

SCUOLA DI DOTTORATO DI RICERCA IN SCIENZE BIOCHIMICHE, NUTRIZIONE 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**

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**Dott. Giovanni Lombardi**

**Materiale 07244**



# CASEINPHOSHOPEPTIDES



$\alpha_{s1}$ -Casein

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

$\alpha_{s2}$ -Casein

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

$\beta$ -Casein

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



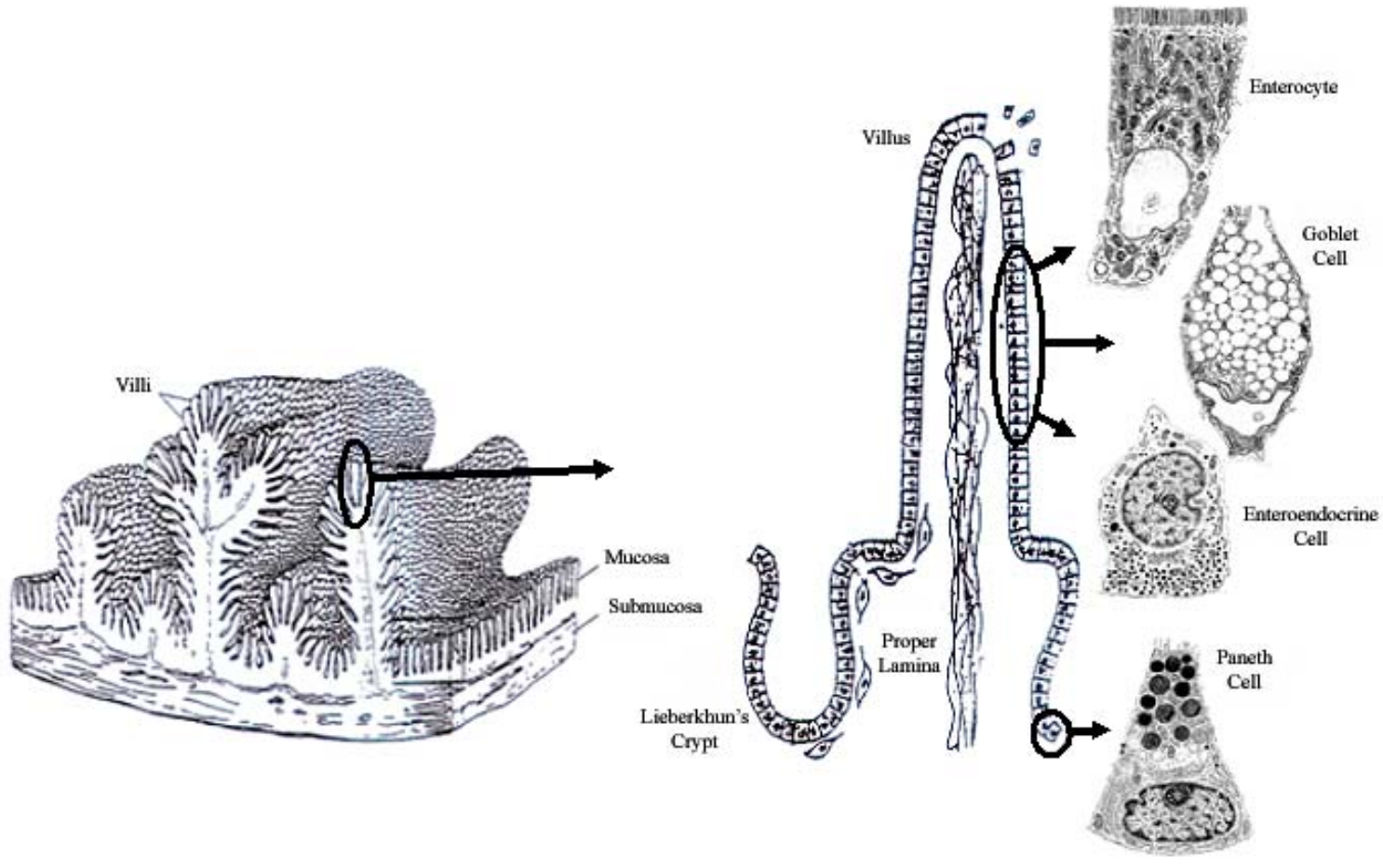
# AIM OF THE STUDY (1<sup>th</sup>)

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

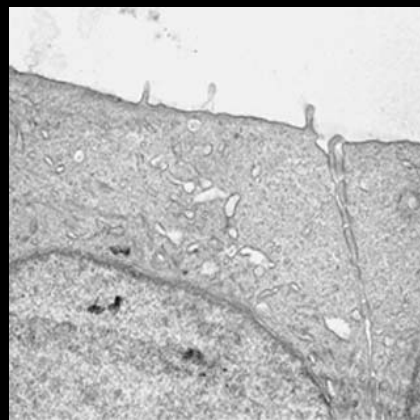
# THE INTESTINAL MUCOSA AND INTESTINAL CELL LINES



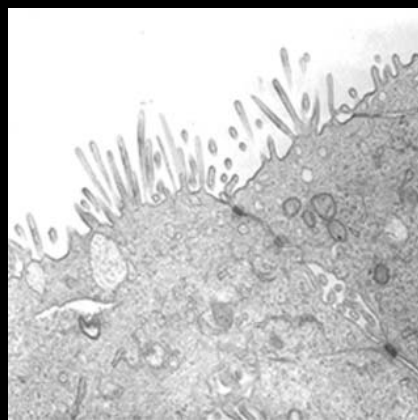
➔ HT-29 human adenocarcinoma cell line

➔ Caco-2 human adenocarcinoma cell line

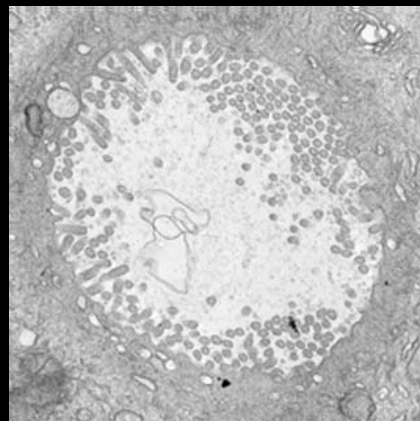
# EVALUATION OF HT-29 CELL DIFFERENTIATIONS



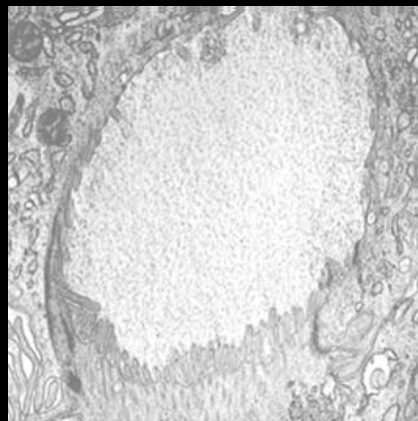
**HT-29 DMEM**  
**10000x**



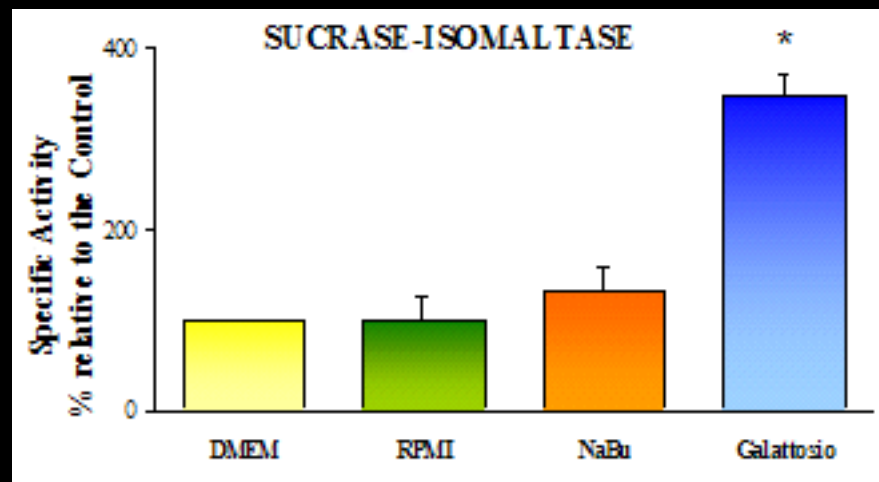
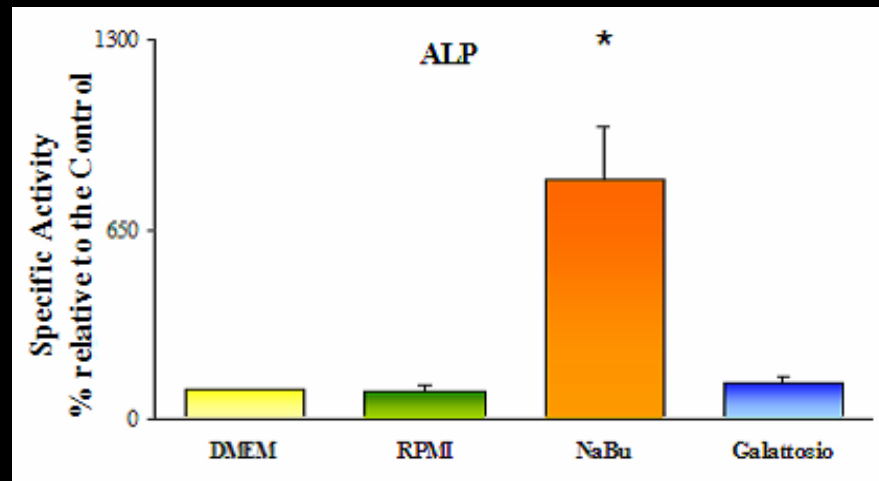
**HT-29 RPMI**  
**10000x**



**HT-29 NaBu**  
**10000x**

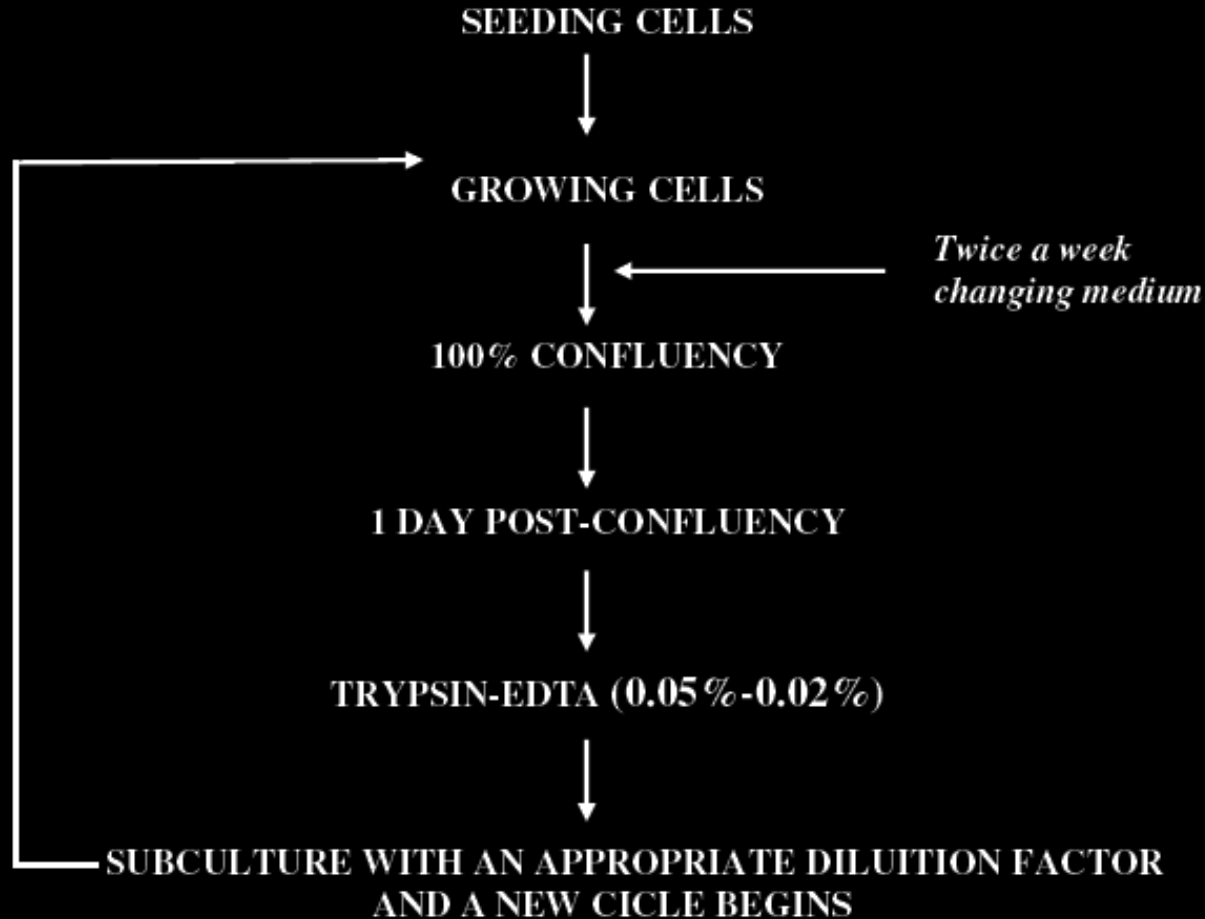


**HT-29 Gala**  
**14000x**



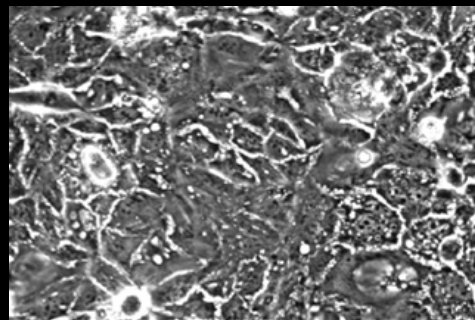


# Caco-2 CELL DIFFERENTIATION: A NEW METHOD

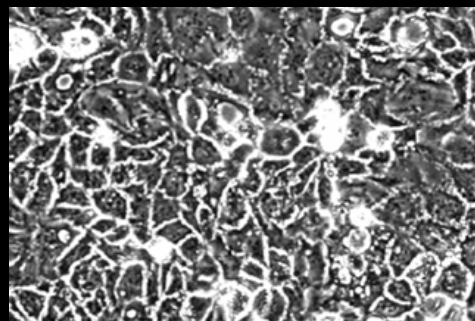




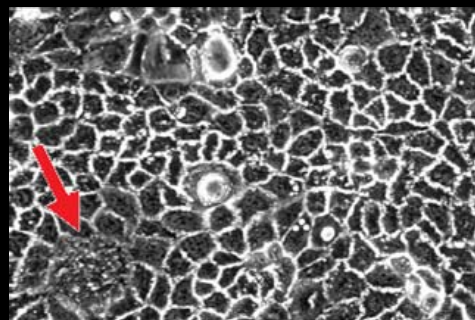
# MORPHOLOGICAL EVALUATION OF Caco-2 CELL DIFFERENTIATION



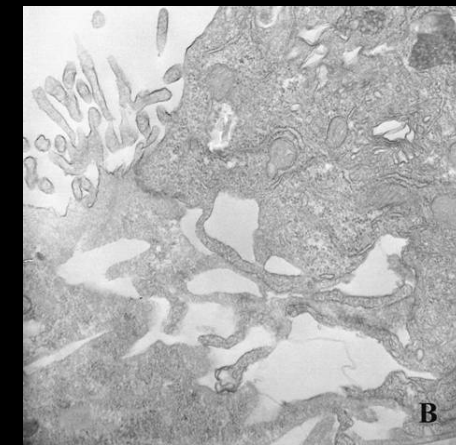
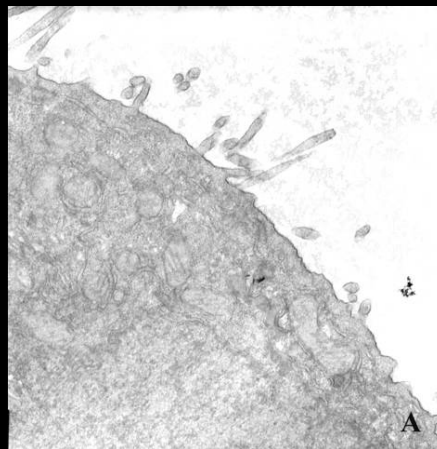
Low Passages  
(6<sup>th</sup>P)



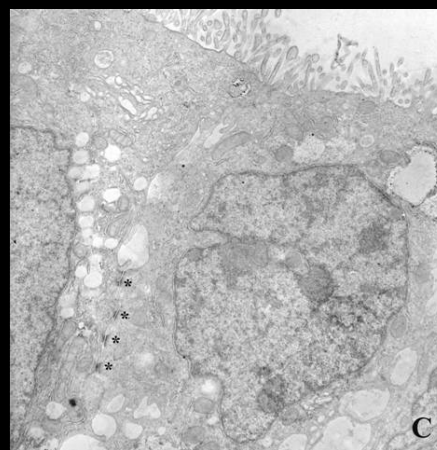
Intermediate  
Passages (24<sup>th</sup>P)



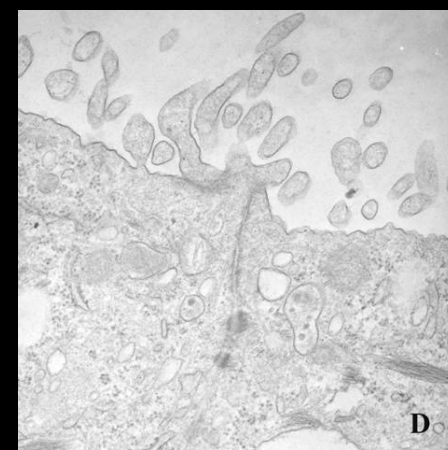
High Passages  
(41<sup>th</sup>P)



11<sup>th</sup> Passage 14000x



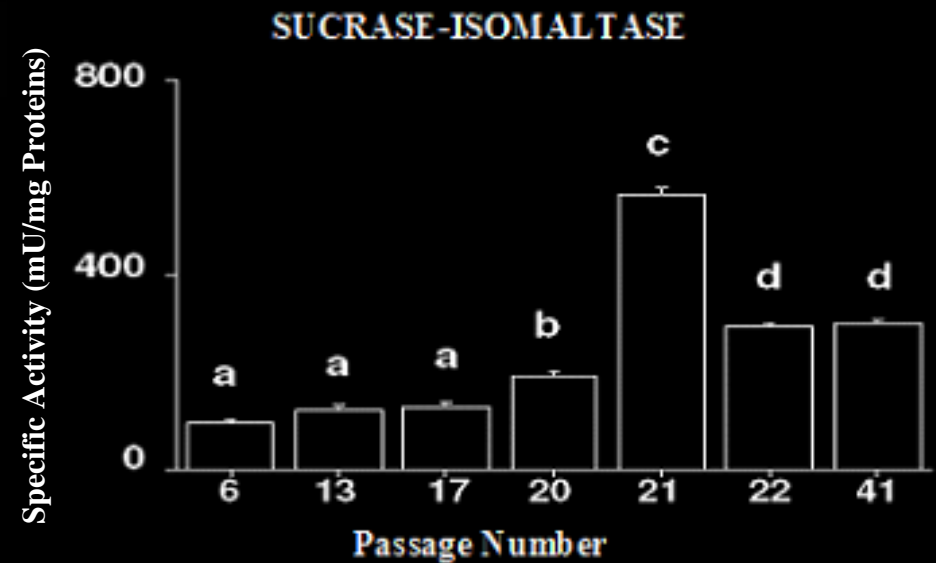
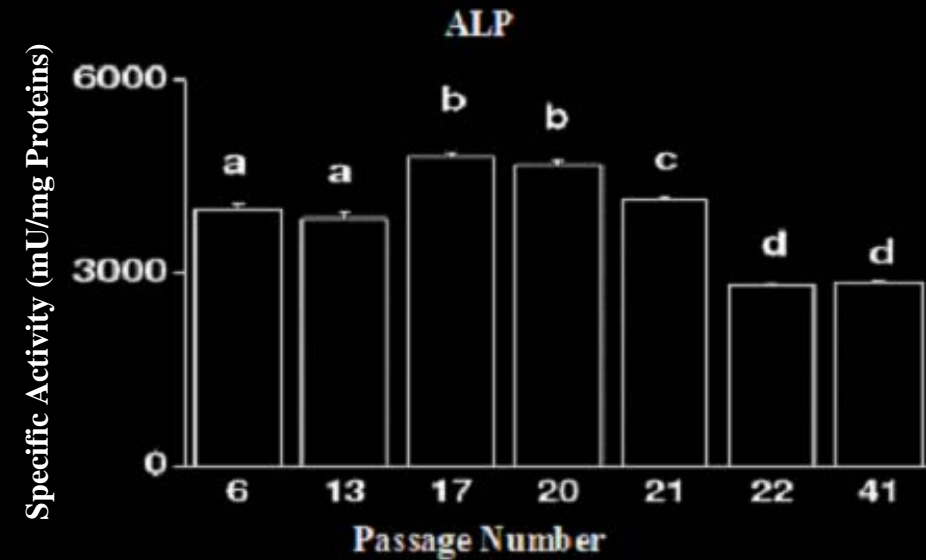
18<sup>th</sup> Passage 4800x



40<sup>th</sup> Passage 20000x



# FUNCTIONAL EVALUATION OF Caco-2 CELL DIFFERENTIATION







# **Caco-2 CELL DIFFERENTIATION: A NEW METHOD**

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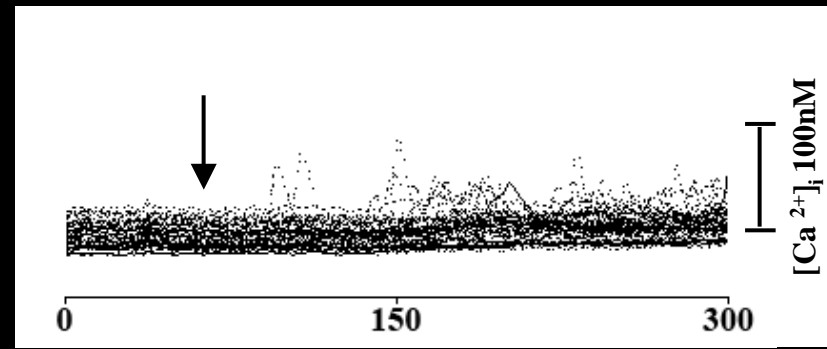
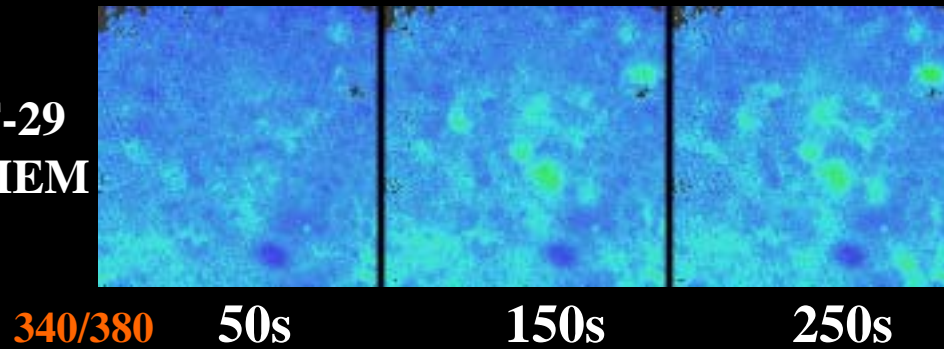
**OUR NEW METHOD OF DIFFERENTIATION FOR  
Caco-2 CELLS IS EFFECTIVE IN REPRODUCING  
THE MORPHOLOGY AND THE FUNCTIONALITY  
OF THE INTESTINAL EPITHELIUM**



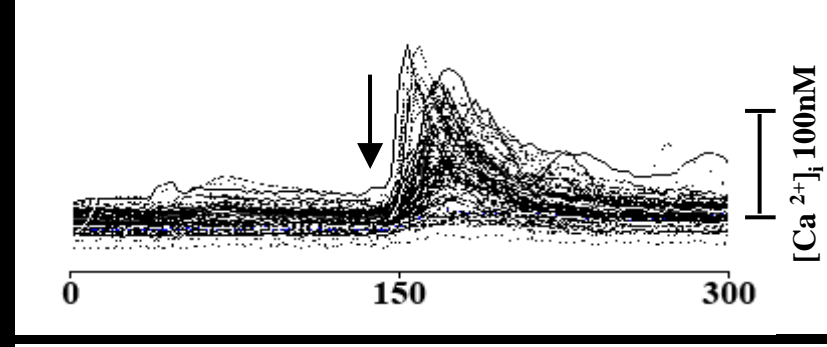
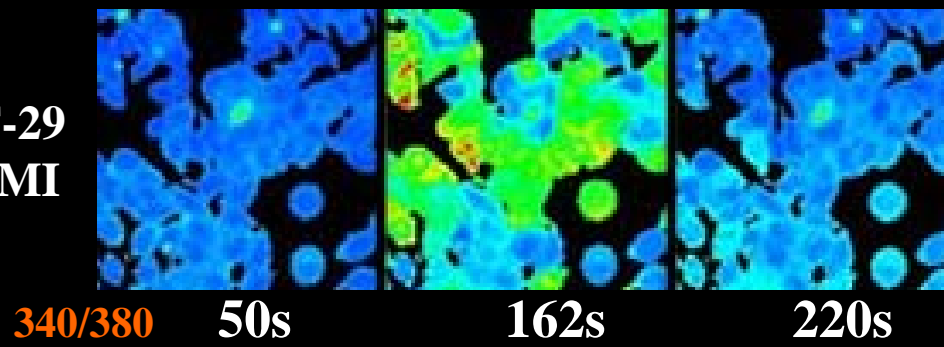
# EFFECT OF CPP ADMINISTRATION ON $[Ca^{2+}]_i$



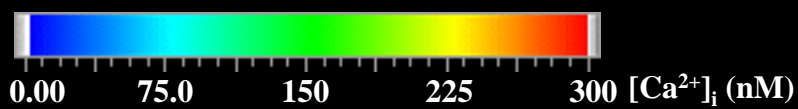
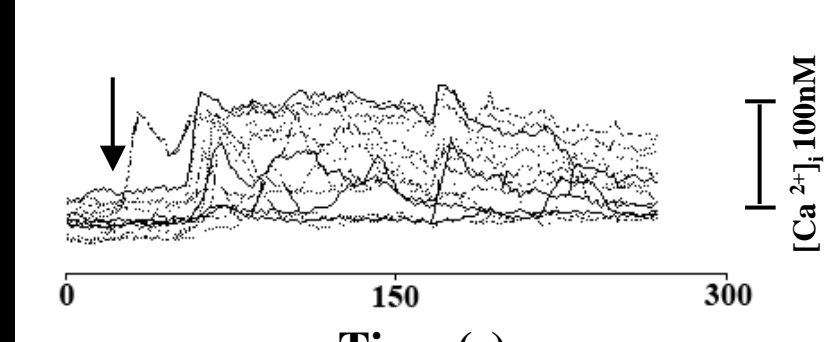
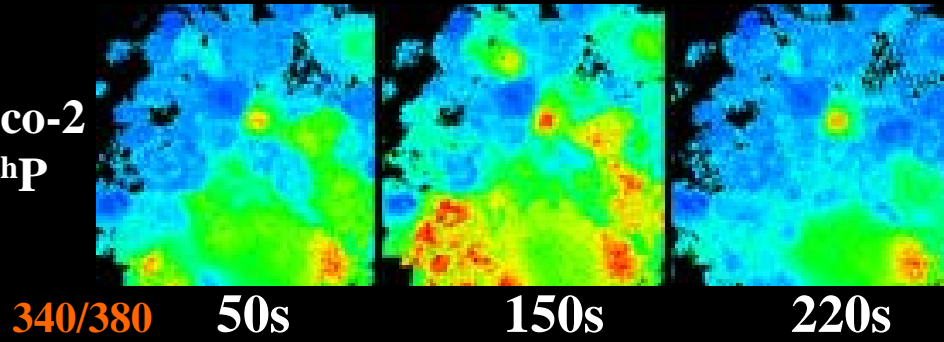
HT-29  
DMEM



HT-29  
RPMI



Caco-2  
18<sup>th</sup>P



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 (2<sup>nd</sup>)



- To study the responses, to CPPs administration, of differentiated and undifferentiated intestinal cells
- **To study the effects of 1,25-(OH)<sub>2</sub>D<sub>3</sub> on cell differentiation and responsiveness to CPP administration**
  - ➔ **Pretreatment with 1,25(OH)<sub>2</sub>D<sub>3</sub> 100nM for 48h**



# 1,25-(OH)<sub>2</sub>D<sub>3</sub>



- 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)

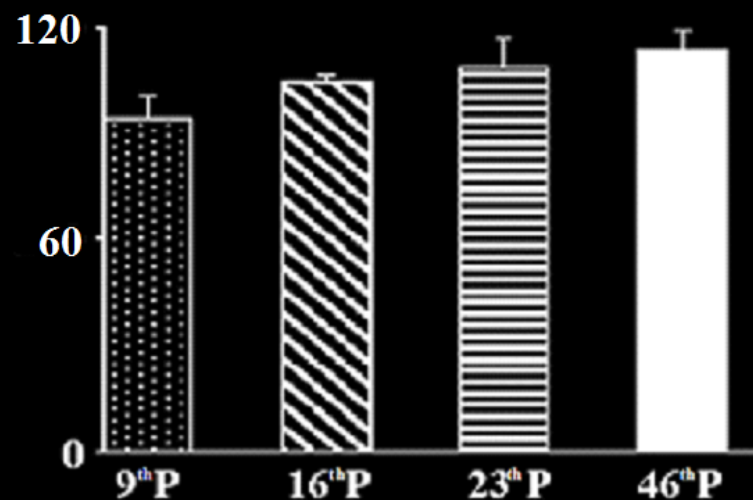
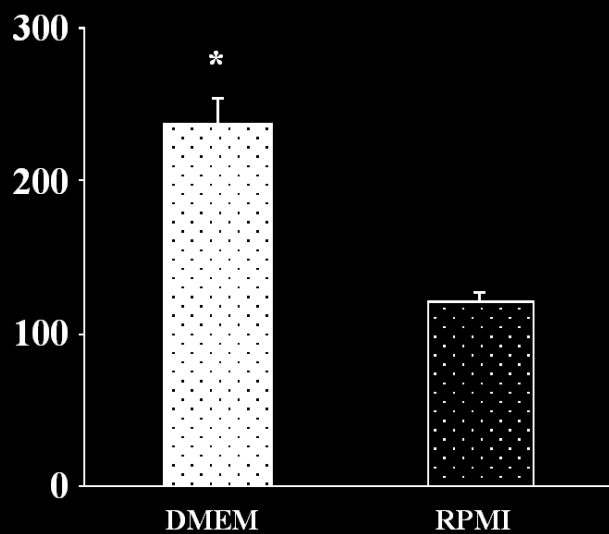
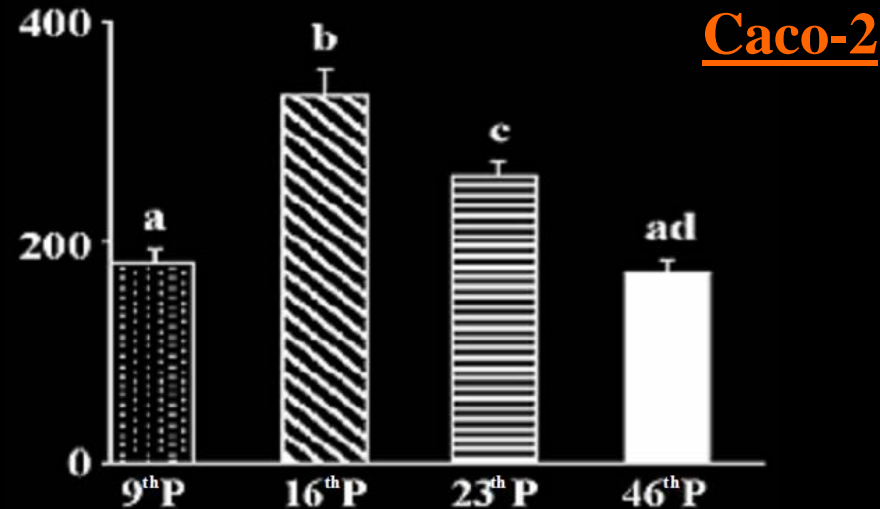
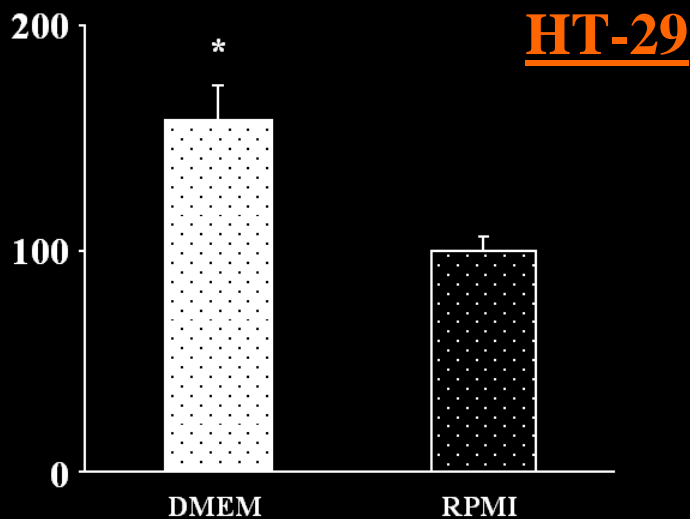


# EFFECT OF 1,25-(OH)<sub>2</sub>D<sub>3</sub> ON CELL DIFFERENTIATION

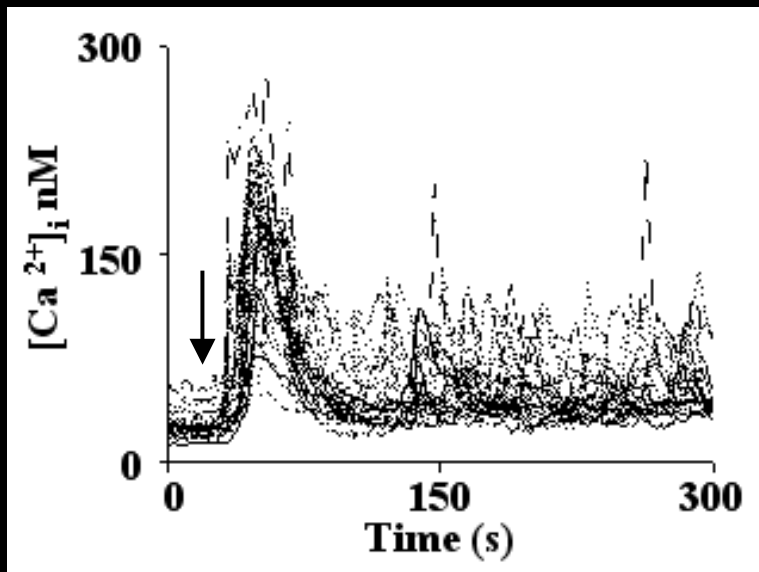


ALP Specific Activity  
(% of Control)

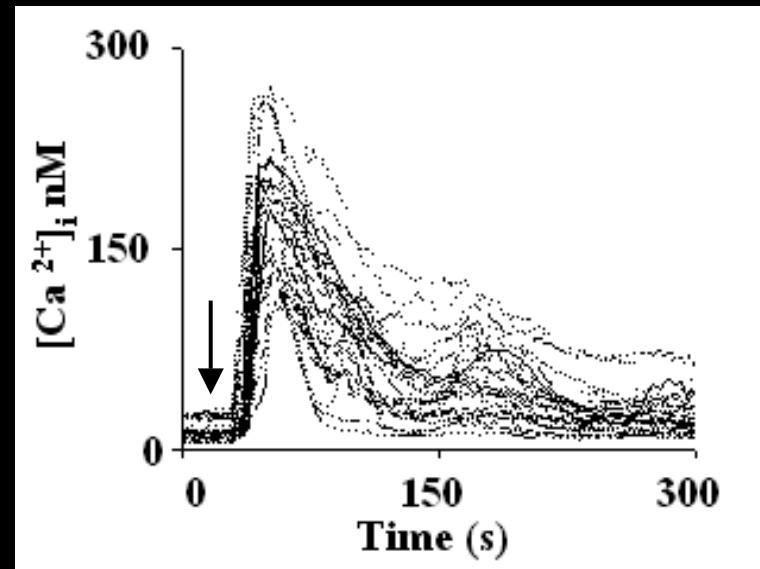
SUCRASE-ISOMALTASE  
Specific Activity (% of Control)



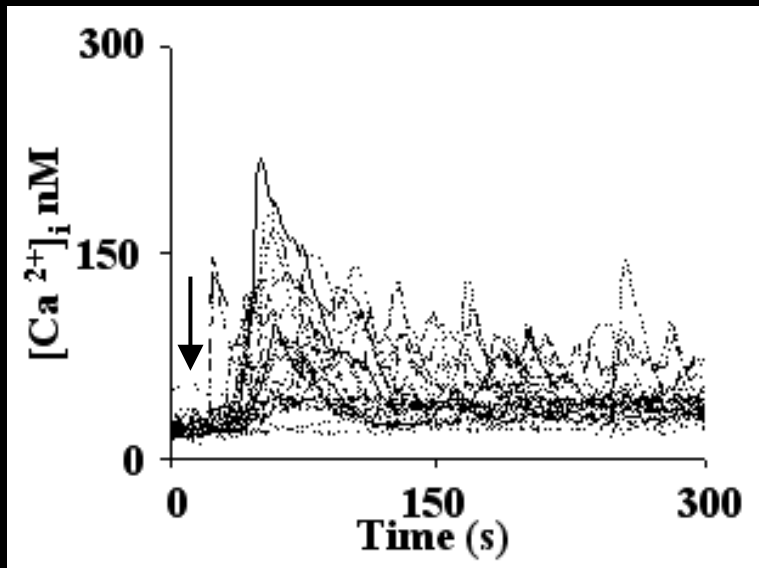
# EFFECT OF CPP ADMINISTRATION ON $[Ca^{2+}]_i$ AFTER 1,25-(OH) $_2$ D $_3$ PRETREATMENT



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



Caco-2 12<sup>th</sup>P  
+ 1,25-(OH) $_2$ D $_3$



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



# EFFECT OF 1,25-(OH)<sub>2</sub>D<sub>3</sub> PRETREATMENT



- **THE 1,25-(OH)<sub>2</sub>D<sub>3</sub> 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)<sub>2</sub>D<sub>3</sub> TREATMENT IS NOT EFFECTIVE ON THE FUNCTIONAL DIFFERENTIATION IN DIFFERENTIATED HT-29 AND Caco-2 CELLS**
- **UNDIFFERENTIATED CELLS PRETREATED WITH 1,25-(OH)<sub>2</sub>D<sub>3</sub> RESPOND TO CPP ADMINISTRATION**





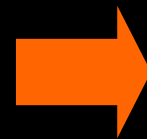
# EFFECT OF $1,25\text{-(OH)}_2\text{D}_3$ PRETREATMENT



- **CELLULAR RESPONSES TO CPP ADMINISTRATION IN PRETREATED CELLS DIFFER WITH RESPECT TO THEIR CONTROL FOR:**
  - **PRESENCE OF  $[\text{Ca}^{2+}]_i$  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

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## 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)<sub>2</sub>D<sub>3</sub> IN THESE PHYSIOLOGIC PROCESSES**