preliminary studies of the growth properties of the papillomas in cell culture. Statistical analysis of the data was done using the two-sided WILCOXON Rank Sum Test[17].

Results and discussion. Figure 1 shows the effect of EGF treatment on the induction of skin tumors by MCA in Swiss-Webster mice. The time required for 50% of the mice to develop tumors was 19 weeks in the control group (PBS injections) but only 14 weeks in the animals injected with EGF. The time interval from initiation of the experiment to the appearance of the first tumor was significantly greater for the control group as compared with the experimental group ($p < 0.01$). In addition to shortening the latency period for the emergence of papillomas, EGF administration also caused a dramatic increase in the average number of papillomas per mouse throughout the course of this experiment (Figure 1, bottom). When the experiment was terminated at 21 weeks the EGF treated mice had an average of 6.0 times as many papillomas per mouse as the control animals. At 21 weeks the control Swiss-Webster mice all had between 0 and 5 papillomas per mouse. In the experimental group, one animal had 3 tumors while the rest of the group had between 10 and 20 tumors per mouse. The mean number of tumors were significantly greater for the experimental group as compared with the controls ($p < 0.01$).

Figure 2 shows the results of a similar experiment in C3HeB/FeJ mice. In this strain 50% of the experimental mice had developed tumors by 13 weeks (at which time none of the control mice had any tumors) while 50% tumor incidence in the control mice occurred at 18 weeks. For the C3HeB/FeJ experiment the time interval for papilloma appearance in the control group was also significantly greater than for the experimental group ($p < 0.02$). When this experiment was terminated the EGF treated mice carried an average of 2.6 times as many papillomas per mouse as the controls. For this strain at 21 weeks the control mice had from 0 to 6 papillomas per mouse, while the experimental animals had from 2 to 14 papillomas per mouse with the mean number of tumors being significantly greater for the experimental group ($p = 0.01$).

These studies clearly indicate that in both the Swiss-Webster and C3HeB/FeJ strains of mice EGF can enhance the carcinogenic activity of MCA, both in terms of the time of appearance of skin tumors and the average number of tumors which develop. Although our experiments do not exclude the possibility that EGF may itself be a carcinogen, attempts by others[18] to obtain papillomas in mice treated with EGF alone have been unsuccessful.

Our primary objective in these tumor induction studies has been to obtain EGF-sensitive tumors which could be adapted to growth in cell culture. We hope to obtain EGF dependent cell lines with appropriate selection procedures. The tumor induction studies reported here are necessary to define the time course for the appearance of tumors in EGF + MCA treated animals and to verify that there is, indeed, a significant difference between EGF + MCA treated mice and the MCA treated controls.

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Calcium and Magnesium Deficiency-Induced Atrophy of Muscle and Calcium Accumulation in the Spinal Cord

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Summary. After severe dietary calcium-magnesium deficiency in rats, succinic dehydrogenase and acetylcholinesterase enzyme activity of gastrocnemius muscle showed a neurogenic atrophy. This alteration was associated with a high concentration of calcium in the spinal cord.

Recent clinical reports have suggested an interrelationship between a disturbance in calcium metabolism and the pathogenesis of neuromuscular disease[2,3]. Lacking reports on the neuropathology of the nervous system and muscles in the calcium and magnesium (Ca and Mg) deficient animal, we previously reported the changes in nervous system and related organs of experimental animals fed a Ca-Mg deficient diet[4].

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Effect of dietary calcium and magnesium restriction on concentration of calcium and magnesium in serum, spinal cord, gastrocnemius and kidney of rats

	Calcium		Magnesium	
	Control	Ca-Mg def.	Control	Ca-Mg def.
Serum (mg/100 ml)	8.68 ± 0.20	6.30 ± 0.29[a]	3.26 ± 0.19	1.54 ± 0.09[a]
Spinal cord (μg/g wet weight)	91.0 ± 10.7	286.6 ± 90.2	155.0 ± 5.2	149.1 ± 5.9
Gastrocnemius (μg/g wet weight)	46.9 ± 2.8	52.7 ± 6.9	277.3 ± 11.2	250.8 ± 10.8
Kidney (μg/g wet weight)	62.3 ± 4.7	1908 ± 245[a]	204.8 ± 8.8	184.2 ± 7.2

Each value is the mean ± SE of the mean of 14. [a] $p < 0.001$.

In this paper we describe the Ca-Mg deficiency-induced chemical alterations in spinal cord, kidney and gastrocnemius muscle tissue of the experimental animal and evaluate the changes seen in the gastrocnemius muscle. The possible relationship between these alterations in gastrocnemius muscle and associated accumulation of calcium in the spinal cord will be discussed.

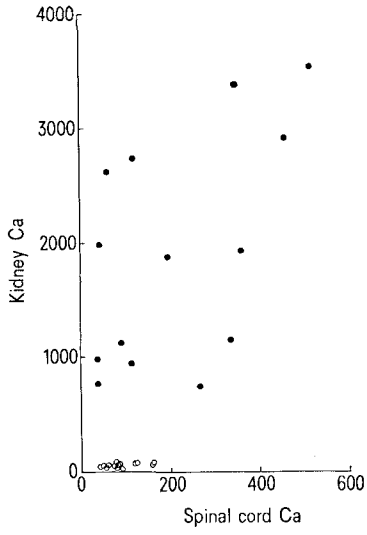

Fig. 1. Levels of Ca in the kidney compared with those in spinal cord, Ca-Mg deficient (●) and control (○). (μg/g wet wt.)

Materials and methods. 14 juvenile albino rats of Wistar strain were fed a Ca-Mg deficient diet with deionized water provided ad libitum. Except for low calcium (0.01%) and magnesium (0.003%) content, the Ca-Mg deficient diet was nutritionally balanced (supplied by the Oriental Yeast Co. Ltd., Tokyo). 14 control animals were fed the Oriental MF diet, containing 2.0% calcium and 0.34% magnesium, with tap water.

After 6 weeks of diet feeding, the experimental and control animals were sacrificed with ether anesthesia and samples of blood, spinal cord, gastrocnemius muscle and kidney were rapidly removed. Spinal cord, gastrocnemius muscle and kidney were ashed in a mixture containing concentrated nitric acid and perchloric acid prior to analysis, while sera were analyzed without ashing following appropriate dilution. Calcium and magnesium content were determined by atomic absorption spectrophotometry. Student's *t*-test was used to determine significance of differences between experimental and control values.

Other parts of the gastrocnemius muscle were frozen and cut in the cryostat for section staining to observe the histochemical reactions of acetylcholinesterase[5] and succinic dehydrogenase[6].

Results and discussion. Macroscopic observations of the Ca-Mg deficient rats at autopsy showed depletion of subcutaneous adipose tissue, bone porosity and atrophy of muscles.

Quantitative data of tissue content of calcium and magnesium in experimental and control animals are shown in the Table. In rats fed Ca-Mg deficient diets, the levels of calcium and magnesium in serum were markedly and significantly lower ($p < 0.001$) than those of control animals. Despite hypocalcemia, the calcium content of

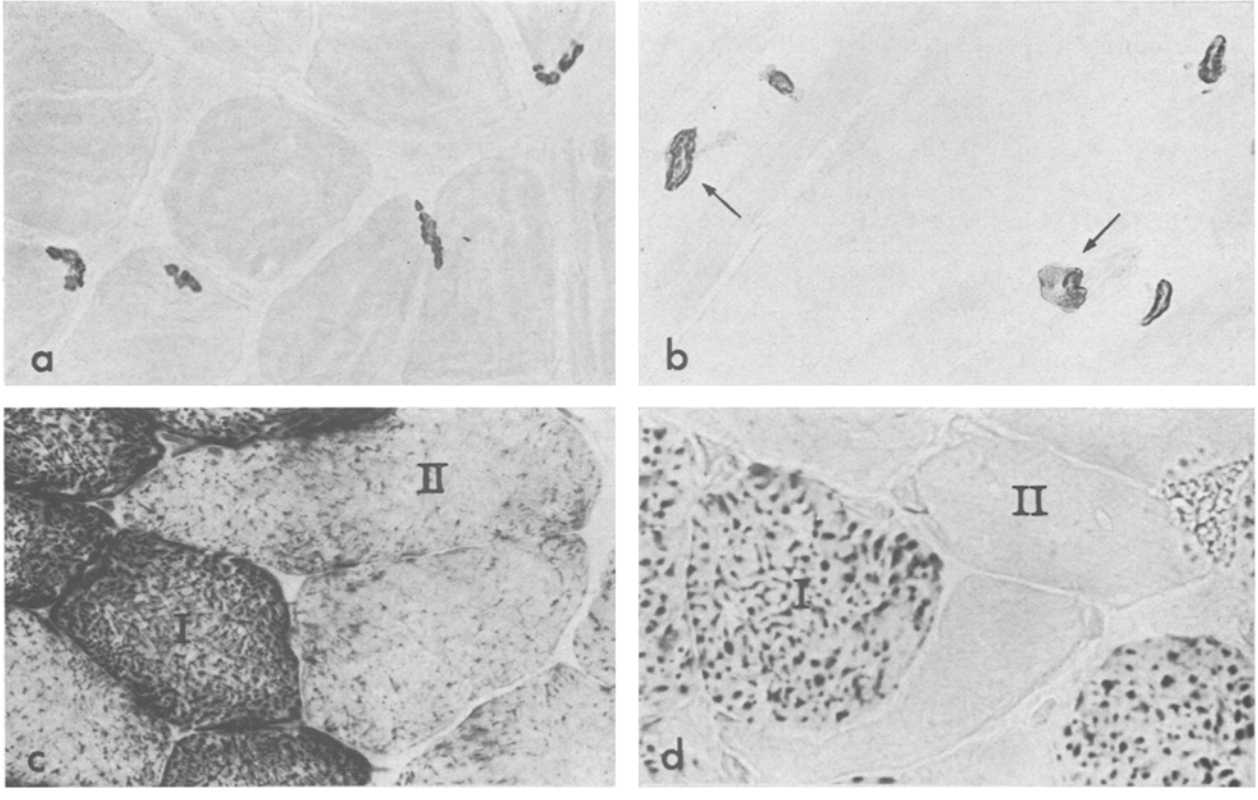

Fig. 2. a) Gastrocnemius of the control rats. AChE reaction. × 320. b) Gastrocnemius of Ca-Mg deficient rats. AChE reaction. Note the swelling of motor end-plate (arrow). × 320. c) Gastrocnemius of the control rats. SDH reaction. Type I fibre (I), Type II fibre (II). × 320. d) Gastrochemius of Ca-Mg deficient rats. SDH reaction. Type I fibre (I), Type II fibre (II). × 320.

the kidney was strikingly increased ($p < 0.001$) when compared with controls. The high content of calcium in the kidney of experimental rats was significantly correlated with a high concentration of calcium in the spinal cord ($r = 0.5592$, $p < 0.05$, Figure 1), while in the control animals, no correlation was noted between the calcium content in spinal cord and kidney. These results suggest a Ca-Mg deficient diet-induced calcium resorption from bone which may account for the appearance of calcium deposits in the spinal cord as well as in the kidney. The reason for calcium deposition in the spinal cord has not been determined but may be related to the Ca-Mg deficiency and/or other metabolic consequence, such as a secondary hyperparathyroidism. A secondary hyperparathyroidism is known to occur after magnesium deficiency[7]. However, we do not known if there is an accompanying variation in calcium concentration of spinal cord. In the gastrocnemius muscle, the calcium content was not significantly different in comparison with the controls.

In general, magnesium content in all organs of the Ca-Mg deficient rat was similar to content in the controls despite a marked fall in concentration of magnesium in serum. The results obtained here agree well with findings known to occur in chronic magnesium deficiency[8]. Soft tissues appear to be capable of maintaining a constant magnesium content even with a concomitant reduction in serum magnesium.

Of greater concern in this study were the marked histochemical changes in the gastrocnemius muscle of the Ca-Mg deficient rat. In the Ca-Mg deficient rat, the gastrocnemius muscle section processed for acetylcholinesterase (AChE) enzyme histochemical study showed a slight reduction in enzyme activity and the motor end-plate appeared swollen (Figure 2b). In the control gastrochemius muscle section, there was an intense AChE activity at the motor end-plate (Figure 2a). It is not inconceivable that these alterations may be attributed to a metabolic disturbance of the motor neuron due to the calcium accumulation in the spinal cord. Our previously reported investigation[4] demonstrated a decrease in succinic dehydrogenase activity of the neuronal perikaryon and a slight swelling of the motor neuron in the spinal cord tissue of the Ca-Mg deficient rat.

Succinic dehydrogenase (SDH) reaction in the Ca-Mg deficient gastrocnemius muscle showed a low enzyme activity, particularly in the sarcolemma and subsarcolemmal region of Type I fibres associating with a disruption of regular SDH reaction pattern in fibres (Figure 2d). Control sections showed a striking variation in the SDH reaction of individual fibres; One group (Type I), usually of small diameter, gave a strong reaction and the other group (Type II), usually of large diameter, gave a consistently weaker reaction (Figure 2a). These findings of atrophy of Type II fibres and slight hypertrophy of Type I fibres, which demonstrated in the Ca-Mg deficient gastrocnemius muscle (Figure 2d), are similar to the characteristic changes in experimental denervation of gastrocnemius muscle[9]. As spinal cord calcium content increased this change became more prominent.

These chemical and histochemical results suggest that calcium deposits in the spinal cord may be of some particular relationship to the manifestation of neurogenic degeneration of muscle in the Ca-Mg deficient rats.

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Sister Chromatid Differential Staining Pattern in Prematurely Condensed Chromosomes[1]

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Summary. Application of sister chromatid differential (SCD) procedure on G_1, S and G_2 prematurely condensed chromosomes (PCC) of cells in the second and third cycle of DNA replication in medium containing BrdU reveals differential staining patterns characteristic of their respective stages in the cell cycle. These findings also suggest a structural similarity between PCC and metaphase chromosomes.

Recent developments in cell biology have offered some high resolution methods for studies of DNA and chromosomal replication[2,3] and detection of sister chromatid exchanges[4-7]. Latt[8] first demonstrated that fluorescent staining with the *bis*benzimidazole dye Hoechst 33258 was quenched in a chromatid if both strands of its DNA had incorporated 5-bromodeoxyuridine (BrdU). When a cell has gone through 2 semiconservative DNA replication periods in a medium containing BrdU, one chromatid contains BrdU in both DNA strands while its sister chromatid has BrdU in only one of its DNA strands. This is reflected in differential fluorescence of the sister chromatids with Hoechst 33258, or differential staining with Giemsa after treating the chromosomes with some mild proteinases[7] or buffers at a high temperature[4,6].

When an interphase cell is fused with a mitotic cell, the interphase chromosomes are induced to condense into discrete units, known as prematurely condensed chromosomes (PCC)[9-11]. The PCC morphology is characteristic of the cell cycle phase of the interphase nucleus.

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