nisms of this effect remain to be elucidated. [Moon et al (1992) *Cancer Detect Prev* 16, 73-79; Fontana et al (1992) *Cancer Res* 50, 1977-1982]. In the present study, we examine the effect of all-trans retinoic acid on insulin-induced cyclin D1 gene expression, in parallel with cell growth, in T47D breast cancer cell line. Cyclin D1 is a cell cycle regulator and a candidate proto-oncogene implicated in mammary tumorigenesis the expression of which is associated with changes in the proliferation rate of breast cancer cells.

T47D breast cancer cells in exponential growth phase were grown for 19 h in serum-deprived RPMI medium for northern-blot analysis. All-trans retinoic acid dissolved in ethanol was then added in culture medium at concentrations over the range 10^{-10}-10^{-6} M, in presence of insulin 10 µg/mL. The final concentration of ethanol in medium was 0.1% and control cells received vehicle only. After 5 h, RNA was extracted from triplicate petri dishes by a guanidium-isothiocyanate procedure and northern-blot analysis was performed with 20 to 30 µg of total RNA per lane. Filters were hybridised with a human cyclin D1 cDNA (obtained from D Beach, Cold Spring Harbor, NY, USA), labelled with α-^{32}P dCTP by random primer extension. mRNA abundance was quantitated by electronic autoradiography (Packard Instant Imager). For growth experiments, T47D cells were plated in 3% fetal calf serum (FCS) supplemented medium, in 24-well plates. Twenty-four hours later, cells were treated with retinoic acid and insulin 10 µg/mL, as described above, in medium containing 1% FCS during 7 days. Cells were fixed with methanol and the amount of DNA was evaluated in situ by measuring the specific reaction fluorescent product of desoxyribose and diaminobenzoic acid [DABA assay; Kissane and Robins (1958) *J Biol Chem* 233, 184-188].

Evaluation of DNA cell content showed that retinoic acid (RA) inhibited insulin-induced T47D cell proliferation in a dose dependent manner:

	0	ins	RA10^{-10}	RA10^{-9}	RA10^{-8}	RA10^{-7}	RA10^{-6}
µg DNA per well	3.5	5.9	5.4	4.9	2.3	2.2	1.1

After northern-blot analysis, we observed that retinoic acid caused a concentration-related inhibition of insulin-induced cyclin D1 gene expression. Results are expressed as a percentage of cyclin D1 mRNA signal from insulin treated cells:

	ins	RA10^{-10}	RA10^{-9}	RA10^{-8}	RA10^{-7}	RA10^{-6}
% of mRNA cyclin D1	100	90	83	65	61	51

In conclusion, we have shown that retinoic acid was able to inhibit cyclin D1 gene expression in parallel with its inhibition action on cell growth. Current studies are focused on characterizing the retinoic acid effect on cyclin D1 gene expression, in order to determine if it acts at transcriptional or translational level. We will compare the RA response of several human breast tumor cell lines.

Influence of high concentrations of extracellular calcium on the proliferation and differentiation of porcine osteoblasts in culture. E Eklou-Kalonji, I Denis, A Pointil-

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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% Ultroser 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-