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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Each G_1-PCC consists of a single chromatid, whereas each G_2-PCC contains 2 sister chromatids. The S-PCC appear to be pulverized, yet depending upon the extent of DNA replication, both G_1 and G_2 fragments can be observed. Chromosome banding[12,13] and electron microscopy[14,15] studies on PCC revealed less condensed structures than the metaphase chromosomes. In the present studies, we have combined the sister chromatid differential (SCD) procedure with the phenomenon of PCC in order to observe the SCD pattern on PCC throughout the cell cycle.

Both male (Don) and female (CHO) Chinese hamster cells were routinely grown as monolayers in McCoy's 5a medium supplemented with 16–20% fetal calf serum. For the PCC experiments, the interphase cells were incubated in the dark in medium containing 15 μg/ml BrdU for about 2 cell cycles (22 h). These cells were trypsinized and mixed with an equal number of mitotic CHO cells which had been collected by selective detachment technique[16]. The mixture was washed twice in Hanks' balanced salt solution and resuspended in 0.5 ml phosphate buffered saline (PBS) containing 2000 hemagglutinating units of UV-inactivated Sendai virus. The fusion mixture was held for 15 min at 4 °C and then incubated for 45 min at 37 °C. By this time the PCC were formed and the cells were fixed in methanol-acetic acid (3:1) for air dried preparations. For SCD staining, a modified procedure of the Korenberg-Freedlender method was employed. Chromosomes were first treated with 50% acetic acid at 37 °C for 4 min and then with 1.0 *M* phosphate buffer

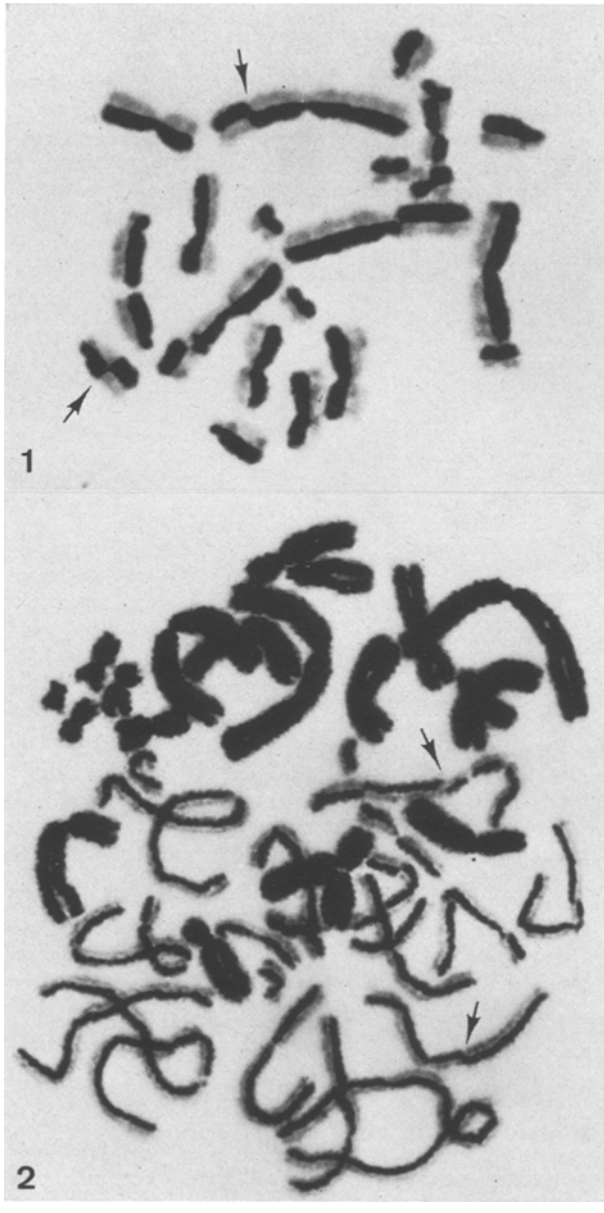

Fig. 1. SCD staining in metaphase chromosomes of a Don cell. Solid arrows indicate sites of sister chromatid exchanges. × 2000.

Fig. 2. SCD staining of G_2-PCC of a CHO cell. Solid arrows indicate sites of sister chromatid exchanges. × 2000.

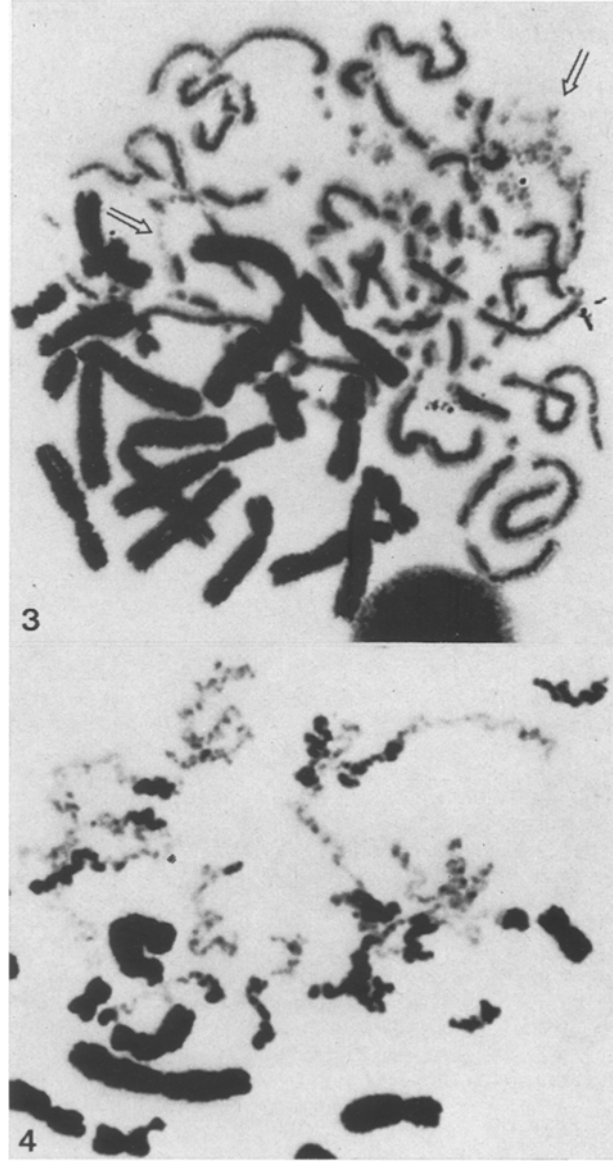

Fig. 3. SCD staining in S-PCC of a CHO cell in the relatively late phase of the 2nd replication period. Replicated fragments show G_2-PCC differential pattern. Hollow arrows indicate regions that were in DNA synthesis at the time of PCC induction. × 2000.

Fig. 4. G_1-PCC of a CHO cell. The G_1 phase is either in the 2nd or 3rd cell cycle after BrdU addition (see text) and shows both intra and inter chromosome differential staining. × 2000.

(pH 8.1) at 89–91 °C for 15 to 45 min. The slides were rinsed in water and stained with 2% Giemsa in distilled water for 15 min.

Metaphase chromosomes of cells which had completed 2 replication cycles in BrdU showed excellent differential staining between sister chromatids (Figure 1). The G_2-PCC cells which had finished the second DNA synthesis period showed SCD patterns similar to those of the metaphase chromosomes (Figure 2). The S-PCC (Figure 3) showed differential staining between sister chromatids of the replicated fragments and 'pulverized' regions indicating DNA synthetic activity immediately before the induction of PCC. The G_1-PCC (Figure 4) showed 2 types of staining patterns. Some chromosomes had differential staining along their lengths while others did not. The G_1-PCC pattern can be interpreted in the following ways. The cells of the cultures used were not synchronized and there is considerable variation in generation time, therefore the intra-chromosome differential staining seen in the cell in Figure 4 could be the result of sister chromatid exchanges in the 2 previous generations. Alternatively, the cell might have been in the S phase of the first cycle at the time of BrdU addition and progressed to the G_1 phase of the 3rd cell cycle at the time of PCC induction. This would result in parts of the G_1 chromosome(s) containing doubly substituted BrdU regions. The inter-chromosome differential suggests random sister chromatid segregation in the preceding anaphase.

Although the mechanism of SCD is still unknown, there is some evidence that unifilarly and bifilarly BrdU substituted chromatids have different packing densities observable by both phase and electron microscopy[6]. Since we have demonstrated that SCD can be induced in PCC much the same way as for mitotic chromosomes, it is probable that a similar density difference, as observed in electron micrographs, may exist among such differentially BrdU incorporated chromatids in PCC. This suggests an organizational similarity between PCC and metaphase chromosomes.

The combination of these 2 techniques will now allow greater precision in DNA synthesis[17] and chromosome aberration studies[18,19].

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Localization of Bovine Pancreatic Polypeptide (BPP)-Like Immunoreactivity in Rat Pancreatic Monolayer Culture

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Summary. Antiserum to bovine pancreatic polypeptide (BPP) has been used for immunofluorescent staining in the light microscope. With this technique it is possible to detect the presence of specific cells in monolayer culture from neonatal rat pancreas which contain BPP or a closely related peptide.

During the isolation of chicken insulin, Kimmel et al.[2] found a polypeptide containing 36 amino acid residues in a straight chain[3] and grouped in a sequence distinct from that of insulin, glucagon, somatostatin, secretin and gastrin. This avian pancreatic polypeptide (APP) has pronounced biological effects[4] and is a normal constituant of circulating plasma in chicken[5]. It was therefore postulated that APP represents a new pancreatic hormone[4,5] present in the cytoplasm of numerous cells disseminated in the exocrine chicken pancreas[6].

Recently, a mammalian counterpart of APP was isolated from the bovine pancreas and named accordingly bovine polypeptide (BPP)[7]. BPP has a sequence of 16 amino acid residues in common with APP and some similarities in biological action[7,8]. In the course of a systematic immunohistochemical study of the endocrine cell population of rat pancreatic monolayer culture, we were able to identify cells reacting to anti-BPP.

Neonatal rat pancreatic monolayer cultures were prepared as described elsewhere[9]. After 3 days of culture, tissue was fixed with a solution of 0,2% picric acid and 2% paraformaldehyde[10] in 320 mOs*M* phosphate buffer (pH 7,4)[11] during 2 to 90 h. After fixation, the cultures were washed in phosphate buffered saline, quickly dehydrated through increasing concentrations of ethanol, rehydrated and processed for indirect immunofluorescence[12]. Cultures were exposed to rabbit anti-BPP

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