathyroidism. The secondary hyperparathyroidism is known to occur after hypocalcemia[6] or hypomagnesemia[7], and is of interest because of the findings of Arieff and Massry[8] who reported that an increment of calcium content in the cerebral gray matter of dogs was found after the administration of parathyroid extract, but did not occur following a vitamin D-induced hypercalcemia.

The calcium accumulation may be attributed to a metabolic malfunction of the spinal motoneuron. A description of renal mitochondrial swelling induced by calcium increment has already been reported by Scarpelli[9]. Judging from the changes which occur in skeletal muscle tissues of denervated animals[10], it is conceivable that the alteration of the metabolism of the spinal motoneuron may produce muscle degeneration, especially, as was seen in the previous studies[3,4], an atrophy of type 2 muscle fibres.

This study suggests that an increase in calcium in the spinal cord of the Ca-Mg-deficient animals has a special significance and is related to the neuromuscular abnormality, which may include neurogenic muscular atrophy, such as the type 2 muscle fibre atrophy.

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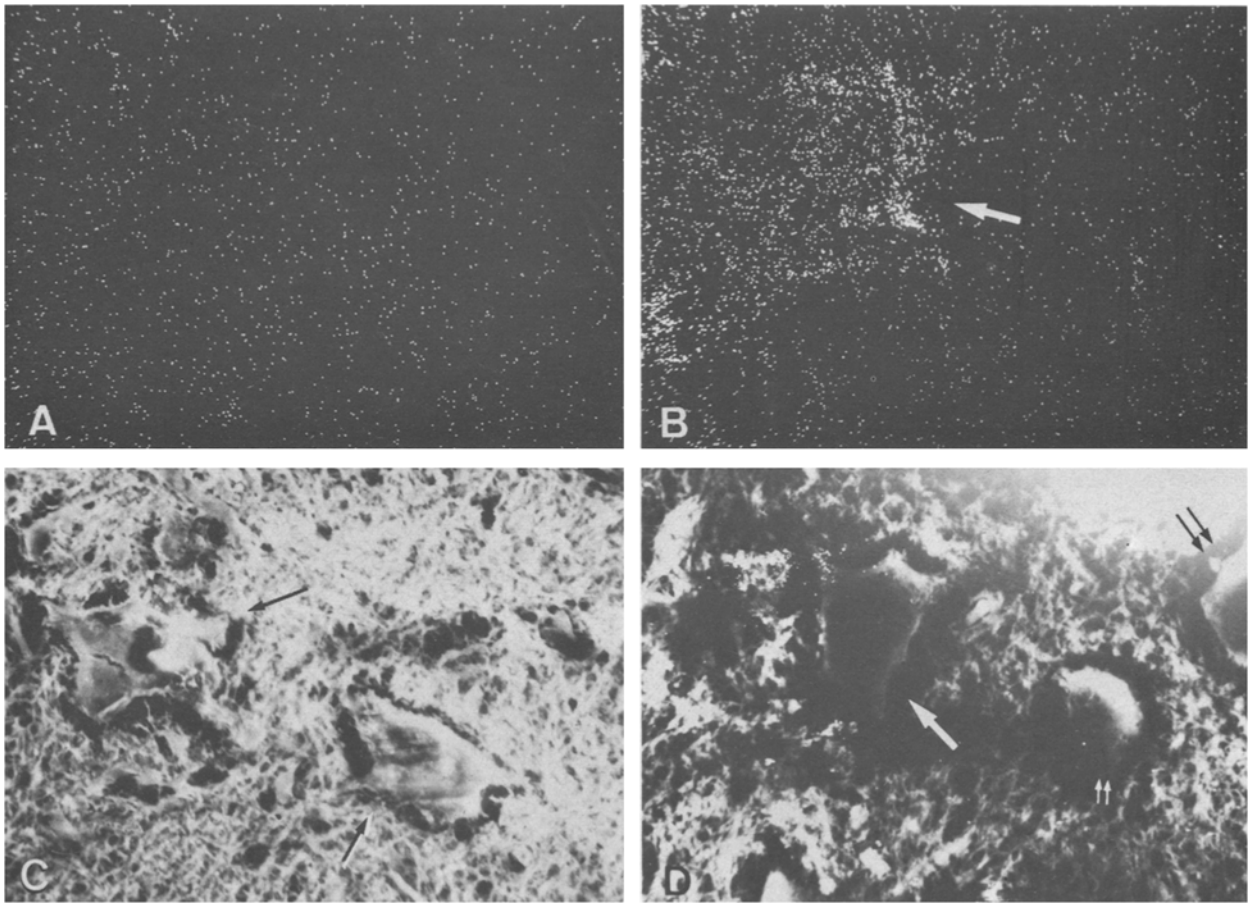

X-ray and secondary electron images of specimens of anterior horn of control and Ca-Mg-deficient rats (×600). *A* is the calcium LαX-ray image of control section. The small individual spots distributed throughout the entire X-ray image are due to background. *C* is the secondary electron image of the same sections. 2 spinal motor neuron (arrow) are observed. *B* is the calcium LαX-ray image from the spinal cord preparation of a Ca-Mg-deficient animal. The area containing excess calcium is marked by an arrow. The location of the calcium can be determined by referring to the secondary electron image of the same section. *D* Ca is situated in and around the region of perikaryon of spinal motoneuron (single arrow). In the other neurons (double arrows), there is no excess Ca.