Roma, 8 e 9 Novembre 2019

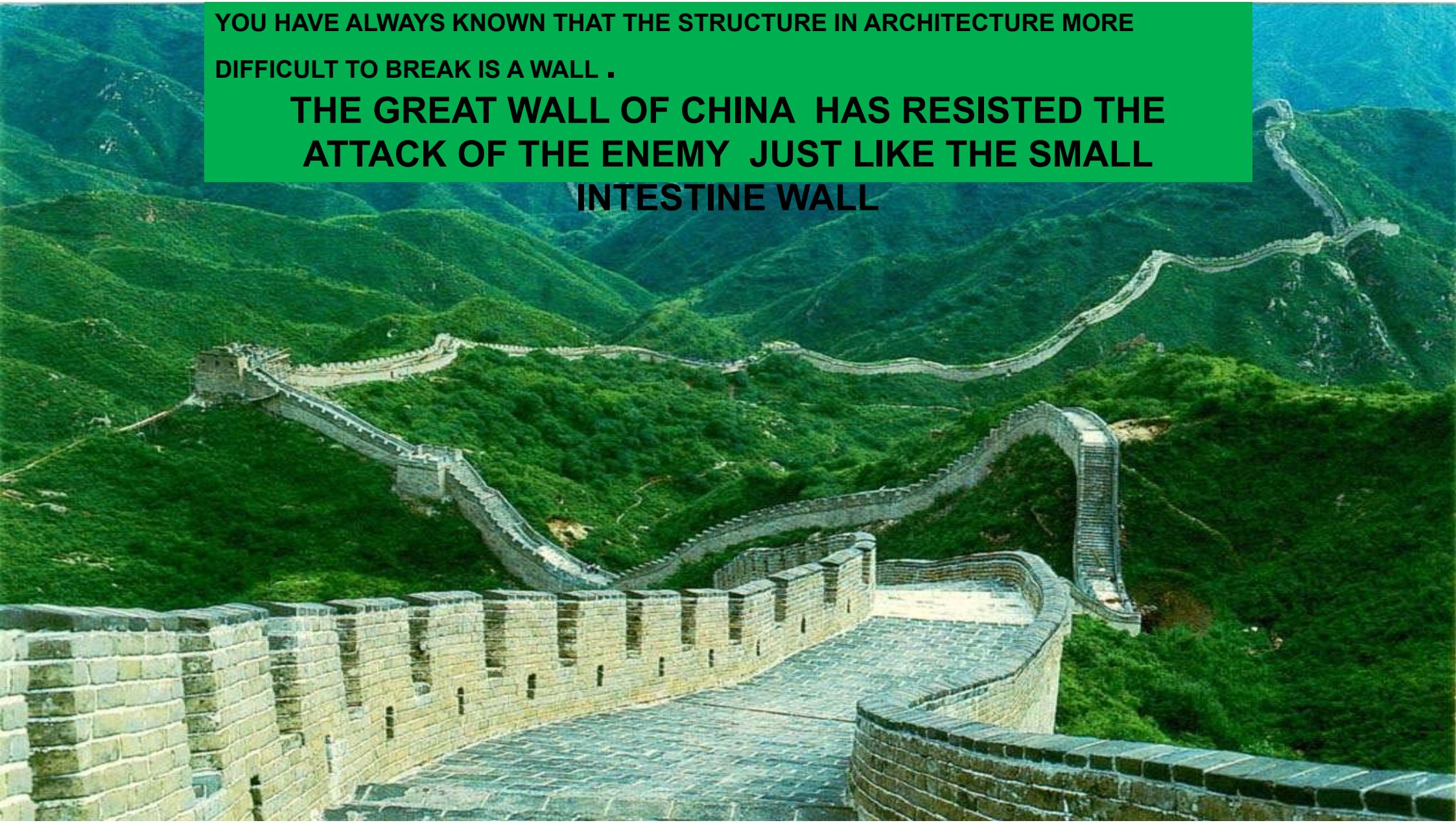
L' INTESTINO PERMEABILE: LA BARRIERA ALTERATA.

LA BARRIERA MUCOSA INTESTINALE: MORFOLOGIA E FUNZIONI NEL NORMALE, NELLE ALTERAZIONI DELLA PERMEABILITA' E NEL «LEAKY GUT»

GIOVANNI GASBARRINI

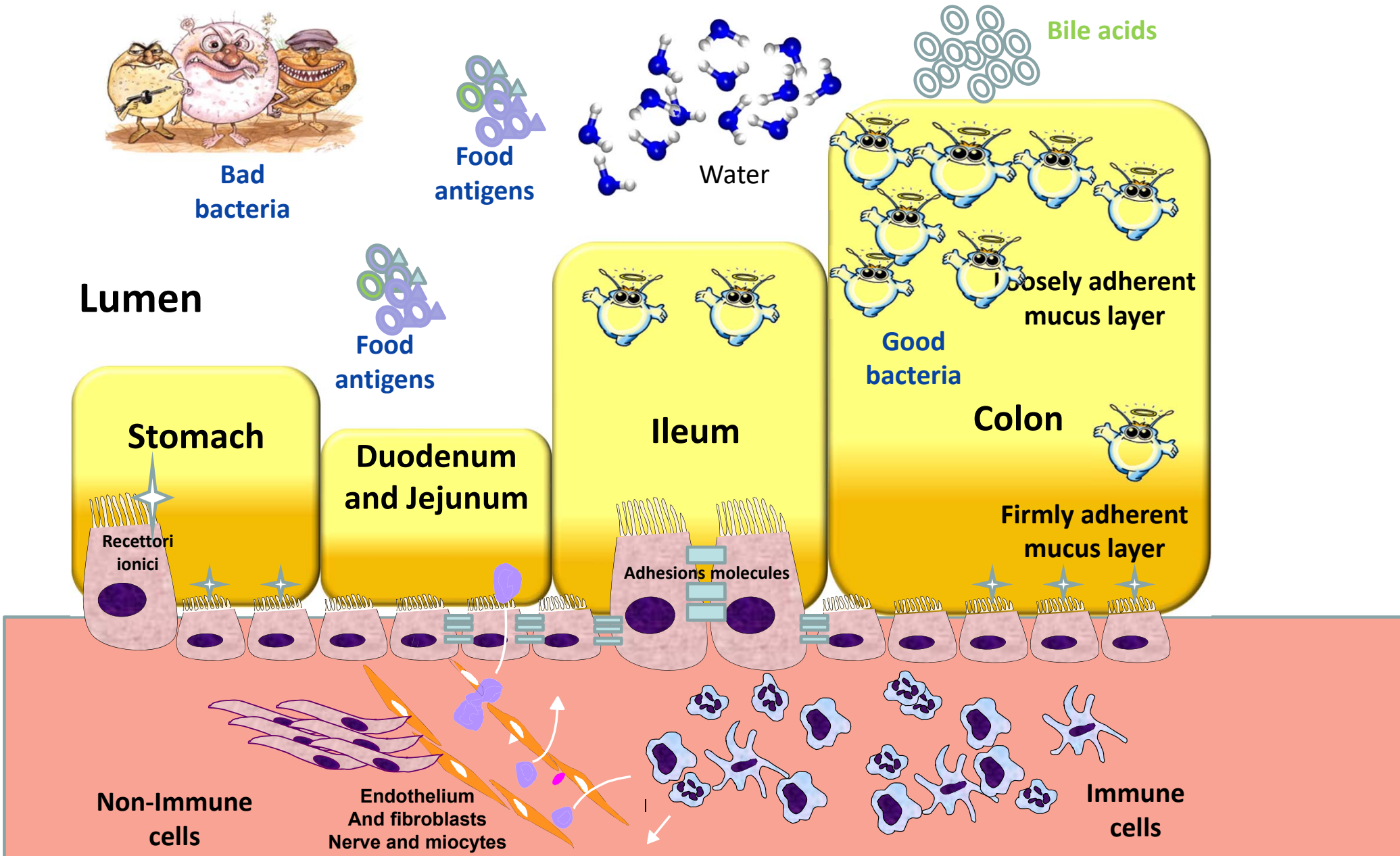
Professore Emerito di Medicina Interna, Roma

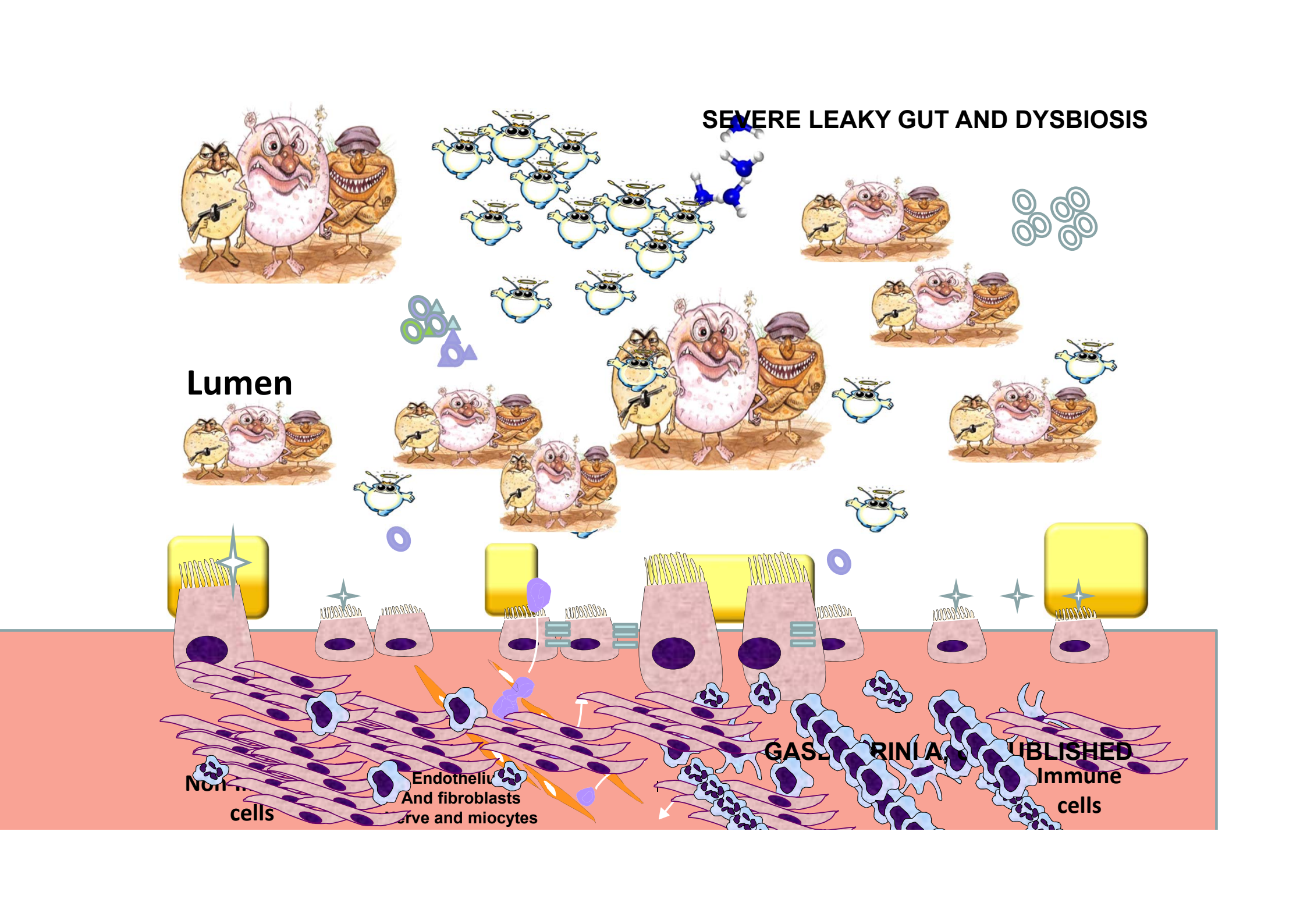
LA BARRIERA MUCOSA



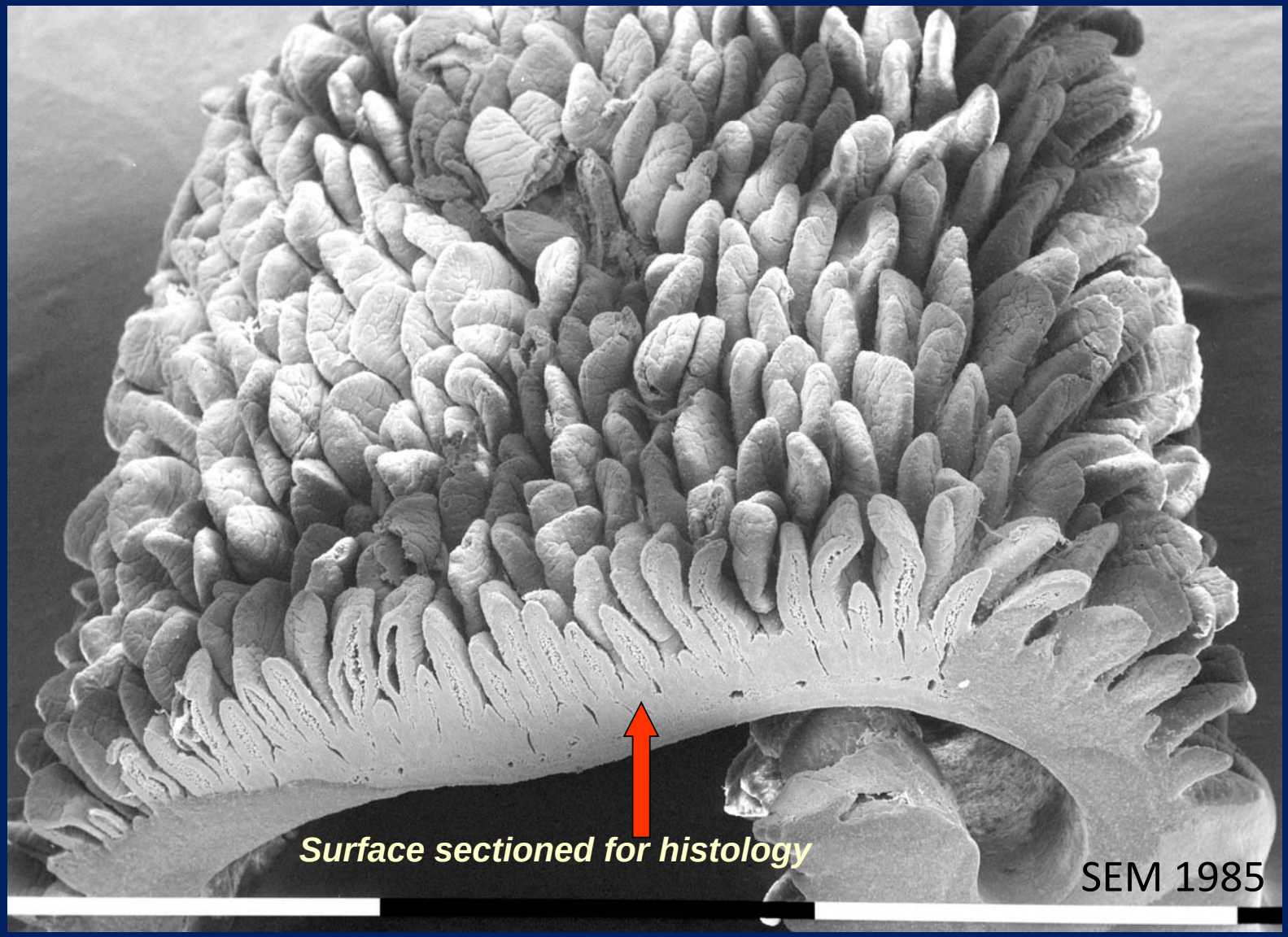
YOU HAVE ALWAYS KNOWN THAT THE STRUCTURE IN ARCHITECTURE MORE DIFFICULT TO BREAK IS A WALL .

THE GREAT WALL OF CHINA HAS RESISTED THE ATTACK OF THE ENEMY JUST LIKE THE SMALL INTESTINE WALL


Bad bacteria
Food antigens
Water
Bile acids
Lumen
Food antigens
Stomach
Duodenum and Jejunum
Ileum
Colon
Good bacteria
Loosely adherent mucus layer
Firmly adherent mucus layer
Recettori ionici
Adhesions molecules
Non-Immune cells
Endothelium And fibroblasts Nerve and miocytes
Immune cells


SEVERE LEAKY GUT AND DYSBIOSIS
Lumen
Endotheliu
And fibroblasts
rve and miocytes
cells
Immune
cells

L’ INTESTINO TENUE


Surface sectioned for histology
SEM 1985

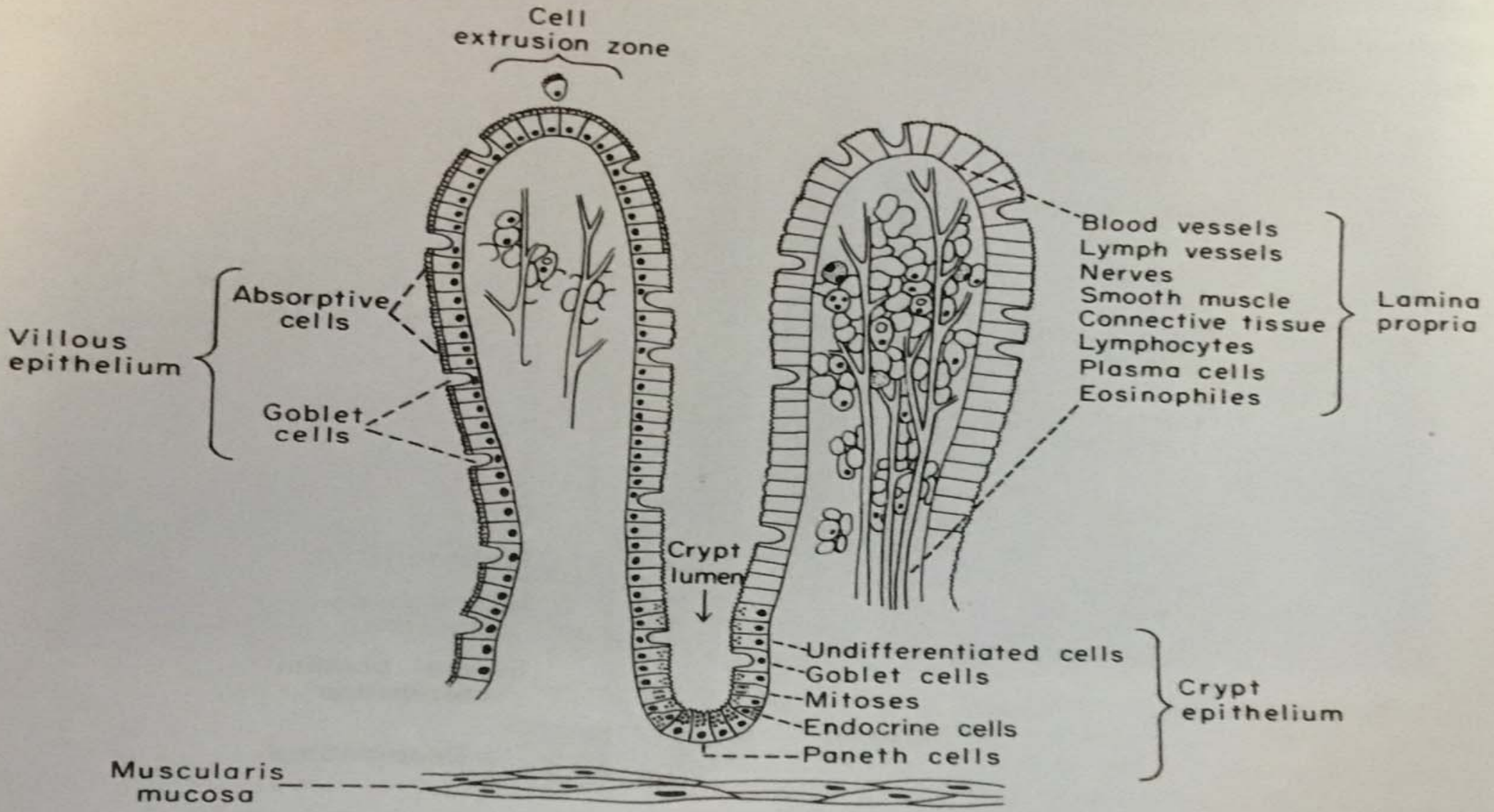


Figure 1 Schematic diagram of two sectioned villi and a crypt illustrating the histological organization of the mucosa of the small intestine.

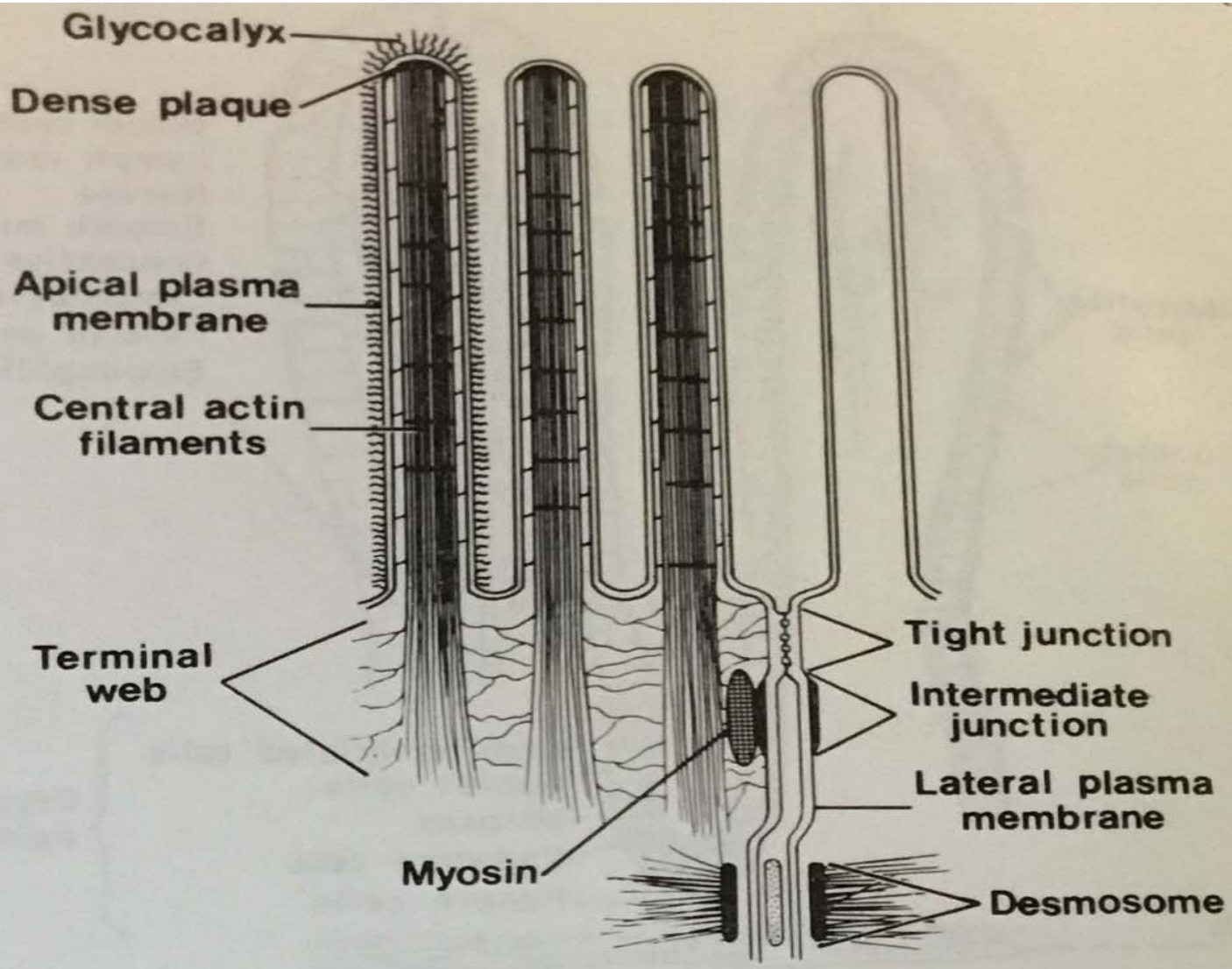


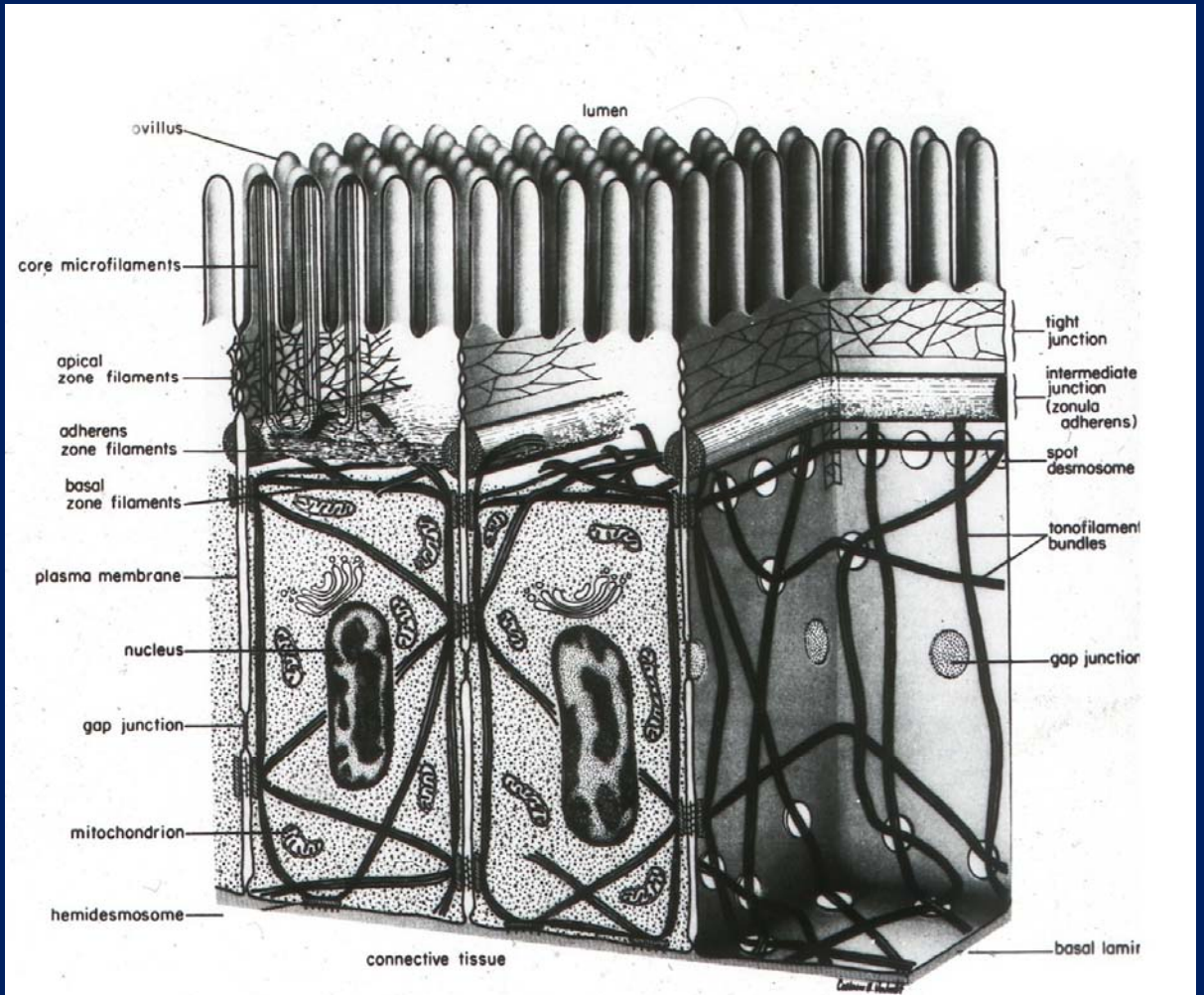
Figure 2 Schematic diagram of the structural features of the apical plasma membrane, the microvilli, the terminal web and the junctional complexes of intestinal absorptive cells.

Figure 3 Freeze-fracture replicas of the apical surface of a villus absorptive cell (left) and an undifferentiated crypt cell (right). Many more intramembrane protein particles are associated with the convex protoplasmic membrane face (P) which covers the microvillus core than with the concave external membrane face (E). P face particles are more numerous in microvillus membranes of absorptive cells (left) than in those of undifferentiated crypt cells (right). E face particle density is comparable in both sites. The upper portion of the absorptive cell tight junction with its cross-linked strands is evident at the bottom of the micrograph on the left (83,000 X).

Cytoskeleton interacts with extracellular matrix, promotes :
-enterocyte plasma membrane homeostasis,
-enterocyte migration

Tight junctions promote adhesion between the enterocytes

- Actin filaments connected to apical junctional complex allow microvilli contraction
- Desmosome tonofilaments give stability to the cellt



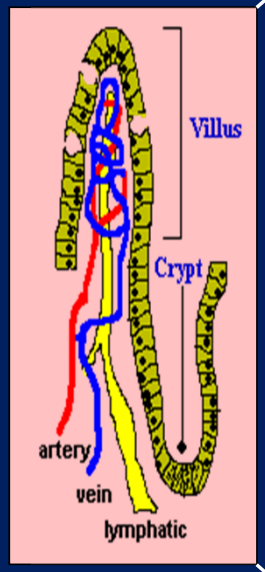
Schematic Diagram of Columnar Epithelial Cells

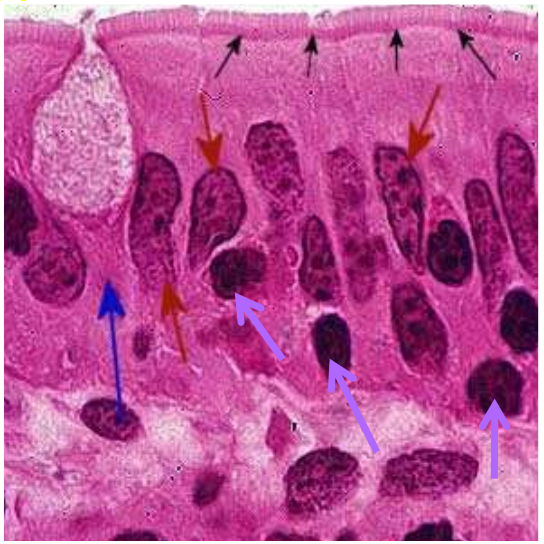
MUCOSAL BARRIER: INTEGRITY OF ENTEROCYTES AND BRUSH BORDER

GASBARRINI G. 1965

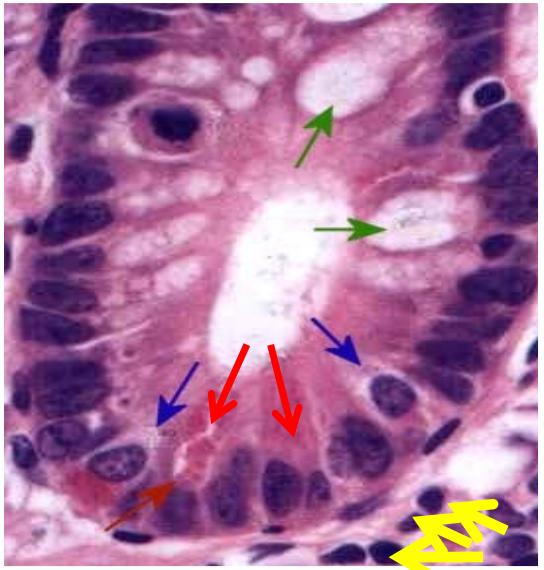
GASBARRINI G. 1966

SPECIALIZED CELLS OF VILLUS AND CRYPT



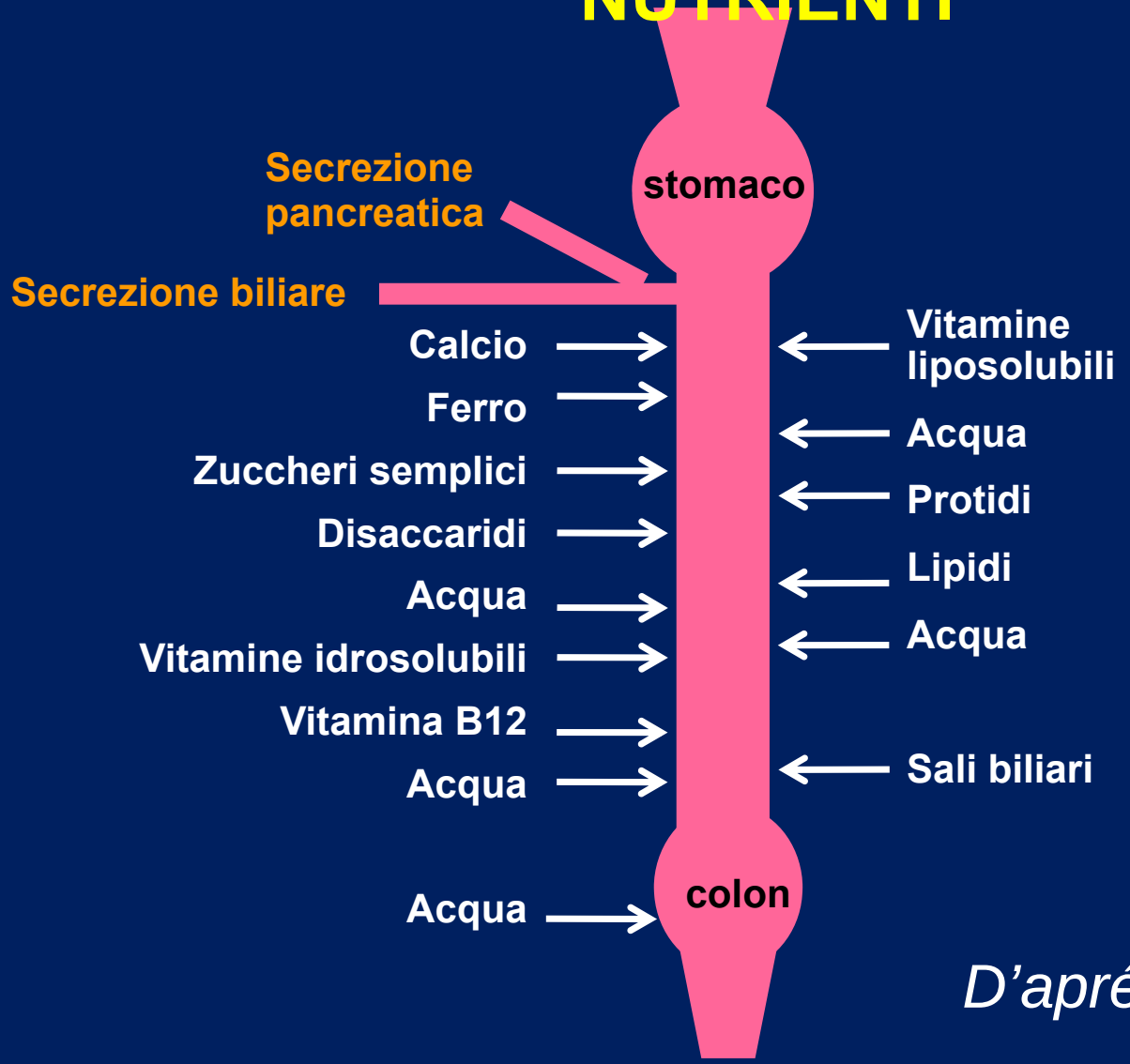


- ENTEROCYTES
- INTRAEPITHELIAL LYMPHOCYTES
- GOBLET CELLS



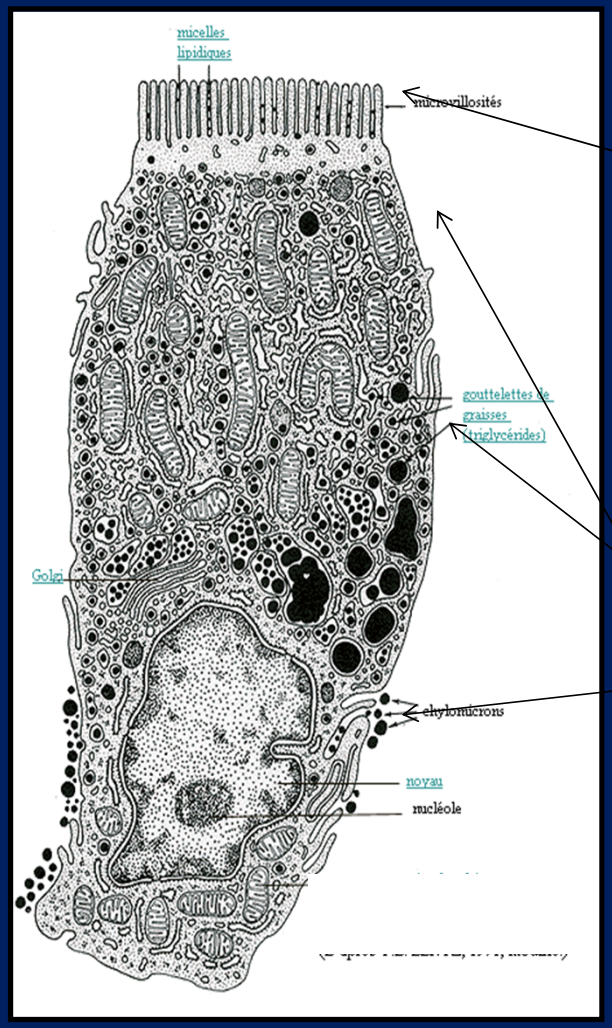
- ENDOCRINE CELLS
- GOBLET CELLS
- PANETH CELLS
- LAMINA PROPRIA LYMPHOCYTES

SEDI DELL'ASSORBIMENTO DEI NUTRIENTI

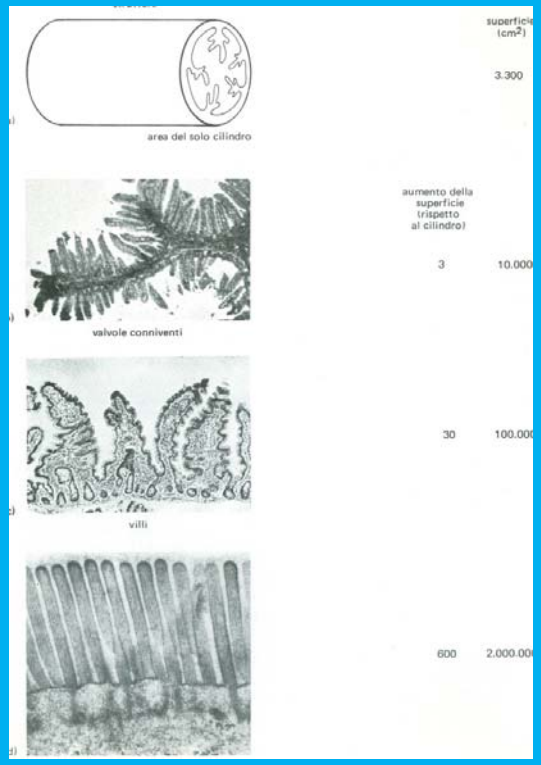


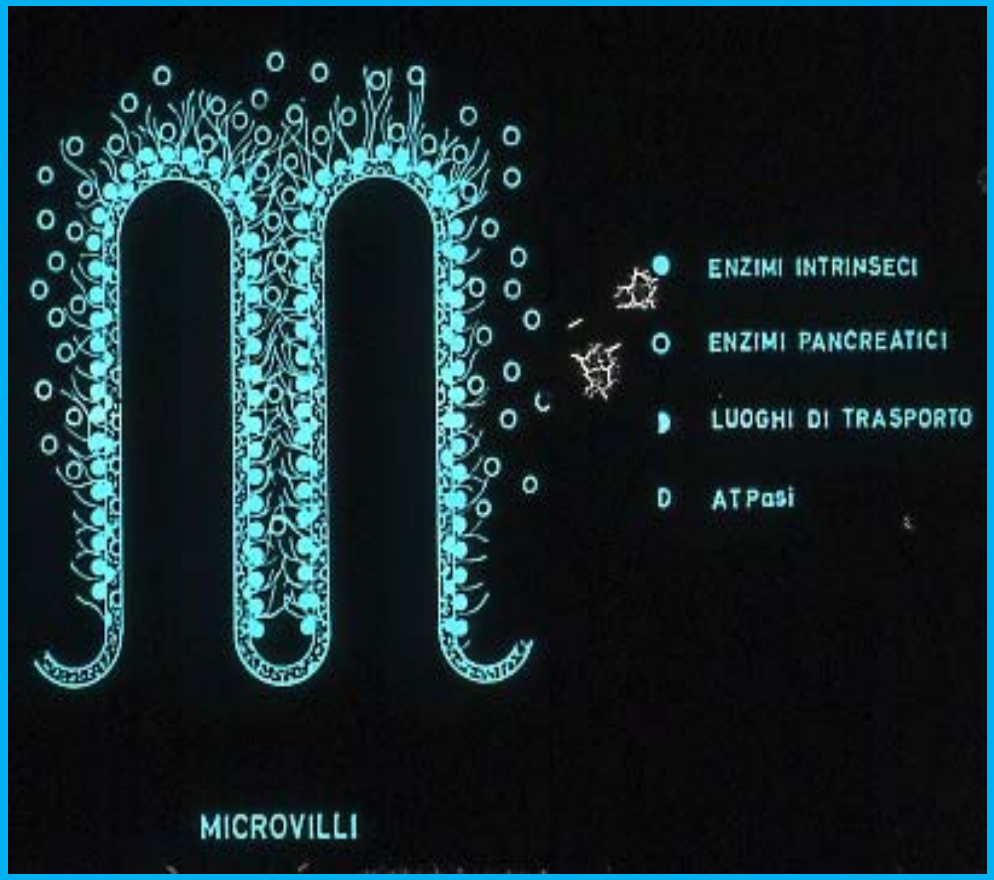
D'aprés C.C., 1968

Gli enterociti

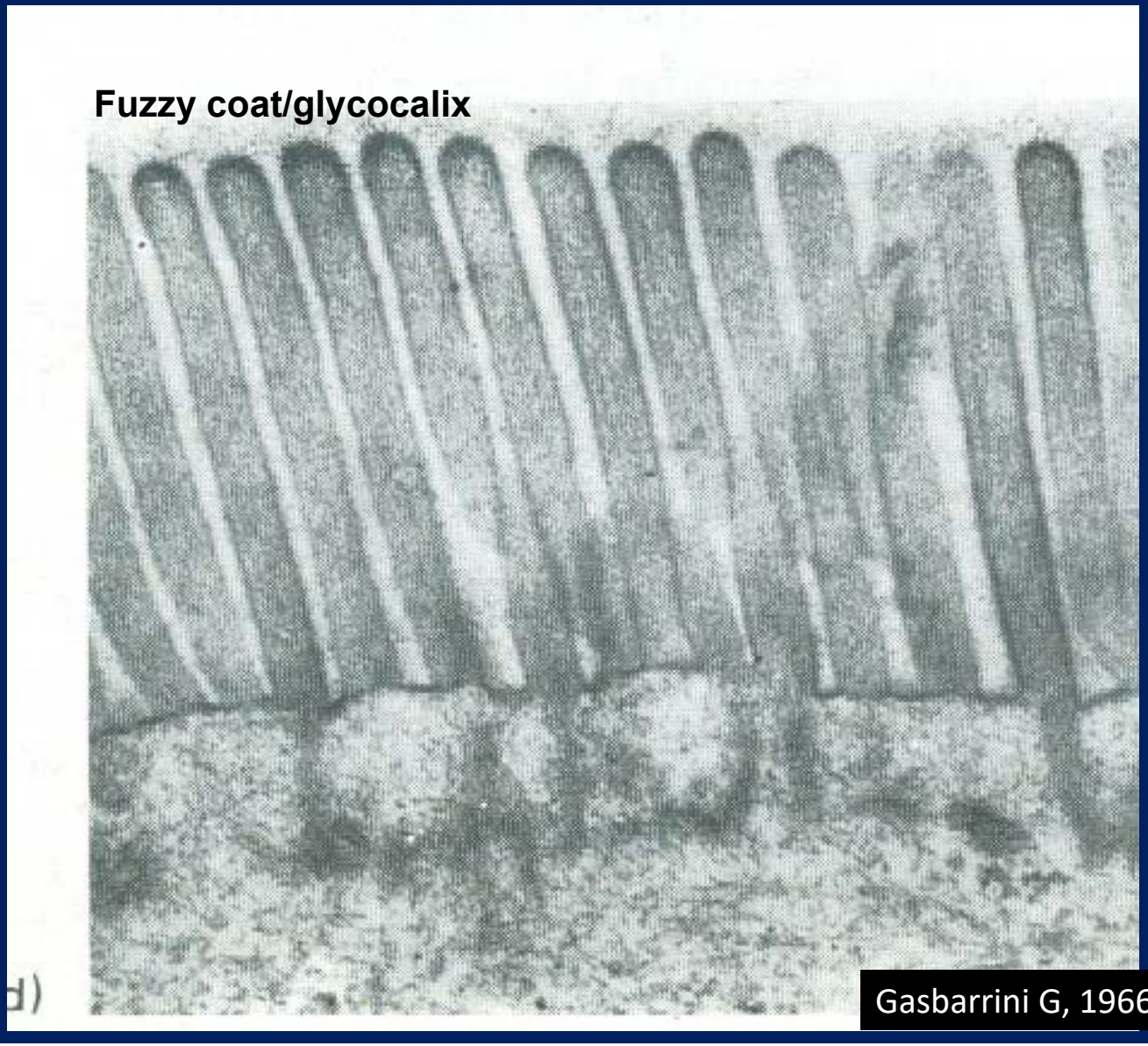


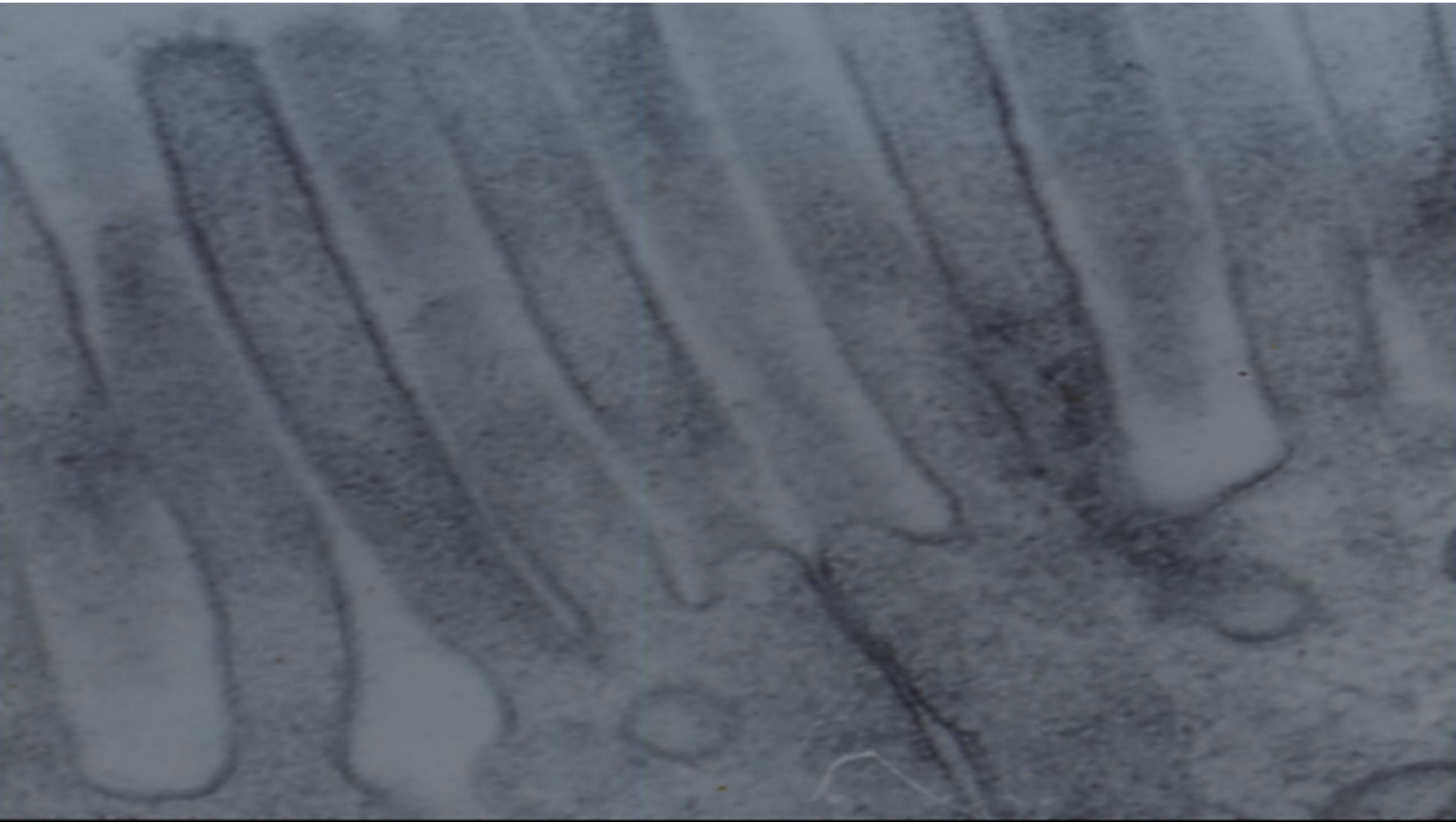
- Cellule colonnari altamente specializzate
  - Membrana luminale ripiegata in numerosi microvilli che incrementano di 600 volte la superficie intestinale (brush border)
  - Ruolo chiave nei processi di assorbimento dei nutrienti, vitamine e d elettroliti
  - Strutture di adesione cellulare
  - Recettori per ormoni e neurotrasmettitori
  - Hanno un rapido turnover cellulare (3-4 gg) che costituisce un importante meccanismo protettivo


superficie (cm^2)
3.300
area del solo cilindro
aumento della superficie (rispetto al cilindro)
3
10.000
valvole conniventi
30
100.000
villi
600
2.000.000

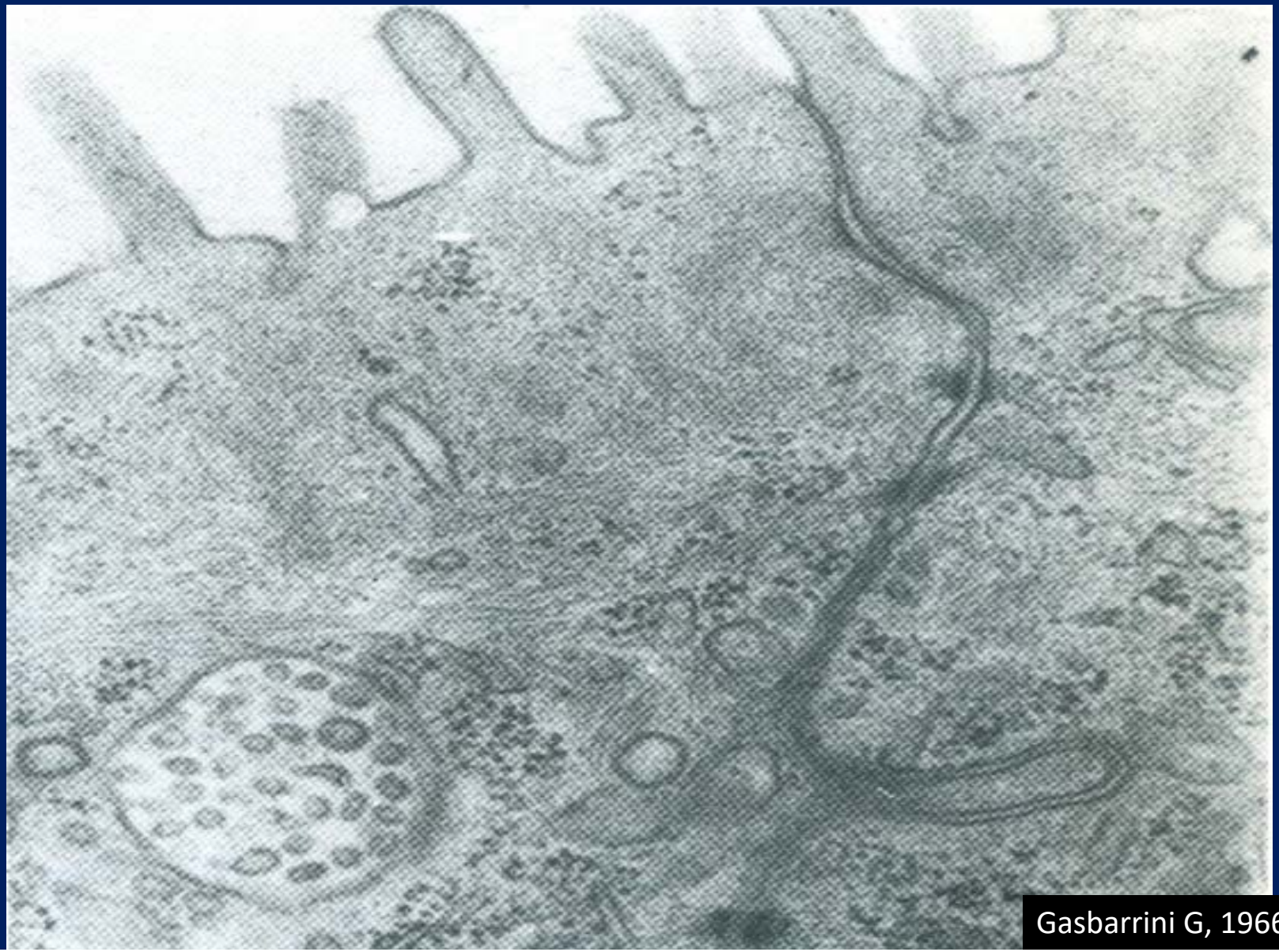

ENZIMI INTRINSECI
ENZIMI PANCREATICI
LUOGHI DI TRASPORTO
ATPasi
MICROVILLI

MV membrane : LIPID BILAYER stained by osmium tetroxide





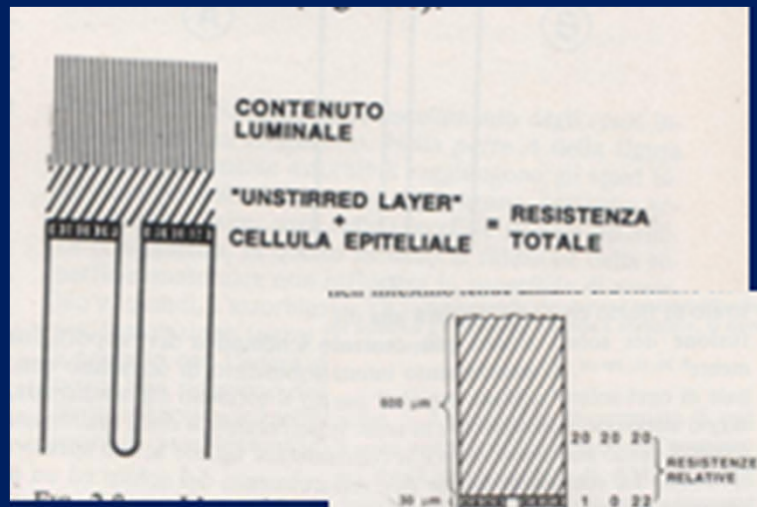
CRYPT CELL

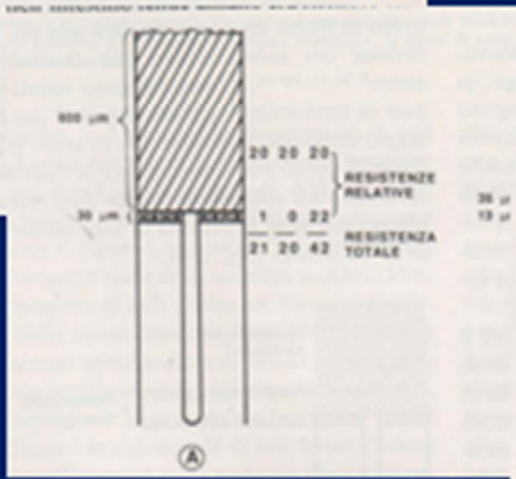


Gasbarrini G, 1966

Gasbarrini G,1966

Gasbarrini G.et al.,Int J Immunopathol Pharmacol,2009





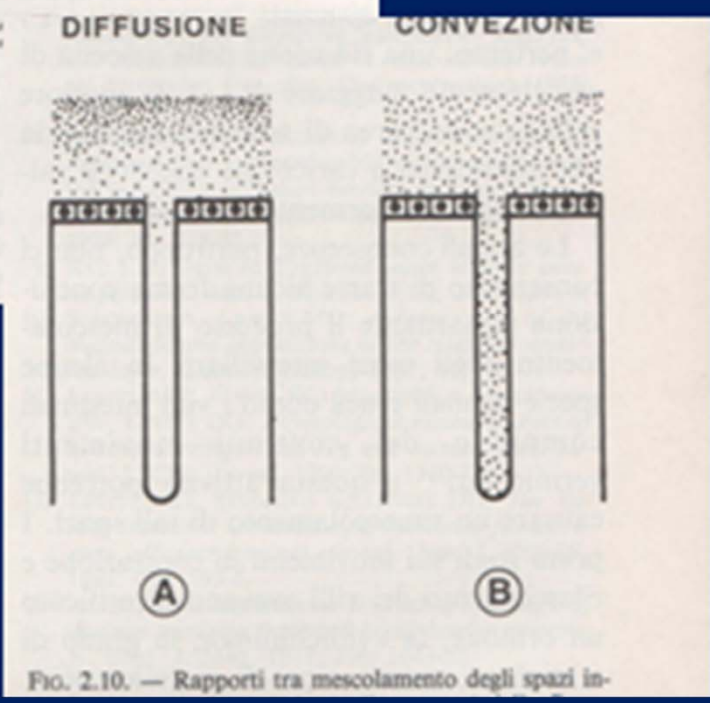
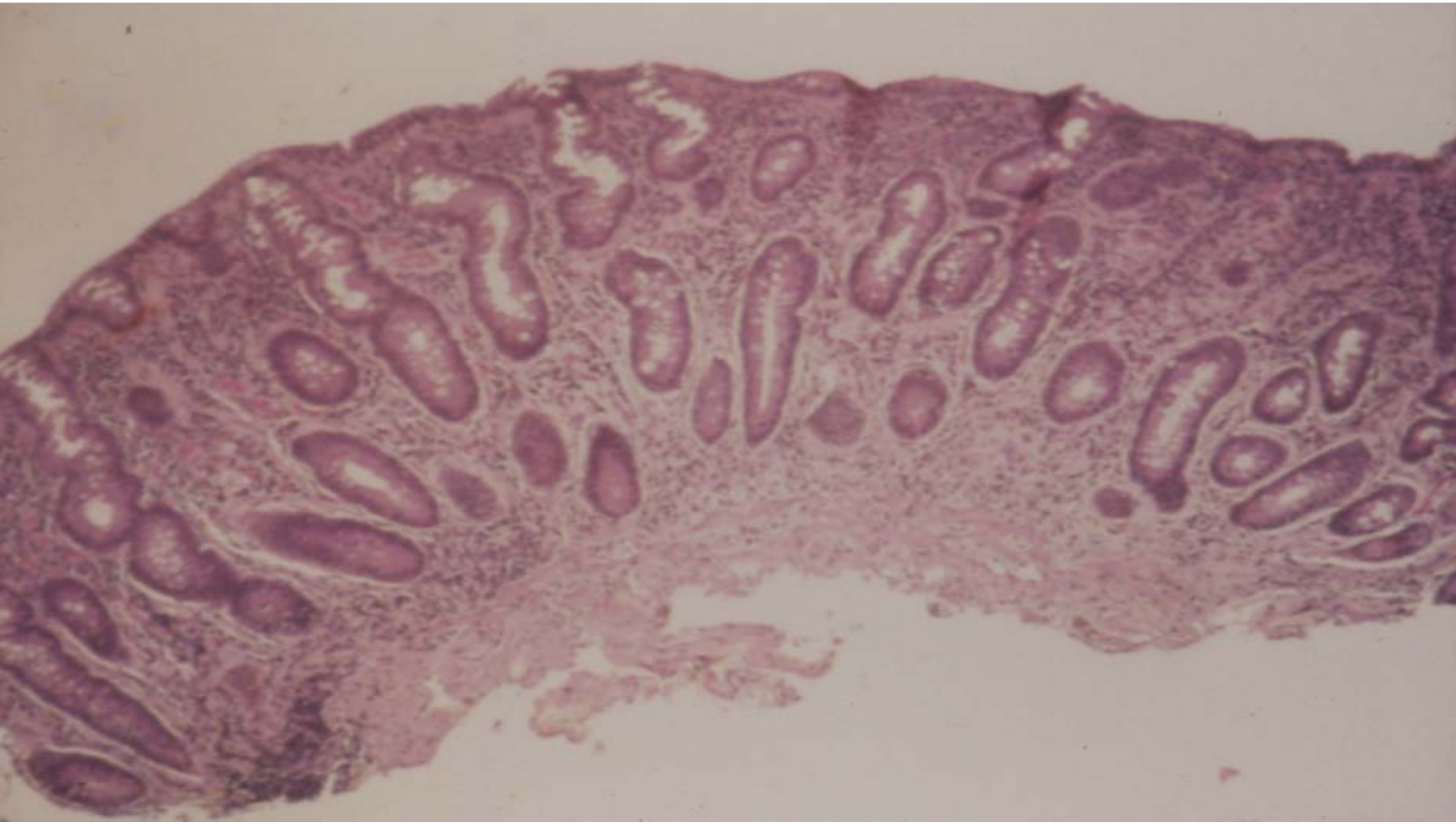


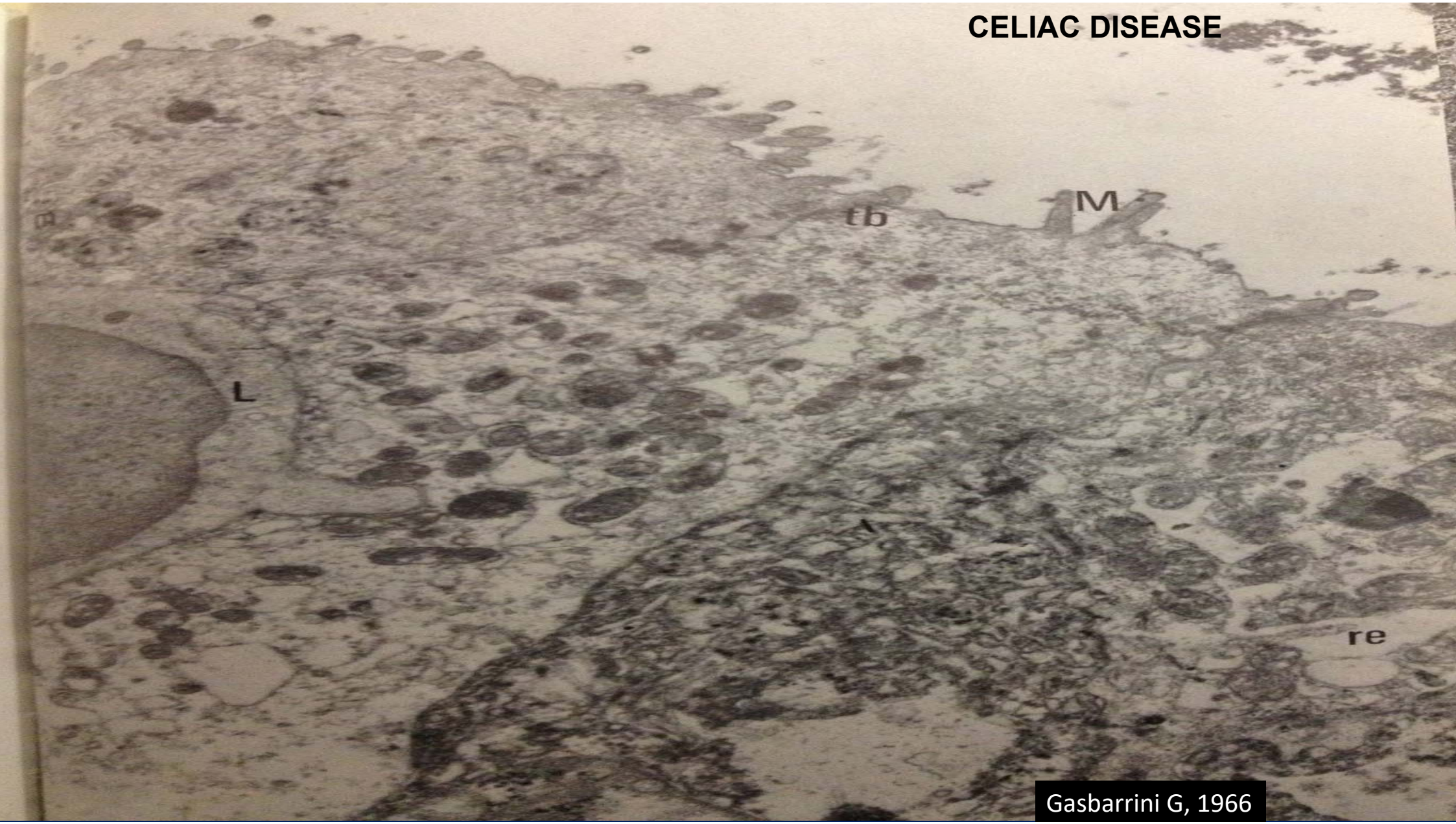
FIG. 2.10. — Rapporti tra mescolamento degli spazi in-

Levitt, Strocchi… Corazza, Gasbarrini, AmJGastr, 1986
Gasbarrini, Corazza, SIMI, Pozzi Ed. 1990



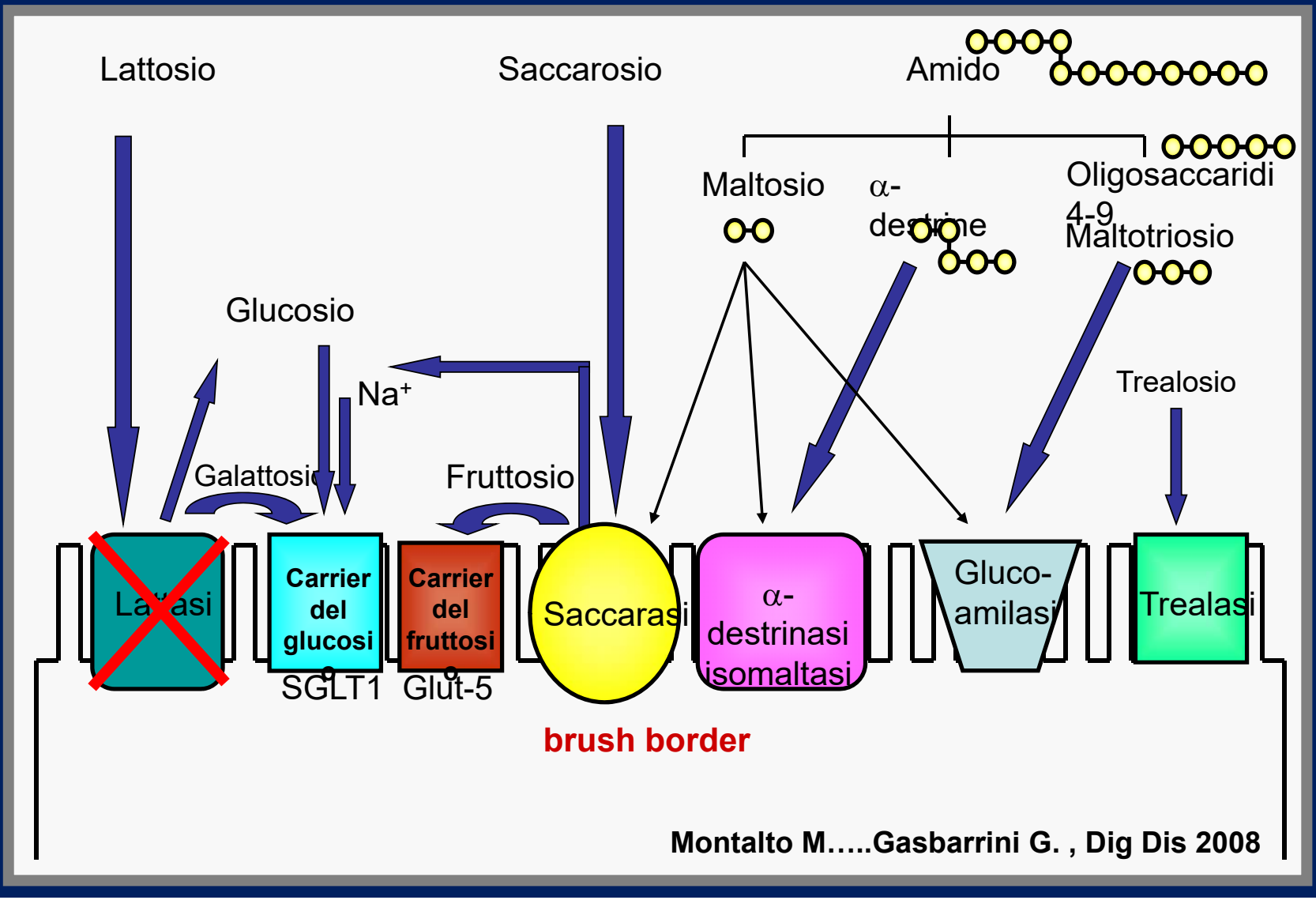
BRUSH BORDER DISCONTINUOUS IN CD

CELIAC DISEASE



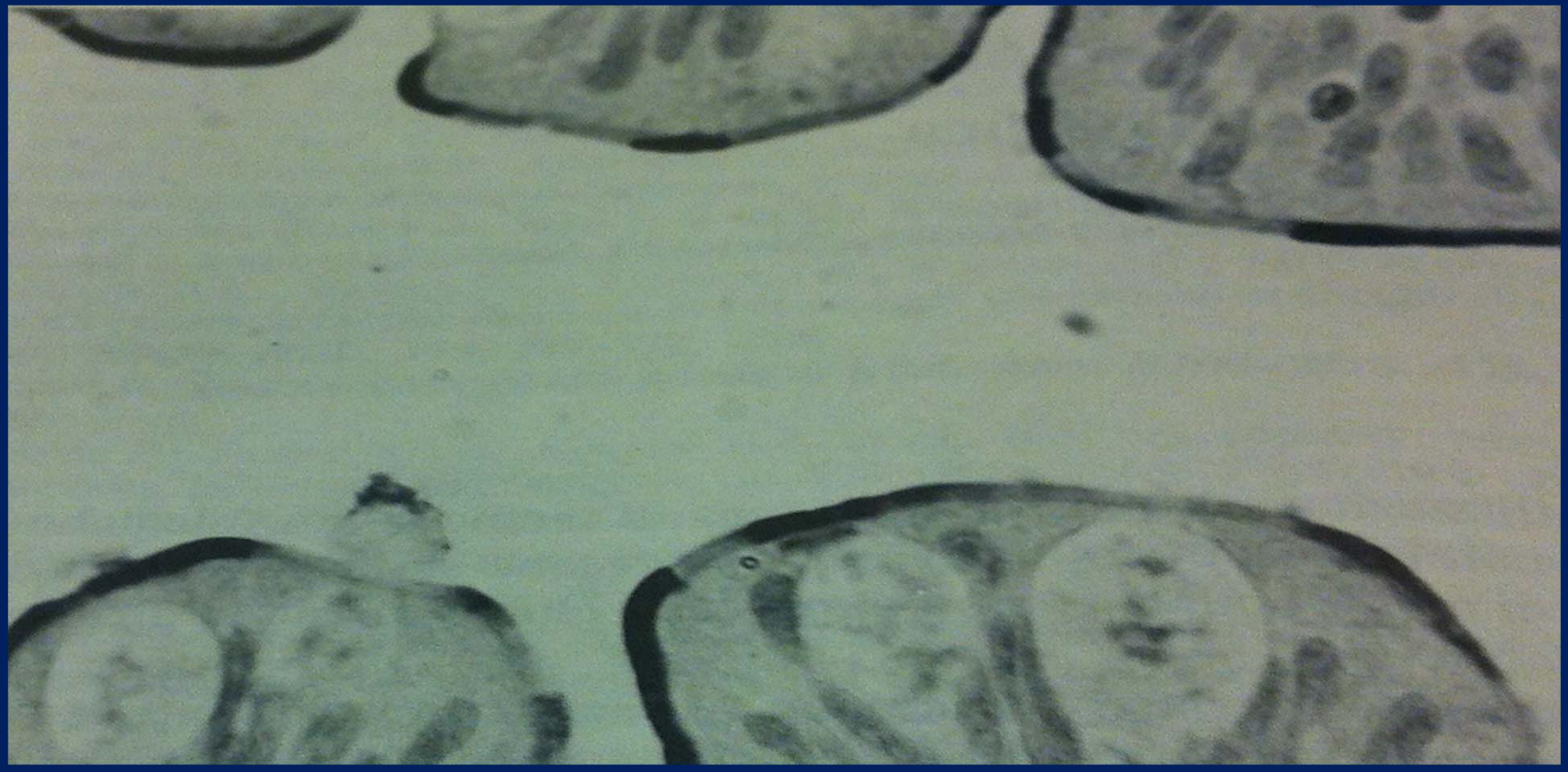
Gasbarrini G, 1966

ENZIMI PER L'ASSORBIMENTO DEI CARBOIDRATI



Montalto M…..Gasbarrini G. , Dig Dis 2008

The deficiency of enzymes of the brush border or malfunction of carriers can be the basis of selective malabsorption of carbohydrate, as in the case of the most common
lactase deficiency



G. Gasbarrrini et al, Morfologia dell'intestino tenue, RORER ED., 1995

FAILED TRANSPORT OF NUTRIENTS

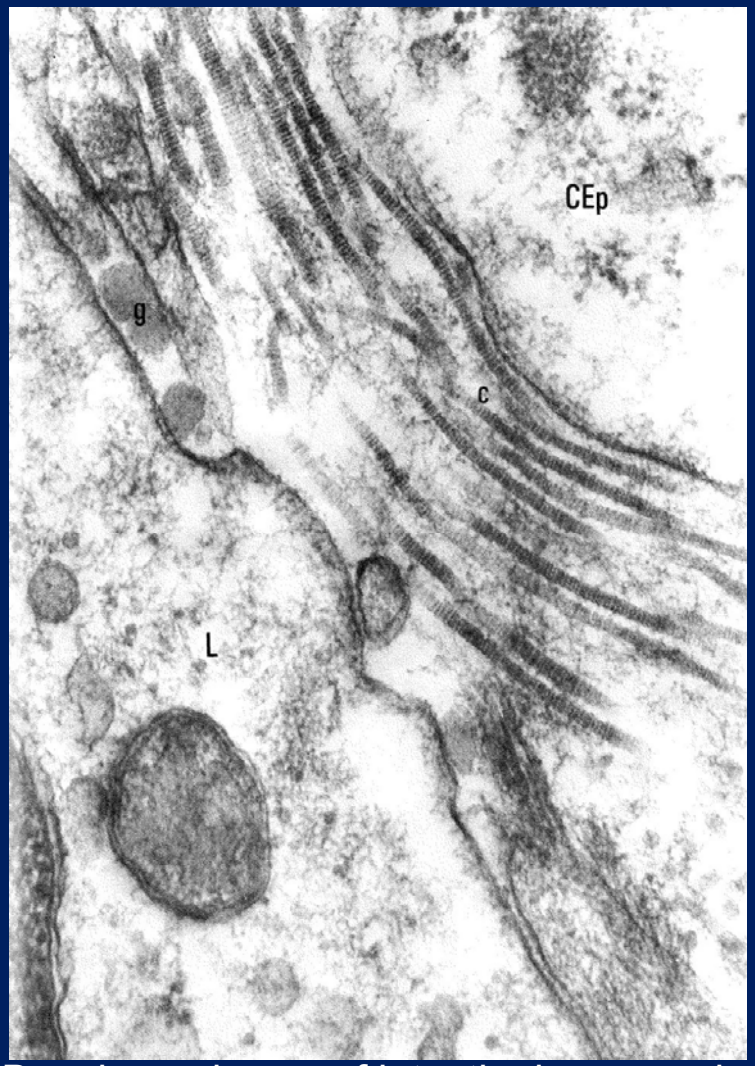
A-beta lipoproteinemia

Bassen Kornzweigh cells / ENEROCYTES FILLED WITH LIPIDS

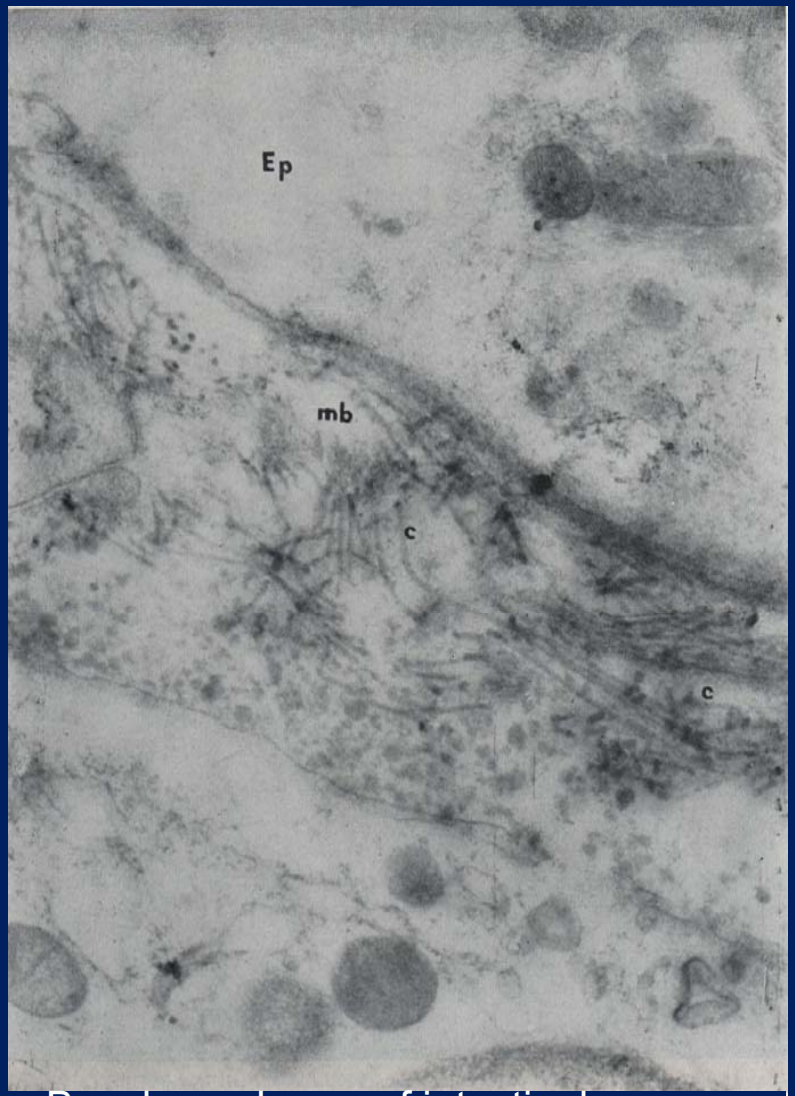
Gasbarrini G, Corazza GR,

Arch. Med.Int. 1990

Laschi R, Gasbarrini G
Sperimentale, 1963



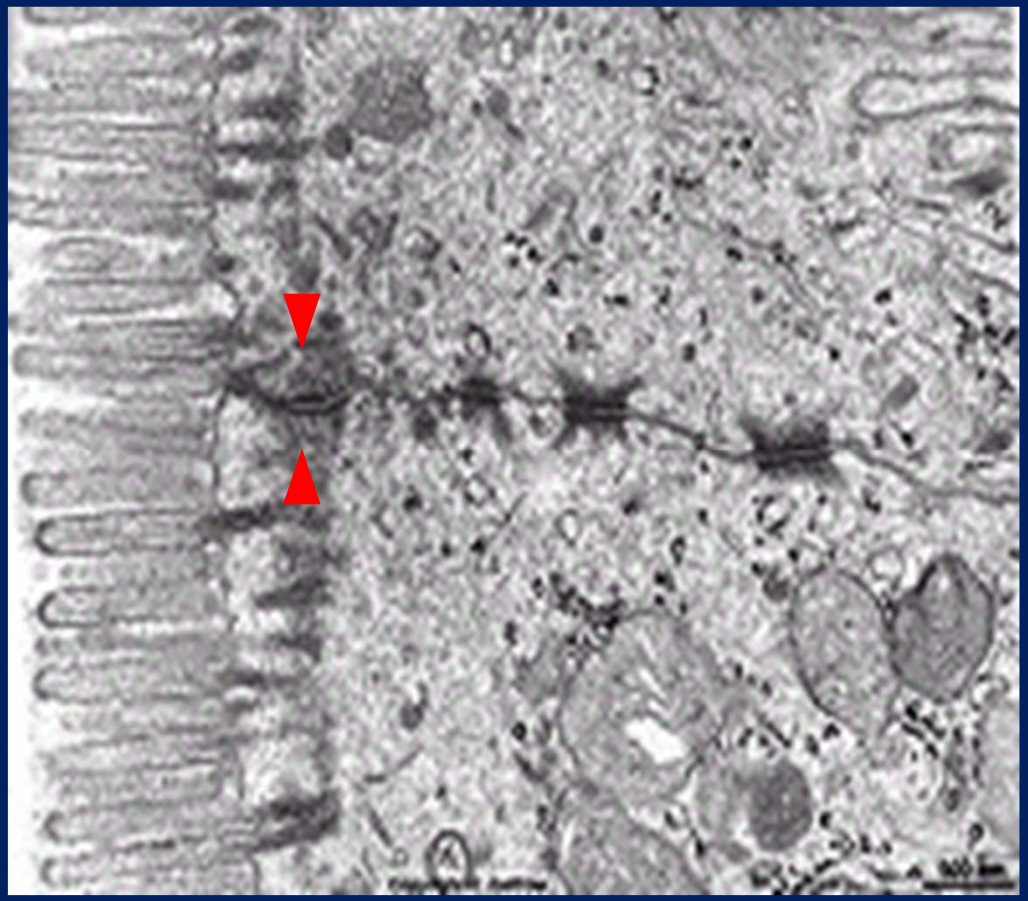
Basal membrane of intestinal mucosa in sclerodermia with collagenous sprue.
Gasbarrini G, Arch Mal App Dig. 1976

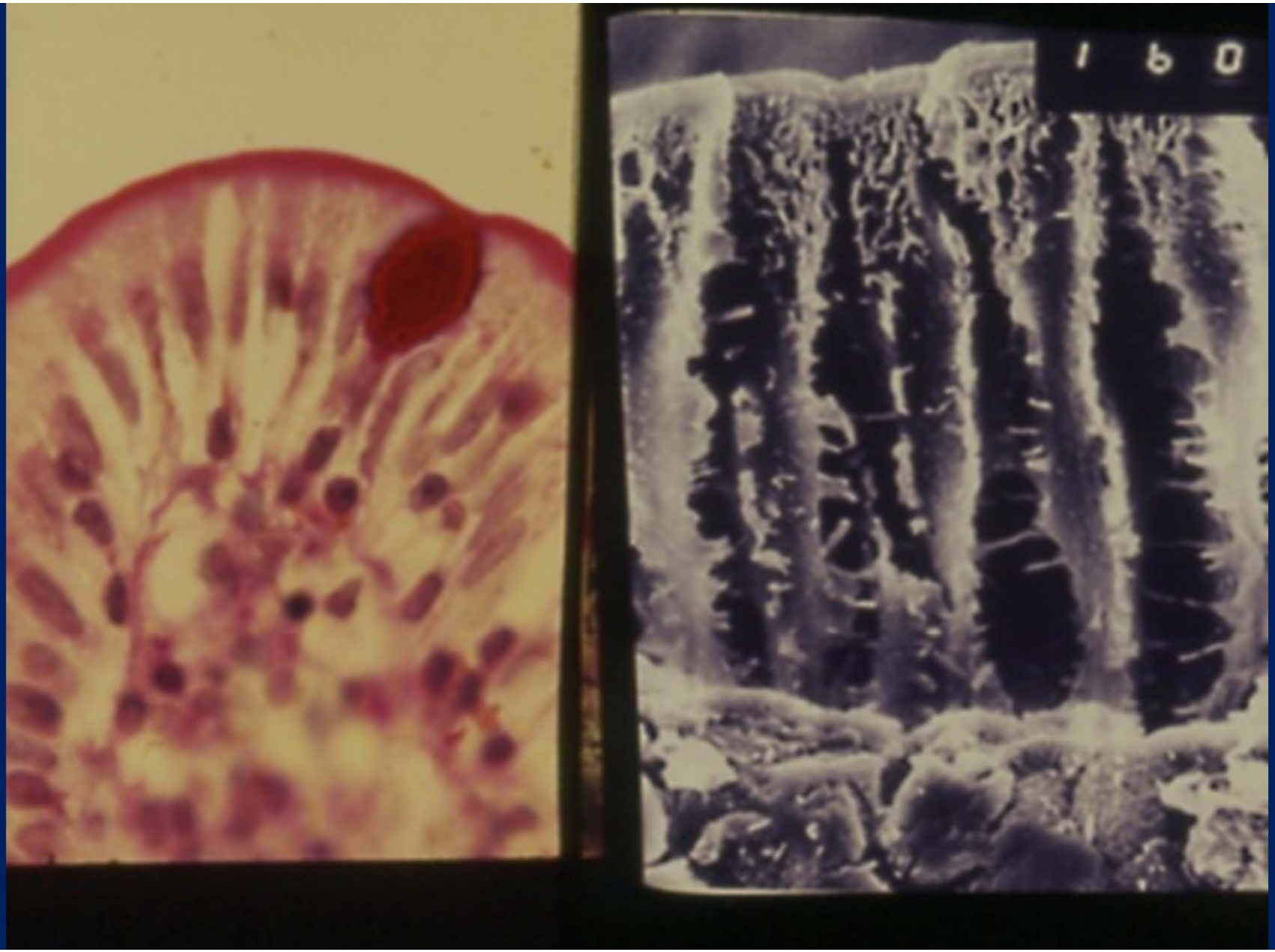


Basal membrane of intestinal mucosa in aging. Gasbarrini G, Arch Mal App Dig. 1976

Tigth junction

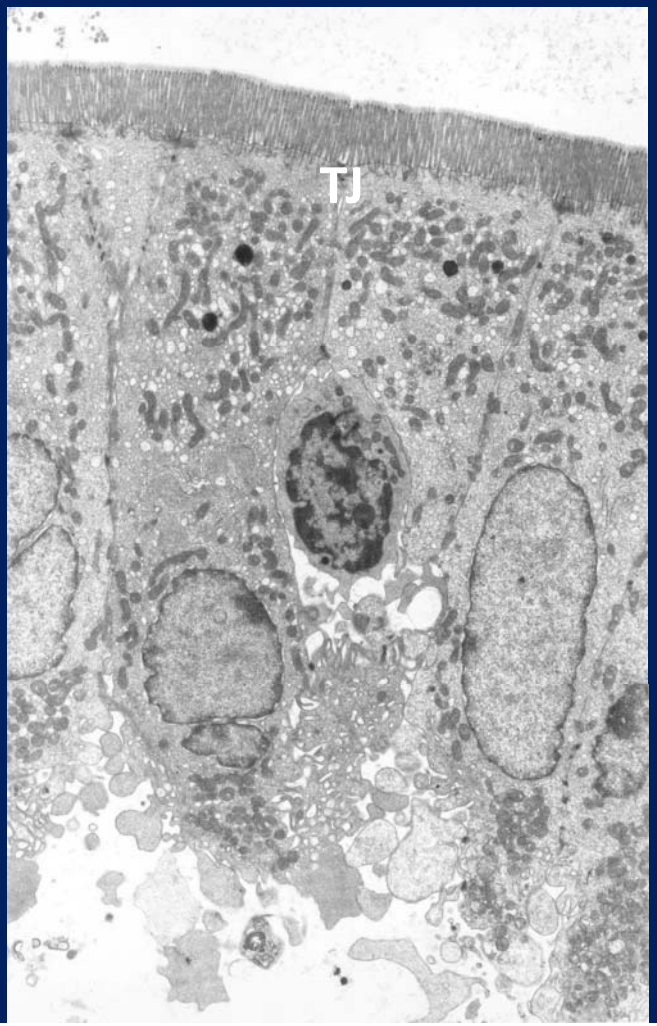
Le tight junction presenti tra gli enterociti sono strutture coinvolte nella funzione di barriera intestinale e sono formate da un complesso di numerose proteine che possono essere danneggiate nel corso di diverse malattie intestinali



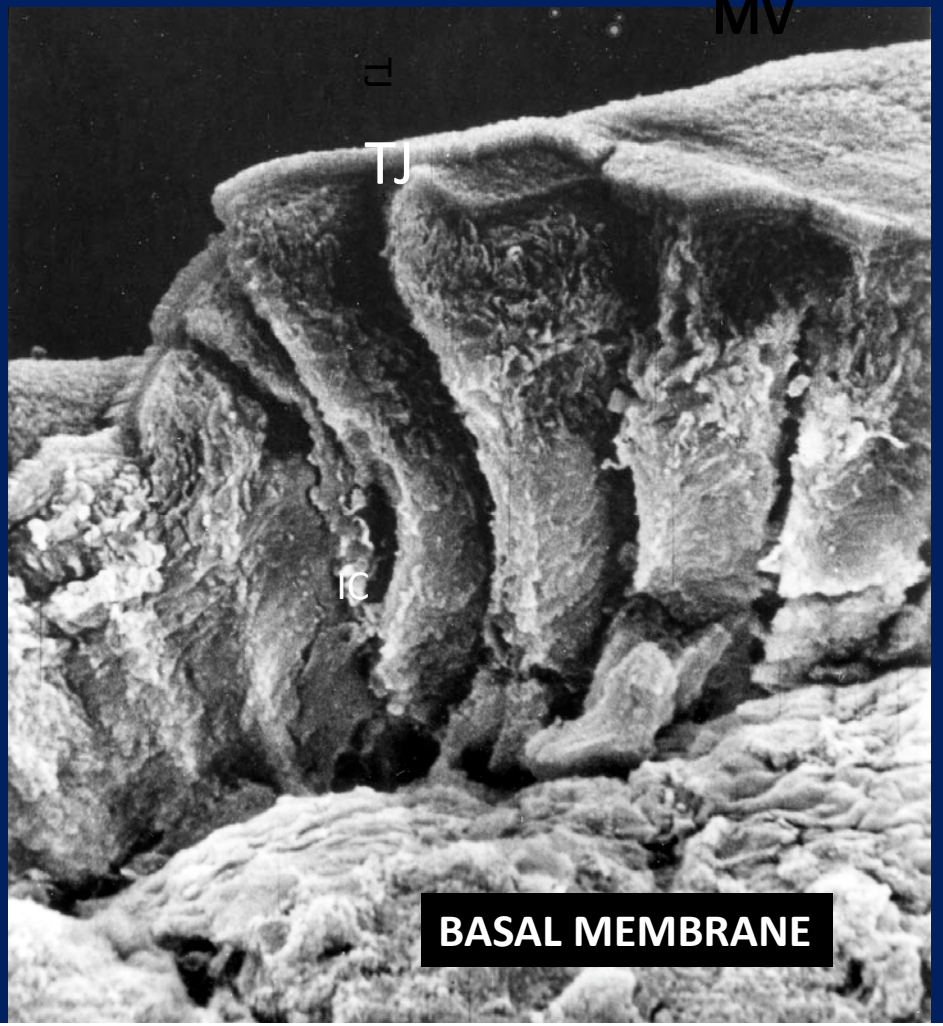

160

The apical junctional complex controls cell polarity and paracellular permeability

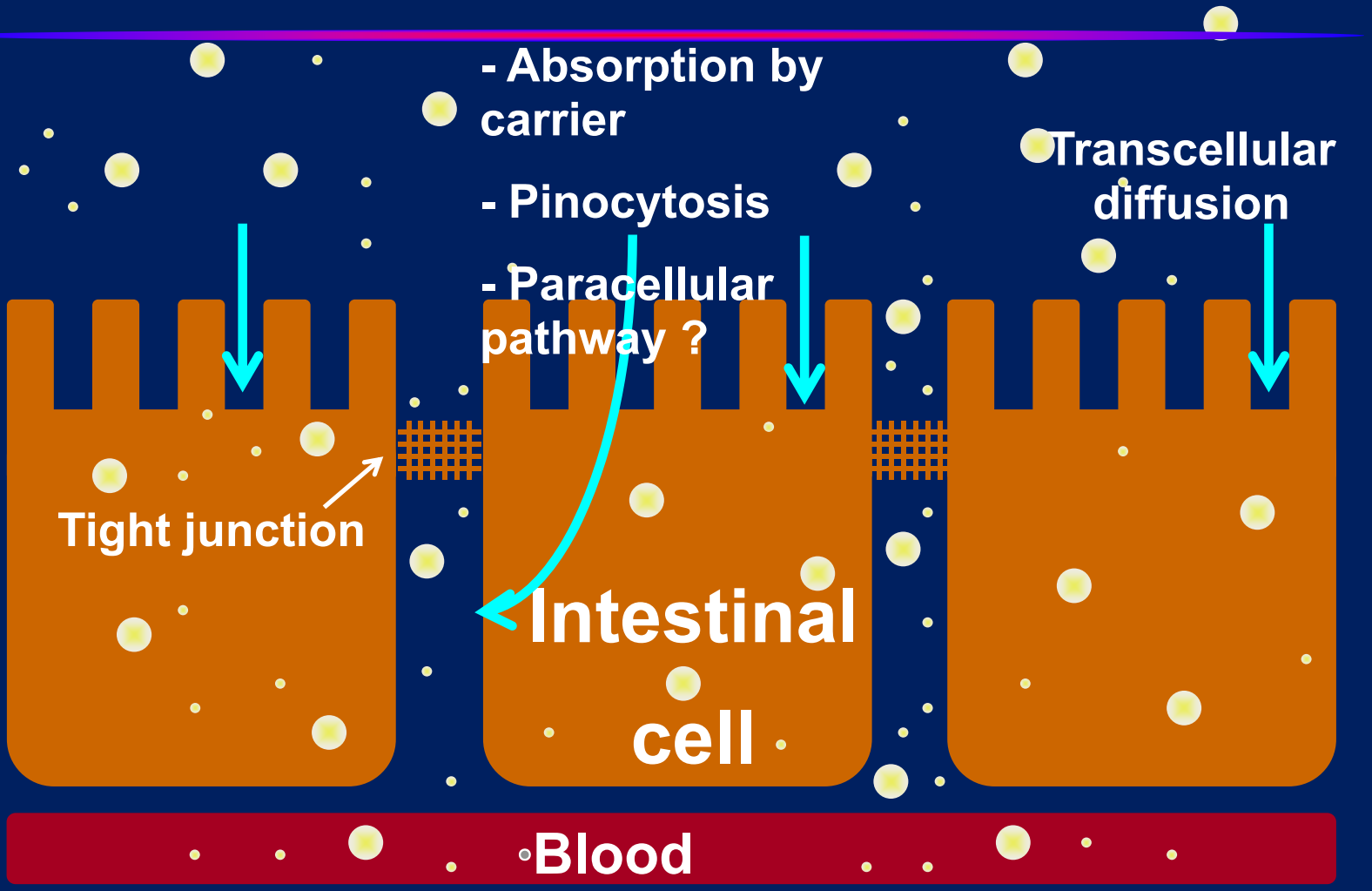
Transmisssion EM



Scanning EM

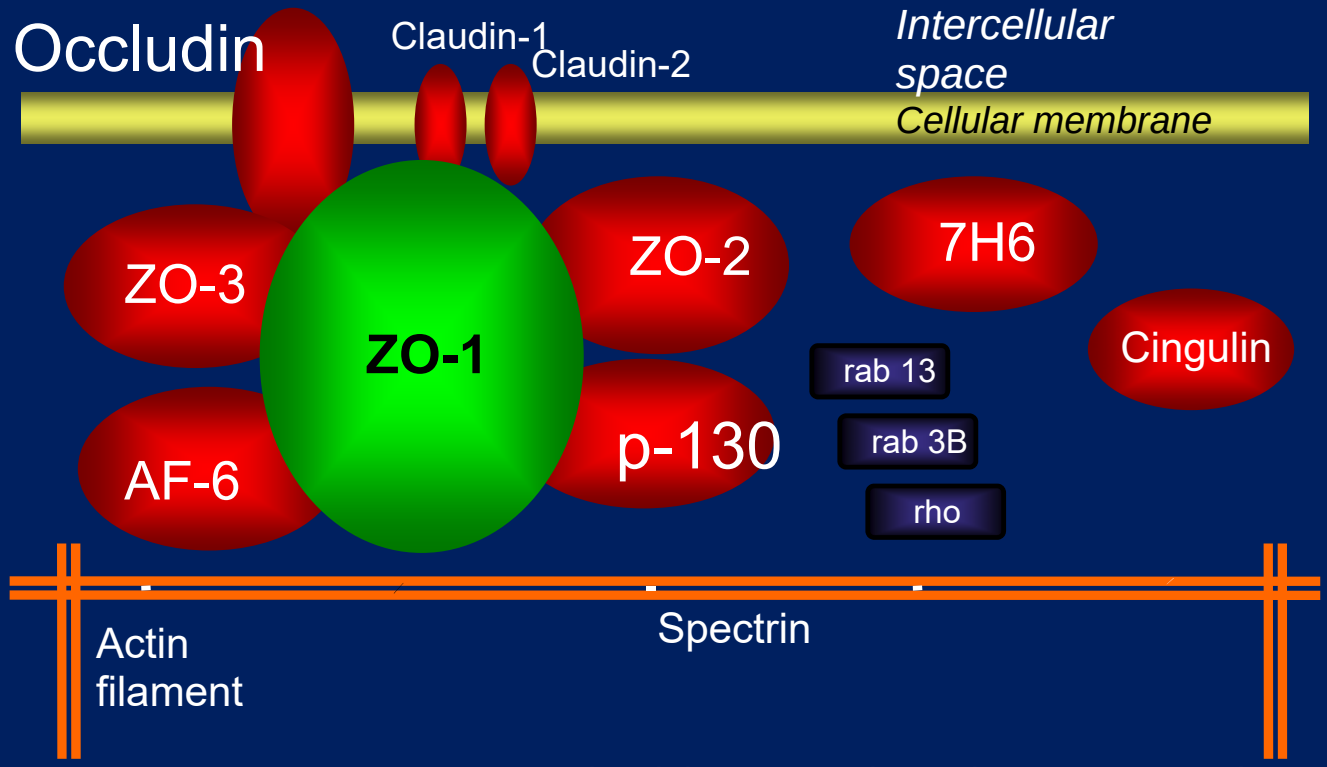


Intestinal Absorption



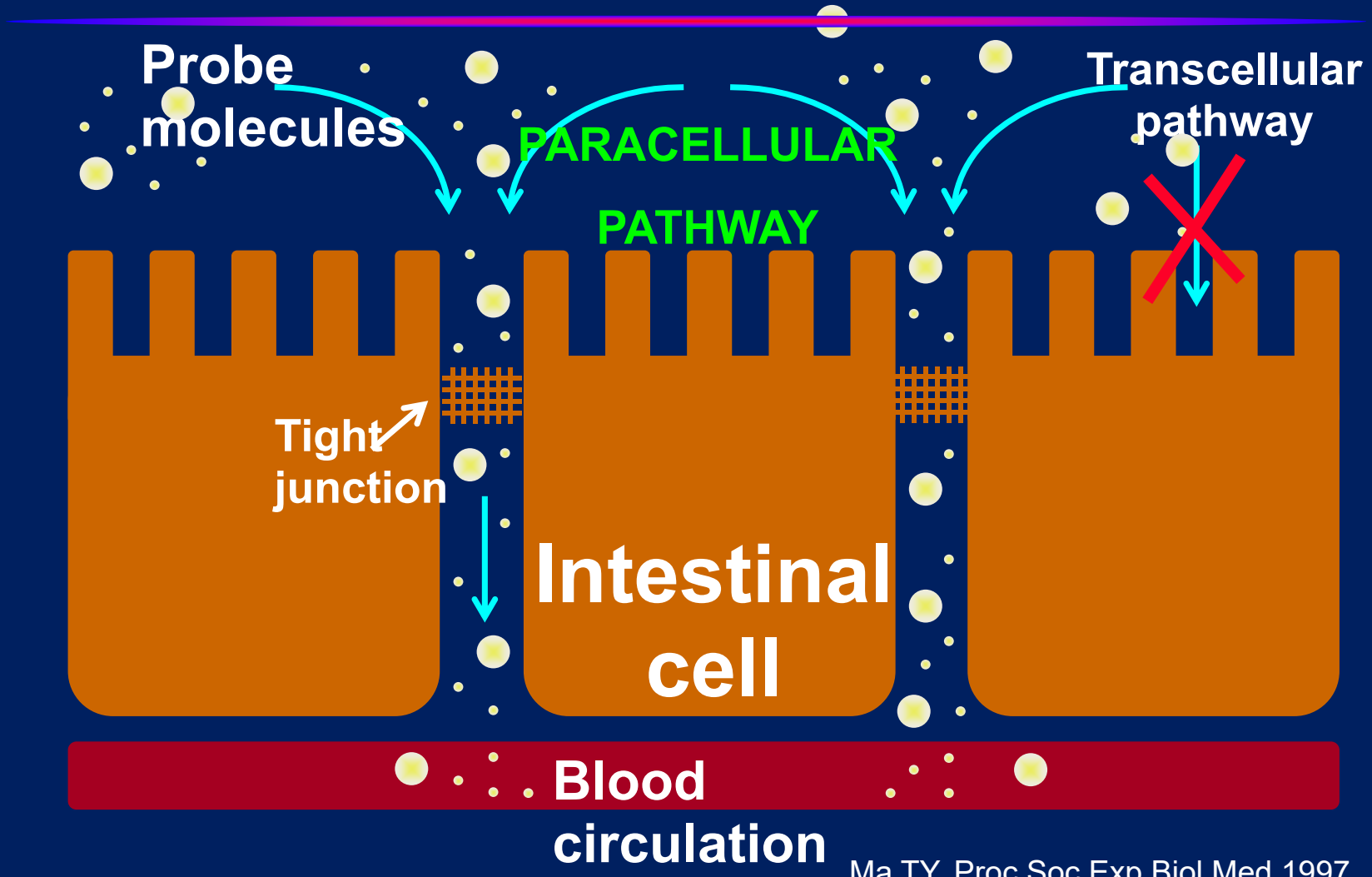
Gasbarrini G, Wirchow Arch Pat, 1963

La ZO-1 è stata la prima di tali proteine ad essere identificata; è considerata un valido marcatore dell'integrità delle tight junction ma, essendo presente anche nel nucleo, è coinvolta nei meccanismi di sviluppo e maturazione cellulare



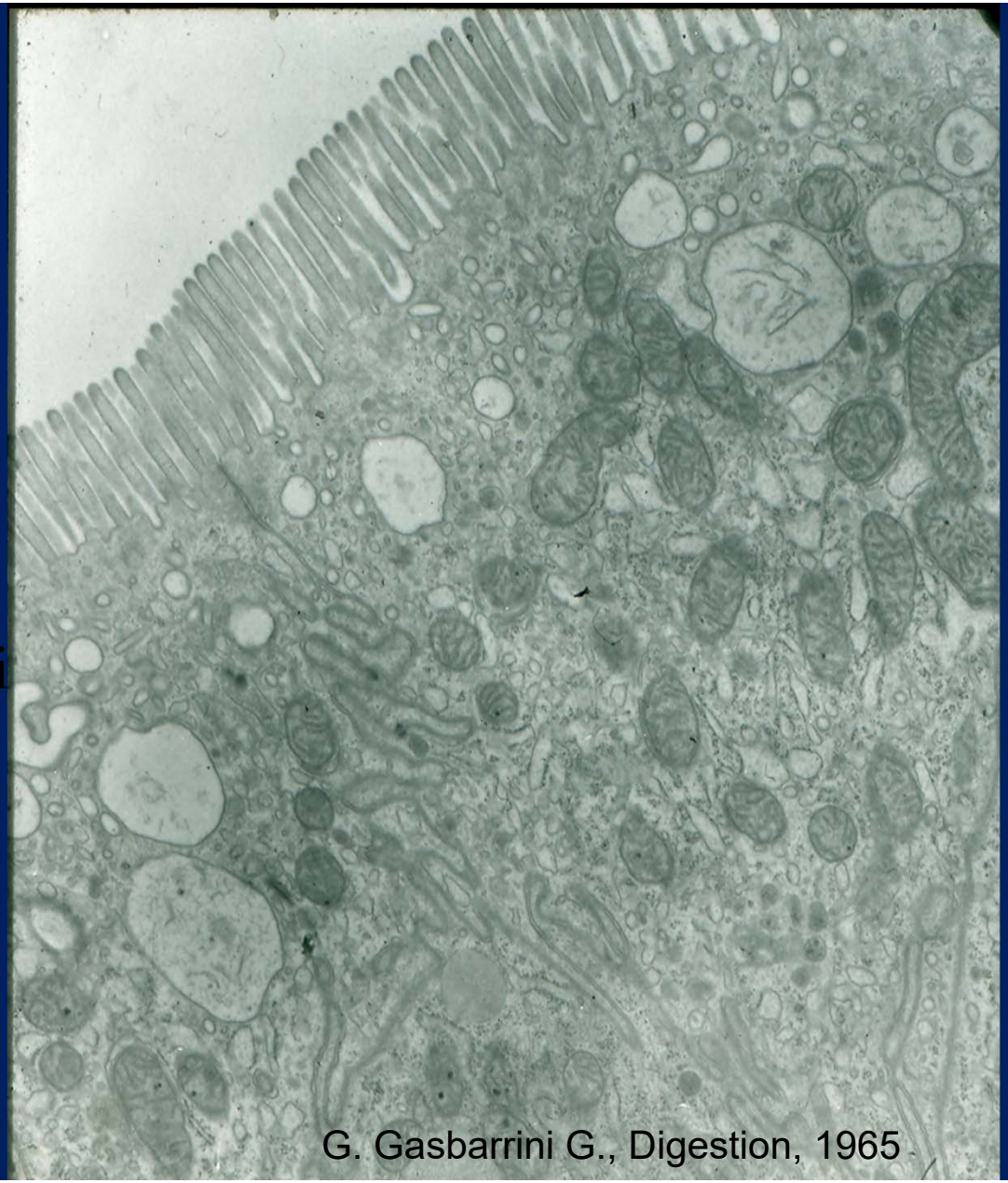
G. Gasbarrini, M. Montalto. Ital J Gastroenterol Hepatol 1999

Permeation pathways

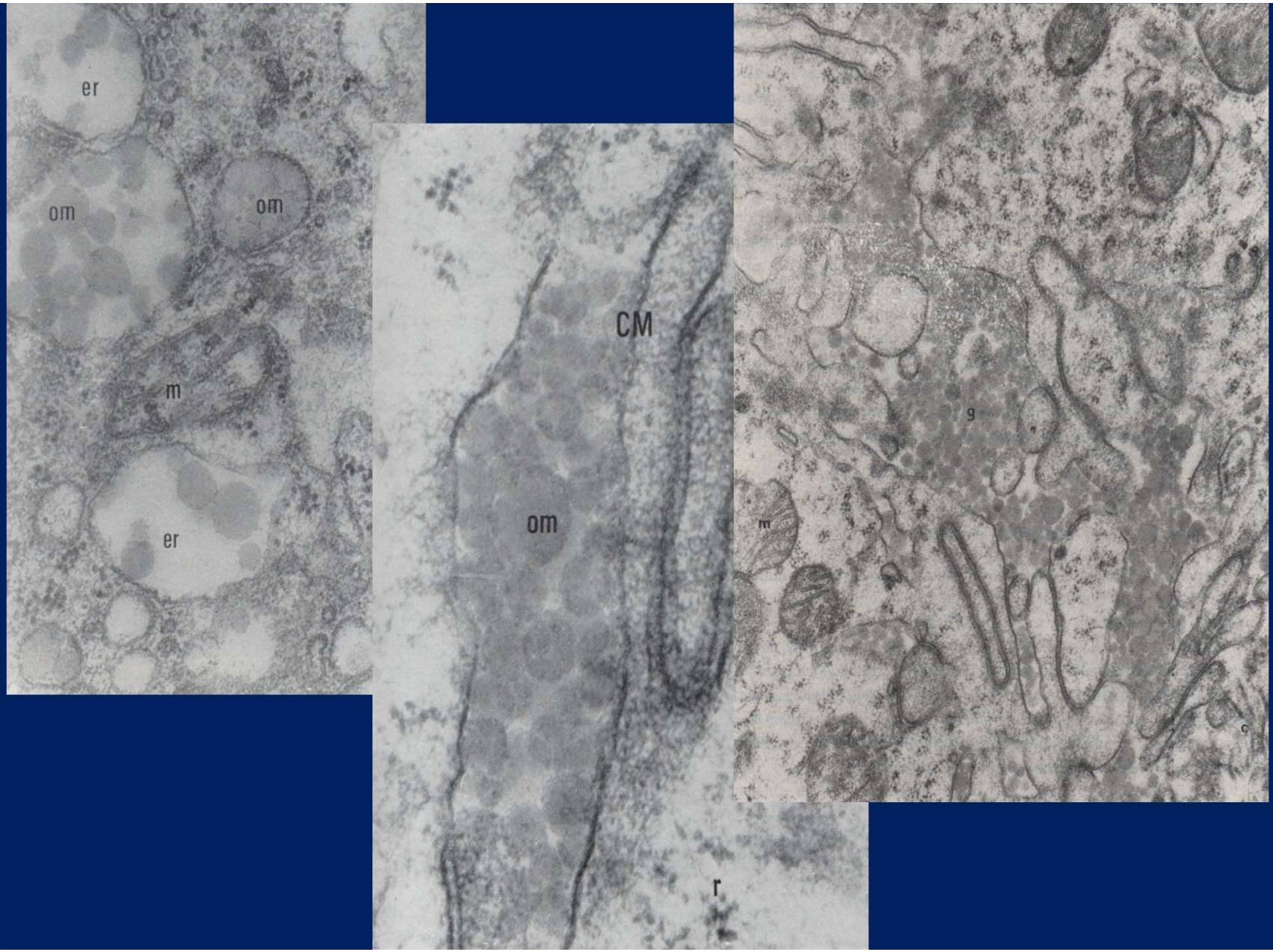


Ma TY, Proc Soc Exp Biol Med 1997

Gasbarri



G. Gasbarrini G., Digestion, 1965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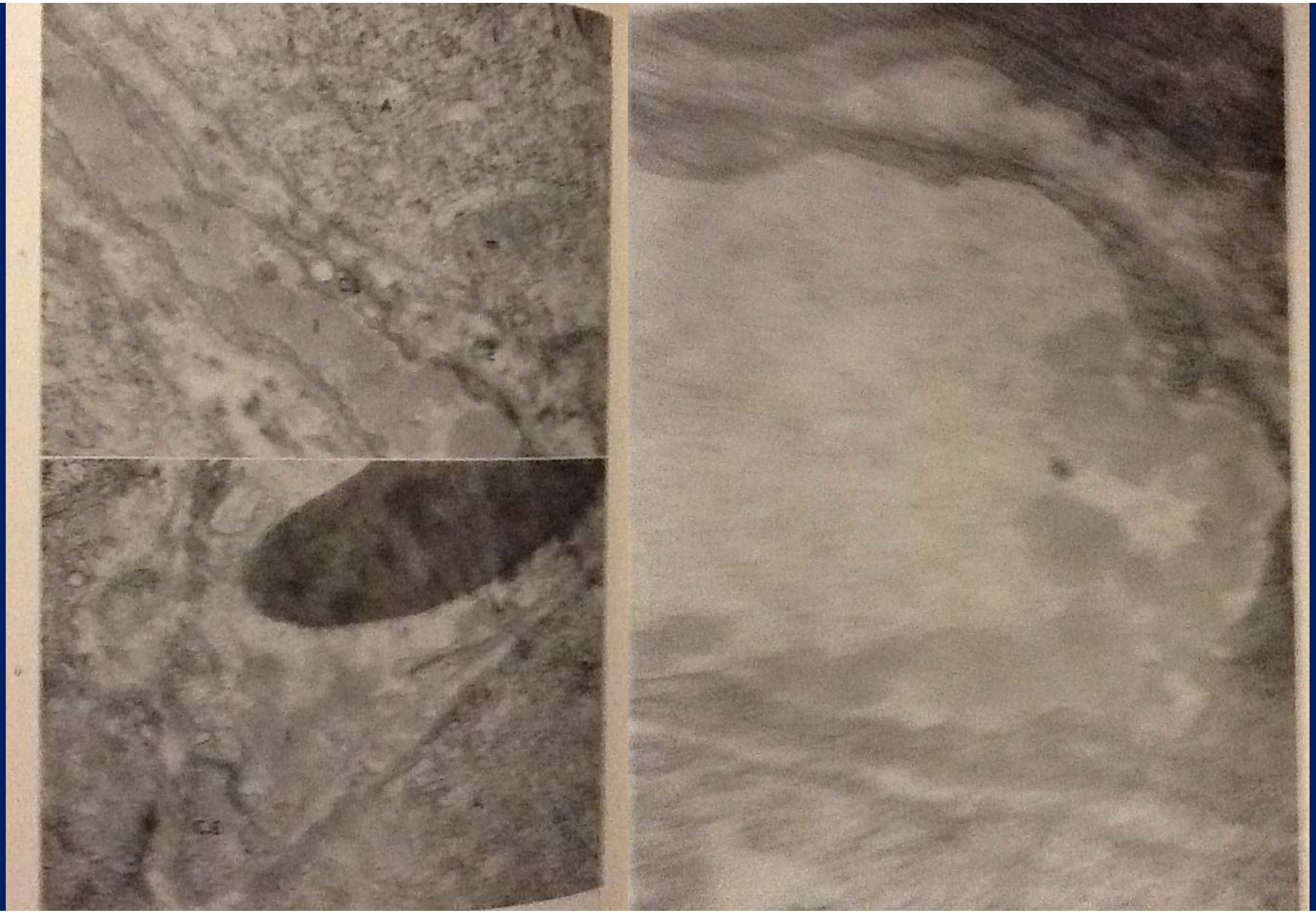
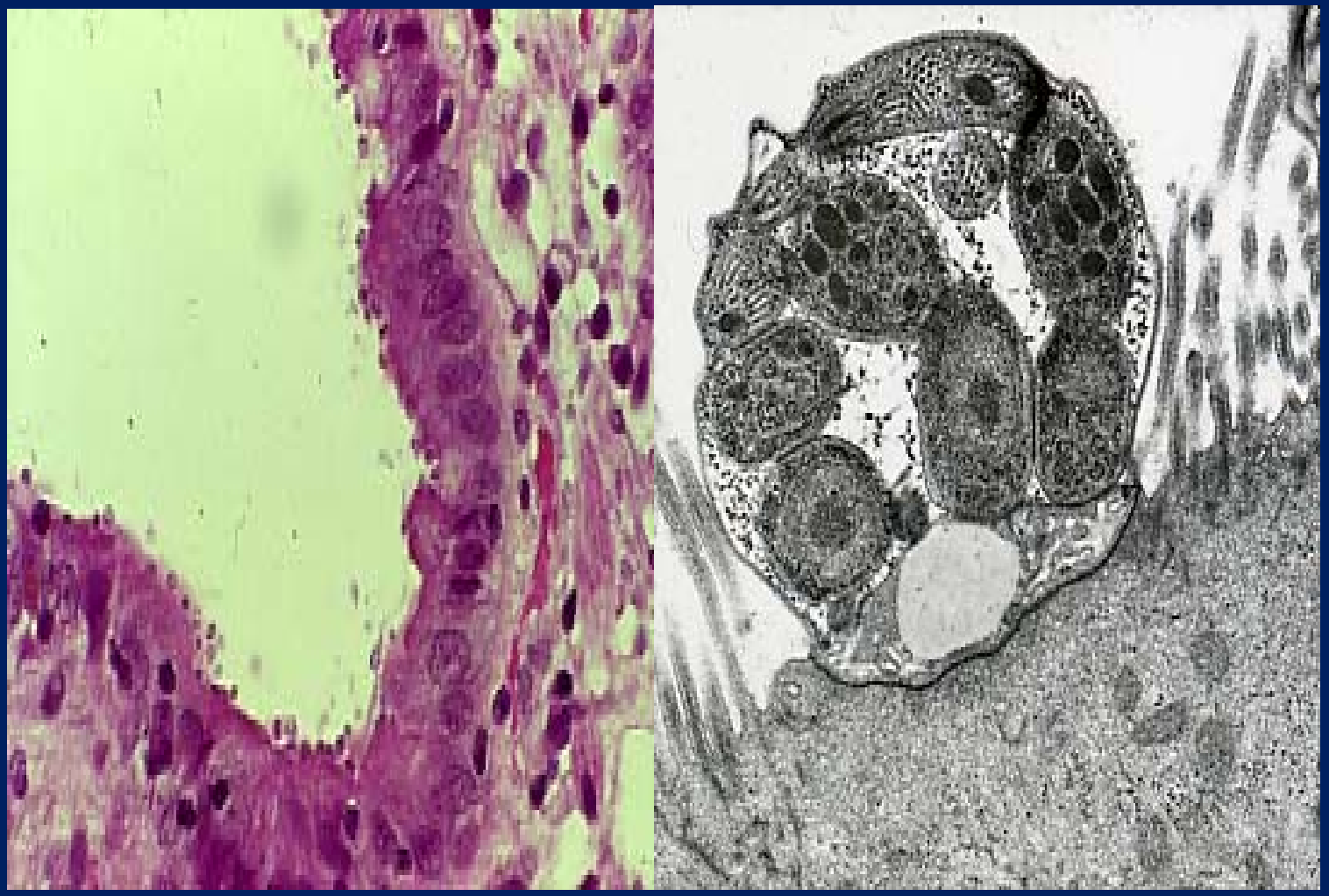
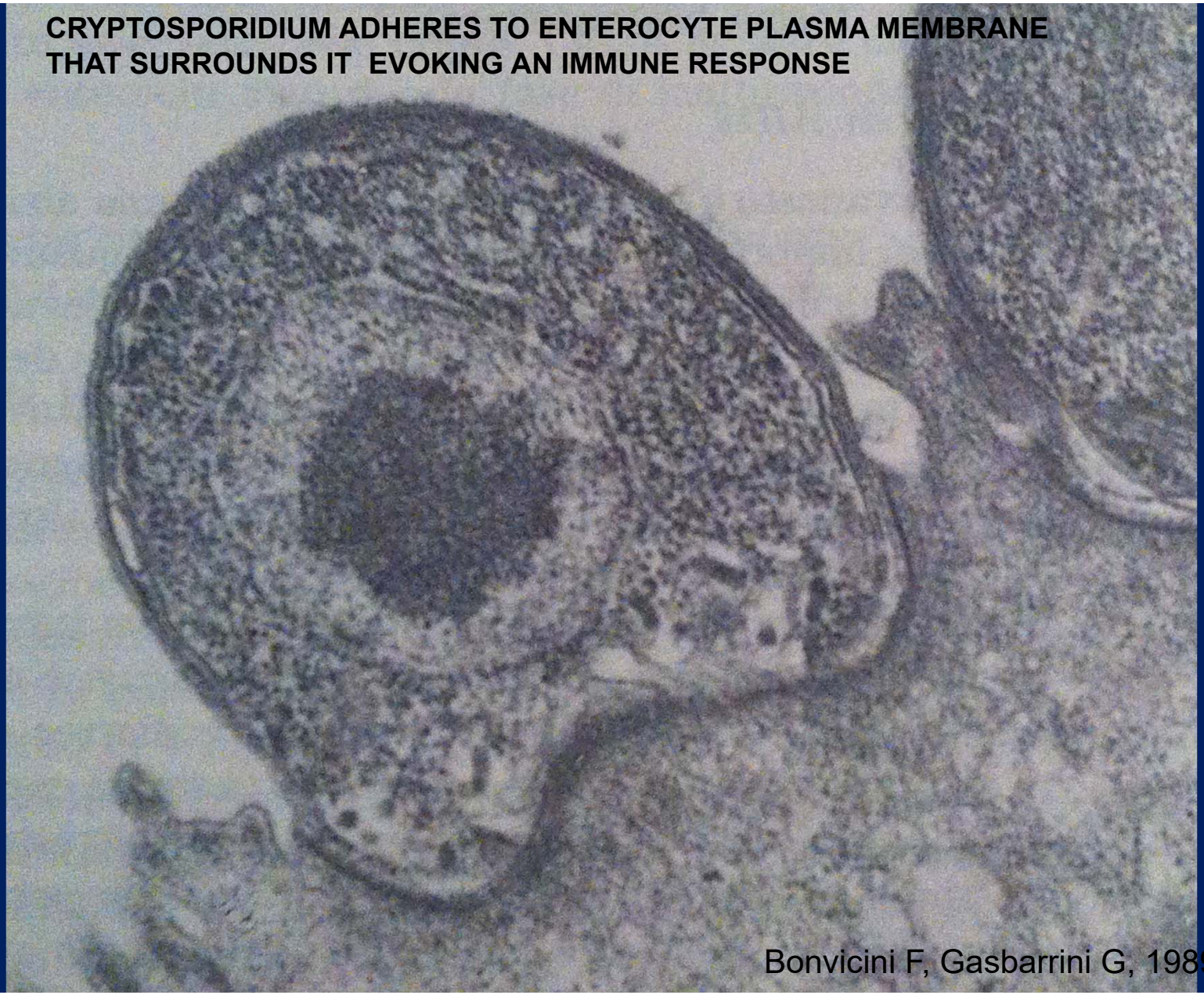
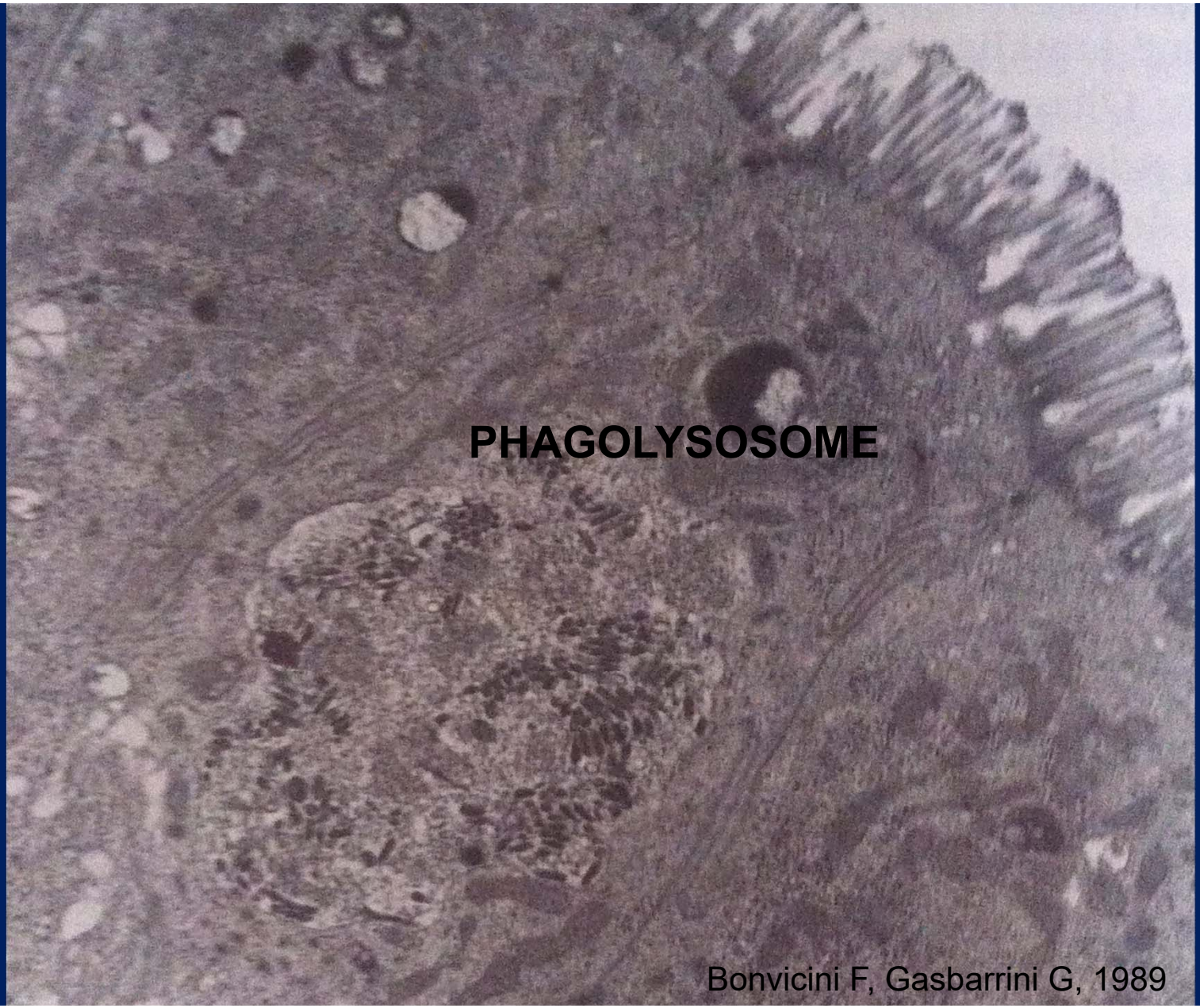




CRYPTOSPORIDIUM ADHERES TO ENTEROCYTE PLASMA MEMBRANE THAT SURROUNDS IT EVOKING AN IMMUNE RESPONSE



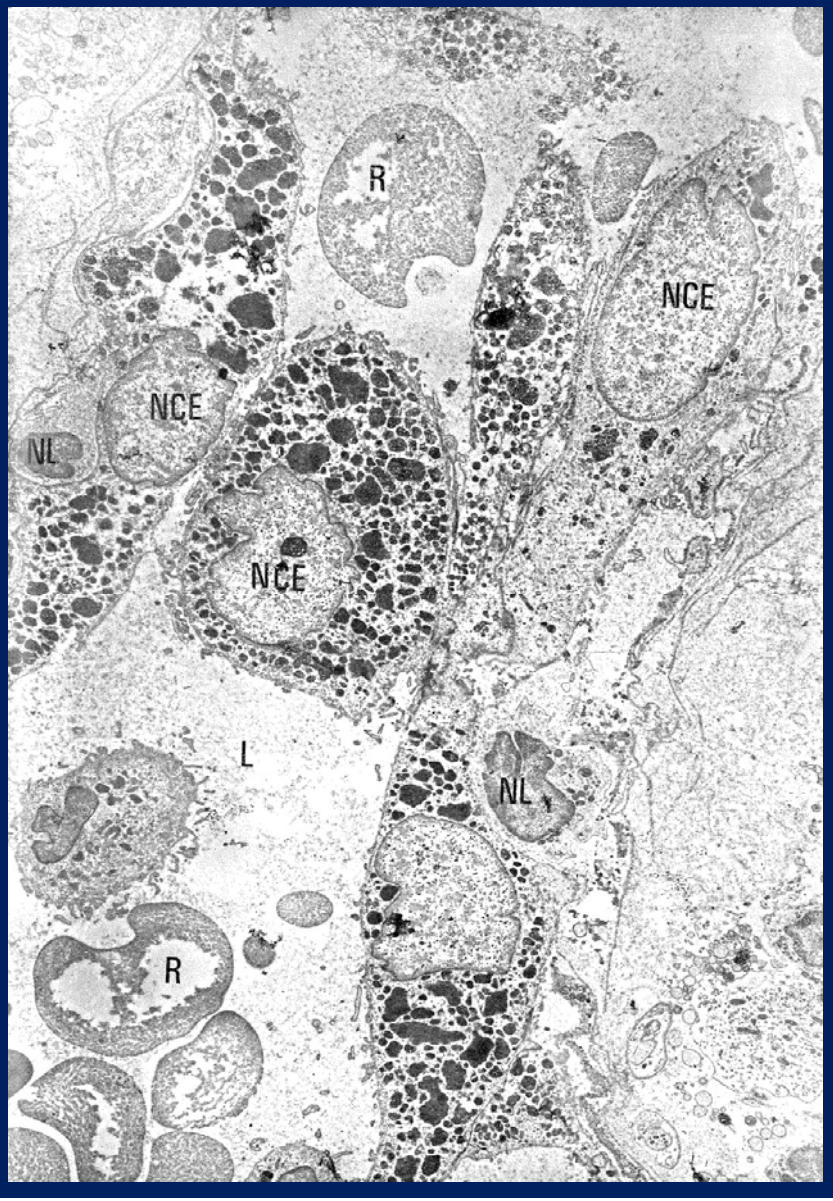
Bonvicini F, Gasbarrini G, 1989



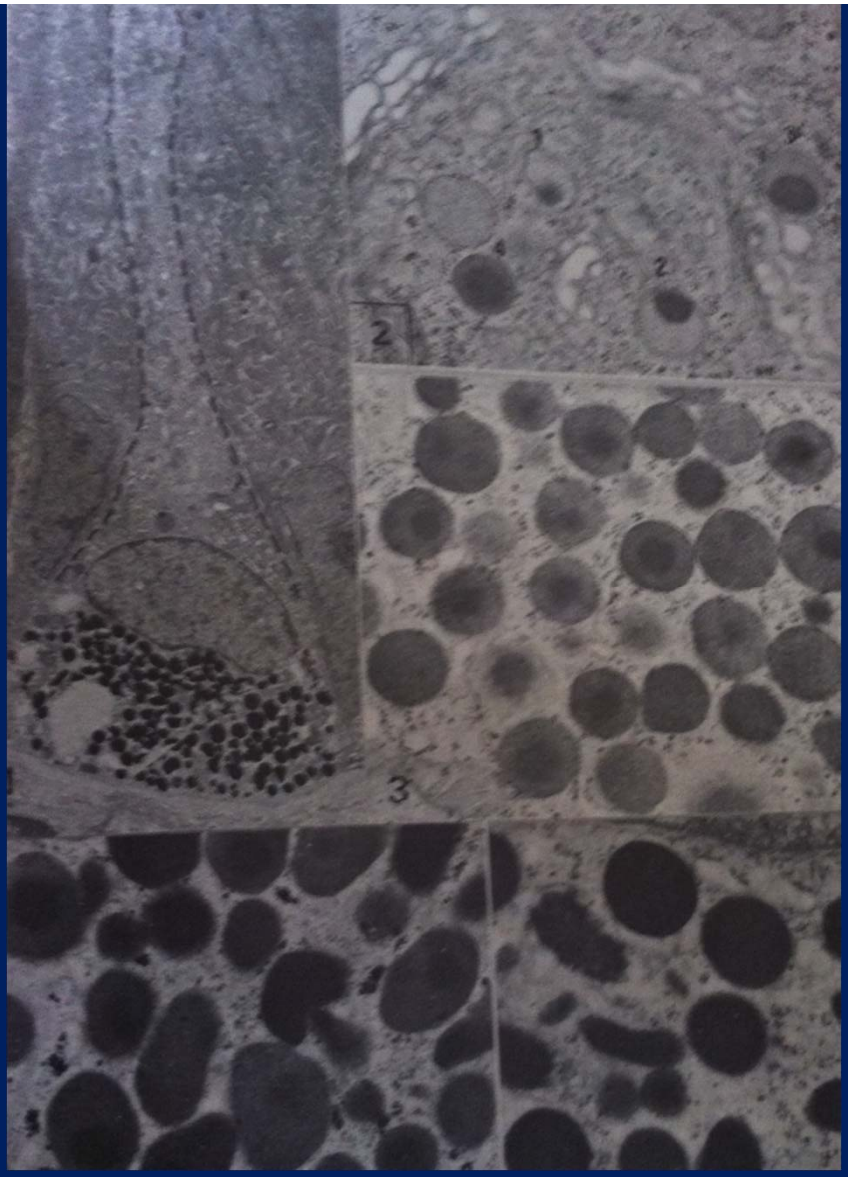
Bonvicini F, Gasbarrini G, 1989

DEFICIENCY OF IMMUNE RESPONSE OF MACROPHAGES AND T CELLS IN WHIPPLE DISEASE

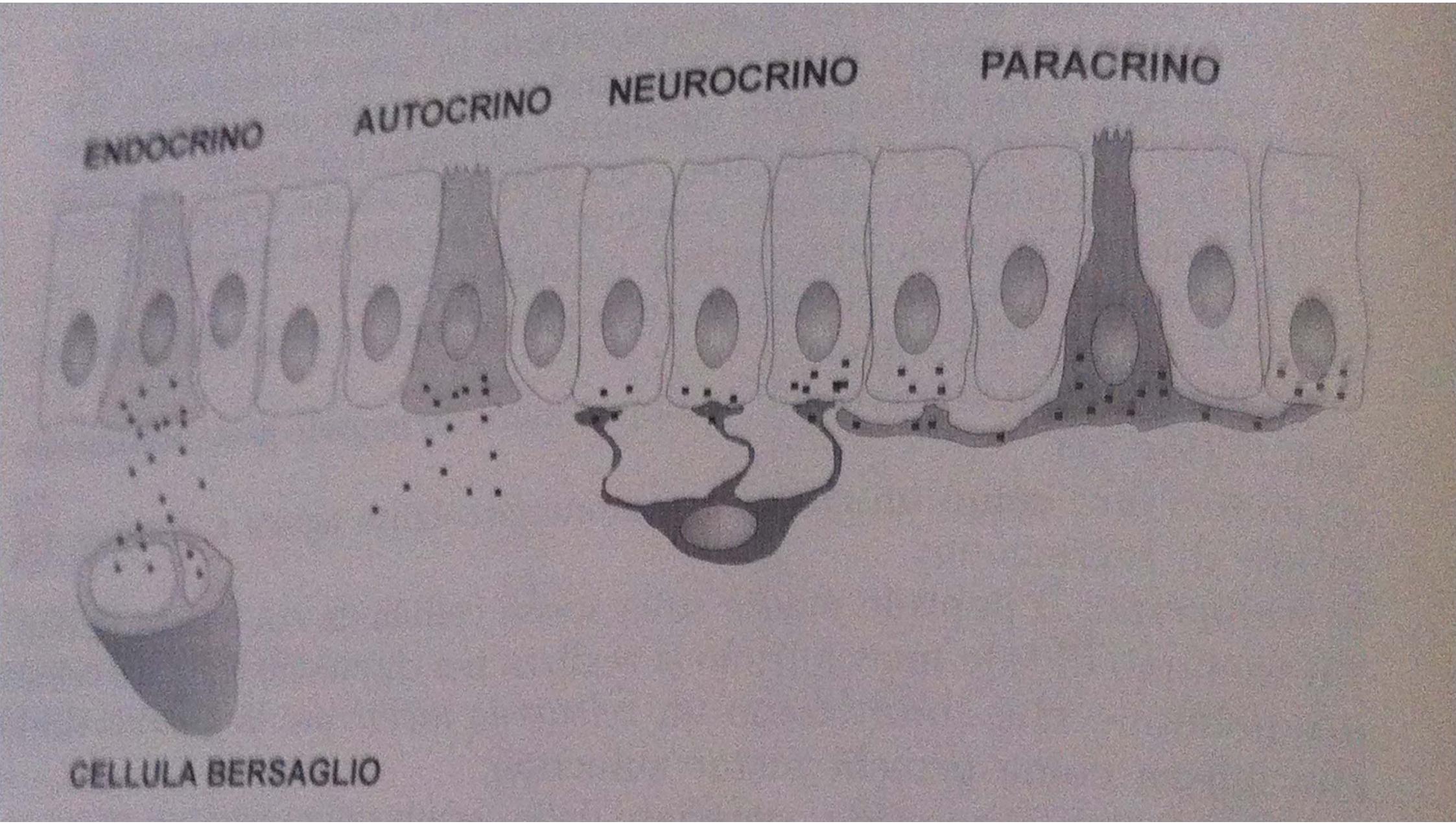
Gasbarrini e Coll. Bull.Sci.Med.Chir. Bo. 1972 – SIMI, Pozzi Ed. 1989



Gasbarrini G,
Sprue Celiaca
Capelli Ed. 1966



G.Gasbarrini ,1972

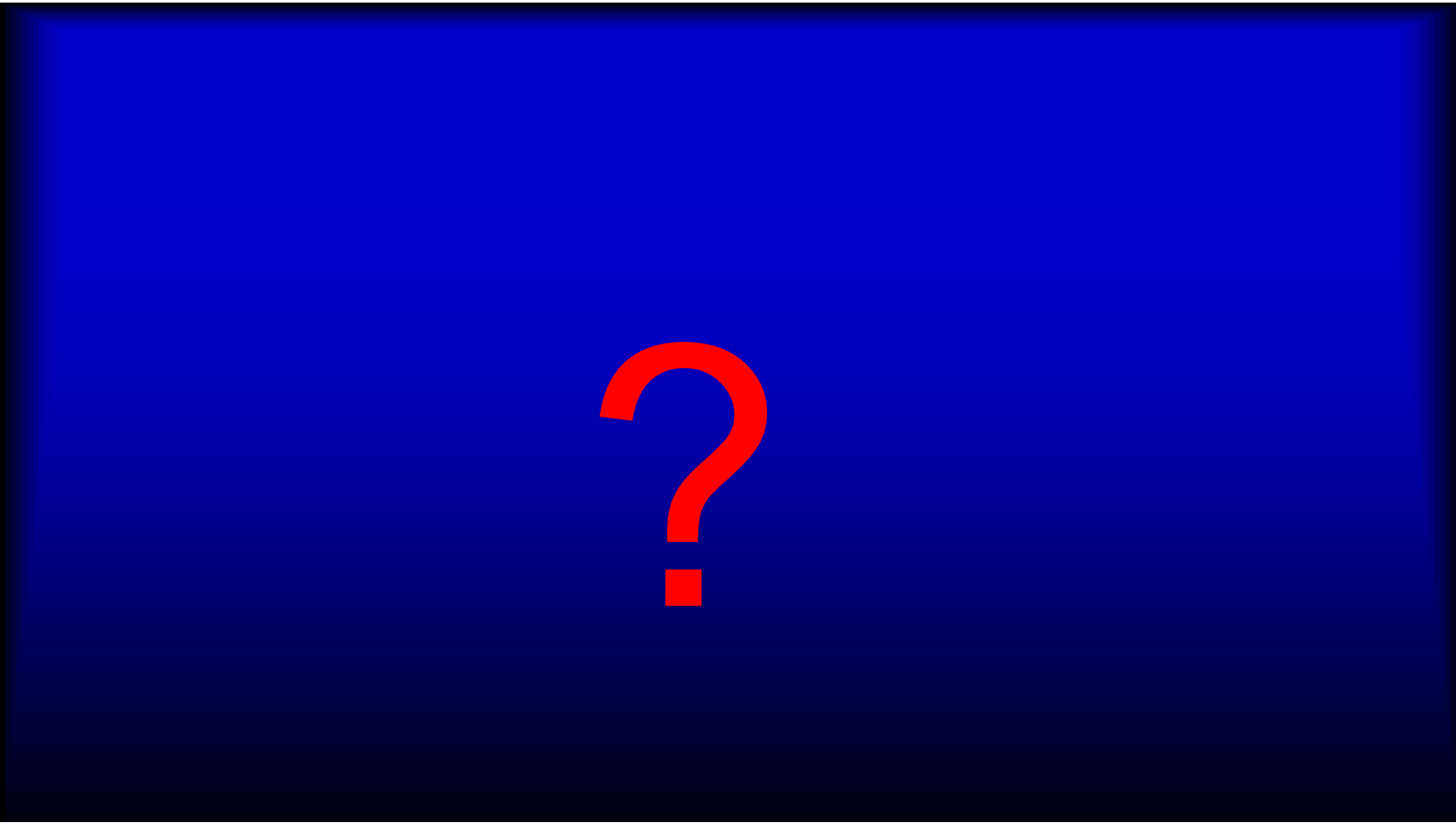

ENDOCRINO
AUTOCRINO
NEUROCRINO
PARACRINO
CELLULA BERSAGLIO

ORGANO ASSORBENTE

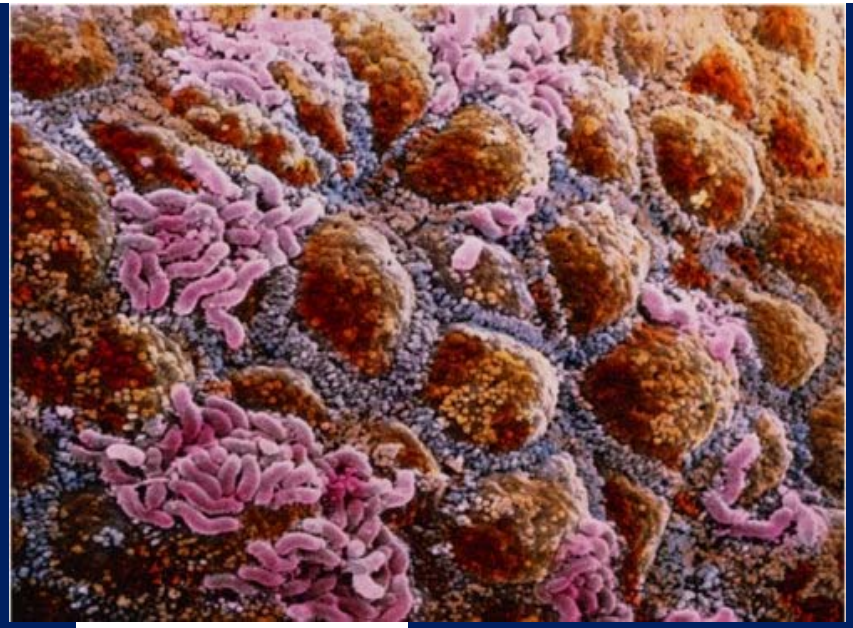
INTESTINO TENUE : condiziona il benessere di tutto l' organismo

XI° ORGANO ATTIVO

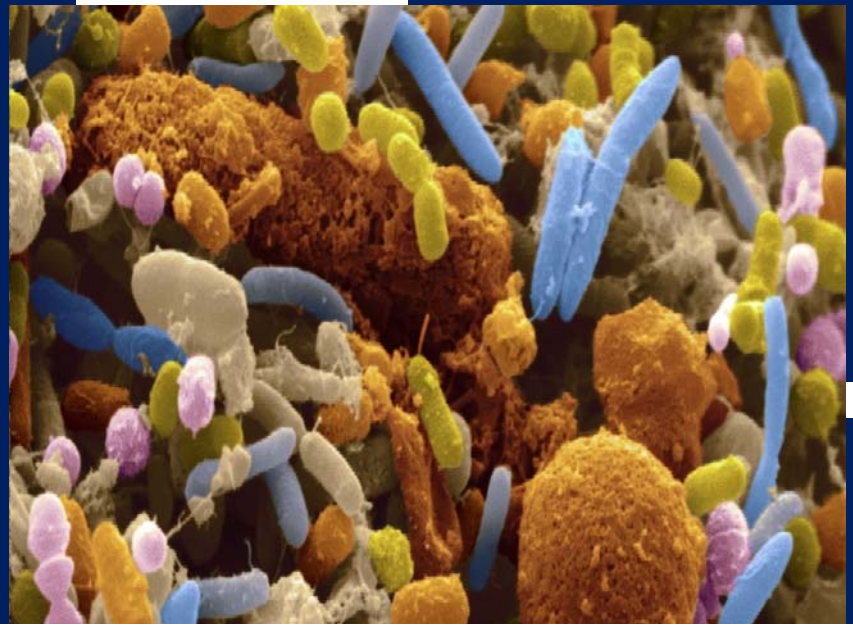
MICROBIOTA INTESTINALE : induce la vita, la malattia, la morte

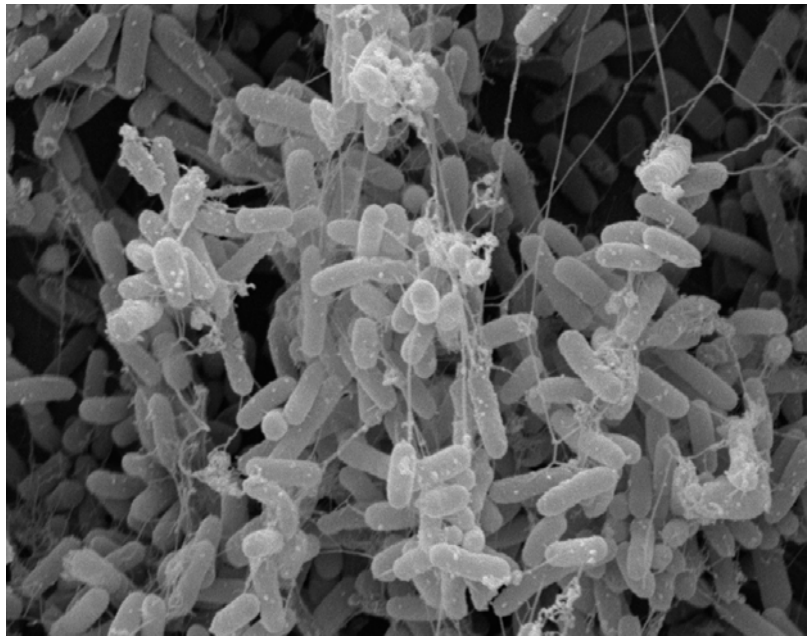


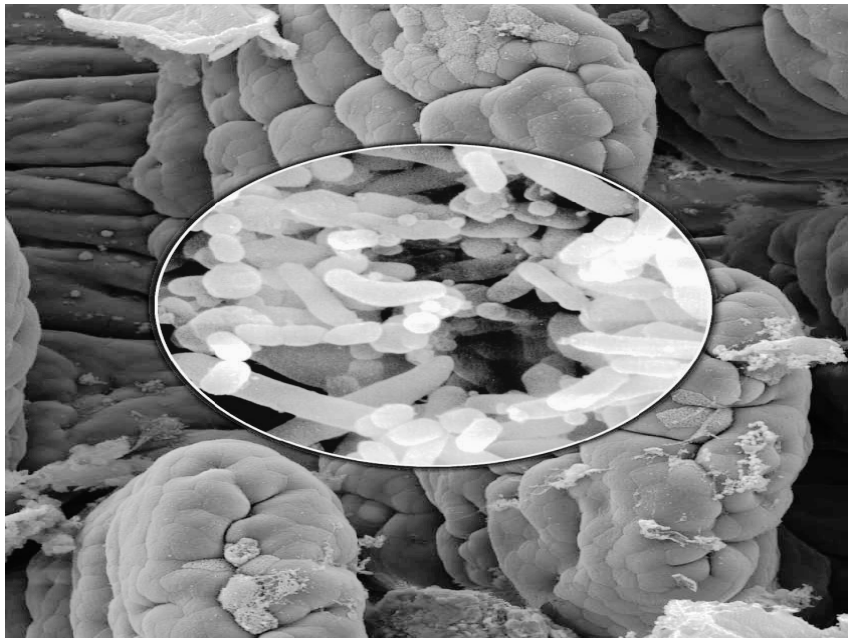
IL MICRIOBIOTA INTESTINALE

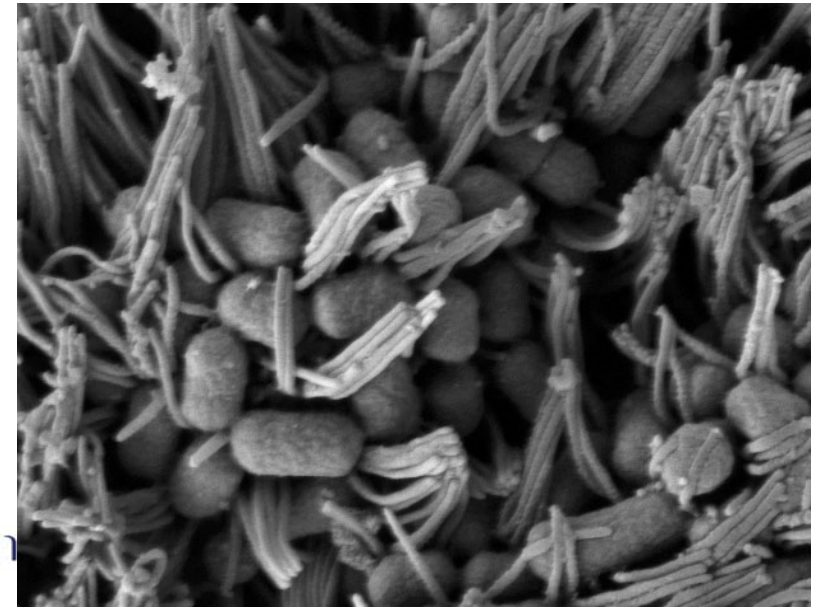



10 µm



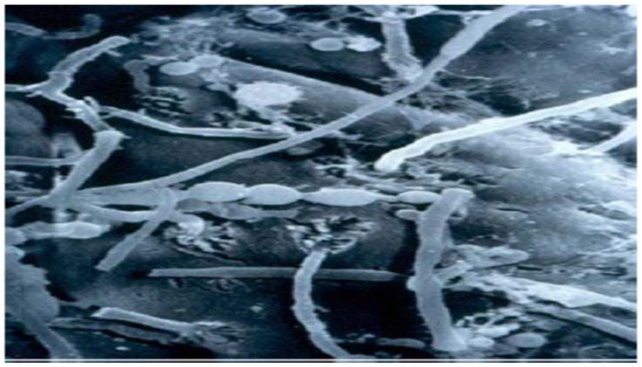






Gem

Yeast: GUT MYCOME



- **Commensal to the gut at low concentrations**
- **In the healthy gut dominant species are:**

Wallemia, Trichocomaceae, Rhodotorula, Saccharomycetaceae, Pleosporaceae, Agaricaceae, Metschnikowiaceae, Cystofilobasidiaceae, Ascomycota, Amphisphaeriaceae...

- **Yeast's functions in the gut it is not clear**
- **Yeasts overgrowth/dysbiosis (related to host genetic and physiology, lifestyle, antibiotic usage, immune system disfunction) could be pathogenic fo the gut**

Dollive S, et al. Genome Biol 2012 Cui et al. Genome Medicine 2013 Thewes S, Mol Microbiol 2007

Virus/phages: GUT VIROME

Berg Miller et al., Environ Microbiol 2012, 14 (1) 207-27

- ***Collection of all viruses of the gut***
- ***Harmful viruses are a minority compared to benign viruses***
- ***Bacterial virus are called bacteriophages***

Random pyrosequencing of virus-enriched metagenomes have been isolated from bovine rumen. In the bovine rumen have been isolated up to 28.000 different viral genotypes; the majority (~78%) of sequences did not match any previously described virus

***Pro phages* outnumbered *lytic phages* approximately 2:1**

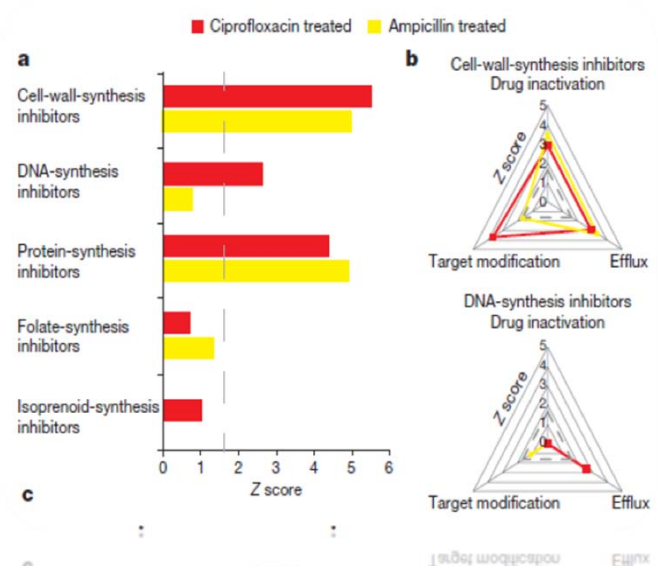
Metabolic profiling revealed an enrichment of sequences with putative functional roles in DNA and protein metabolism, but a low proportion of sequences assigned to carbohydrate and amino acid metabolism

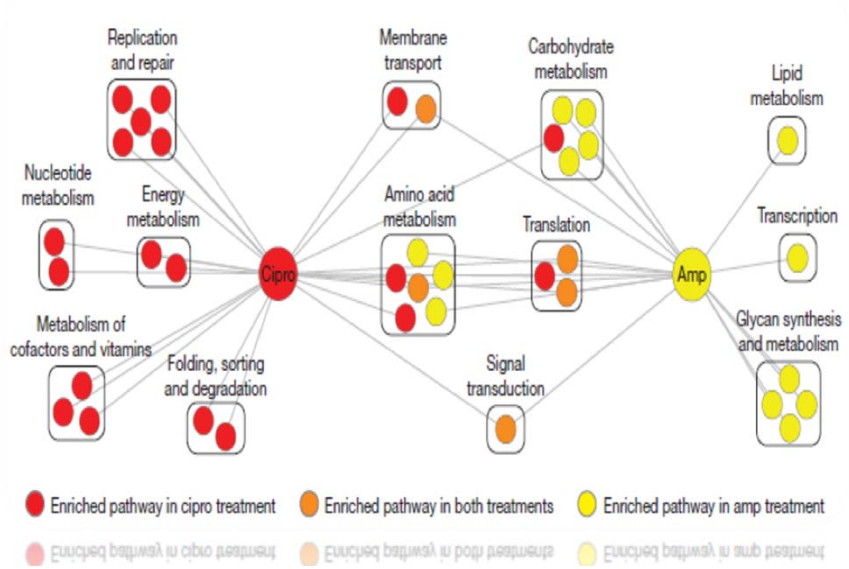
GUT VIROME

Phage-bacteria relationships

Modi SR et al., Nature 2013;499(7457):219-22

Virus metagenome is a resource for the acquisition of additional genes to maximize adaptation to the changing gut environment following antibiotic or diet-mediated perturbation

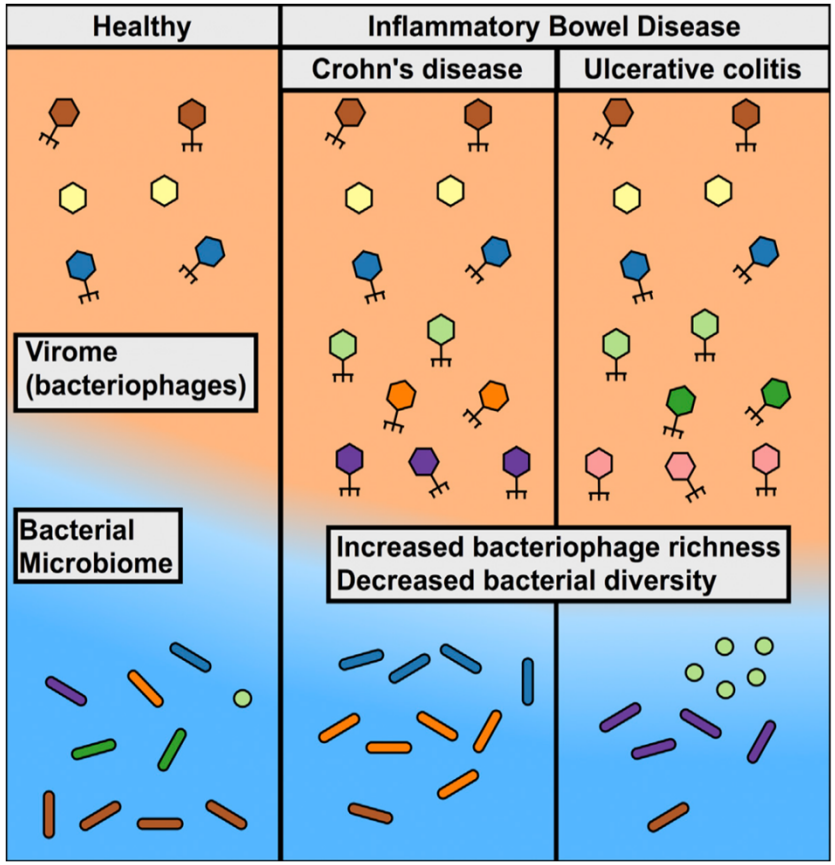




Cell

Disease-Specific Alterations in the Enteric Virome in Inflammatory Bowel Disease

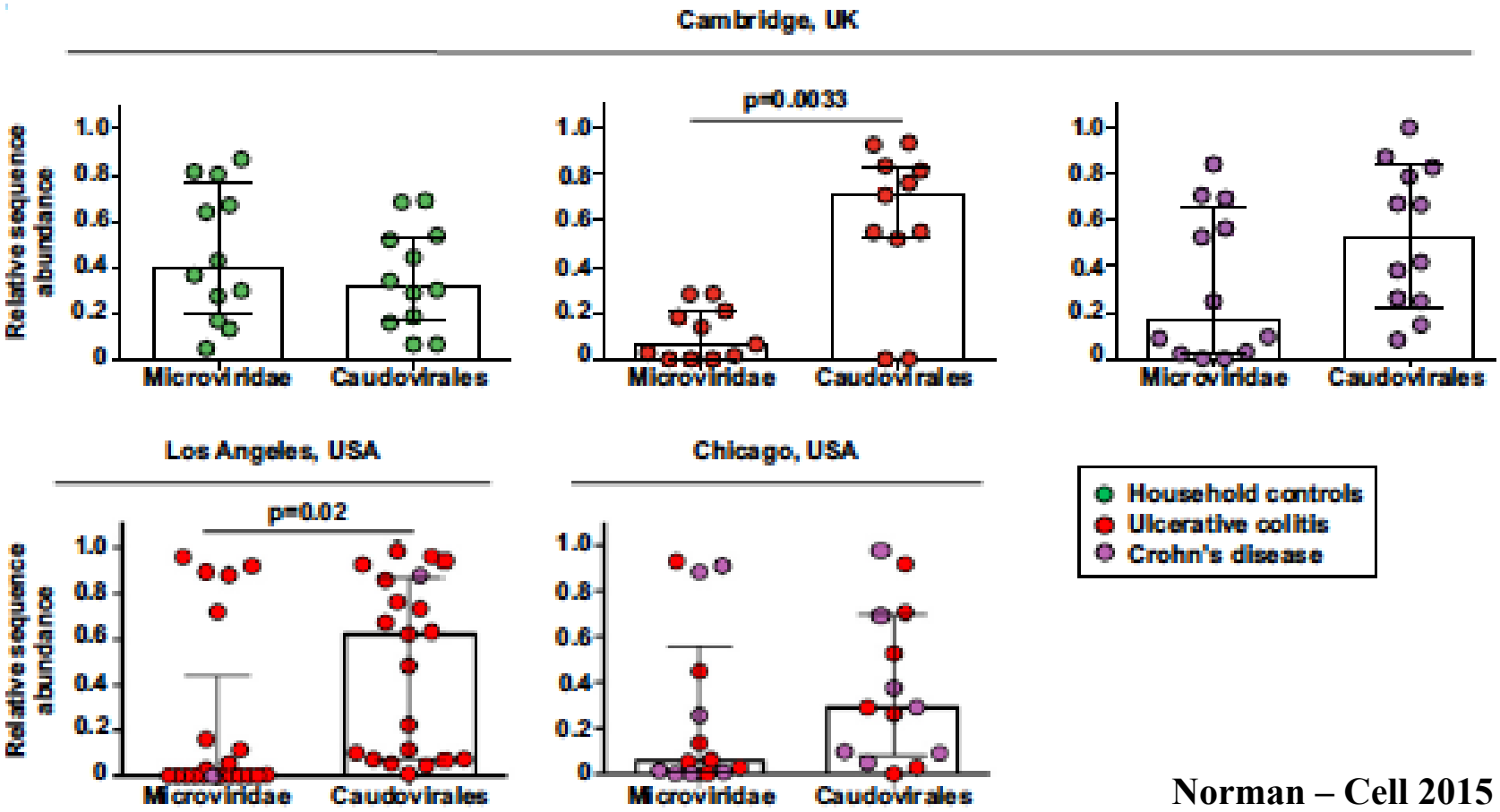
- The enteric virome is abnormal in multiple IBD patient cohorts
- The enteric virome richness increases in Crohn Disease and Ulcerative Colitis
- Decreases in bacterial diversity and richness in IBD do not explain virome changes
- Virome changes in CD and UC are disease-specific



Norman – Cell 2015

IBD patients have an altered bacteriophage abundance

↑ Caudovirales ↓ Microviridae



Norman – Cell 2015

Gasbarrini G. et al.
SIMI 1991

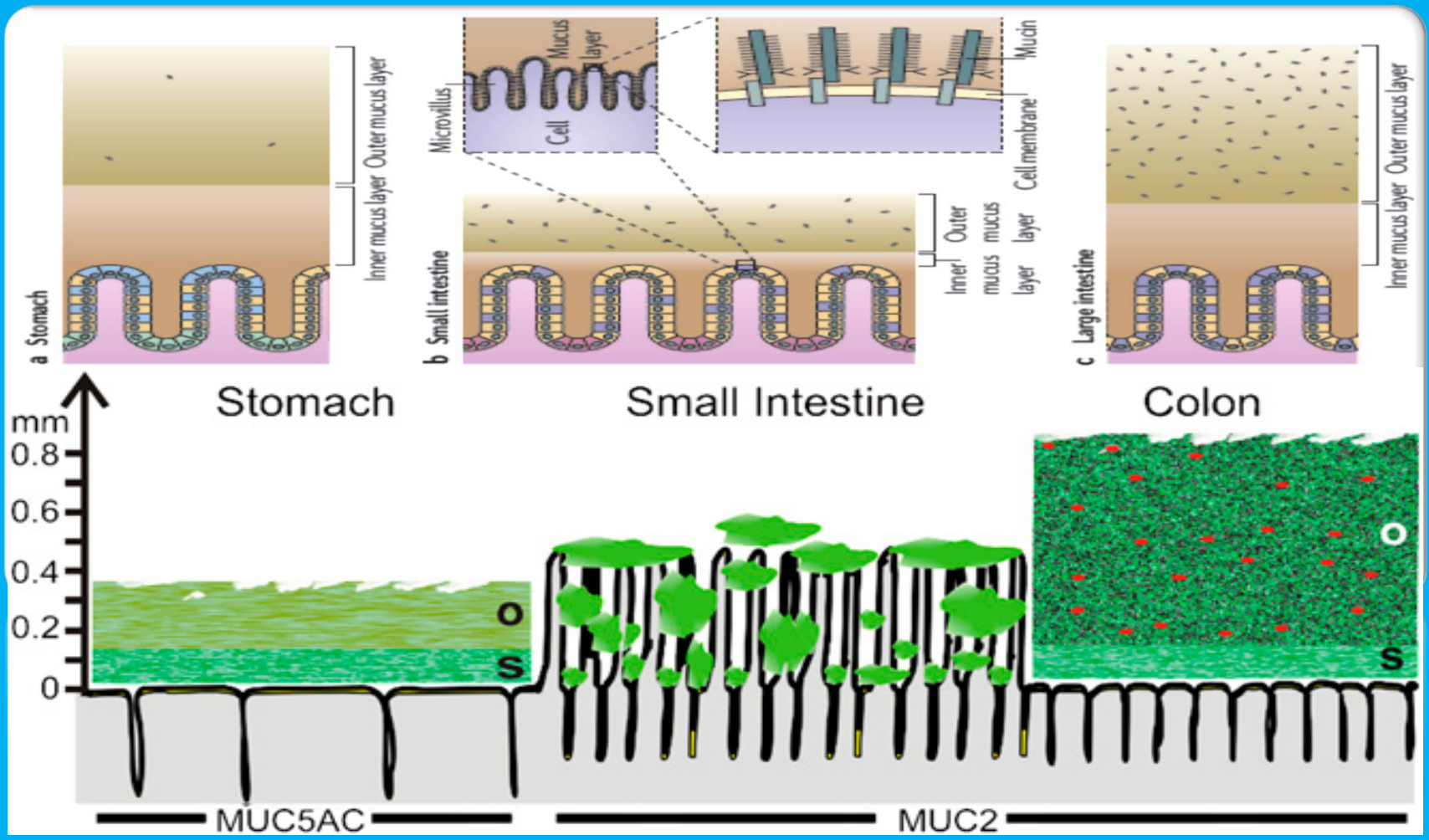
ORGANO ASSORBENTE

INTESTINO TENUE : OSPITANTE : condiziona il benessere di tutto l' organismo

XI° ORGANO ATTIVO

MICROBIOTA INTESTINALE : OSPITE : induce la vita, la malattia, la morte

MUCUS DISTRIBUTION: OUTER LAYER THIN AND PATCHY ON SMALL INTESTINE WHERE MUCOUS CELLS ARE FEWER

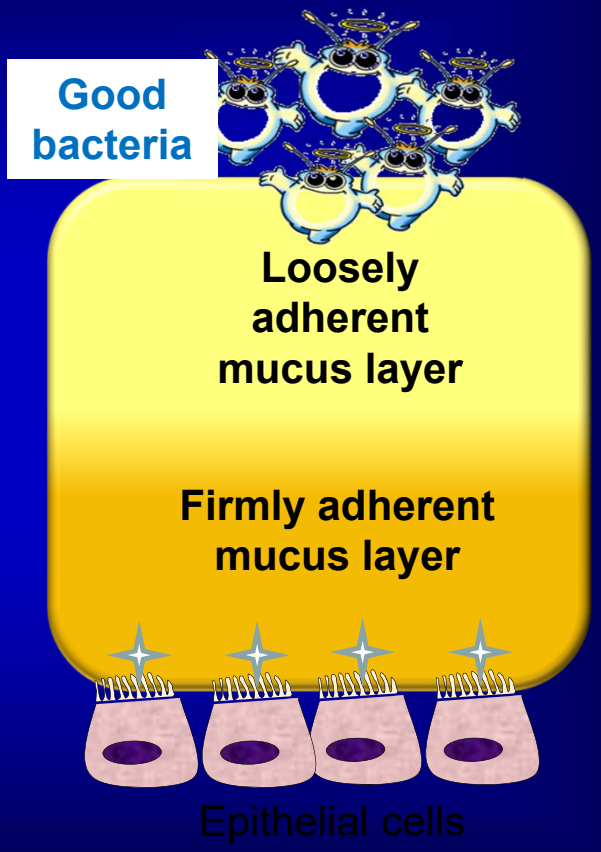


McGuckin et al, Inflamm Bowel Dis 2011

McGuckin et al, Nat Rev Microb 2010

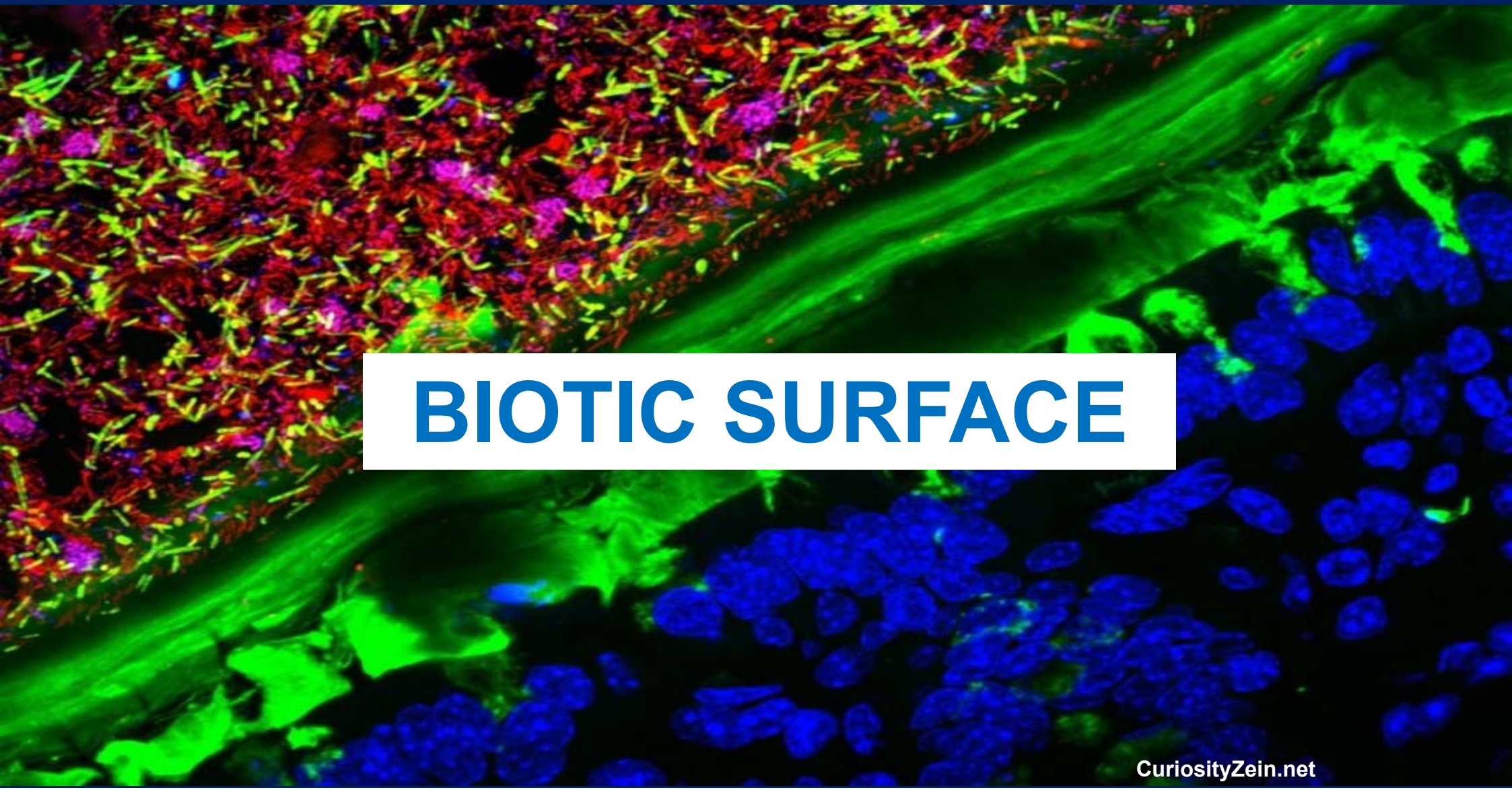
IMPORTANCE OF THE MUCUS LAYERS

- The inner mucus layer is dense and does not allow bacteria to penetrate, thus keeping the epithelial cell surface free from bacteria
- The inner mucus layer is converted into the outer layer, which is the habitat of the commensal flora

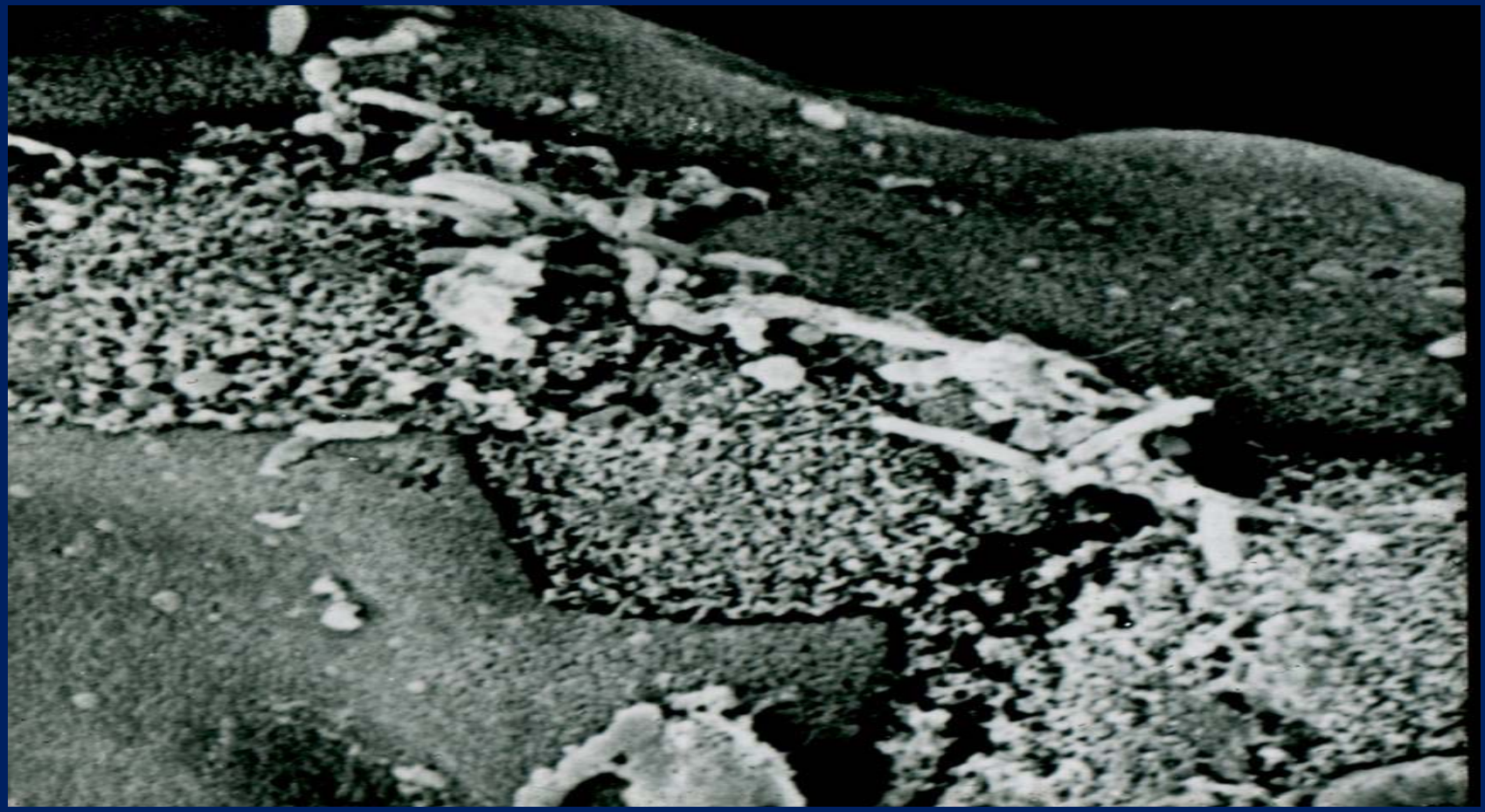


Johansson, M.E., Proc Natl Acad Sci U S A, 2010

THE ANATOMO-MICROBIOLOGICAL GUT BARRIER

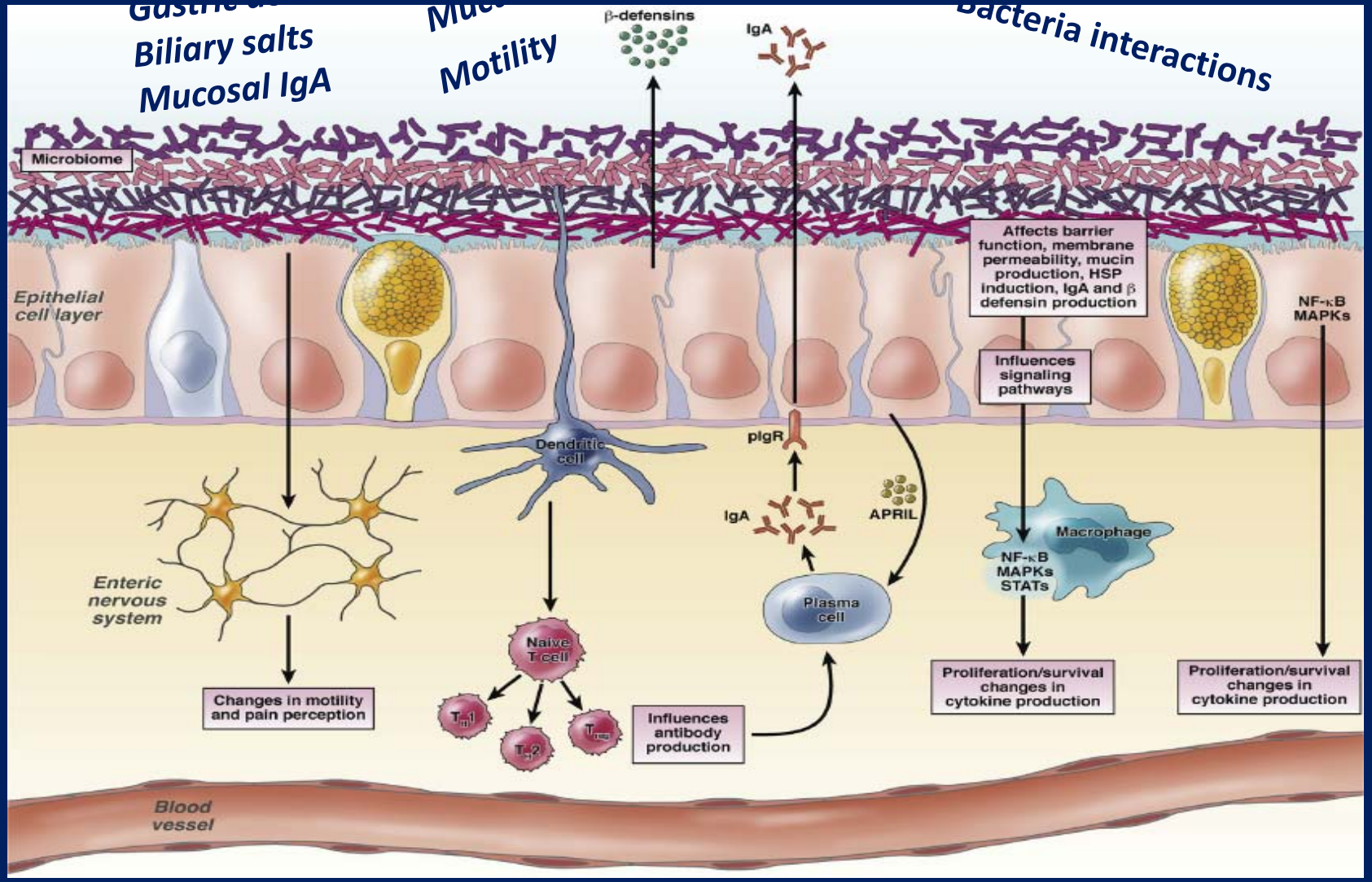


Effect of the mucolytic activity of bacteria on the surface of enterocytes

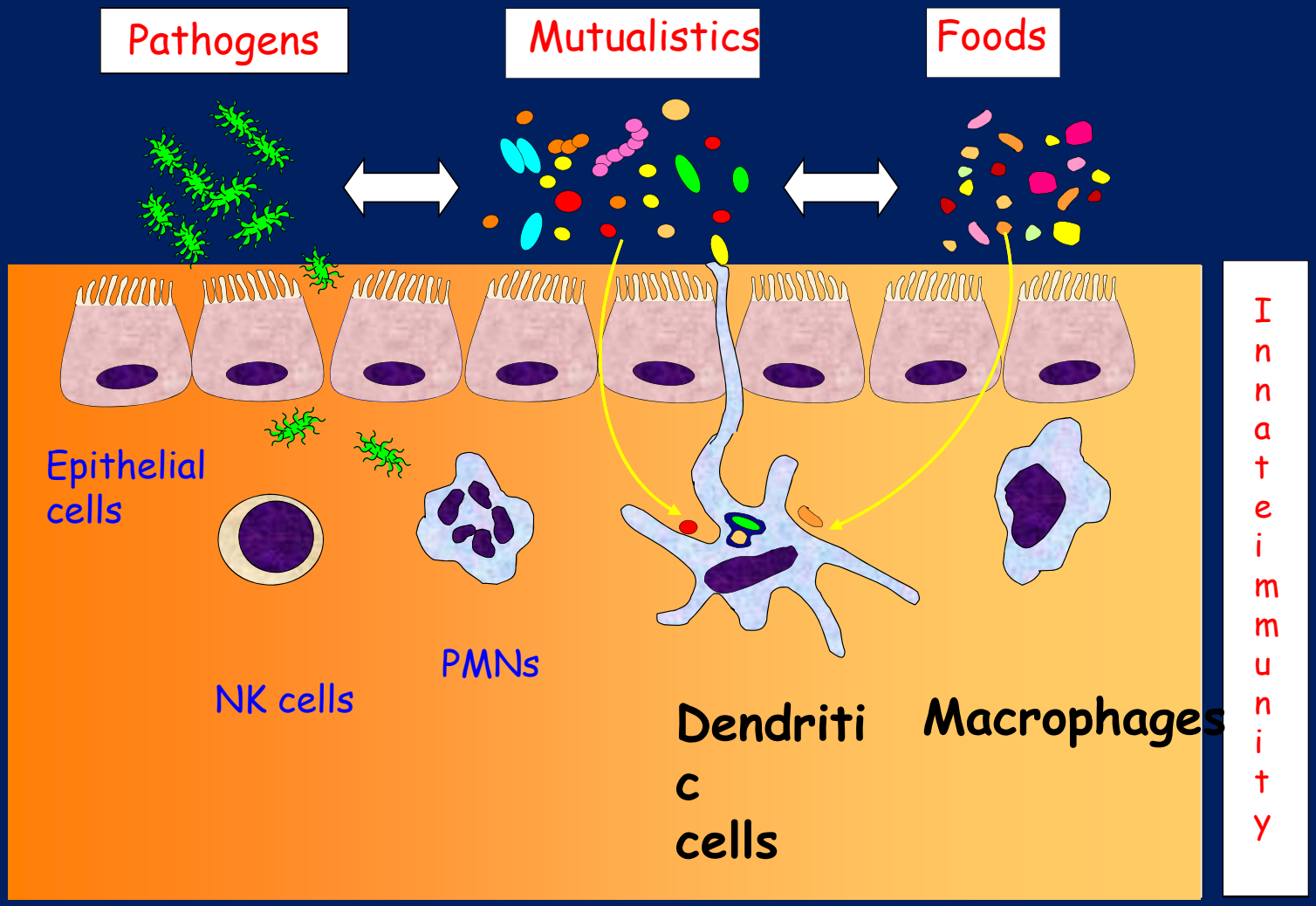


Gasbarrini G Et al., SEM, 1985

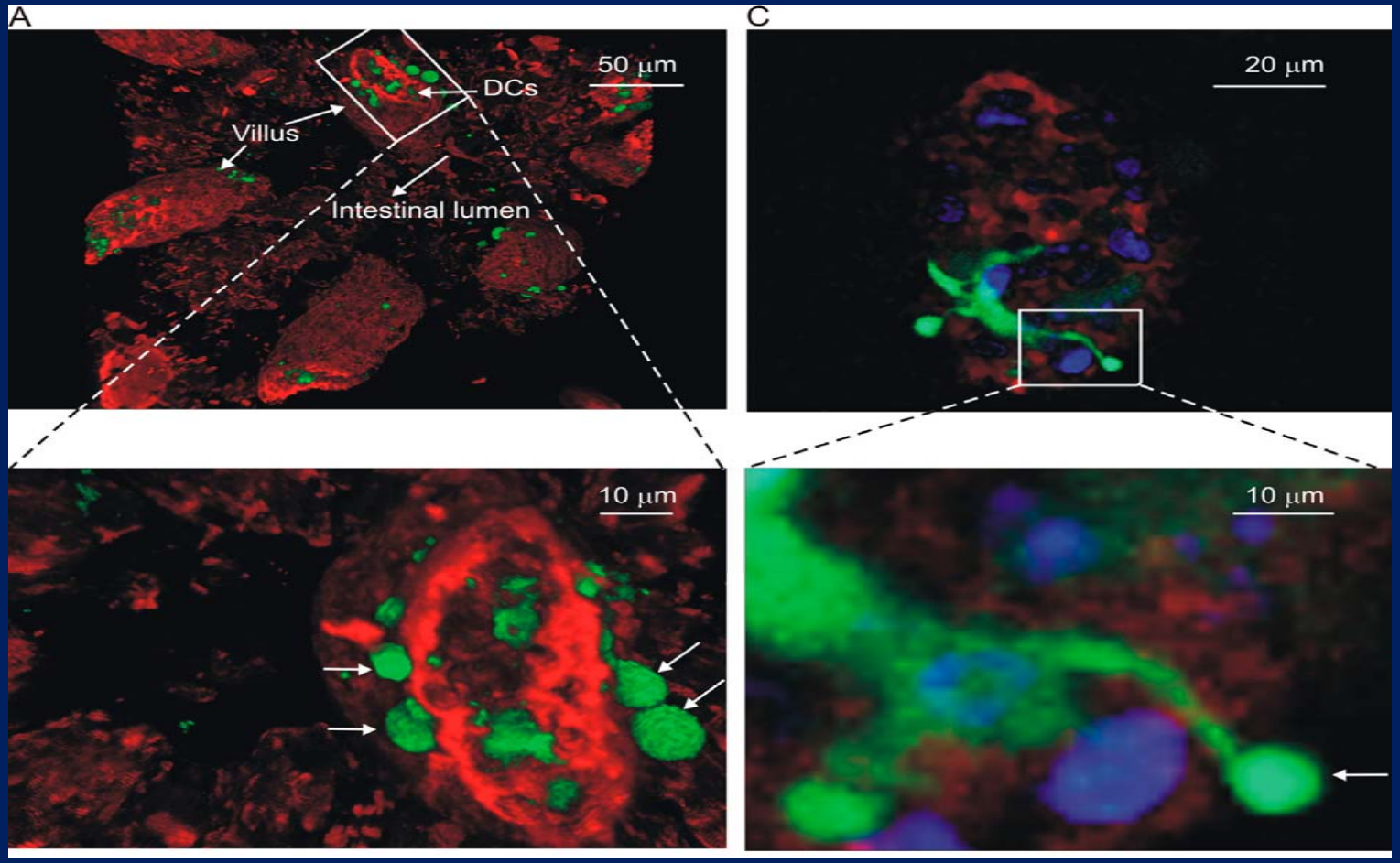
GUT BARRIER INTEGRITY



A continuous gut cross-talk



DENDRITIC CELLS form PSEUDOPODS that reach the intestinal lumen and capture dangerous molecules - Immunofluorescence



Chieppa M, JEM 2006

The intestinal epithelium is the largest of the body's mucosal surface that separates mammalian host from the external environment, and, like all the wallsis attached by enemirs and is defended by friends

ROME MONUMENTS OF THE IMPERIAL FORUMS DID NOT RESIST AS…

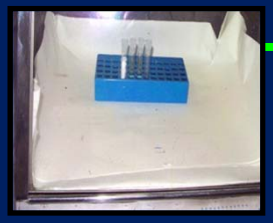
^{51}Cr-ethylenediaminetetra-acetate (^{51}Cr-EDTA)

100μCi (1.85 MBq) ^{51}Cr-EDTA (*Amersham Health, England*) diluted with water up to 10 ml volume

24/hrs urine collection

Preparation of two 3-ml urine samples

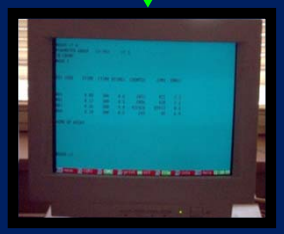
Preparation of the standard: collection of 0.2 ml (1/50) of the dose and dilution with water up to 3 ml volume



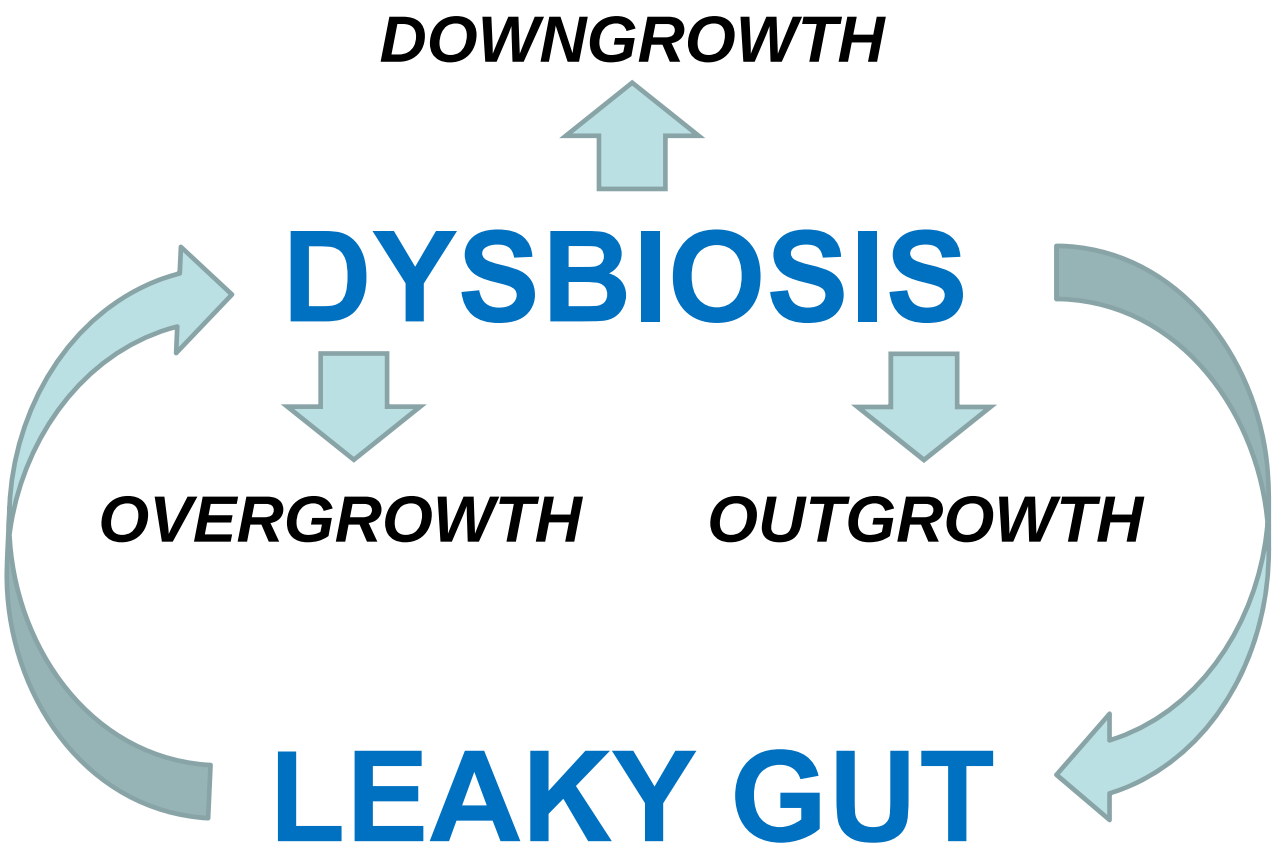
Standard, urine samples and background

Gamma-counter counts

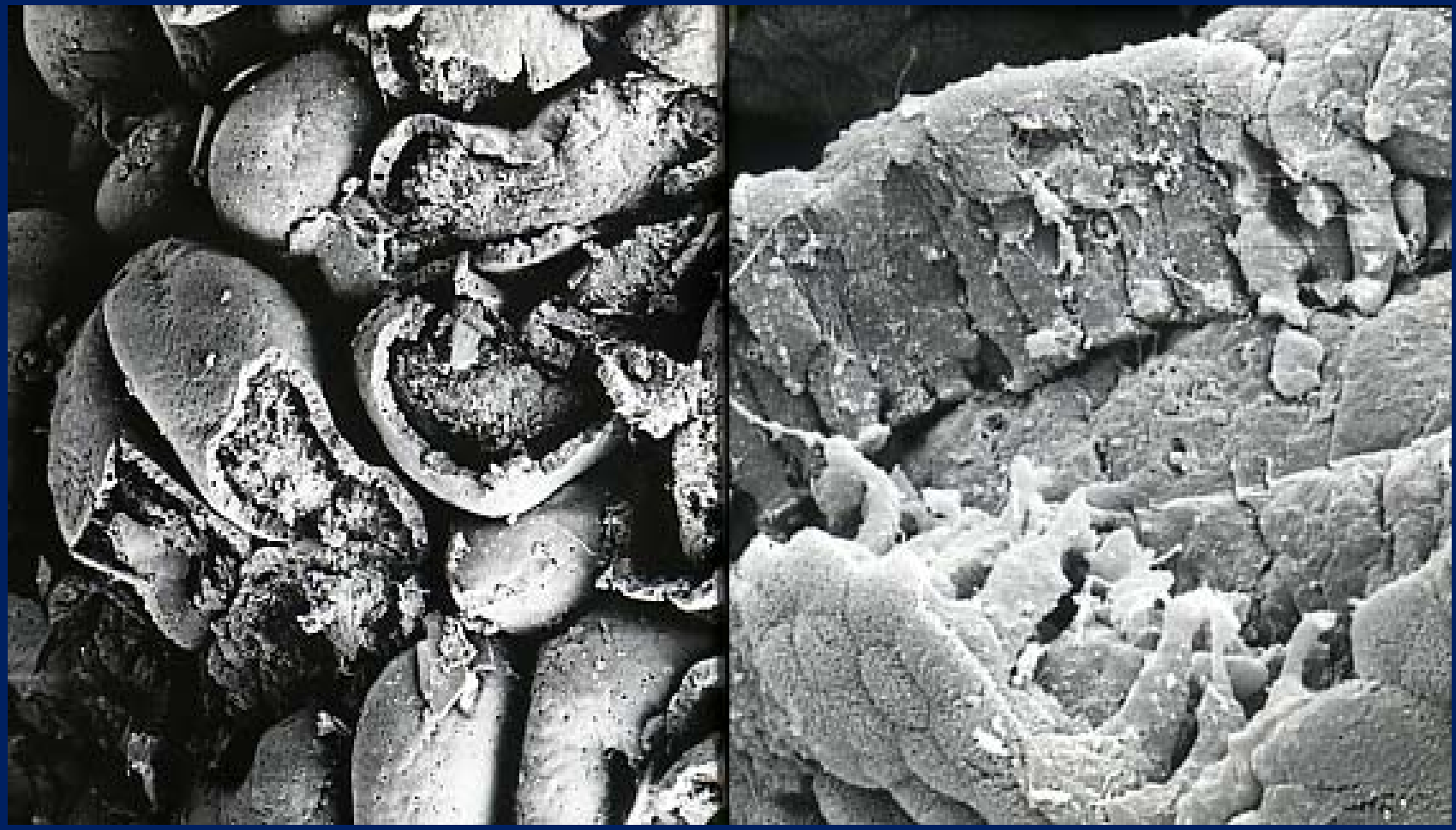
(*LKB-Wallac 1282 Compugamma, Turku, Finland*)



Final results espressed as percentage of administered dose according the formula: [Mean Urinary Counts x Urinary Volume] x [Standard counts x 50]$^{-1}$


DOWNGROWTH
DYSBIOSIS
OVERGROWTH
OUTGROWTH
LEAKY GUT

A NORMAL CYTOSKELETON ENABLES ENTEROCYTES TO MIGRATE AND HEAL MICROEROSIO



Gasbarrini G, Bonvicini F, SEM 1985

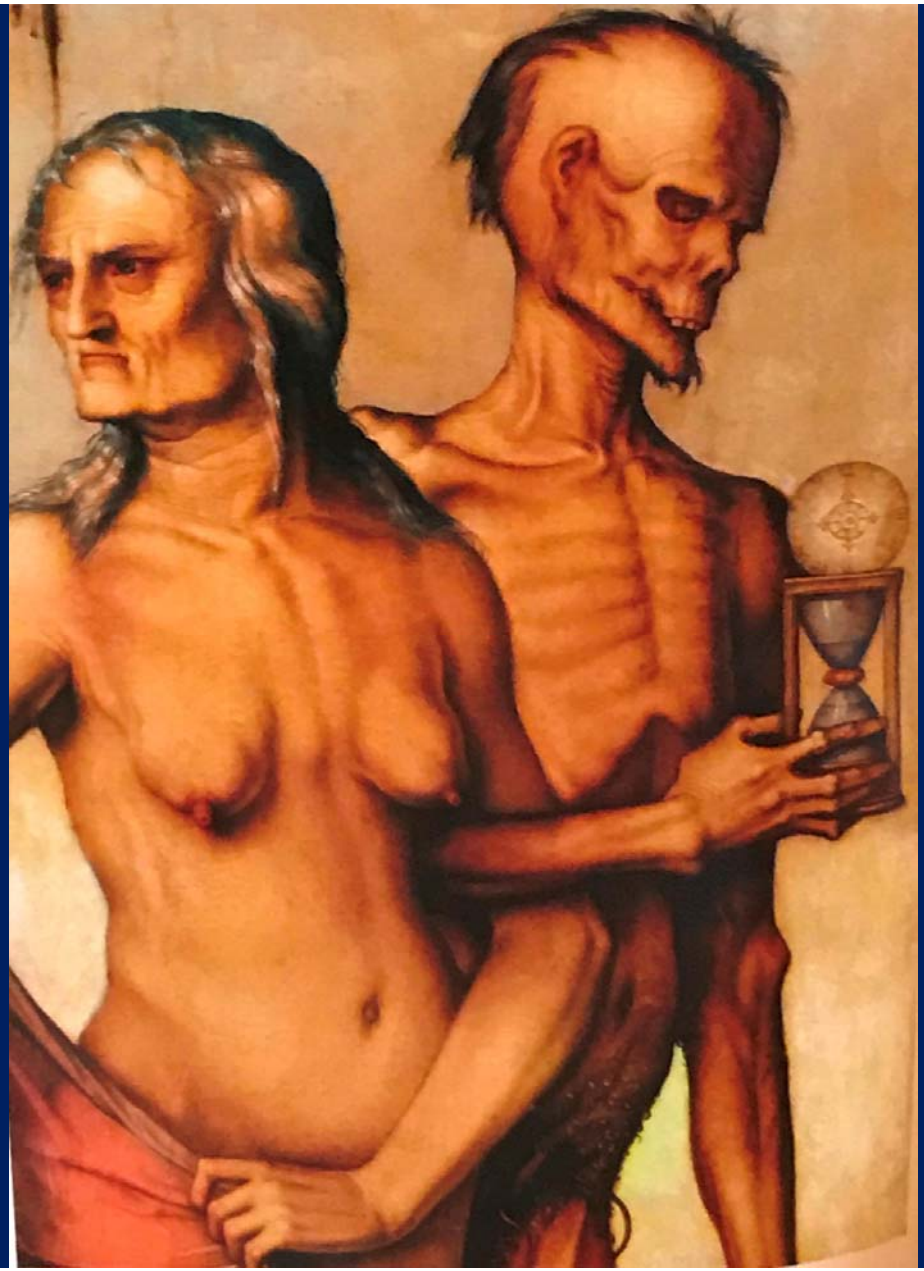
-

MANGIARE

ASSORBIRE

NUTRIRSI

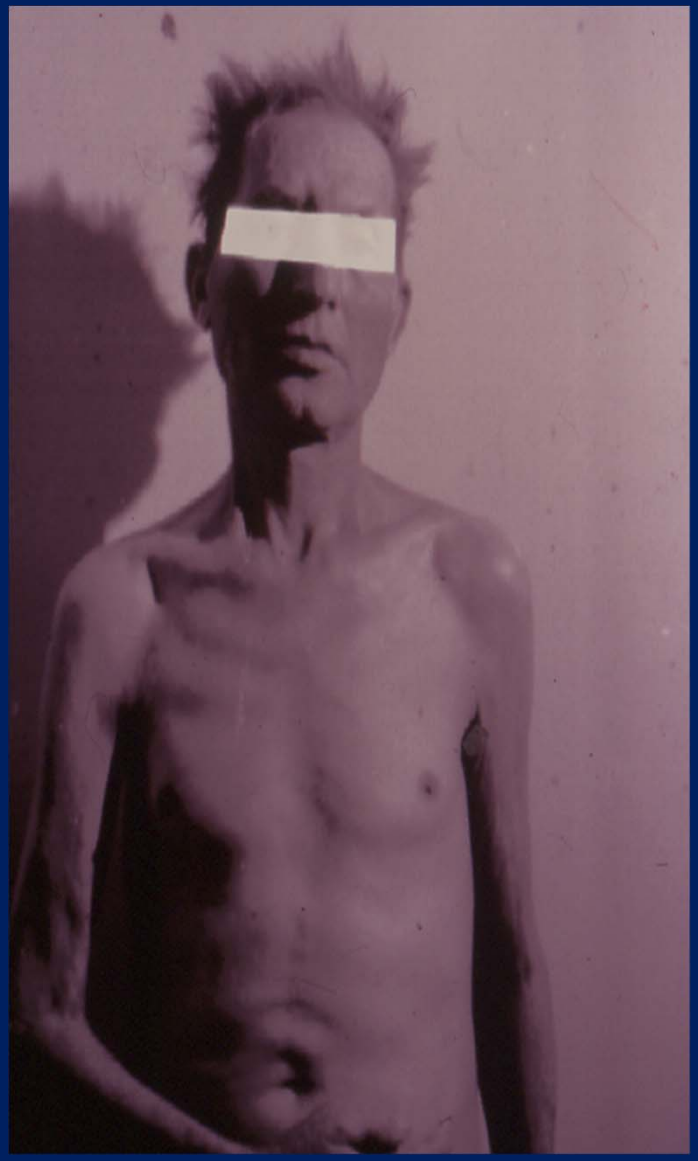
MAGREZZA DA RIDOTTO APPORTO

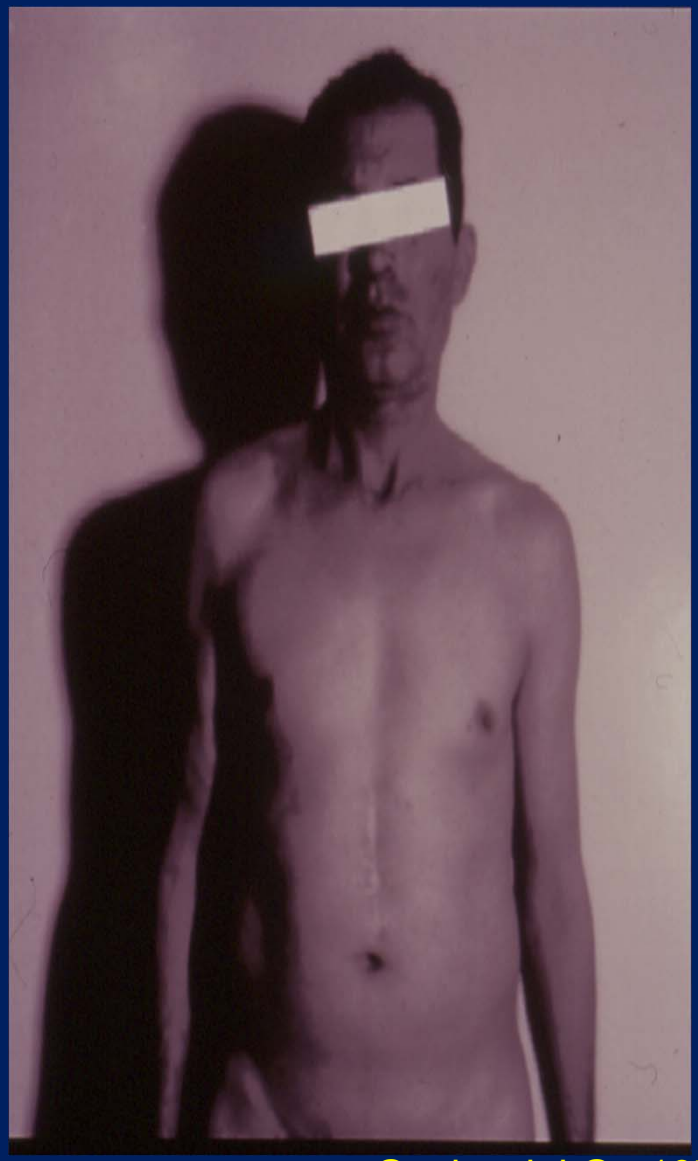


MAGREZZA DA MALASSORBIMENTO

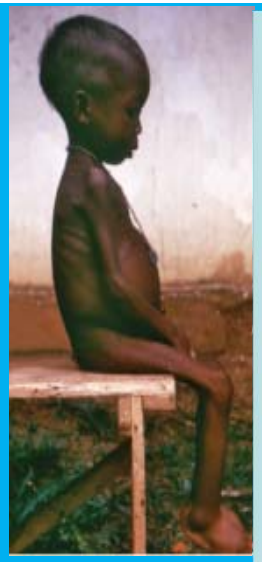

GASBARRINI G. , 1963

MAGREZZA (?) DA CONTAMINAZIONE BATTERICA NELL' INTESTINO TENUE





Gasbarrini G., 1975



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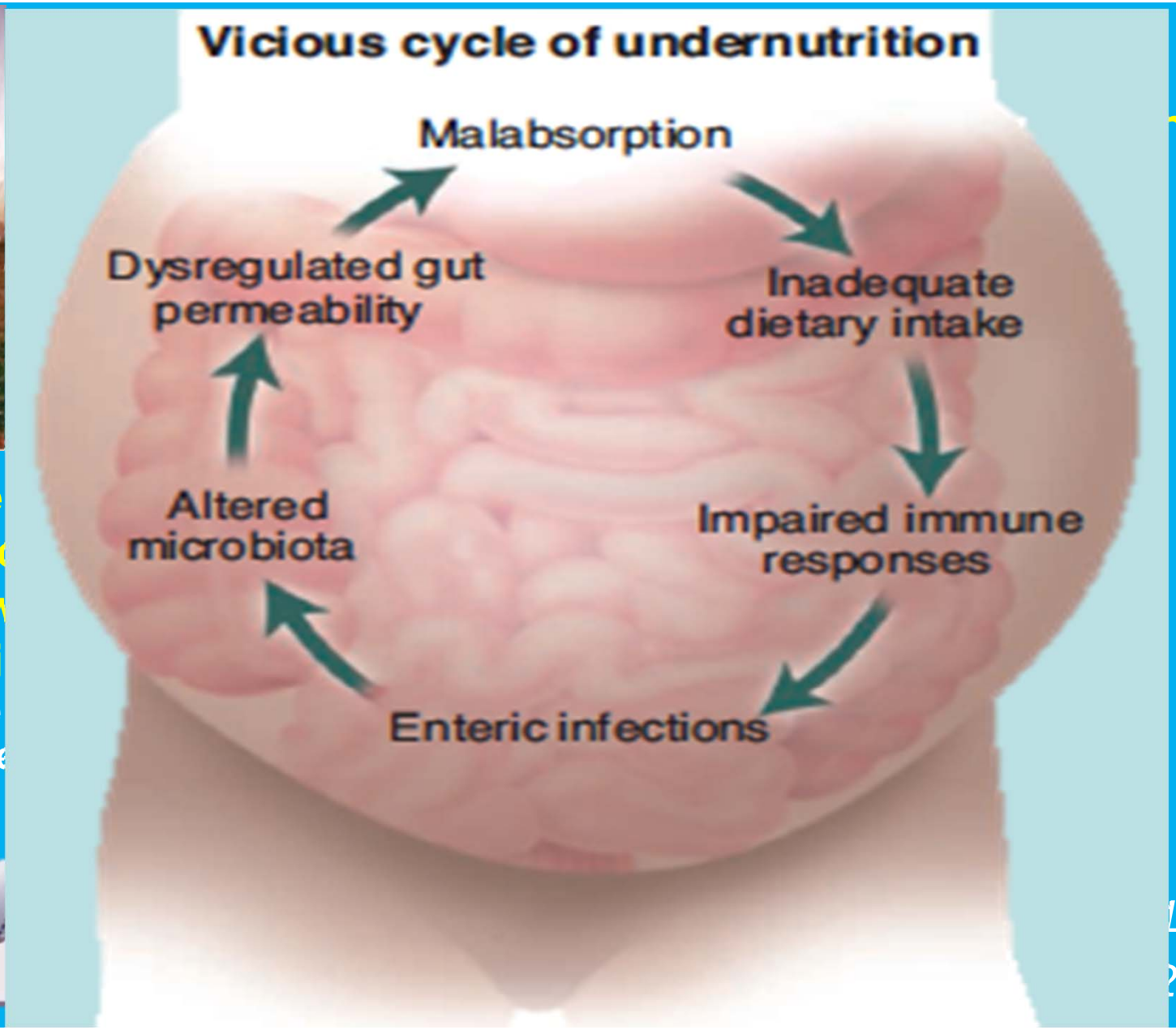
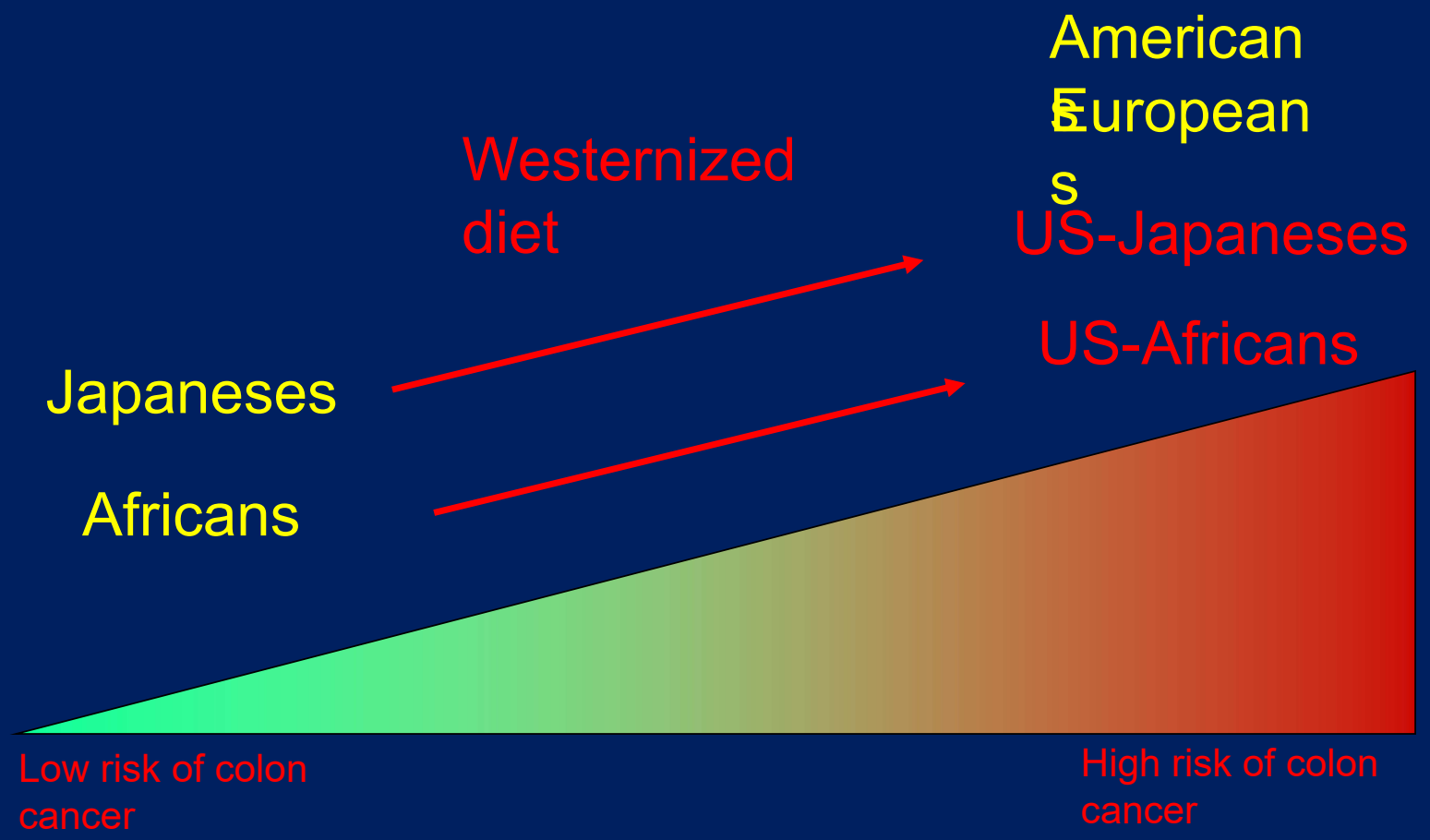


ny

13
2

Epidemiological studies

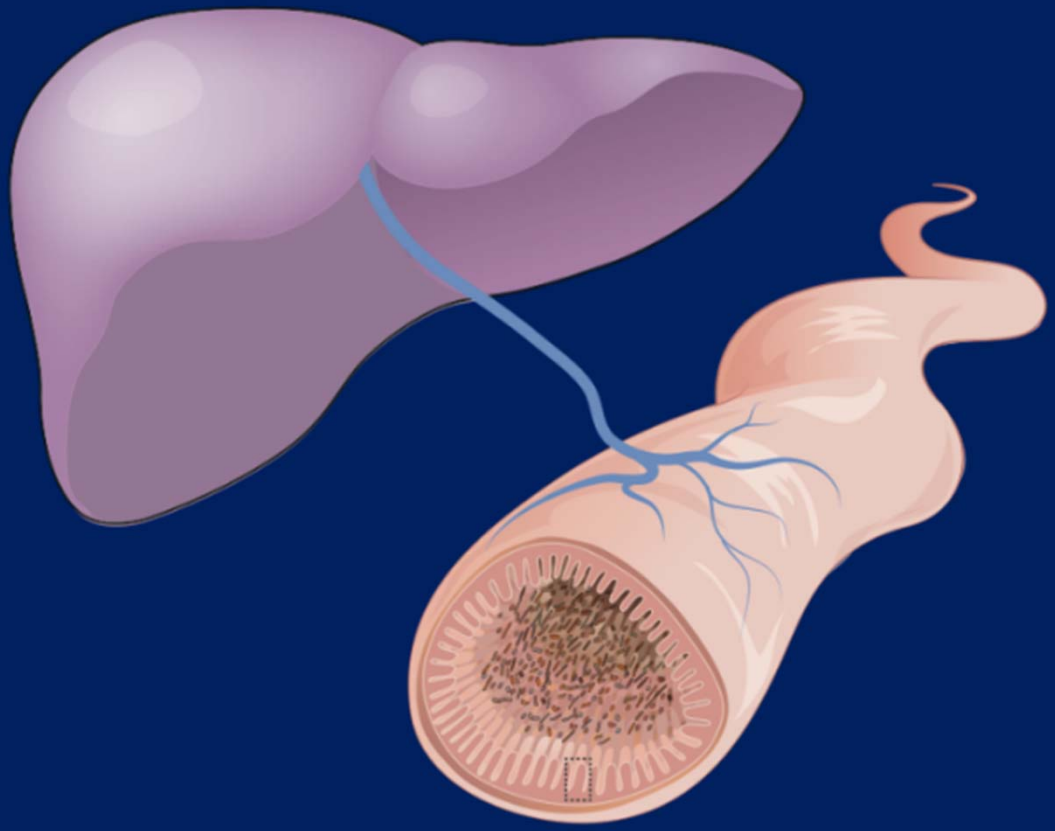


HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases

EDITORIALS

WAJAHAT Z. MEHAL HEPATOLOGY, June 2012

The Gut-Liver Axis: A Busy Two-Way Street



Gut microbiota and NAFLD/NASH

HIGH FAT DIET

Altered gut microbiota
(↑ Firmicutes/Bacteroidetes, ↓ diversity)
SIBO (50% of NASH pts)

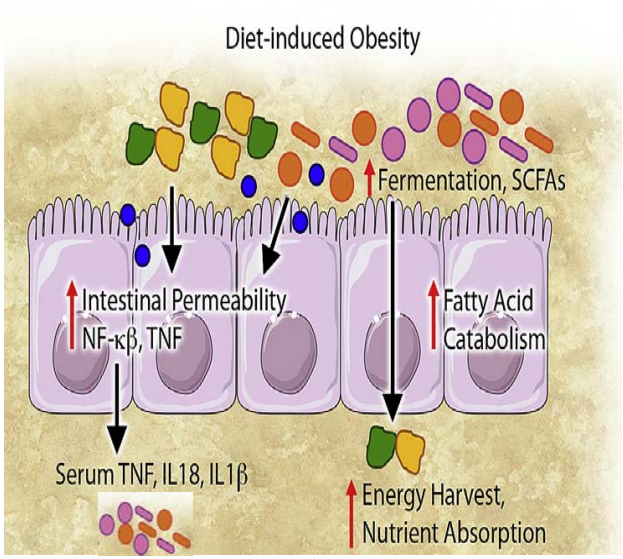
↑ Intestinal permeability

↓ Satiety

↑ Energy harvest
↑ Lipogenesis
↑ Gluconeogenesis

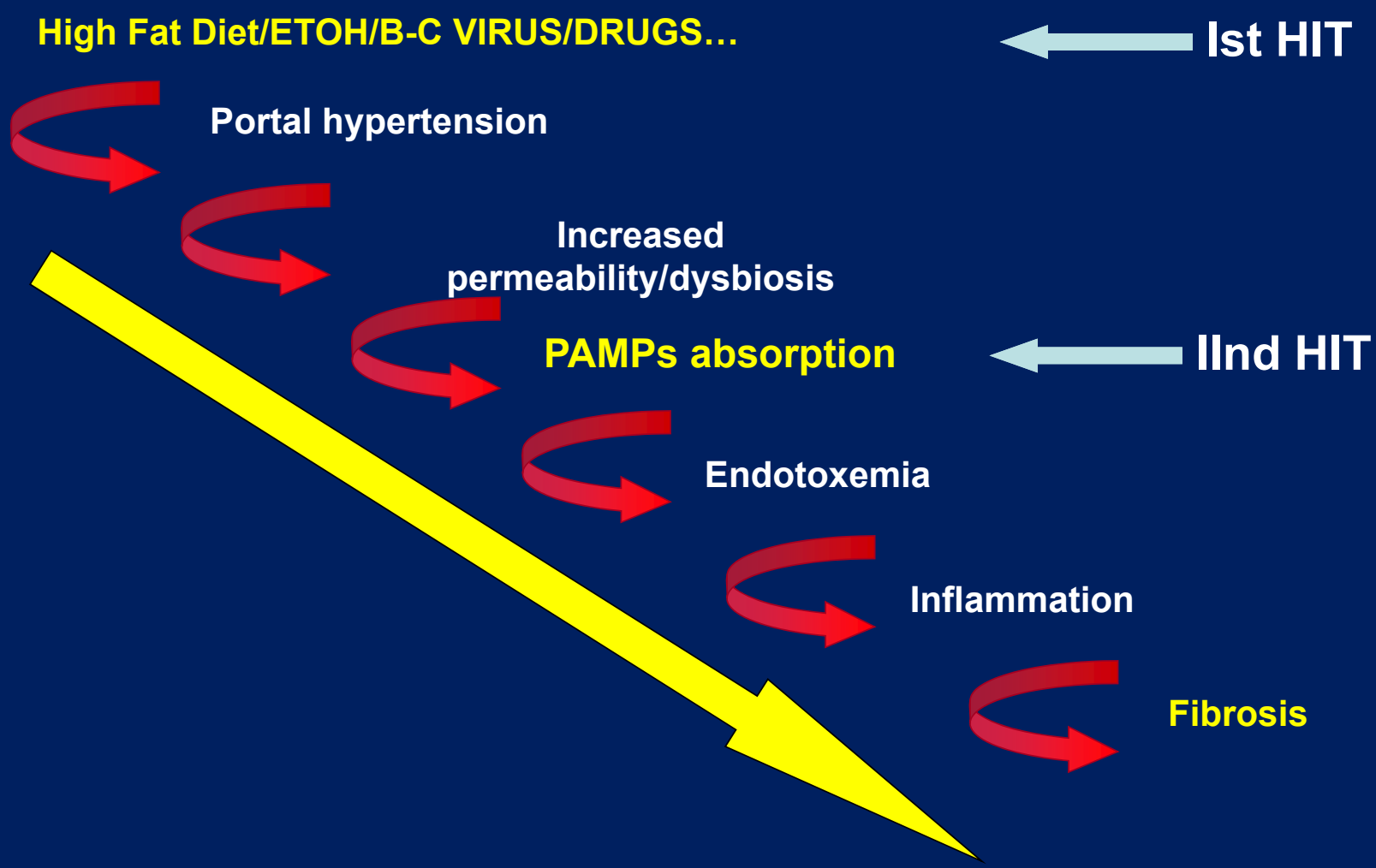
↓ Hepatic VLDL secretion

↑ LPL ↓ beta-oxidation=
↑ FFA uptake in liver/adipose tissue



Delzenne et al., Nat Rev Endocrinol 2011
Leung DY et al. J Allergy Clin Immunol 2013
Schwabe RF et al. Gastroenterology 2012
Ponziani FR et al. Exp Rev Gastroenterol Hepatol 2015

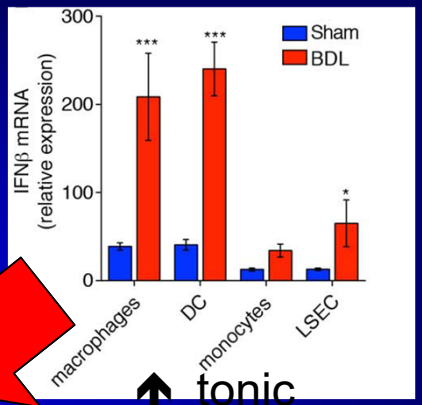
ROLE OF MICROBIOTA IN LIVER DISEASE PROGRESSION



Gut bacteria translocation & immune dysfunction

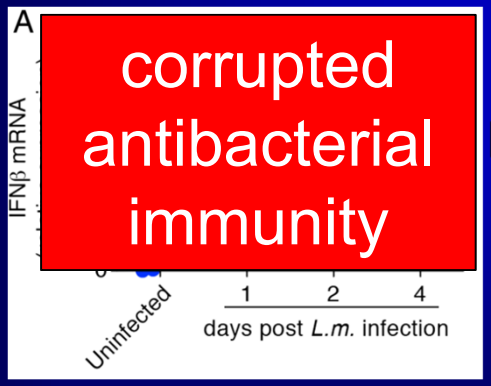
MURIN MODEL OF LIVER FIBROSIS

Gut microbiota translocation

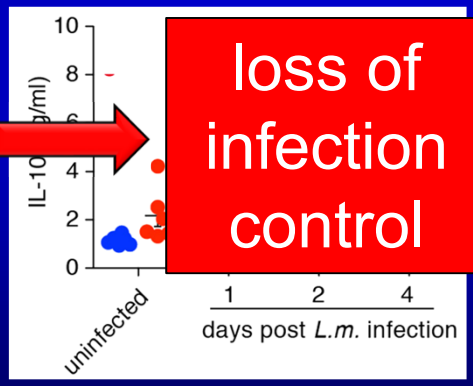


tonic type I IFN

AFTER BACTERIAL INFECTION (L. monocytogenes)



corrupted antibacterial immunity



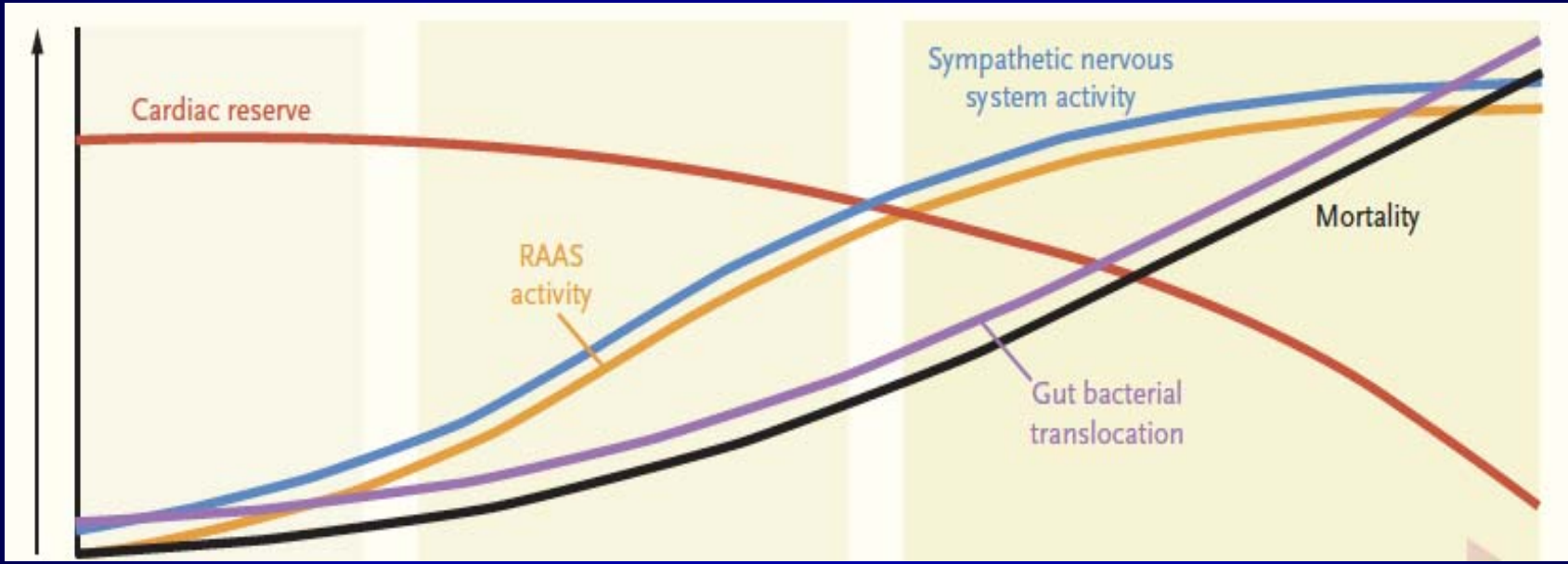
loss of infection control

MORTALITY

increased myeloid cells production of high concentrations of IFN and IL-10

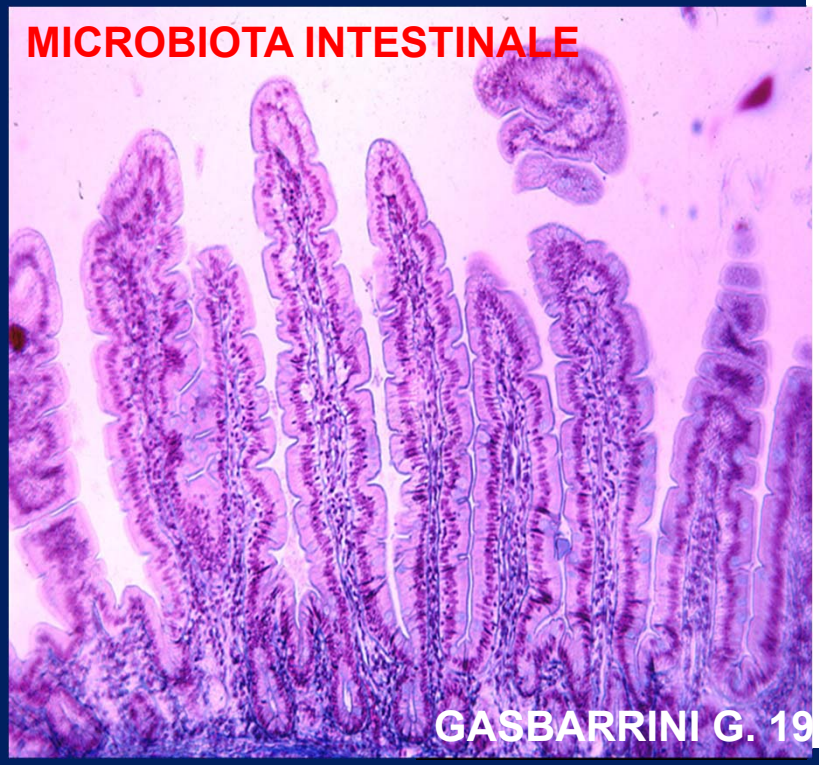
Hackstein CP et al. Gut 2016

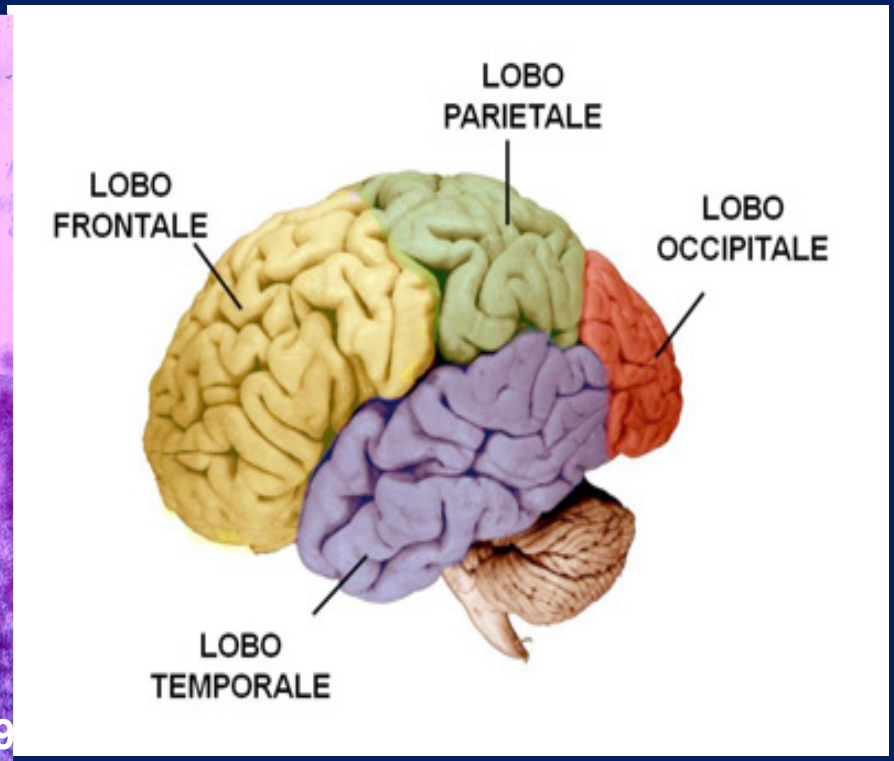
TODAY…
GUT MICROBIOTA HAS A PROGNOSTIC VALUE IN LIVER CIRRHOSIS



DISEASE PROGRESSION → **DISEASE PROGRESSION** → **DISEASE PROGRESSION**

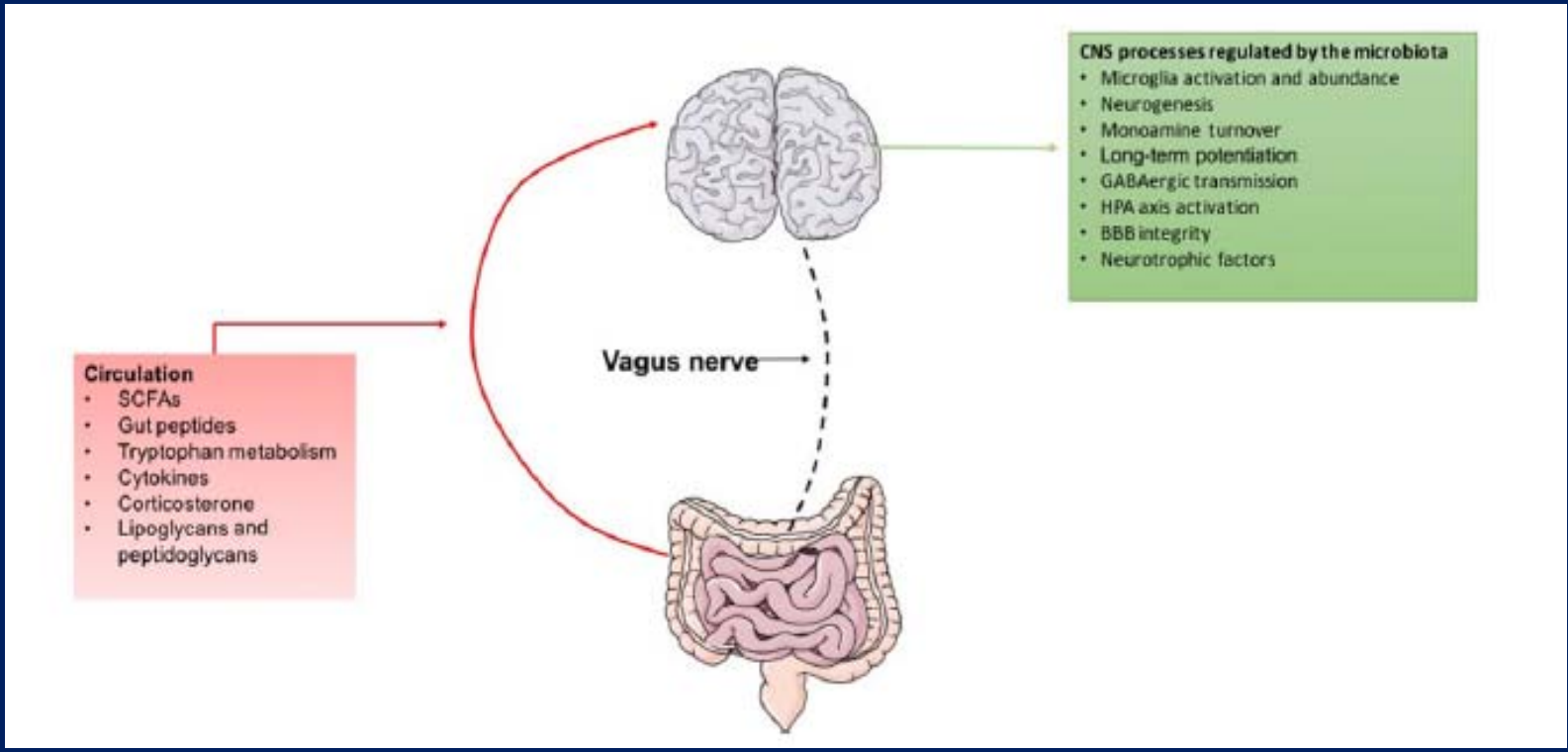
Ge PS et al. NEJM 2016


MICROBIOTA INTESTINALE
GASBARRINI G. 19


LOBO
PARIETALE
LOBO
FRONTALE
LOBO
OCCIPITALE
LOBO
TEMPORALE

2 ⇄ ? ⇄ 1

MICROBIOTA-GUT-BRAIN AXIS



Sherwin E et al, Recent developments in understanding the role of the gut microbiota in brain health and disease. Ann N Y Acad Sci. 2017 Aug 2. doi: 10.1111/nyas.13416.

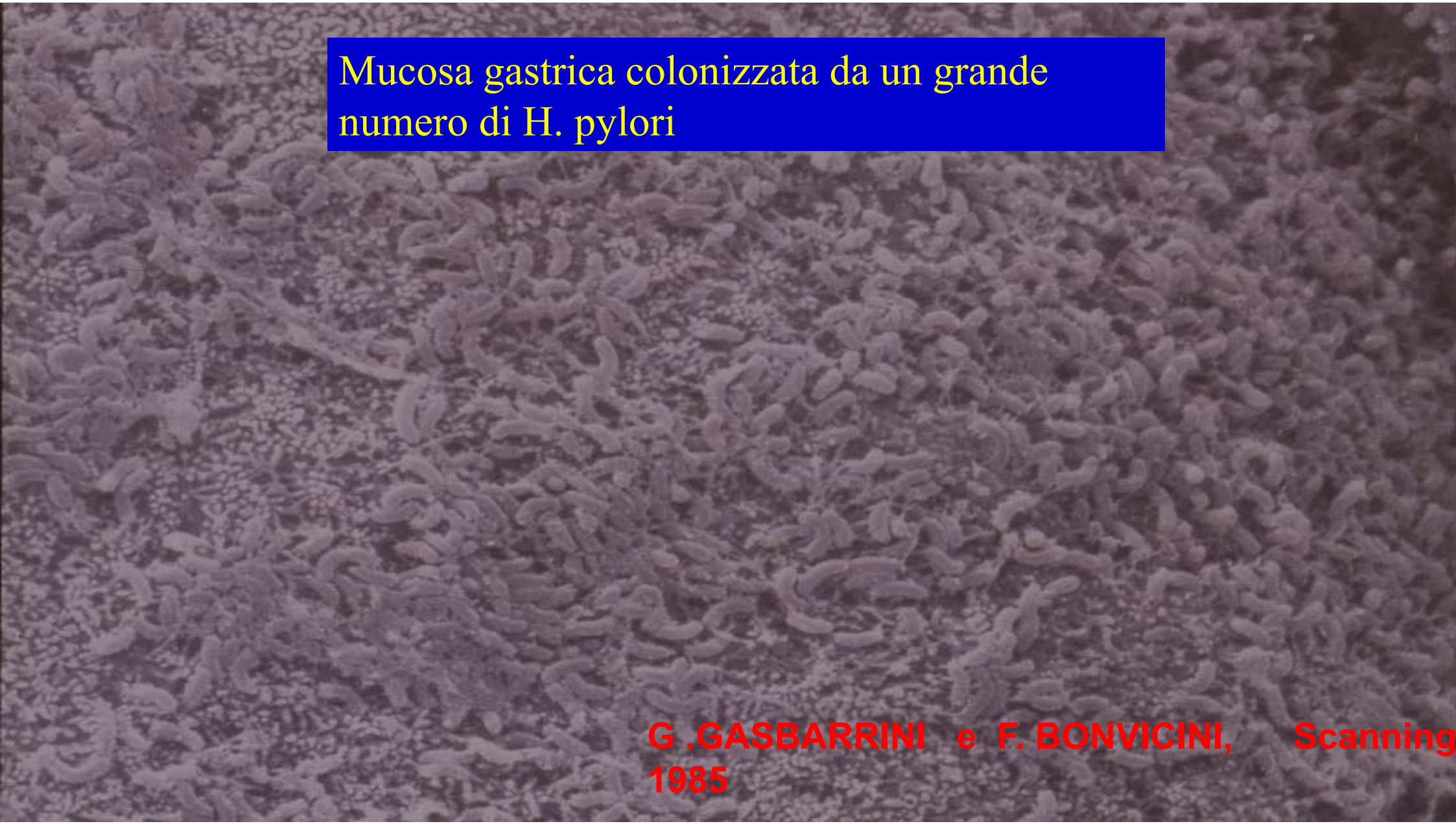
GUT MICROBIOTA ↔ NEUROLOGIC DISEASES

- Parkinson's disease (PD)
- Alzheimer's disease (AD)
- Cerebrovascular diseases
- Affective disorders
- Alcohol addiction

- Autism spectrum disorders
- Multiple Sclerosis

} Not typical of the elderly

Sherwin E et al, Recent developments in understanding the role of the gut microbiota in brain health and disease. Ann N Y Acad Sci. 2017 Aug 2. doi: 10.1111/nyas.13416.


Mucosa gastrica colonizzata da un grande numero di H. pylori
G .GASBARRINI e F. BONVICINI, Scanning 1985

MICROBIOTA-GUT-BRAIN AXIS

- Neurotransmitters synthesis
- Modulation of the immune system
- Neuroactive metabolites production
- Vagus pathway
- Modulation of key dietary amino-acids (TRP)

- Neuroinflammation
- Stress
- Neurotransmission
- Neurogenesis
- Modulation of complex behaviors

Sherwin E et al, Recent developments in understanding the role of the gut microbiota in brain health and disease. Ann N Y Acad Sci. 2017 Aug 2. doi: 10.1111/nyas.13416.

E’ IL MICROBIOTA INTESTINALE

(ospite)

O L’ INTESTINO TENUE

(ospitante)

IL NOSTRO SECONDO CERVELLO ?

- CIBO E STILE DI VITA,

- FATTORI AMBIENTALI E GENETICI (INDIVIDUALI E MICROBIOMA)

Gut microbiota composition correlates with diet and health in the elderly

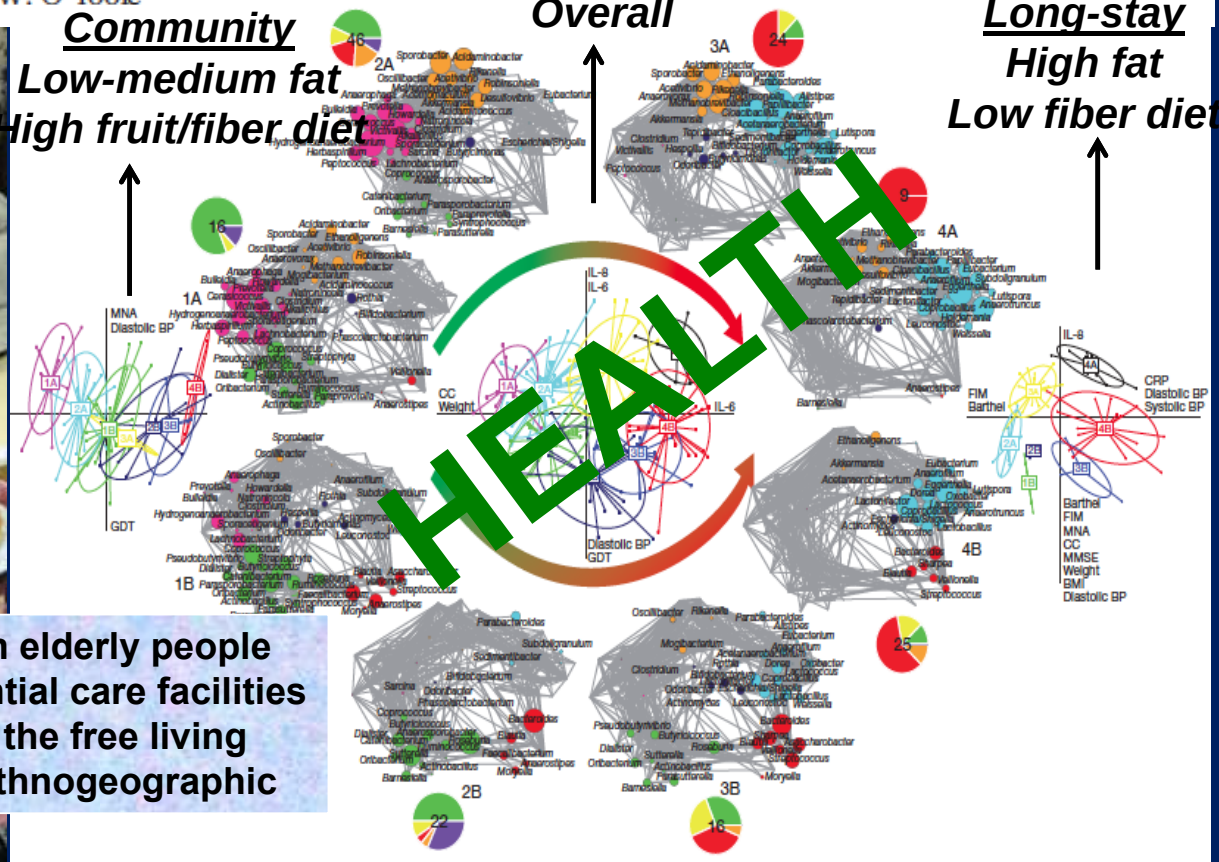
178 | NATURE | VOL 488 | 9 AUGUST 2012

Marcus J. Claesson[1,2]*, Ian B. Jeffery[1,2]*, Susana Conde[3], Susan E. Power[1], Eibhlís M. O'Connor[1,2], Siobhán Cusack[1], Hugh M. B. Harris[1], Mairead Coakley[4], Bhuvaneswari Lakshminarayanan[4], Orla O'Sullivan[4], Gerald F. Fitzgerald[1,2], Jennifer Deane[1], Michael O'Connor[5,6], Norma Harnedy[5,6], Kieran O'Connor[6,7,8], Denis O'Mahony[5,6,8], Douwe van Sinderen[1,2], Martina Wallace[9], Lorraine Brennan[9], Catherine Stanton[2,4], Julian R. Marchesi[10], Anthony P. Fitzgerald[3,11], Fergus Shanahan[2,12], Colin Hill[1,2], R. Paul Ross[2,4] & Paul W. O'Toole[1,2]

Community
Low-medium fat
High fruit/fiber diet

Overall

Long-stay
High fat
Low fiber diet



Microbiota composition in elderly people living in long-stay residential care facilities was different from that of the free living elderly, within the same ethnogeographic region.

Gut microbiota composition correlates with diet and health in the elderly

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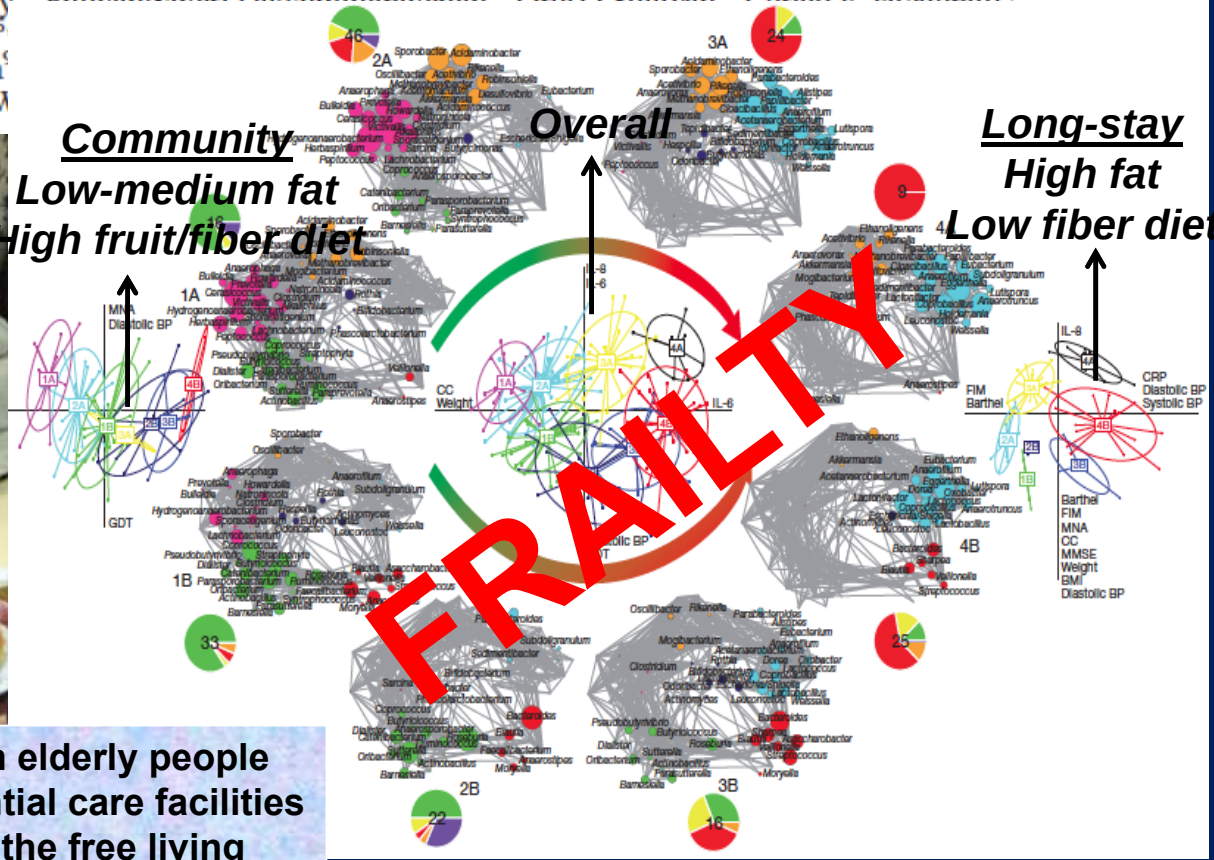


Cell Reports
Article

The Gut Microbiota of Rural Papua New Guineans: Composition, Diversity Patterns, and Ecological Processes

Asaro and Suasi (Papua Nuova Guinea populations):

-live in traditional settings

-no sewage, wastewater, or drinking water treatment facilities exist, drinking water is derived primarily from rivers, streams, or rainwater and is mainly consumed without boiling or any other treatments

-both communities rely on subsistence agriculture for their food supply, with households having their own gardens
-under-nutrition is rare in PNG, as carbohydrate sources are generally available.

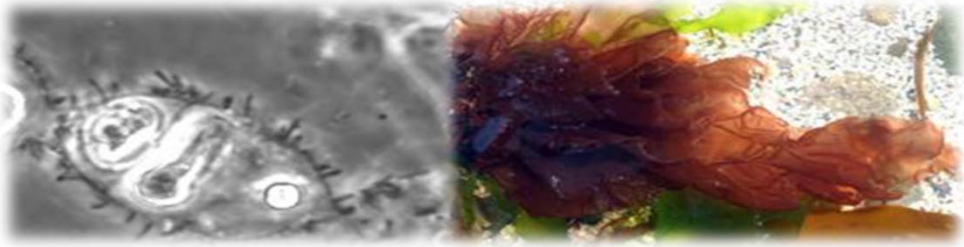
-staple foods are sweet potato, taro, and plantain, which are traditionally cooked in open fires and meat-derived protein (principally pork and fish) is consumed less frequently

-antibiotic use is high due to the high burden of infectious diseases, poorly regulated administration, and the lack of diagnostic capacity, which leads to empirical treatments.

Martinez I et al. Cell Press 2015

Diet and gut bacteria

"A special gift for sushi eaters"



Zobellia galactanivorans is a marine Bacteroidetes able to metabolize the polysaccharide porphyran from marine red algae. Metagenomics revealed that porphyranases were common in the Japanese population but absent in North American individuals.

In Japan, the genome of the gut bacterium Bacteroides plebeius revealed presence of a porphyran utilization locus upstream of genes for conjugative DNA transfer, acquired by horizontal gene transfer from sea-weed-associated bacteria found in sushi.

Heheann JH et al. Nature 2010

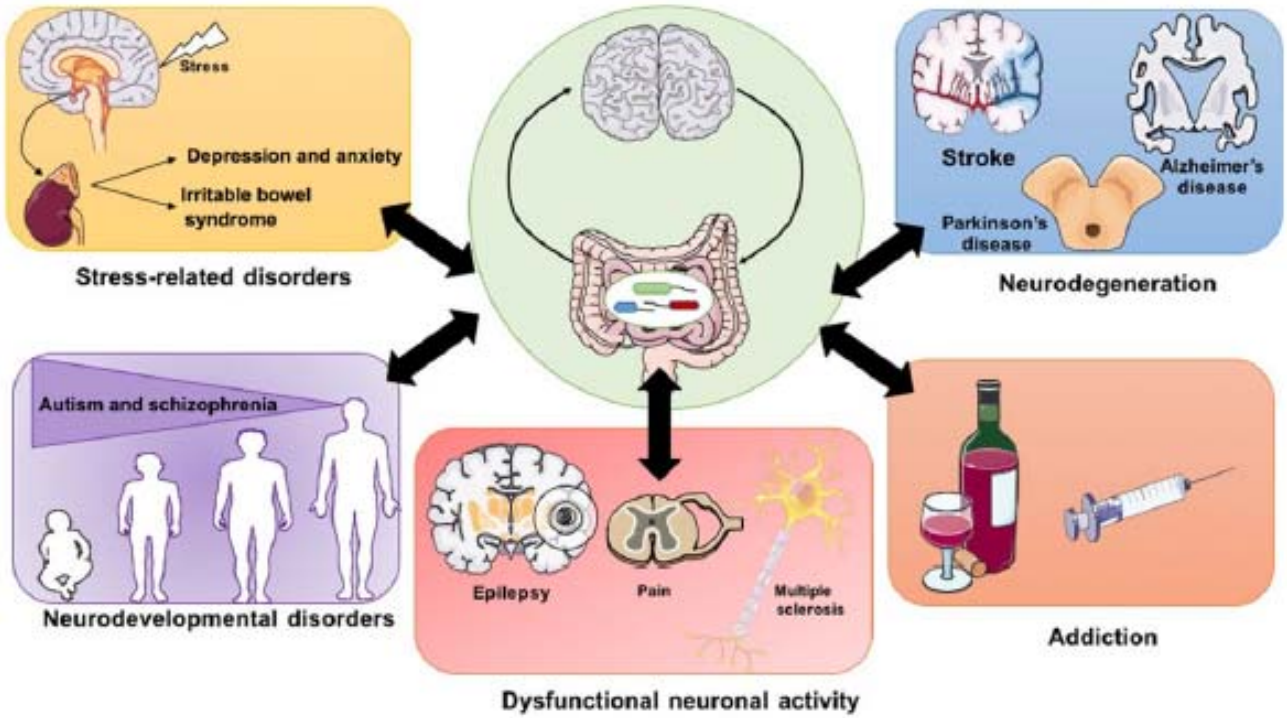
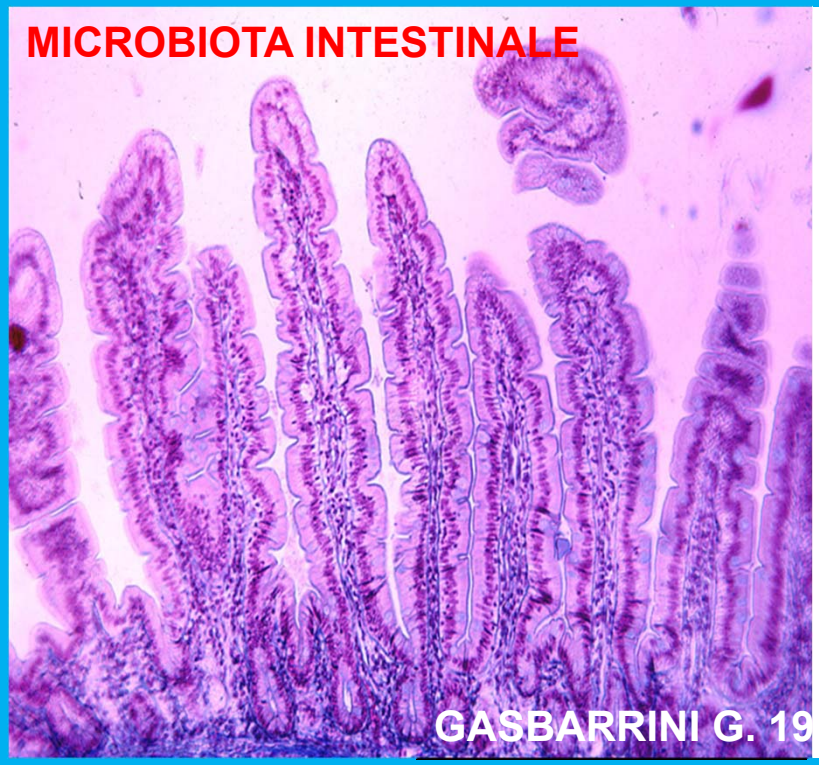
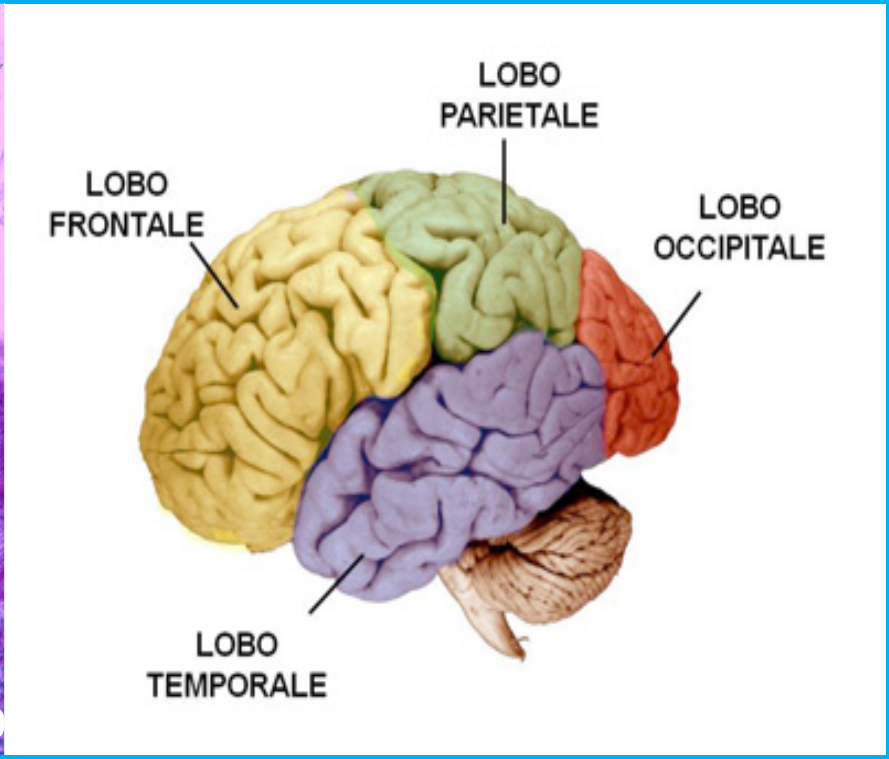


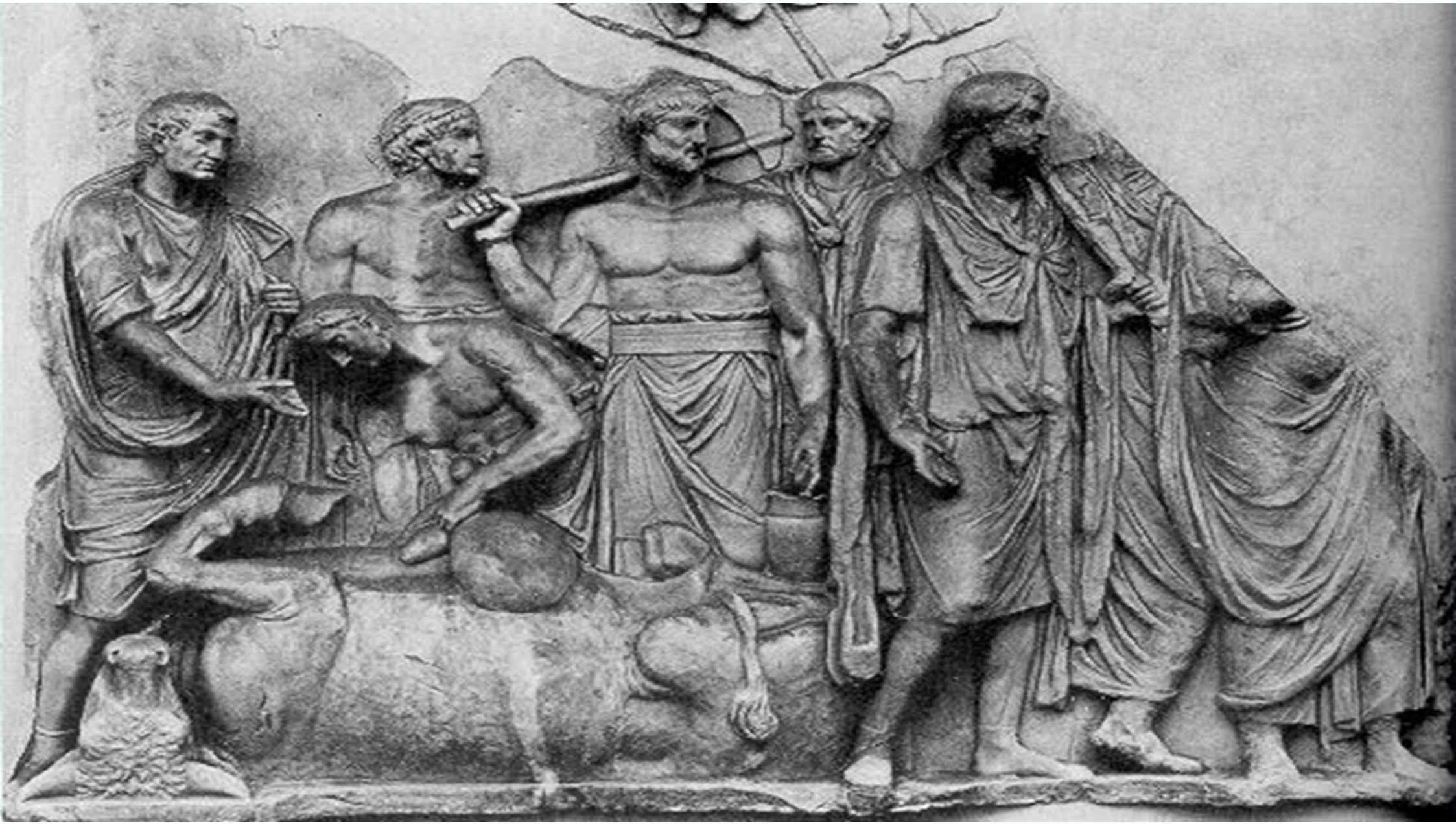
Figure 3. Overview of the gut–microbiota–brain axis in neurology and psychiatry. Evidence now suggests that the microbiota–gut–brain axis is involved in a variety of neurological and psychiatric conditions, such as depression, addiction, stroke, and Parkinson's disease. Moreover, modulating the microbiota in these conditions using probiotics, prebiotics, or through diet has displayed efficacy in preclinical studies, with some clinical studies also demonstrating efficacy. However, a greater understanding of how the intestinal commensals are affected in these various conditions will allow for the rational development of microbiota-based therapies in these various disorders.

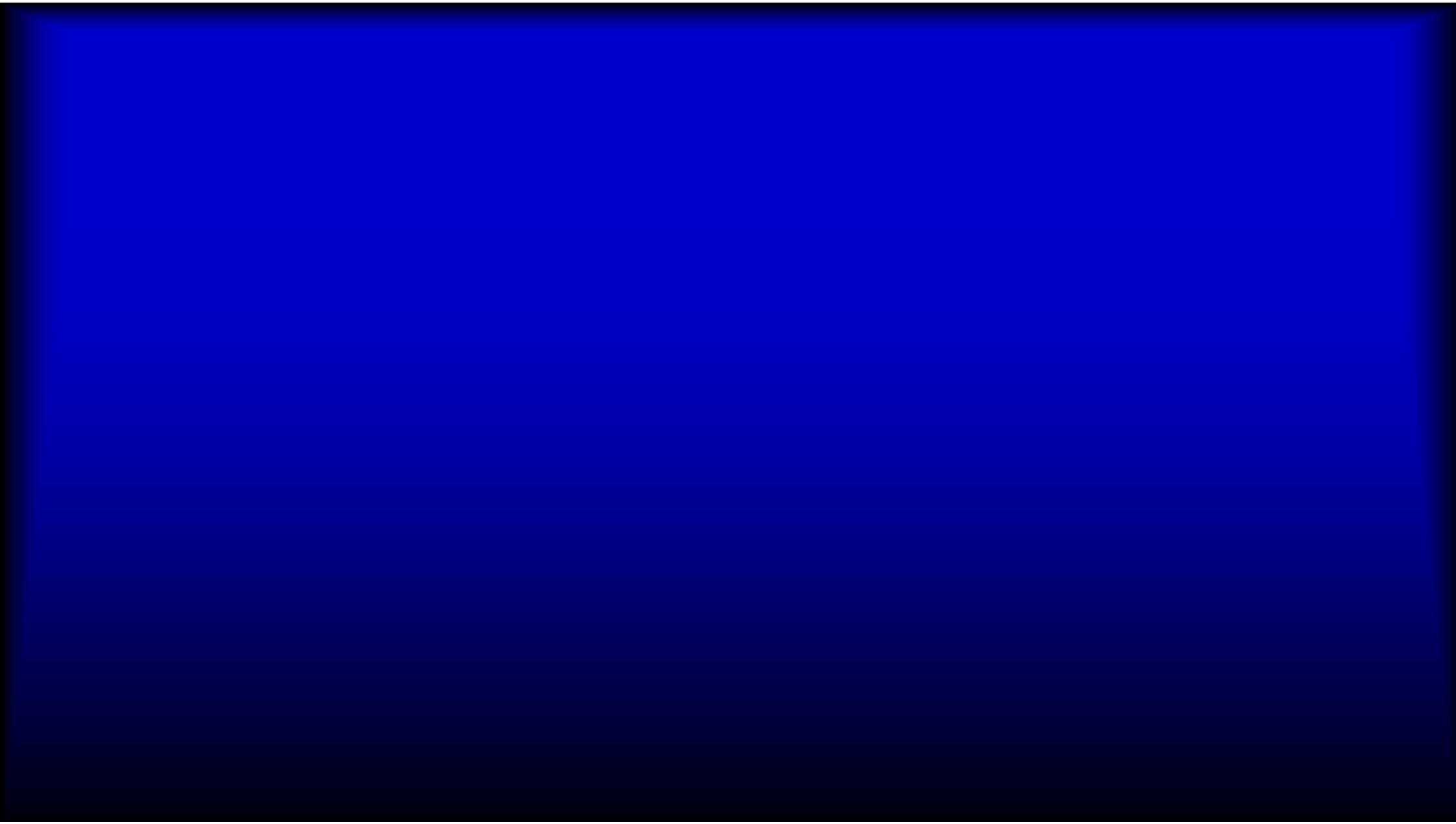
Sherwin E et al, Recent developments in understanding the role of the gut microbiota in brain health and disease. Ann N Y Acad Sci. 2017 Aug 2. doi: 10.1111/nyas.13416.


MICROBIOTA INTESTINALE
GASBARRINI G. 19


LOBO
PARIETALE
LOBO
FRONTALE
LOBO
OCCIPITALE
LOBO
TEMPORALE

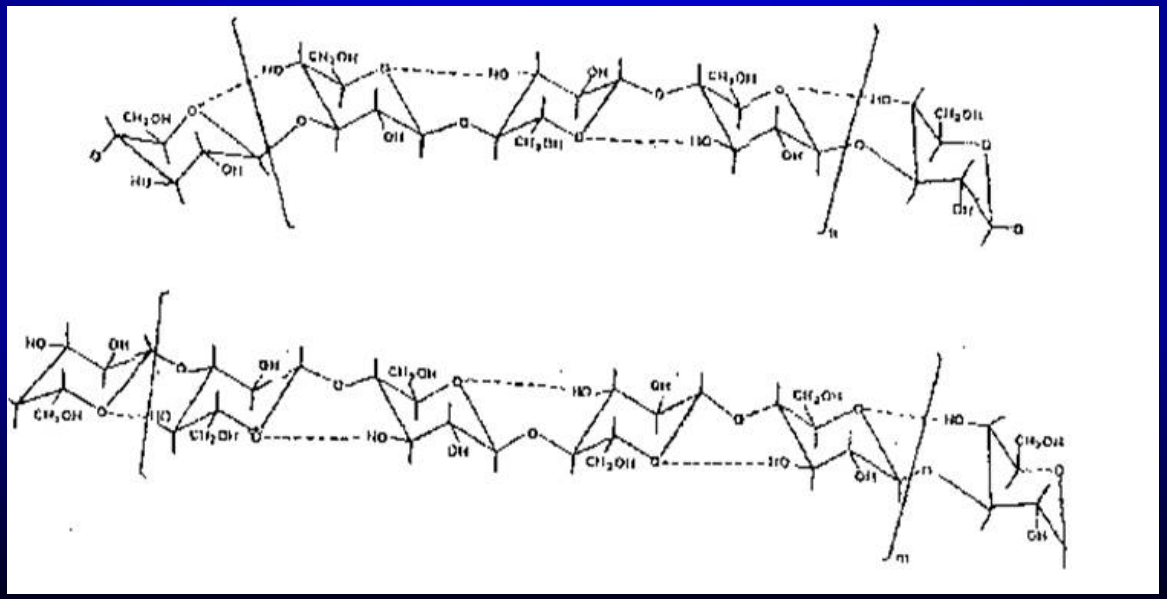
2 ⇄ ? ⇄ 1





Beta-Glucano

- Il Beta-glucano modula la risposta immunitaria riducendo l'attivazione della cascata citochinica e l'attivazione delle cellule NK
- Riduce, inoltre, la permeabilità intestinale



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«Se uno vuole essere il primo, sia l'ultimo di tutti e il servitore di tutti».
25 ord B
VANGELO di MARCO


Roma Termini
Binari 1 e 2 Est
Terminal Fiumicino
Biglietteria
Coca-Cola
Li invita a vedere il Trofeo della Coppa del Mondo FIFA


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ORGANO ASSORBENTE

INTESTINO TENUE : condiziona il benessere di tutto l' organismo

XI° ORGANO ATTIVO

MICROBIOTA INTESTINALE : induce la vita, la malattia, la morte

E’ IL MICROBIOTA INTESTINALE O L’ INTESTINO TENUE

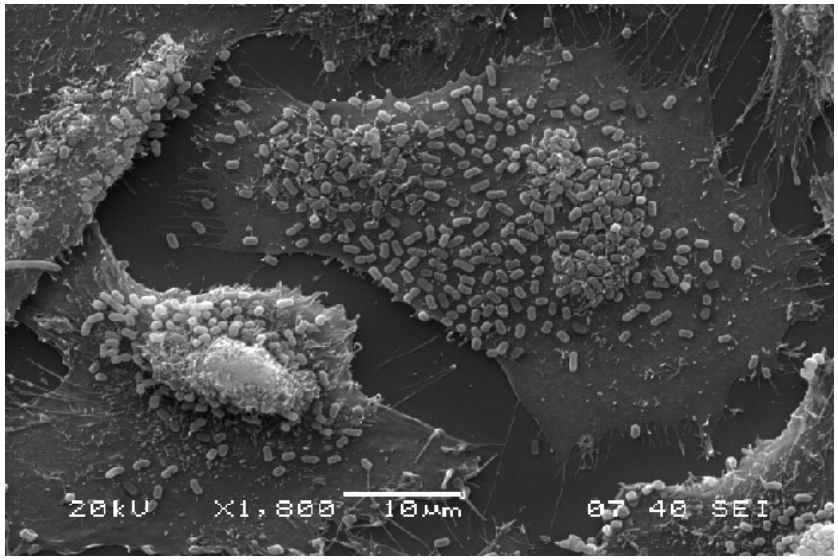
(ospite) *(*

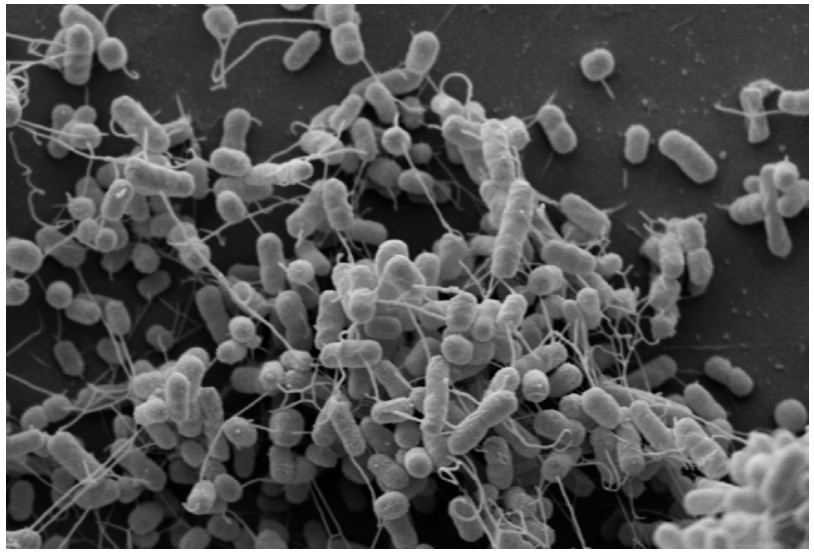
ospitante)

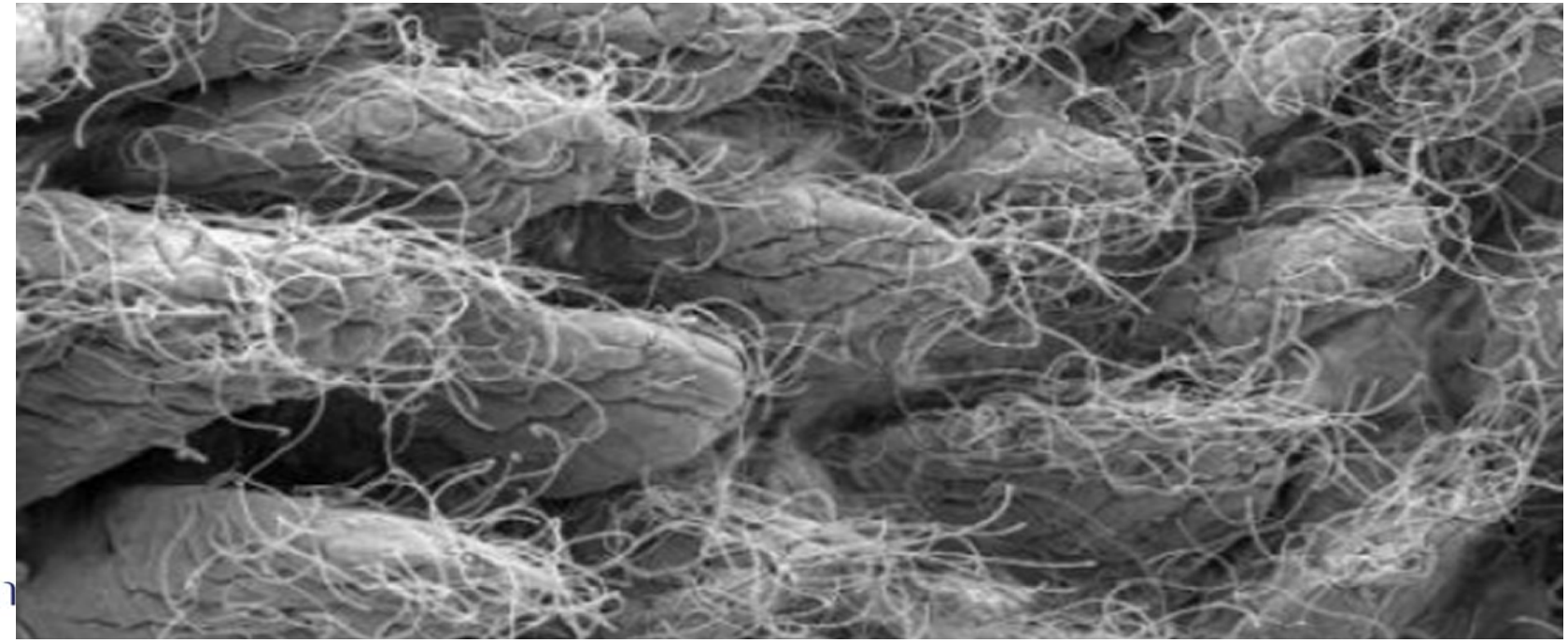
IL NOSTRO SECONDO CERVELLO ?

ED E’PROTAGONISTA O GREGARIO (« SERVITOR CORTESE

QUELLO CENTRALE?







Gem