

**SCUOLA DI DOTTORATO DI RICERCA IN SCIENZE BIOCHIMICHE, NUTRIZIONALI E
METABOLICHE**

**DOTTORATO DI RICERCA IN BIOCHIMICA
XXII CICLO**

**Role of the Caseinophosphopeptides and Vitamin D on
calcium uptake and cell functions in human cancer
intestinal cell lines differentiated in culture: a possible
correlation between nutrients and colon cancer**

Docente Guida: Chiar.ma Prof.ssa Vanna CHIGORNO

Dott. Giovanni Lombardi

Matr.: R07244



CASEINPHOSHOPEPTIDES



α_{s1} -Casein

45 63
-Gly-Ser-Glu-Ser-Thr...Glu-Ser-Ile-Ser-Ser-Ser-Glu-Glu-
P P P P P P

α_{s2} -Casein

55
-Gly-Ser-Ser-Ser-Glu-Glu-Ser-Ala-Glu-Val-Ala-Thr-Glu-Glu-Val-Lys-
P P P P

β -Casein

13 34
-Val-Glu-Ser-Leu-Ser-Ser-Ser-Glu-Glu...Gln-Ser-Glu-
P P P P P

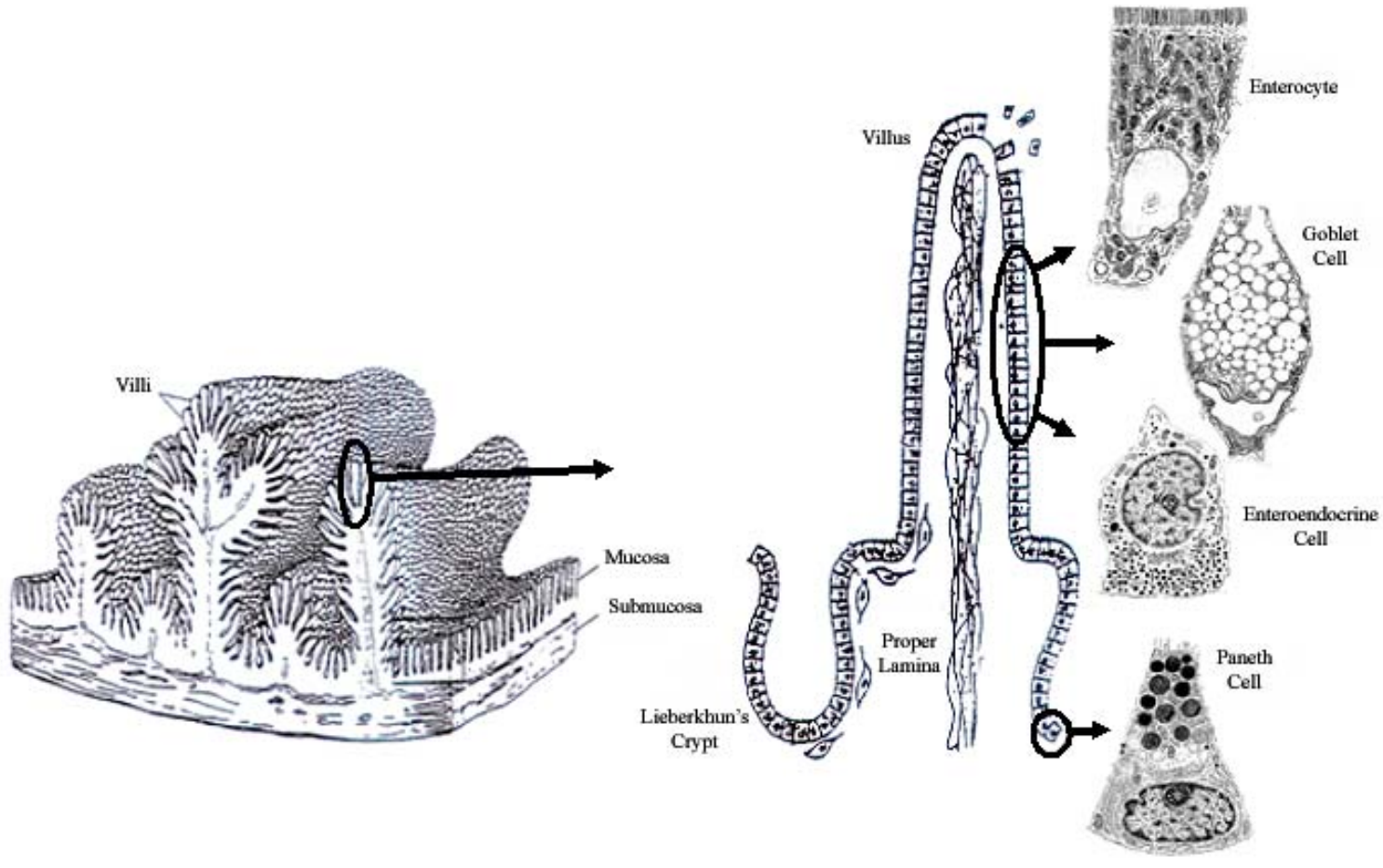


AIM OF THE STUDY (1th)



- **To study the responses, to CPP administration, of differentiated and undifferentiated intestinal cells**
 - ➔ **Necessity of intestinal epithelium in vitro models**

THE INTESTINAL MUCOSA AND INTESTINAL CELL LINES

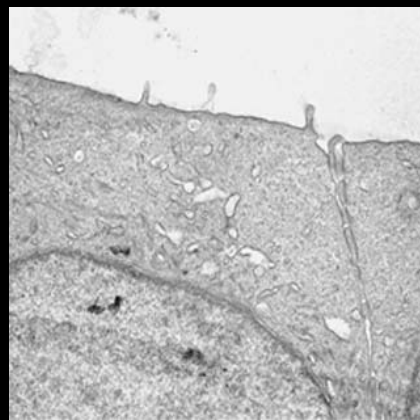


➔ HT-29 human adenocarcinoma cell line

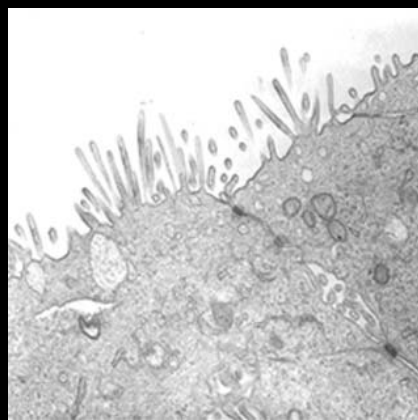
➔ Caco-2 human adenocarcinoma cell line



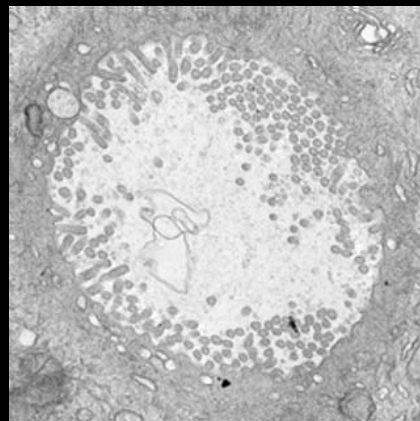
EVALUATION OF HT-29 CELL DIFFERENTIATIONS



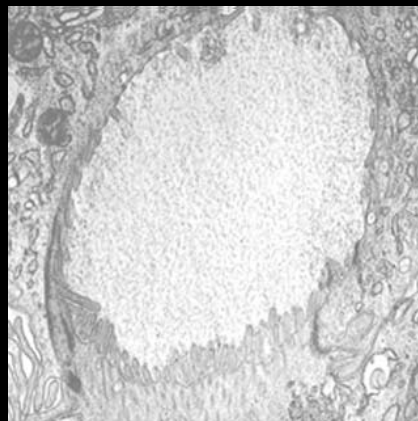
HT-29 DMEM
10000x



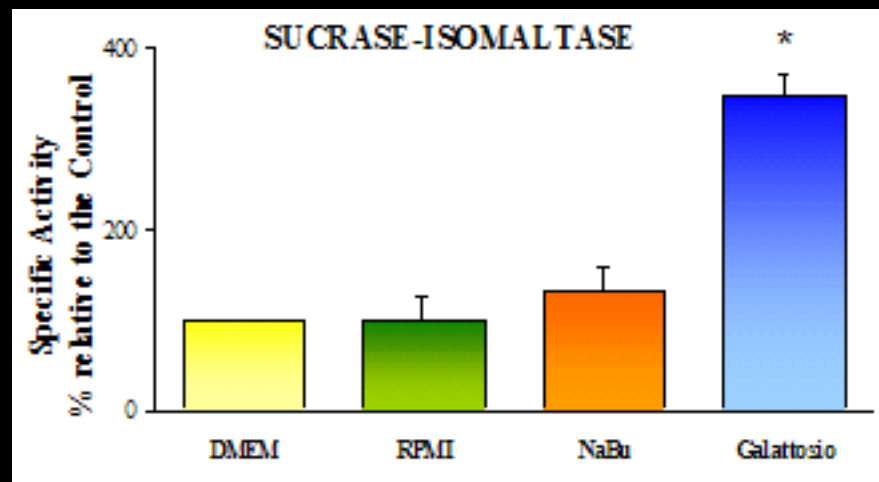
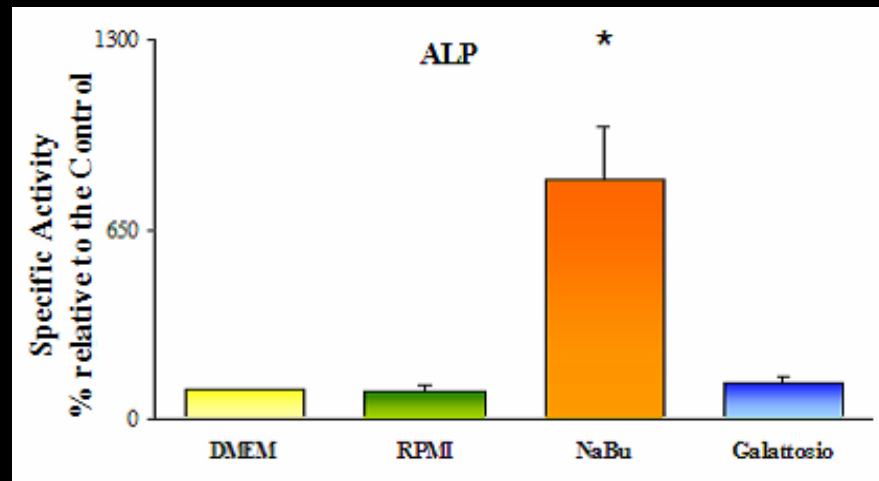
HT-29 RPMI
10000x



HT-29 NaBu
10000x

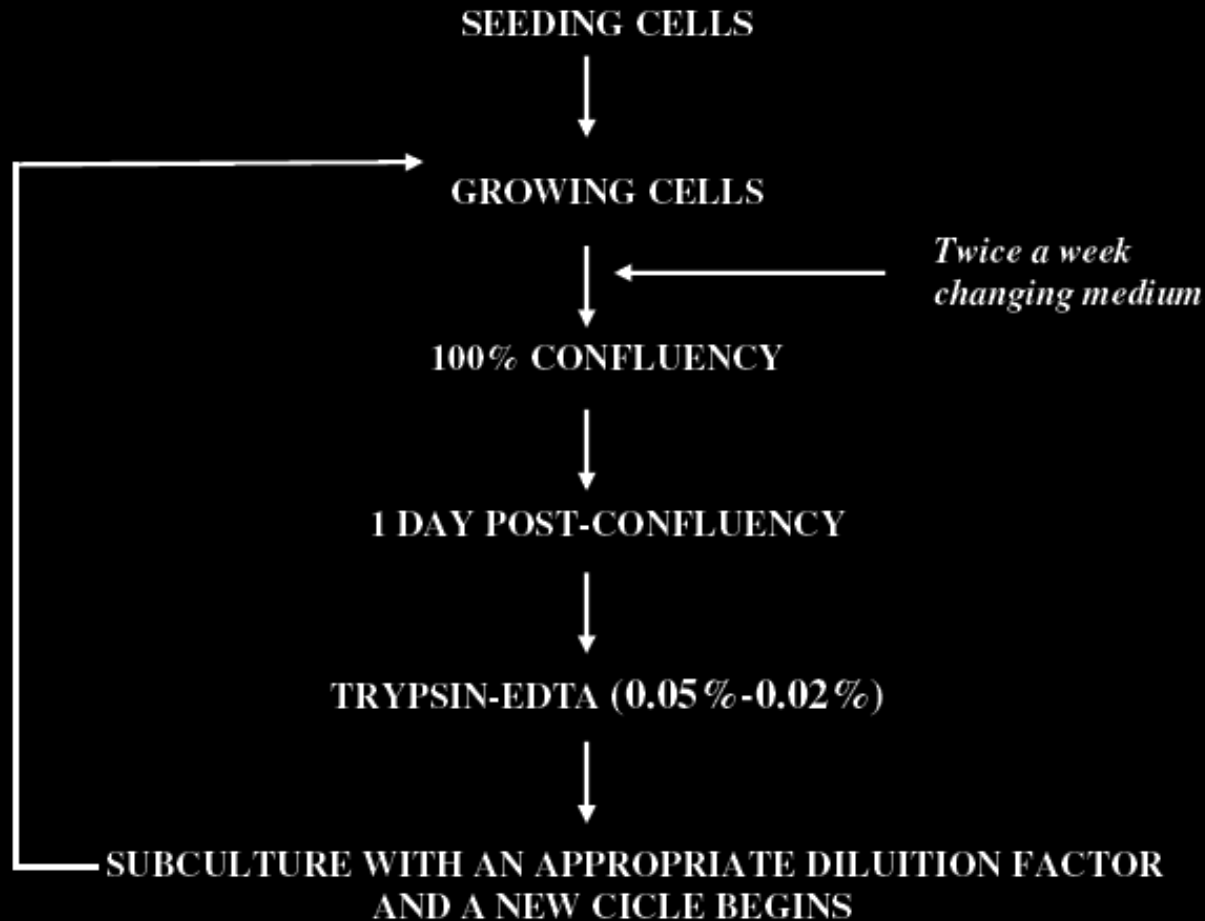


HT-29 Gala
14000x



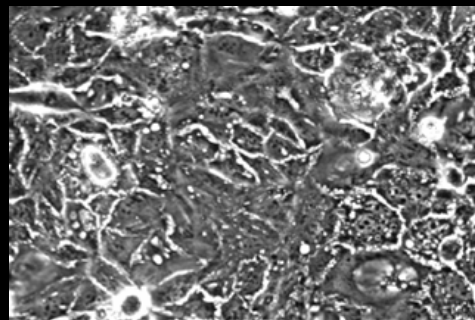


Caco-2 CELL DIFFERENTIATION: A NEW METHOD

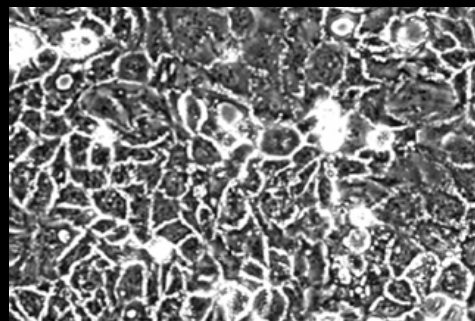




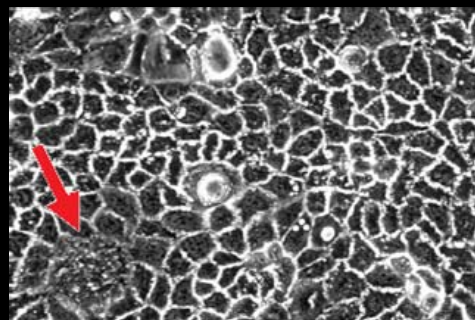
MORPHOLOGICAL EVALUATION OF Caco-2 CELL DIFFERENTIATION



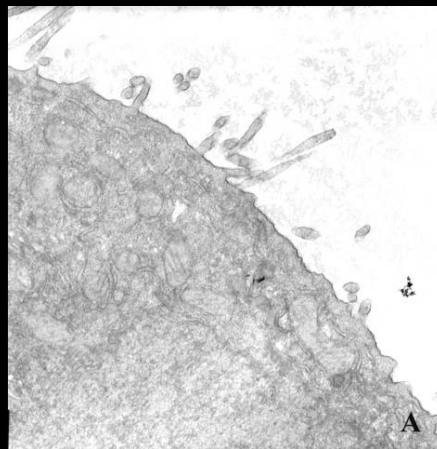
Low Passages
(6thP)



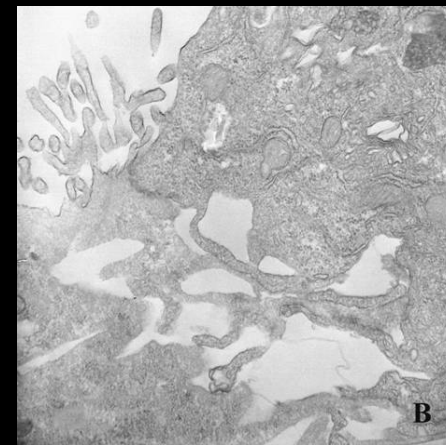
Intermediate
Passages (24thP)



High Passages
(41thP)

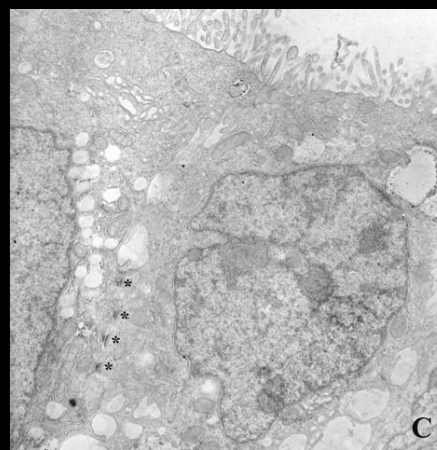


A



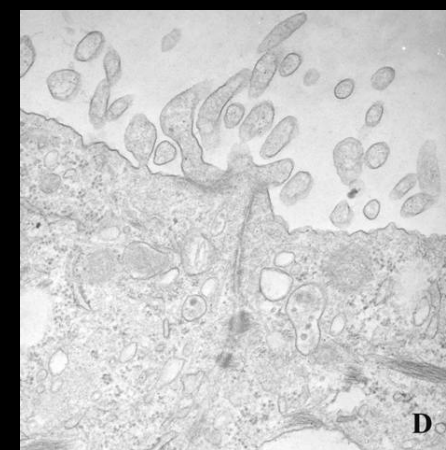
B

11th Passage 14000x



C

18th Passage 4800x

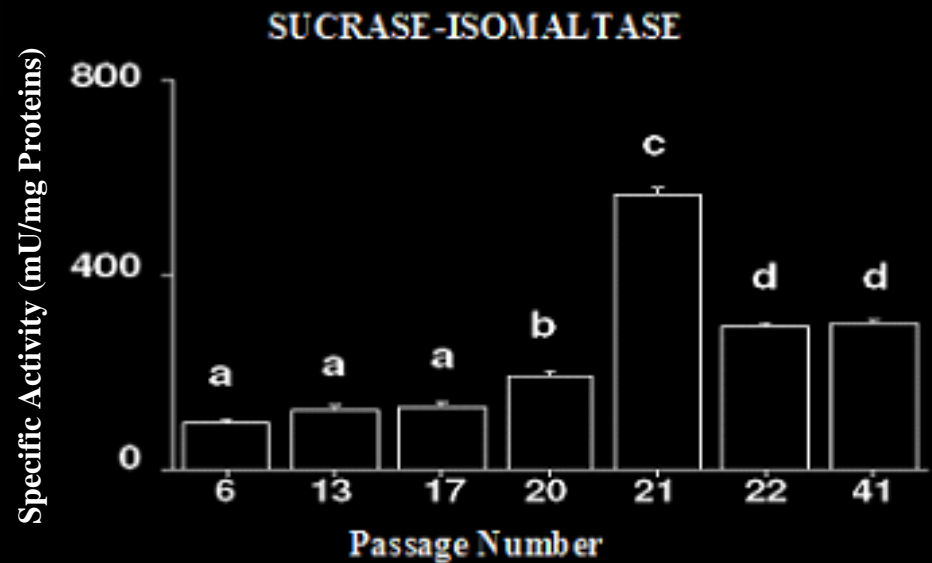
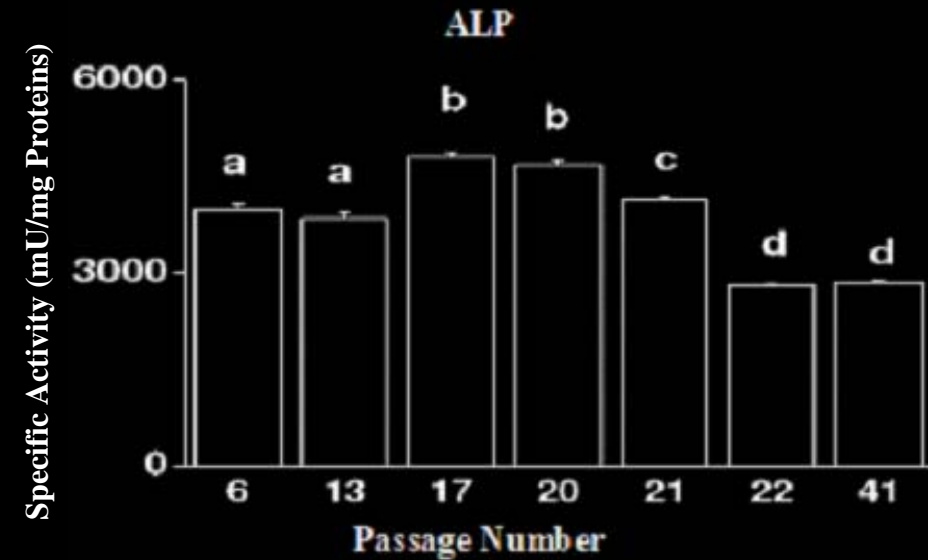


D

40th Passage 20000x



FUNCTIONAL EVALUATION OF Caco-2 CELL DIFFERENTIATION





Caco-2 CELL DIFFERENTIATION: A NEW METHOD



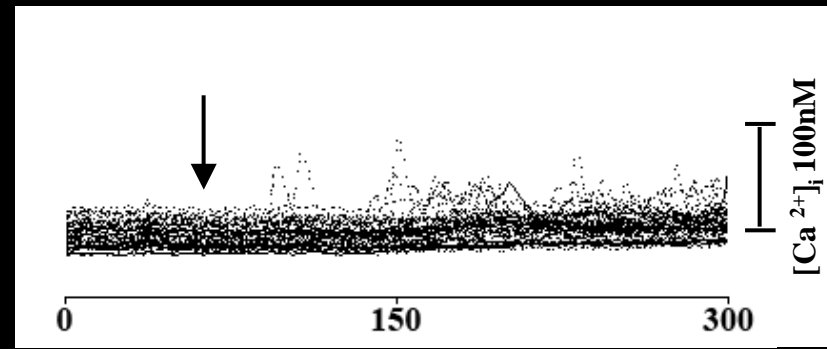
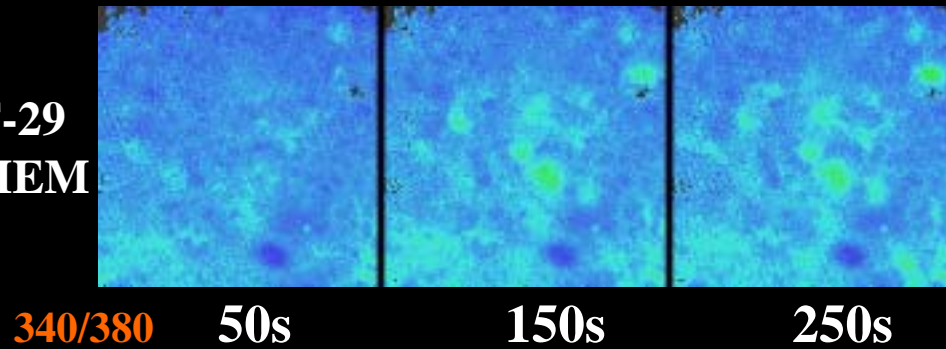
**OUR NEW METHOD OF DIFFERENTIATION FOR
Caco-2 CELLS IS EFFECTIVE IN REPRODUCING
THE MORPHOLOGY AND THE FUNCTIONALITY
OF THE INTESTINAL EPITHELIUM**



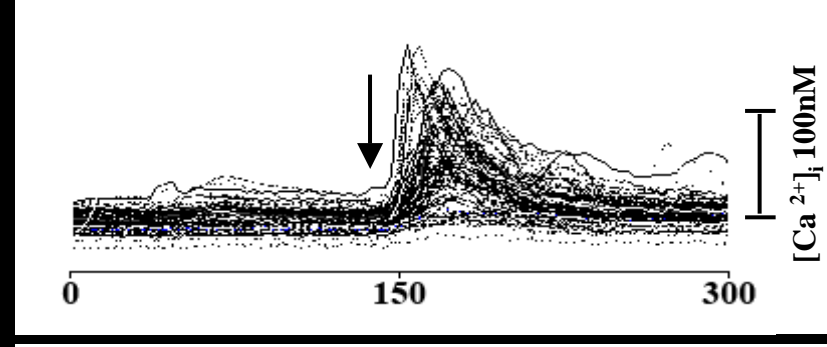
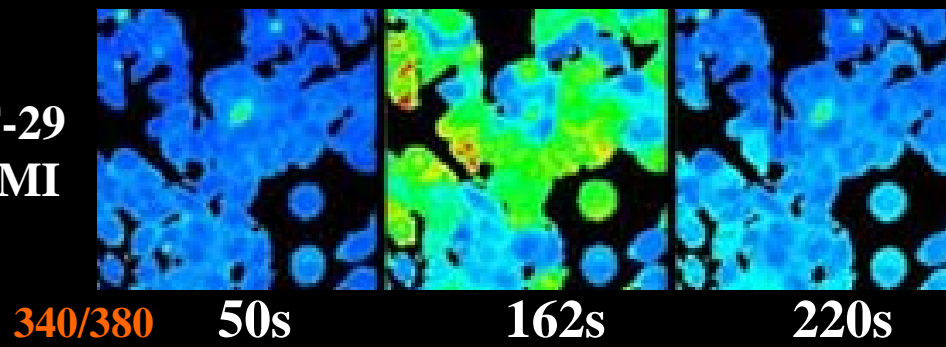
EFFECT OF CPP ADMINISTRATION ON $[Ca^{2+}]_i$



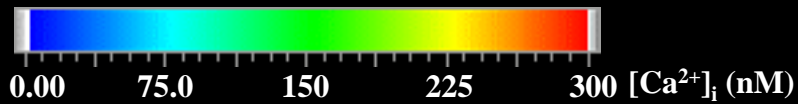
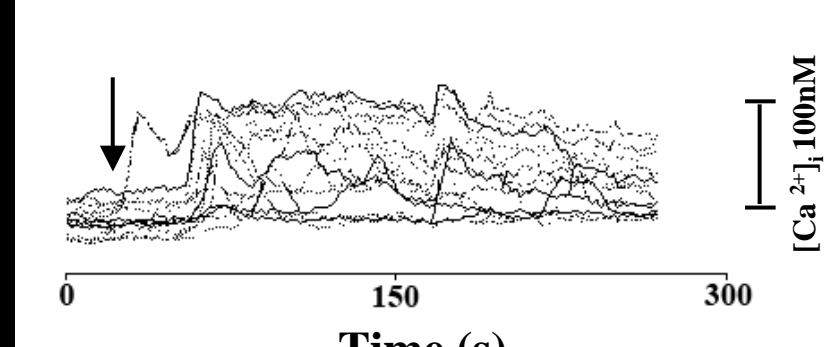
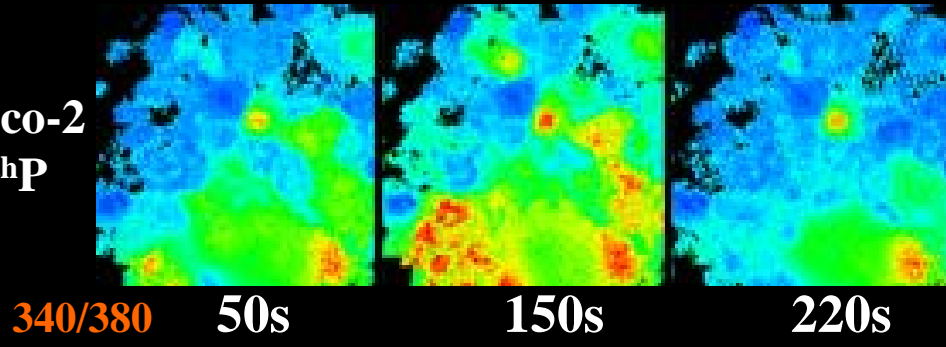
HT-29
DMEM



HT-29
RPMI



Caco-2
18thP



Time (s)



EFFECT OF CPP ADMINISTRATION ON $[Ca^{2+}]_i$



CPP ADMINISTRATION INDUCES TRANSIENT $[Ca^{2+}]_i$ INCREMENTS ONLY IN DIFFERENTIATED INTESTINAL CELLS

- HT-29 induced to differentiate (growth in RPMI-1640 medium, treatment with NaBu, gradual adaptation to Galactose)
- Caco-2 grown for above 18 passages before the post-confluence stage



AIM OF THE STUDY (2nd)



- To study the responses, to CPPs administration, of differentiated and undifferentiated intestinal cells
- **To study the effects of 1,25-(OH)₂D₃ on cell differentiation and responsiveness to CPP administration**
 - ➔ **Pretreatment with 1,25(OH)₂D₃ 100nM for 48h**



1,25-(OH)₂D₃



- It is a differentiating agent for intestinal cells
 - Well defined antineoplastic activity
- It is the main regulator of intestinal calcium absorption:
 - Regulation of the transcellular calcium transport
 - Regulation of the epithelial paracellular permeability
- It is an inductor of the extracellular calcium signalling (CaSR)

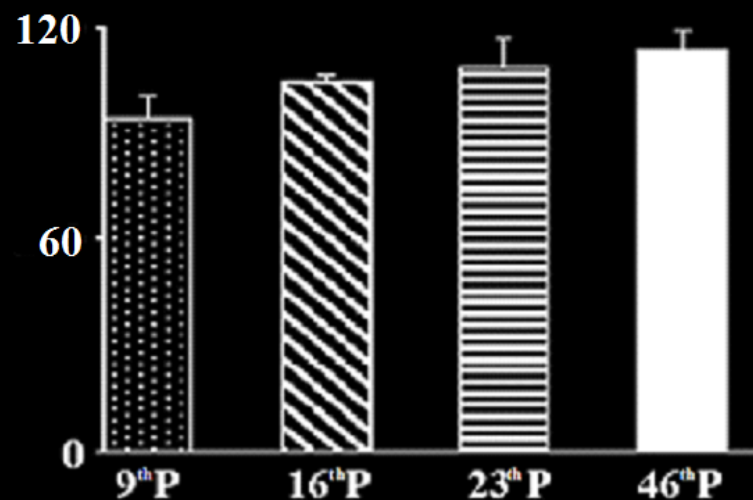
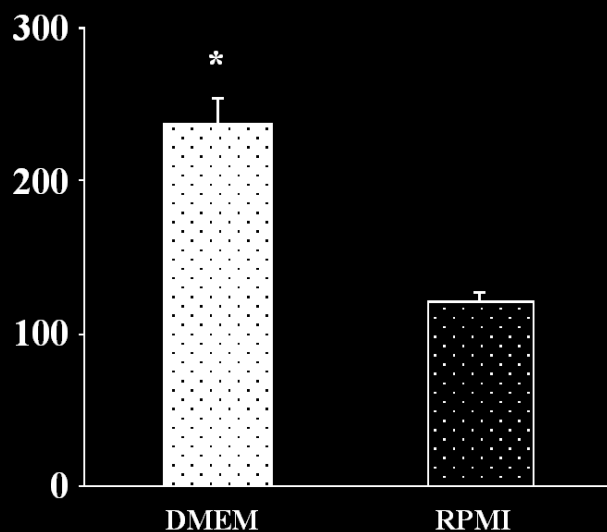
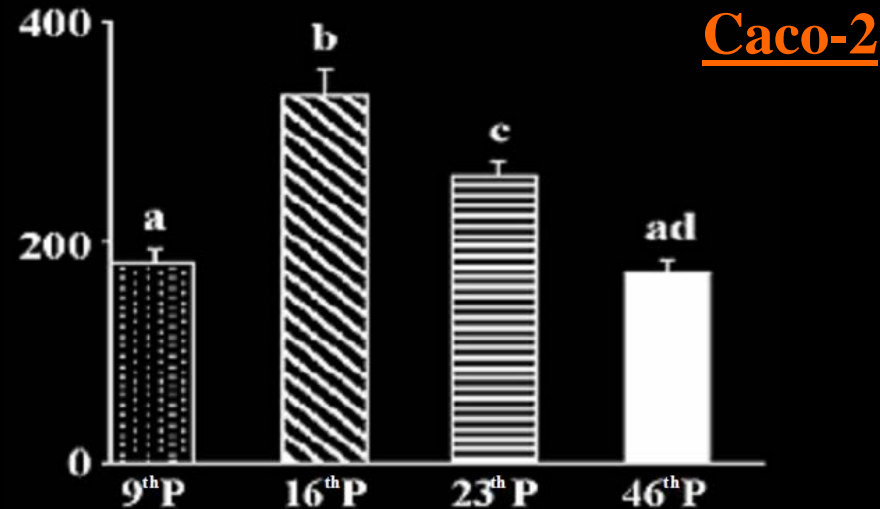
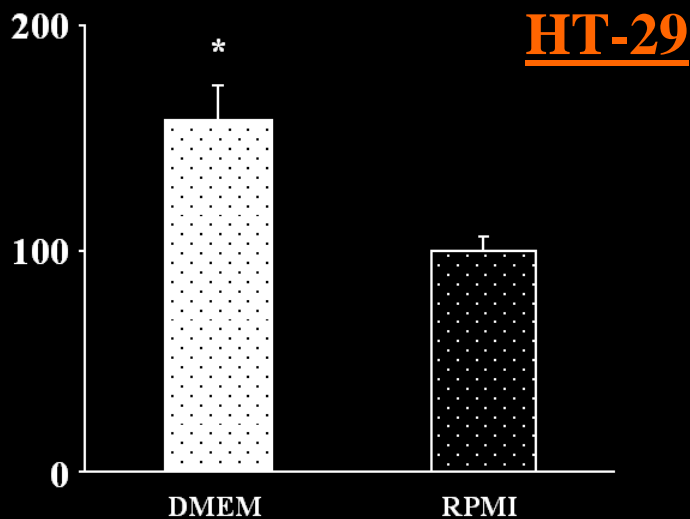


EFFECT OF 1,25-(OH)₂D₃ ON CELL DIFFERENTIATION

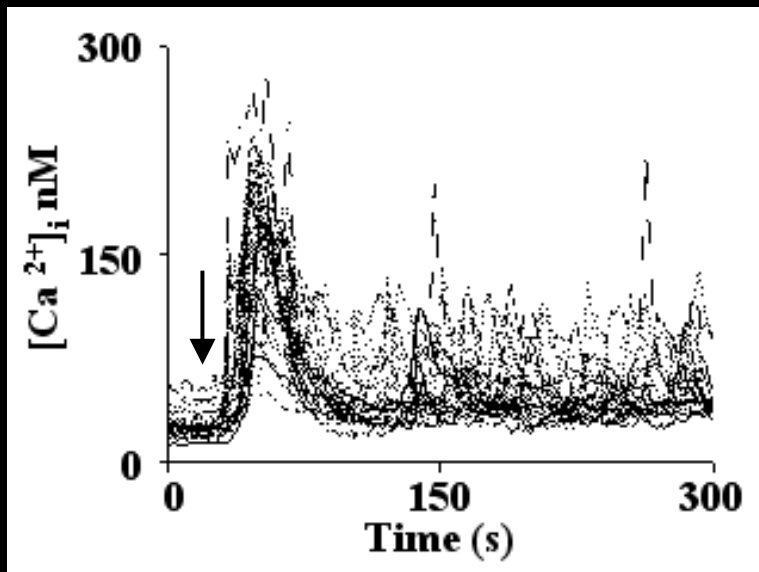


ALP Specific Activity
(% of Control)

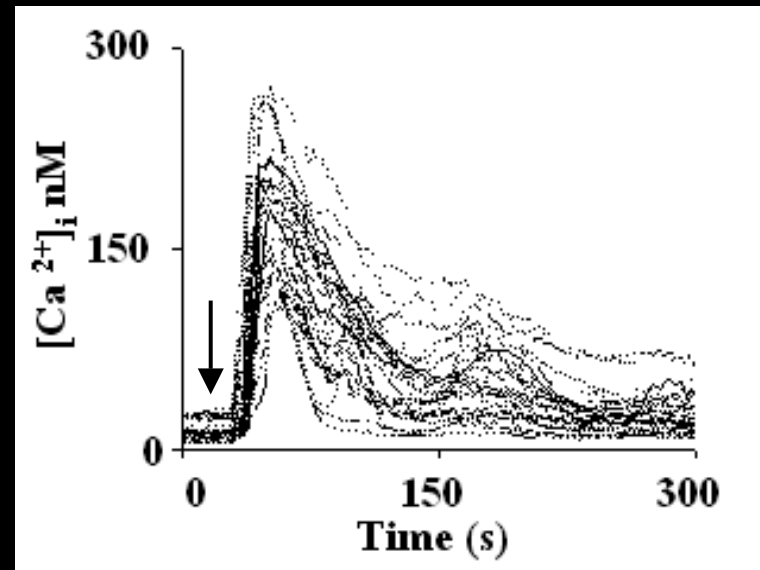
SUCRASE-ISOMALTASE
Specific Activity (% of Control)



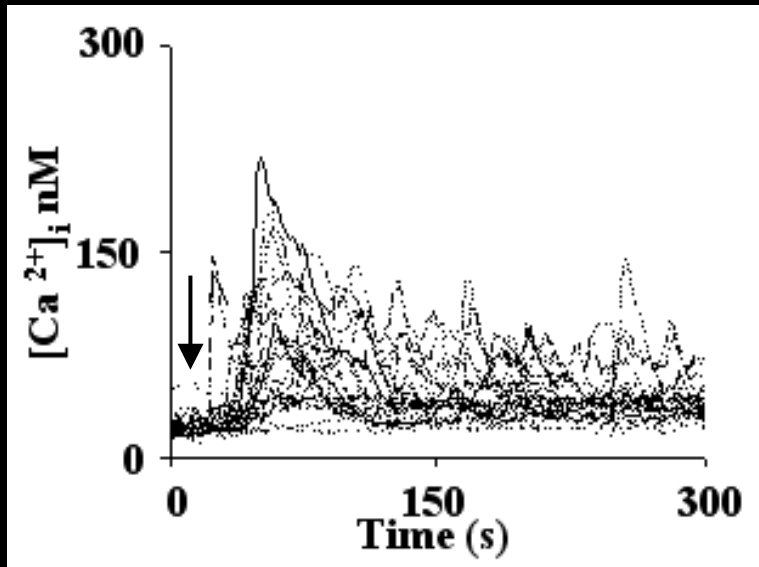
EFFECT OF CPP ADMINISTRATION ON $[Ca^{2+}]_i$ AFTER 1,25-(OH) $_2$ D $_3$ PRETREATMENT



HT-29 DMEM
+ 1,25-(OH) $_2$ D $_3$



Caco-2 12thP
+ 1,25-(OH) $_2$ D $_3$



HT-29 RPMI
+ 1,25-(OH) $_2$ D $_3$



EFFECT OF 1,25-(OH)₂D₃ PRETREATMENT



- **THE 1,25-(OH)₂D₃ IS ABLE TO INDUCE A DIFFERENTIATED PHENOTYPE IN INTESTINAL TUMOR CELLS**
 - **INCREASED SUCRASE-ISOMALTASE SPECIFIC ACTIVITY IN PRETREATED HT-29 DMEM**
 - **INCREASED ALP SPECIFIC ACTIVITY IN LOW PASSAGES PRETREATED Caco-2**
- **1,25-(OH)₂D₃ TREATMENT IS NOT EFFECTIVE ON THE FUNCTIONAL DIFFERENTIATION IN DIFFERENTIATED HT-29 AND Caco-2 CELLS**
- **UNDIFFERENTIATED CELLS PRETREATED WITH 1,25-(OH)₂D₃ RESPOND TO CPP ADMINISTRATION**



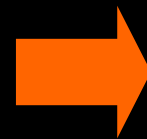
EFFECT OF 1,25-(OH)₂D₃ PRETREATMENT



- **CELLULAR RESPONSES TO CPP ADMINISTRATION IN PRETREATED CELLS DIFFER WITH RESPECT TO THEIR CONTROL FOR:**
 - **PRESENCE OF [Ca²⁺]_i OSCILLATIONS PERSISTING ALL OVER THE EXPERIMENT IN HT-29 CELL LINE**
 - **BIPHASIC SPIKES IN Caco-2 CELL LINE**

CONCLUSIONS

To enhance the dairy calcium fraction ready to be taken up by intestinal cells



CPPs



Usefull tools for the study of the interaction between nutrients and the phenomenon underlying the intestinal calcium absorption processes

→ USE AS FUNCTIONAL FOODS OR NUTRACEUTICS



CONCLUSIONS

DAIRY FOOD COMPONENTS THAT MAY BE PROTECTIVE AGAINST COLON CANCER

Calcium

Vitamin D

Conjugated linoleic acid

Sphingolipidis

Butyric acid

Bacterial cultures

From: *Studies of calcium in food supplements in humans*; P.R. Holt
Ann. NY Acad. Sci. 1999, 889: 128-137.



FUTURE PERSPECTIVES



- **EVALUATION OF THE EFFECTS OF A LONG TERM TREATMENT WITH CPPs ON INTRACELLULAR CALCIUM SIGNALLING**
- **TO TEST THE EFFECTS OF A LONG TERM TREATMENT WITH CPPs ON:**
 - **PROLIFERATION**
 - **DIFFERENTIATION**
 - **APOPTOSIS**
- **EVALUATION OF THE INTERPLAY OF CPPs AND 1,25-(OH)₂D₃ IN THESE PHYSIOLOGIC PROCESSES**