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The gut microbiota: its history, characterization and role in different ages and environmental conditions and in gastrointestinal, metabolic and neurodegenerative pathologies

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Abstract: The ingestion of germs which take part in the constitution of the intestinal flora is well documented in human history; in fact, as shown in several cave paintings, since the Neolithic Age, the changes in human habits to a lifestyle based on farming and breeding led many populations to the consumption of aliments containing fermented milk. The use of dairy products is also confirmed in the Holy Bible, in the Book of Genesis, and in the discovery of a mummy dating 1615 B.C., which was found in the Chinese desert of Taklamakan with some cheese in its mouth. In ancient Greece, Hippocrates described the nutritional importance of cheese for the athletes. Many years later, according to the legend, the prophet Muhammad donated to the ancestors of the mountaineers of Caucasus Kefir, a drink rich in lactic acid, bacteria, and probiotics which is obtained from the fermentation of milk. Marco Polo reported the use of a comparable drink, "Chemmisi", during his journey to China. All these historical evidence suggests that the benefits derived from the consumption of probiotics were somehow noted to our ancestors, but the first scientific studies regarding this issue have been conducted by l'ja Il'ič Mečnikov (Charkiv, 05/16/1845 – Paris, 07/16/1916), who speculated a link between the longevity of the Bulgarian shepherds and the large consumption of yogurt, which contributed to the maintenance of balance in the intestinal flora and the inhibition of pathogen bacteria. It is nowadays documented that the benefits on health determined by probiotics consumption can be partly ascribed to their action on the Gut Microbiota, which also plays a major role in gastrointestinal, metabolic and neurodegenerative pathologies, in different ages and environmental conditions.

Keywords: Gut microbiota, Probiotics, Bacteria.

INTRODUCTION

The ingestion of germs which take part in the constitution of the intestinal flora is well documented in human history; in fact, as shown in several cave paintings, since the neolithic age, the changes in human habits to a lifestyle based on farming and breeding led many populations to the consumption of aliments containing fermented milk.

The use of dairy products is also confirmed in the Holy Bible, in the Book of Genesis, and in the discovery of a mummy dated 1615 B.C., which was found in the Taklamakan Desert in

China with some cheese in its mouth. In ancient Greece, Hippocrates described the nutritional importance of cheese for the athletes.

Many years later, according to the legend, the prophet Muhammad donated to the ancestors of the mountaineers of Caucasus, kefir grains, a leavening agent made of lactic bacteria and mold which is used to prepare a drink rich in lactic acid, bacteria and probiotics of fermentation of milk. Marco Polo reported the use of a comparable drink, "Chemmisi", during his journey to China.

All of this historical evidence suggests that the benefits derived from the consumption of probiotics were somehow recognized by our ancestors, but the first scientific studies regarding this issue were conducted by I'ja Il'ič Mečnikov (Charkiv, 05/16/1845 – Paris, 07/16/1916), who speculated on the existence of a link between the longevity of Bulgarian shepherds and the high consumption of yogurt, which contributed to the maintenance of balance in the intestinal flora and the inhibition of pathogenic bacteria[1].

Yogurt is currently defined as fermented milk, most commonly cow's milk, containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, which convert lactose to lactic acid. During the fermentation process, many biochemical reactions determine the taste of yogurt through the transformation of fats and proteins. The product is also rich in folic acid and niacin.

It is nowadays documented that the health benefits determined by probiotic consumption can be partly ascribed to their action on the gut microbiota.

THE GUT MICROBIOTA

The term "gut microbiota" defines the community of microorganisms that colonize the human intestinal tract.

The main characteristics regarding these germs have been well documented in the research field; it is now known that the gut microbiota contains more than 9 phyla, 1,000 species and 17,000 subspecies; however, in each individual, it is possible to find 4-6 phyla, 130-150 bacterial species, and 800-1,200 subspecies. The weight of the gut microbiota in an average adult is 800-1,000 g, and it contains about 3,000,000 genes, much more than those that are expressed in human DNA.

In such a wide community, a remarkable variability in gut microbiota has also been shown among healthy humans. Much of this diversity is still unexplained, although factors such as diet, environment, host genetics and early exposure to microbes are involved.

This variable microbial population affects the host's life in many ways, influencing the activity of the mucosal barrier, the immune system (mucosal and cell-mediated), food digestion and nutrient absorption, drug metabolism and several metabolic pathways linked to the host's wellness and illness.

A major role in regulating the activity of microorganisms in host health is played by the intestinal epithelium, which represents the largest interface between organs and environment and is involved in nutrient and water absorption, as well as the exclusion of harmful substances transiting in human gastrointestinal tract, providing a protective barrier from pathogenic germs and their metabolites.

In addition to a widely represented wall immune system and antimicrobial peptides, the composition and amount of secreted mucus are essential components of this barrier. In fact, the mucus usually protects the epithelial cells from direct contact with intestinal bacteria. However, pathological conditions such as inflammatory states can lead to a reduction in the amount of mucus, putting the epithelial cells in direct contact with the lumen. This process leads to hyperpermeability and sustained immune activation, with the perpetuation of phlogosis.

The epithelial membrane presents different structures, each with its own specific functions. The main components of this membrane are:

- protofilaments of the apical fuzzy coat of the microvilli [Figure 1];
- terminal bar junctional complex, that ensures contact between the various epithelial cells and can modify their activity according to the molecular content of the lumen [Figure 2];
- the complex of cytoplasmic organelles: mitochondria, with essentially a respiratory function;

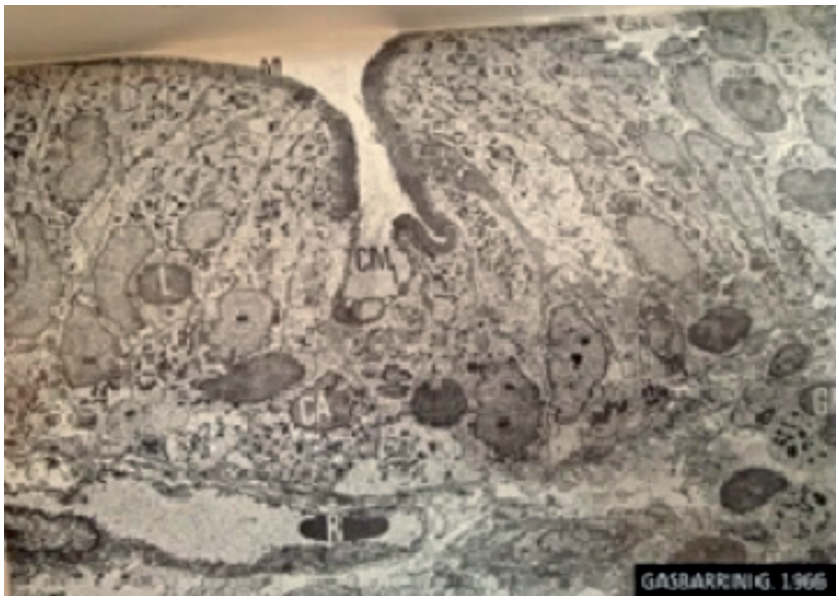

Figure 1. Structural characteristics of intestinal mucosa; M: microvilli, CM: Mucous cell, L intra-epithelial lymphocyte, CA: endocrine cell; R: red cell. TEM, 1500 x. From: Gasbarrini G. Bull. Scs Med. Chir. Bo 1.12.1966.

lysosomes, with a digestive function; the smooth and rough endoplasmic reticulum, rich in ribosomes, dedicated to intracellular transport up to the Golgi apparatus, an organelle made up of cisterns in which the processing of cellular content and its delivery to the intercellular spaces or towards the basal membranes take place. There, the substances absorbed enter into contact with the various migrating cells of the lamina propria or reach the blood or lymphatic vessels;

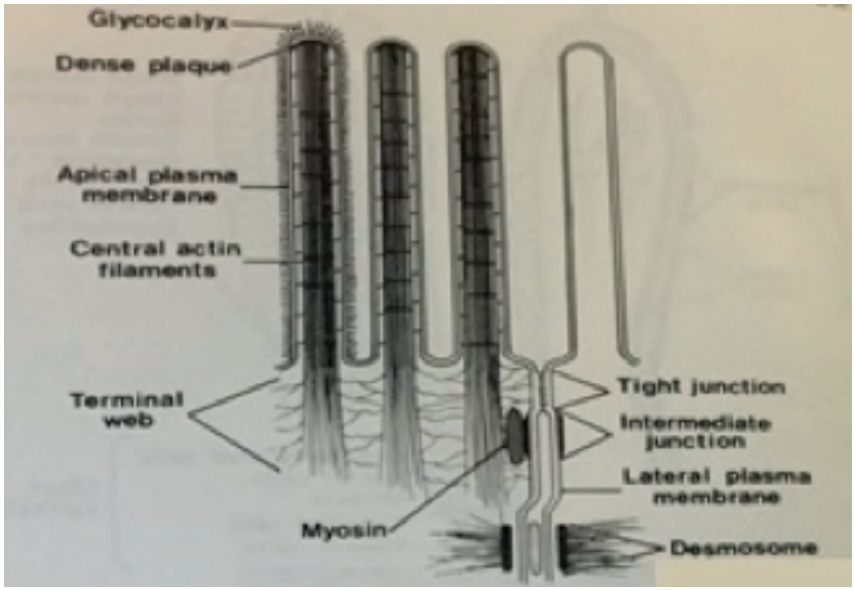

Figure 2. Junctional complex. Gasbarrini G, et al. Morfologia intestinale. Congr Internat Gastroenter, Edizione Compositori, Bologna, 1976.

– the cells of the amine precursor uptake decarboxylase (APUD) system, which, in addition to their endocrine function, have a paracrine activity, allowing the control of each other and an influence on exocrine secretion.

The activity of the gut microbiota is the result of a complex interaction among bacteria, Archaea, viruses, phages, microeukaryots, helminths and parasites. The most prevalent bacteria belong to the phylum of Firmicutes and Bacteroidetes, which together account for about 70% of the bacterial content of the gut[2-4].

The Gut Microbiota of each individual consists of a stable portion, which is determined in the first years of life, and a variable portion, which is under the influence of the amount and the type of food ingested, the drugs taken, especially antibiotics, as well as stress factors, including violence, experienced by the subject since early life.

In such a wide and variegated scenario, it is not possible to clearly define a "eubiotic" Microbiota, considering the major importance of the interaction among germs with respect to the action of a single microorganism. This represents a milestone in medicine, disproving the "single germ theory" postulated by Koch in the XIX century.

In this new polymicrobial vision, health and illness are not influenced by a single germ, but by the equilibrium of thousands of factors.

The concept of "eubiosis" refers to a situation of equilibrium among host factors, including gut microbiota in its wide variability, host's lifestyle and habits, and external factors.

Disturbances in this equilibrium lead to a situation of dysbiosis, with important repercussions on health and an increased risk of digestive and extra-digestive pathologies.

The first influence on gut microbiota is already determined during intrauterine life, as it is now known that the placenta is not completely sterile and harbors a unique microbiota[5].

Furthermore, during labor, the baby comes into contact with vaginal microbes, and, later, through breast feeding, its gastrointestinal tract is reached by thousands of germs.

The ambient surroundings, food and people in contact with the baby in the first 2 or 3 years of life cooperate in the development of its "native core microbiota", which accounts for about one-third of the gut microbiota and is generally stable lifelong[6]. Consequently, stressful events in early life, such as violence, starvation, illness, and drug administration, especially antibiotics, can permanently affect the composition of the microbiota[7].

The presence of a "critical window" in early life, when the gut microbiota can influence the development of persisting metabolic traits[8], has been recently described in animals.

In preclinical models, mice receiving penicillin during weaning gained a total mass and fat mass in adult age. Furthermore, mice receiving penicillin-altered microbiota (transfer of the caecal microbiota from 18 week-old penicillin-treated mice to 3 week-old germ-free mice) gained a total mass and fat mass at a significantly faster rate. In fact, mice who received a penicillin-altered microbiota showed a decreased expression of intestinal immune response genes, similarly to their donors. Indeed, studies showed that these immunologic and metabolic changes may not be caused by direct effects of antibiotics, but rather by derived changes in the gut microbiota[8,9].

Currently, there is no direct evidence for a similar causal relationship in humans and further studies are required.

Surely the products of the interactions between the different intestinal germs and the remaining content of the intestinal lumen are influenced by the wide extension of the intestinal surface and by the response of the structures of the lamina propria, as well as the integrity of the intestinal mucosa and the quantity of mucus.

Numerous research studies, on both animals and humans, have shown the speed with which diet affects the characteristics of the gut microbiota, especially on the germs that make up the most variable quantity, *i.e.,* those acquired after the first years of life[10].

This portion of the microbiota can be altered both quantitatively and qualitatively, inducing the so-called "leaky gut", a condition with unpredictable consequences, in particular, the immunological and metabolic ones.

Therefore the main digestive and extra-digestive diseases assume characteristics of differing importance. A striking example is the experiment that showed how mice transplanted with feces of mice affected with colitis developed colitis just like their donors[11].

Another example is the activity of germs with the ability to transform metabolic fibers into carbohydrates, as occurs in large ruminants (cows, horses, zebras, and giraffes) in which the intake of vegetables induces an increase in muscle mass[12].

Also noteworthy is dysbiosis and the "leaky gut" that can lead to all of the complications of chronic hepatitis, both viral and metabolic, via immunological actions that can also lead to hepatic carcinoma. This is associated, for example, with the consumption of substances with a toxic effect on the liver, such as alcohol.

Considering that the correction of the type of intestinal microbiota can avoid or improve digestive diseases (non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH)), extra digestive diseases (diabetes, other metabolic disorders), and immunosuppression; there are numerous studies seeking to identify antibiotics or even probiotics, prebiotics, symbiotics and post-biotics that can modulate the gut microbiota and its activities.

In particular, fermentation is stimulated by Bifidobacteria and Lactobacilli contained in vegetables and depressed by Bacteroides, Clostridia, Salmonella, etc. contained in milk and dairy products. This occurs through a process of short chain fatty acids (SCFA) and/or inulin production, etc., with a reduction of intestinal permeability[13].

There are numerous studies verifying which of the many probiotics on the market have a better and safer action. It is essential, first of all, to know the species and strain of microorganism. For example, the mechanism of action is different depending on the content, whether single or multi-strain, whether they are alive or dead, and whether they are bacteria or fungi.

Surely the enterotype of the gut microbiota can be characterized according to the type of food ingested, people who eat foods rich in biotin and riboflavin (salami, meat, and dairy products) develop a microbiota characterized by proteolytic bacteria, while people who eat foods rich in thiamin and folate (flour, rice, etc.) show a microbiota characterized by saccharolytic bacteria (Prevotella, Bifidobacteria, Lactobacilli)[14].

Furthermore, interesting studies have been conducted to characterize the influence of the diet on the microbiota and the state of health in the elderly. Results show that the microbiota varies according to whether the population examined lives at home or in health facilities, always with a concordance between the diet and the microbiota[16].

Concerning geriatric age individuals, the type of food taken has a decisive influence on the composition of the intestinal microbiota; therefore, wellness and many pathophysiological changes are closely related to the diet, as well as to environmental factors and individual characteristics[14].

This influence depends on the modulation of the microbiota on molecular mediators of inflammation.

Regarding neurodegenerative diseases, it is particularly interesting to evaluate the influence of intestinal microbiota on their occurrence and development.

In fact, recent evidence suggests that the gut microbiota, playing a major role in the "gut-brain axis", is involved in a bidirectional communication between the gut and the central nervous system through neuroanatomical pathways, immune system and the bacterial synthesis of neurotransmitters and neural regulators[17].

These substances seem to influence brain functions indirectly, because, even if they cross the mucosal layer of the intestine, they would not be capable of crossing the blood-brain-barrier (BBB)[18].

However, changes in the intestinal mucosal barrier function, with consequent translocation of cytokines and inflammatory factors, could increase the permeability of BBB, making it possible for peripherally produced molecules to impact on brain function directly[17].

Gastric infection with *Helicobacter pylori*, and in particular with *cag* pathogenicity island positive strains, has been significantly correlated with Parkinson's disease and with vascular diseases, in particular, those affecting the brain via carotid damage, and/or the heart via coronary diseases. In fact, recent evidence based on electron microscope findings suggests that *H. pylori* can be involved in systemic diseases through direct mucosal damage. The mechanism is based on needle-like structures (pili), membranous appendages and the polar flagella of *H. pylori*, that merge to the apical mucous cell membrane [Figures 3-5] [19]; furthermore, some gastric cell microvilli project towards bacteria, which

finally penetrates in epithelial cells through basal membrane holes[19] [Figure 6], where they stimulate the fusion of phagosomes and lysosomes [Figure 7].

In Parkinson's disease, the eradication of *H. pylori* was followed by a significant improvement of motility disorders and reduction in constipation, probably due to an influence on the autonomous enteric nervous system. Furthermore, in mucosal and submucosal nerve fibers of Parkinsonian patients, it is possible to detect alpha-synuclein, a hallmark of Parkinson's

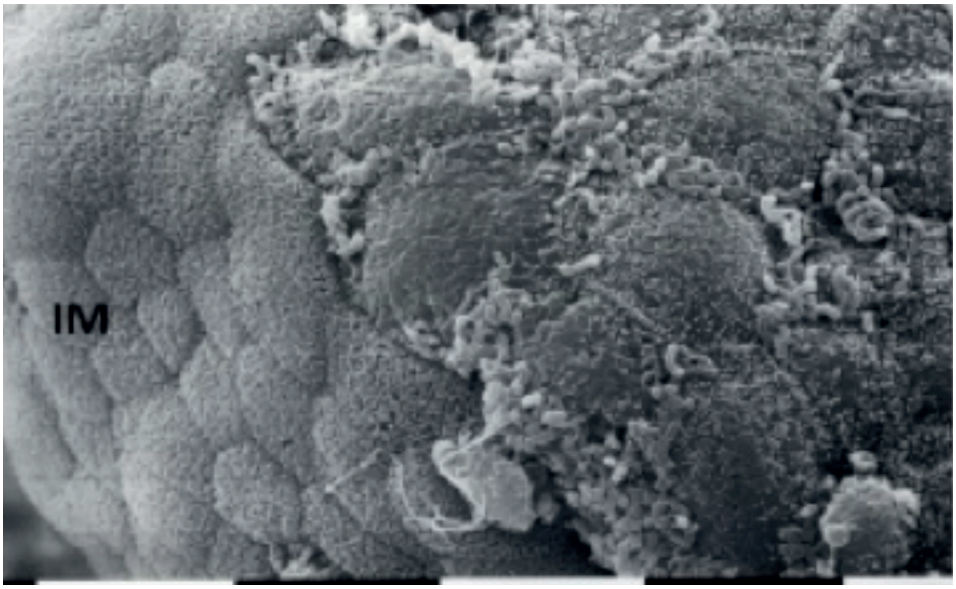

Figure 3. *Helicobacter pylori* crowding and penetrating the intercellular spaces of mucous gastric cells, avoiding areas of intestinal metaplasia (IM). SEM, bar = 10 mm. Gasbarrini G, Bonvicini F. Interaction between Helicobacter pylori and human gastric mucosa revisited by electron microscopy: still something new to debate? Eur Rev Med Pharmacol Sci. 2018; 22: 5312-5316.

disease in the brain. There is some preclinical evidence that alpha synuclein in the nerves can be transported to the brain via the vagus nerve.

The influence of *H. pylori* infection on the carotid plaque and, therefore, on stroke, as well as coronary pathology, has been demonstrated in various clinical and experimental works. The damage produced always occurs through the mechanism of inflammation[20].

For other diseases, namely Alzheimer's disease, affective disorders, alcohol-induced disorders, multiple sclerosis and autism, much research is ongoing.

Figure 4. Hp leans at the top of microvilli of gastric mucosa. SEM, bar = 1 mm. Gasbarrini G, Bonvicini F, Interaction between Helicobacter pylori and human gastric mucosa revisited by electron microscopy: still something new to debate? Eur Rev Med Pharmacol Sci. 2018; 22: 5312-5316.

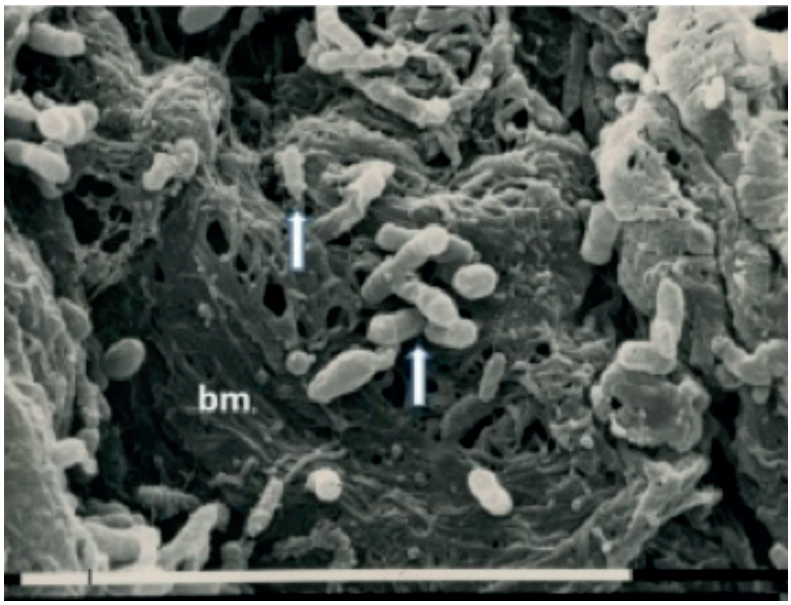

Figure 5. *Helicobacter pylori* penetrates trough damaged mucosa. SEM, bar = 10 mm. Gasbarrini G, Bonvicini F, Interaction between Helicobacter pylori and human gastric mucosa revisited by electron microscopy: still something new to debate? Eur Rev Med Pharmacol Sci. 2018; 22: 5312-5316.

In Alzheimer's disease, the worsening of the clinical features depends on amyloidosis, which occurs primitively or secondarily to chronic infection[20].

In neurodegeneration, the microbiota modulates immunological processes in the central nervous system, and it may induce oxidative toxicity and inflammation that contribute to the evolution of the process. For this reason, many studies are now aimed at finding the main sources of amyloid-inducing microbes and how to prevent it.

With regards to the depressive syndrome, particularly interesting are some studies showing that transplantation of depressed patients' microbiota into microbiota-depleted rats, caused them to develop a depression behavioral phenotype. These rats also show an elevated kynurenine/tryptophan ratio, indicating that perhaps the conversion of tryptophan into kynurenine, a metabolite with negative effects, can be facilitated by this particular microbial pattern[20].

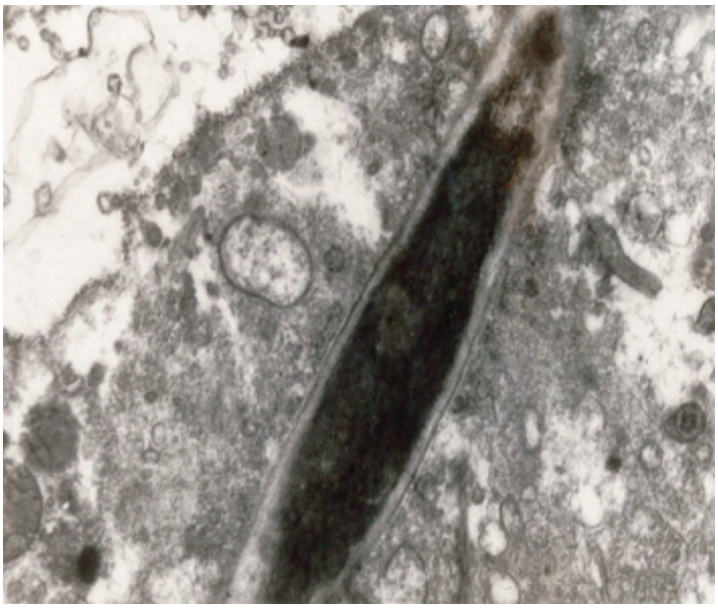

Figure 6. Hp in epithelial gastric cell; TEM, bar = 1 mm

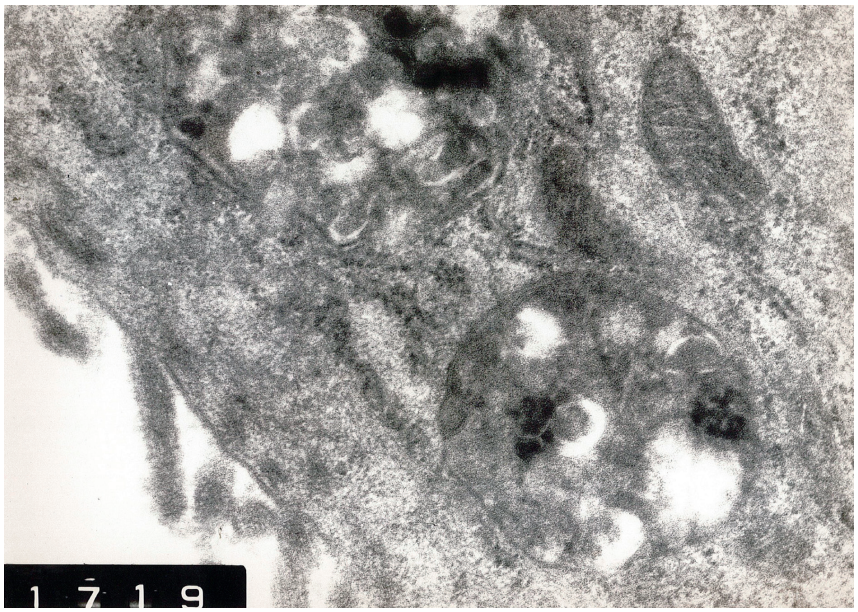

Figure 7. Phagolysosome containing bacterial-derived products in the epithelial cell. TEM, bar = 0.5 mm.

In the pediatric field, recent discoveries concern the role of intestinal microbiota in the development of pediatric acute-onset neuropsychiatric (PANS) and pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) syndromes, pathological situations characterized by the rapid onset of tics, obsessive-compulsive disorder and other behavioral alterations, with the identification of alterations of intestinal bacterial flora in patients affected by one of the two conditions compared to controls. In particular, it would appear that streptococcal infection may result in modifications of the intestinal microbiota, such as inducing a pro-inflammatory immunological state, which tends to remain even after a resolved infection. Furthermore, indirect alterations in behavior may depend on changes in the synthesis of centrally acting metabolites induced by the microbiota[21].

Concerning behavior and lifestyle, it is appropriate once again to enhance physical activity and balanced diets, always remembering the action of probiotics, as well as all factors that can modulate the type of our microbiota, which play key roles in the maintenance of a normal intestinal permeability.

Conflict of interest

All authors have no conflict of interest to report for this manuscript.

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