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The direct involvement of extracellular calcium in the alteration of bone remodeling and mineralization following dietary calcium deficiency remains unclear. The few studies already carried out on osteoblastic responses to extracellular calcium concentrations *in vitro* have used immortalized cell lines [Sugimoto et al (1993), *J Bone Miner Res* 8, 1445-1452]. We found previously that low concentrations of extracellular calcium, reflecting hypocalcemia, do not affect primary porcine osteoblasts (OB) [Eklou et al (1995), *Calcif Tissue Int* 57, 311]. In this experiment we investigated the influence of high concentrations of extracellular calcium mimicking resorption site conditions, on the proliferation and differentiation of primary porcine OB.

OB from trabecular metacarpal bone from pigs were isolated by enzymatic digestion. After one passage, OB were seeded in DMEM supplemented with 10% FCS and 2% Ultrosor containing 2.2 mM total calcium (control medium). The cells were switched to high calcium medium (5, 7 or 10 mM total calcium; $n = 3$) 1 or 3 days after plating for 24 or 48 h then returned to control medium until the end of the culture period. Cell proliferation and cell differentiation were assayed by measuring DNA content, alkaline phosphatase (ALP) activity and carboxyterminal propeptide of type I procollagen (PICP) release, ie, collagen synthesis.

Incubation of the cells in 7 or 10 mM calcium medium on day 1 and for 48 h ($P < 0.01$) slowed the rate of cell proliferation dose-dependently without altering their DNA content at confluency. Whether the change in calcium concentration was made on day 1 or day 3, calcium added for 48 h had no effect on collagen synthesis but ($P < 0.01$) slowed the increment in the ALP activity of porcine OB dose-dependently. This inhibition of the ALP activity persisted even

after the cells were back to the control medium.

Increasing the concentration of calcium strongly inhibits the ALP activity of porcine OB. ALP is involved in the mineralization process probably through crystallization of calcium and phosphate into hydroxyapatite. In physiological terms, the inhibition of the OB ALP activity by high calcium at the resorption site may help prevent the recrystallization of calcium in the vicinity, which would inhibit the release of calcium into the circulatory system.

Primary cultures of proximal and distal colon of Wistar rat: proliferative effect of the growth cofactor heparin. B Kaeffer, C Bénard, HM Blottière, C Cherbut (*Centre de recherche en nutrition humaine de Nantes, Inra et Hôpital Laënnec, BP 1627, 44316 Nantes cedex 03, France*).

The development of a simple and reliable method to cultivate epithelial cells of normal intestinal mucosa will pave the way to assay food fibre effects on the proliferation and differentiation of noncancerous cell phenotypes. Recently, Flint et al (1994) have shown that the addition of heparin to a growth medium containing a low fetal calf serum concentration stimulates the proliferation of epithelial cells from the small intestine of 5-day Wistar rats [*J Cell Sci* 107, 401-411]. We have estimated the proliferative effect of heparin on colonic cell primary cultures.

Cell survival was assayed by trypan blue exclusion. Cellular proliferation was measured by crystal violet staining, calculating a proliferative index on three rats (200 g): ratio between optical densities of extracted stain on three cell monolayers cultivated for 9 days and on three monolayers cultivated for 2 days. Tissular origin of cells was identified by immunostaining (stromal cells expressing vimentin, cytok-

eratin-18 expressed by epithelial). After inoculation, isolated cells were viable at 90% and cellular clusters at 100%. The incorporation of 50 $\mu\text{g}/\text{mL}$ heparin in a growth medium at 1% fetal calf serum has significantly increased the proliferative index of distal colonic cells (5.86 ± 0.77) comparatively to controls (3.26 ± 0.27) according to a Student's *t* test ($P < 0.05$). Alternatively, no significant difference was found between the index for proximal

part (3.41 ± 0.48) and those for controls (2.75 ± 0.29). The cultures were lost after 12 days.

We confirm that heparin stimulates the proliferation of distal colonic cells on 9 days but not those of the proximal compartment. Designing a defined growth medium including heparin will be essential to understand the interactions between food fibres, growth factors and digestive epithelial