The Microfloras Adaptable Changes of Stomach after Total Resection of Colon

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Abstract

The adaptable changes of microfloras of stomach after total resection are observed in dynamics, where one can divide into two periods:

- Early period post operational changes (7 - 30 days)

- Adaptation period (60 - 90 days).

Keywords: Stomach; Microfloras; Total Resection of Colon

Introduction

Currently, in clinical surgery, different volumes of colon resection often take place. They are carried out for nonspecific ulcerative and granulomatous colitis, Crohn's disease, various types of bowel obstruction, its tumor lesions, necrosis due to various reasons and, first of all, mesenteric vessel thrombosis and other pathological conditions of the colon [1,3-5].

At present, it is not advisable to consider compensatory and adaptive processes in the digestive tract, especially in the stomach, without taking into account the ulcerogenic microflora (and fauna), as well as the ratio of defense and aggression factors [7-11].

Total colon resection leads to complex anatomical, histological and physiological changes in the body. After the complete removal of the large intestine, there is a gradual adaptation, adaptation of the functions of individual organs and systems of the body to new conditions of existence. Unfortunately, the available literature data on the structural and functional restructuring of the stomach after total colon resection are few in number and have mainly clinical and physiological orientation, their results are often contradictory. We, in the available literature, have not found any messages revealing the nature of adaptive shifts in the microflora of stomach contents after total colon resection, which allows us to develop pathogenetic methods for correcting dysbacteriosis observed after surgery. Disclosure

of the mechanisms of adaptive and adaptive changes occurring in the stomach has a certain practical value, especially since functional relationships, common origin, blood supply, innervation and relationships of individual representatives of the microflora of the stomach and colon determine the reaction of one of these organs to the removal of the other.

Materials and Methods

The experiments were carried out on 50 adult white outbred male rats with an initial body weight of 150 - 220g, kept in the same vivarium conditions. The experimental animals underwent a total resection of the large intestine according to the Ayletta method [6] and the control animals (10) opened the abdominal cavity and, after revision of the internal organs, were sutured like in experimental animals. The experimental and control animals were slaughtered on days 7, 15, 30, 60, 90 in the morning on an empty stomach under light ether anesthesia and the stomach contents were taken for bacteriological examination under sterile conditions in a phosphate buffer solution in a ratio of 1:10, then the material was diluted in 10 time.

In order to isolate and identify pure cultures of aerobic microorganisms, the test material was inoculated on differential diagnostic nutrient media: Endo and Levin (counting a group of intestinal bacteria - *Escherichia, Proteus*), Kalina's medium (*Enterococci*), blood agar (quantitative accounting of aerobic bacteria), yolk - salt agar (*Staphylococcus* count), Sabouraud's medium (*Candida* and other fungi).

Asporogenic anaerobic microorganisms (*Bifidobacteria*, *Bacteroids*) were isolated by the drop method using watch glasses, described by Lizko., *et al*. [2], and the use of special elective culture media. To enumerate and isolate lactic acid bacteria (lacobacilli), we used MRS-4 medium.

Bifidobacteria, Bacteroids, Lactobacilli and *Enterococci* were identified by cultural, morphological and tinctorial properties.

The results obtained were expressed in decimal logarithms per ml of stomach contents.

For transmission electron microscopy (TEM), tissue samples were fixed with a 2.5% solution of glutaraldehyde in a phosphate or cacodylate buffer, after dehydration in alcohol-acetone, they were poured with an epono-araldite mixture. Ultrathin sections obtained on an Ultracut ultratome were contrasted in an Ultrostainer apparatus and viewed under a Hitachi H-600 electron microscope.

For scanning electron microscopy, the preparations after the above-described fixation were subjected to dehydration in alcohol-acetone, then dried by the critical point method in an HPC-2 apparatus and gold-sputtered in an IB-2 apparatus. Examined in a Hitachi S405A electron microscope.

Results and Discussion

The analysis of the obtained data showed (table) that in the norm in the contents of the stomach *lactobacilli* and asporogenic anaerobic microflora dominate over aerobic autoflora.

During electron microscopic examination in control rats, single microorganisms are found in the glandular lumens at all levels, including in the lower third, where the main cells are concentrated (Figure 1). With total colon resection on days 7-15-30, dysbacteriosis develops, manifested in a decrease in the total number of *lactobacilli* and asporogenic anaerobic microflora and a predominance of aerobic autoflora.
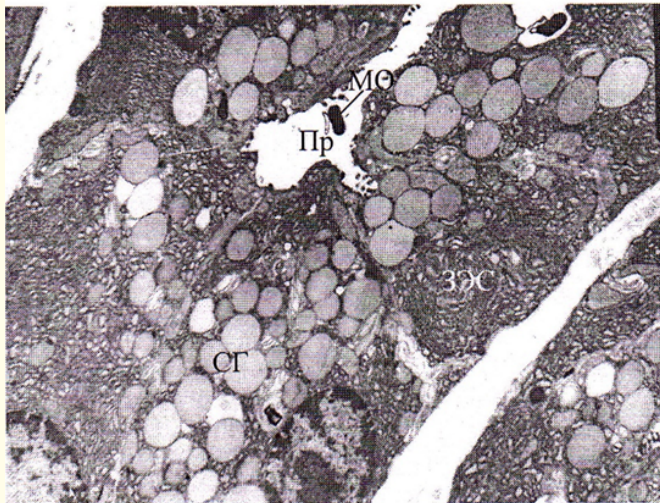
Figure 1: *Lower third of the fundic gland. Main cells. Single microorganisms in the lumen. Control. TEM x 7500. ZES - Granular Endoplasmic Reticulum; MO – Microorganism; Pr – Clearance; SG - Secretory Granule.*

Electron microscopic studies of the fundus of the stomach of the glands 30 - 60 days after total colon resection reveal a significant increase in the number of so-called parietal microorganisms, that is, microbes located in the immediate vicinity of the plasma membrane or directly on its surface. In this case, the apical parts of the cells, in direct contact with microorganisms, undergo pronounced alteration. Their surface becomes uneven. Projections and depressions are noted (Figure 2).
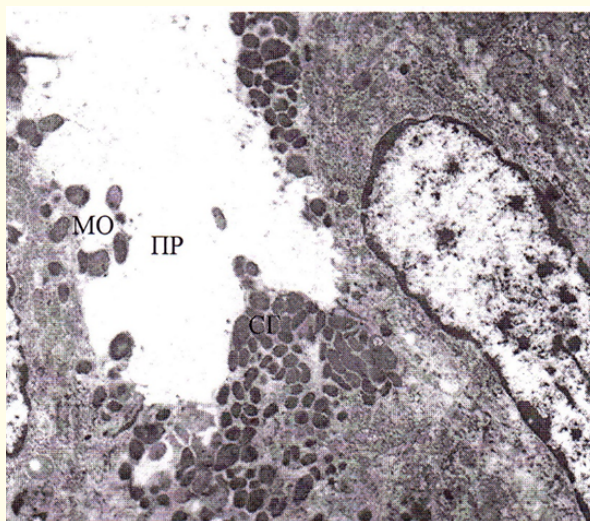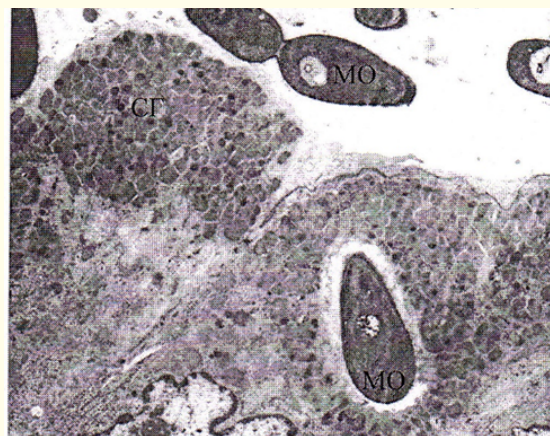

Figure 2: *Microorganisms in the lumen and on the surface of mucocytes 30 days after total colon resection. TEM x 7500. MO - Microorganism; Pr - Clearance; SG - Secretory Granule.*

Microorganisms located both in the lumen (luminal microflora) and on the cell surface have different shapes and sizes. Along with large microorganisms, comparable in size to cell nuclei (apparently fungi of the genus Sandida), there are medium-sized microorganisms, most often cocci (Figure 2 and 3). Often, microorganisms are in a state of division (Figure 3 and 4). Intracellular localization of parietal microorganisms is also rare (Figure 4). At the same time, the microbial bodies that penetrate the cell are surrounded in it by a zone of enlightenment, which is apparently formed due to surfactants on the outer shell of the microbe. There were no reactions in the cytoplasm of cells and no response to microbial penetration was found (Figure 4).


Figure 3: *Microorganisms (Candida type fungi) on the surface of mucocytes. 60 days after total colon resection. TEM x 7500. MO - Microorganism; SG - Secretory Granule.*

Figure 4: *Microorganisms (Candida type fungi) on the surface and in the cytoplasm of mucocytes. 60 days after total colon resection. TEM x 7500. MO - Microorganism; SG - Secretory Granule.*

Scanning electron microscopy also revealed round-oval bodies on the apical parts of the surface-pit cells, which are, apparently, fungi of the genus *Candida* (Figure 5).
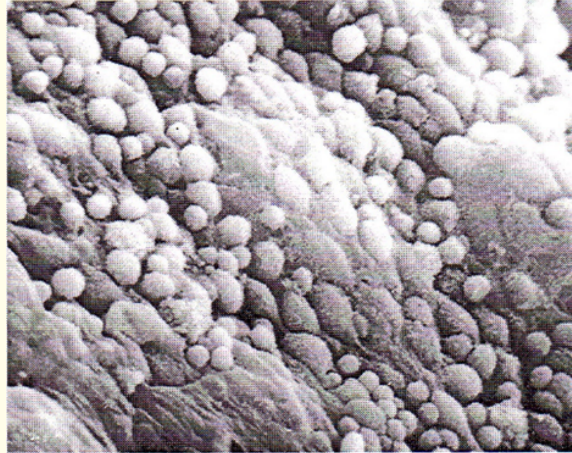
Figure 5: *Microorganisms on the surface of mucocytes of the fundic stomach. 60 days after total colon resection. SEM x 1000.*

Dysbacteriosis persists for 30 - 60 days, and the process of restoring the quantitative composition of the microflora of the stomach contents begins 90 days after the total colon resection.

The results of a comparative study of the quantitative ratios of *Lactobacilli, Bifidobacteria* and *Bacteroids* showed that lactic acid bacteria almost predominate in the norm. In the early stages after total colon resection (7-15-30 days), *Bifidobacteria* and *Lactobacilli* were more sensitive than *Bacteroids* (Table).

Discovered microflora	Control (Norm) n-10	Terms and conditions				
		7 days n-8	15 days n-8	30 days n-8	60 days n-8	90 days n- 8
Bifidobacterium	4,30 ± 0,17	-	-	1,84 ± 0,25	2,18 ± 0,32	3,0 ± 0,46
Lactobacilli	4,90 ± 0,12	-	1,48 ± 0,36	2,08 ± 0,42	2,78 ± 0,54	3,0 ± 0,15
Colibacillus (E. coli)	2,26 ± 0,28	4,81 ± 0,18*	5,62 ± 0,12*	6,49 ± 0,32*	5,20 ± 0,42*	3,63 ± 0,26*
Enterococcus	3,16 ± 0,32	5,37 ± 0,11*	6,35 ± 0,24*	6,84 ± 0,53*	6,05 ± 0,38*	5,18 ± 0,11
Staphylococcus	3,48 ± 0,22	6,20 ± 0,25*	6,93 ± 0,14*	4,46 ± 0,14	4,0 ± 0,54	4,71 ± 0,32
Mushrooms of the genus *Candida*	3,19 ± 0,21	5,90 ± 0,48*	6,16 ± 1,02*	5,20 ± 0,83*	4,53 ± 0,36*	4,0 ± 0,26
Bacteroids	3,20 ± 0,41	4,86 ± 0,27*	5,68 ± 0,12*	5,60 ± 0,44*	4,51 ± 0,14	3,32 ± 0,54
Proteus	2,46 ± 0,30	3,48 ± 0,18*	4,40 ± 0,52*	4,36 ± 0,12*	4,83 ± 0,29*	3,16 ± 0,18*

Table: *The quantitative composition of the microflora of the stomach contents is normal and with total colon resection (M ± m; lg/ml). Note: *: The absolute number of microorganisms is 106;*

n: Number of animals.

According to our observations, the number of *Bifidobacteria* begins to recover by the 30th day after the total colon resection, and on the 60th - 90th days, their number increases, which, apparently, is explained by the presence of a more perfect adaptation mechanism.

In the early stages (7-15-30 days) of observation, relative dysbacteriosis is noted in the stomach contents, characterized by an increased amount of fungi of the genus *Candida* and *Bacteroids* against the background of an unreduced amount of *lactobacilli* and *Bifidobacteria*.

The results of the studies showed that normally in the stomach contents of intact rats, the coccal group of bacteria predominates over the intestinal group, and *Enterococci* and *Escherichia coli* dominate over *Staphylococci* and *Proteus*. The penetration of coccal bacteria with food from the oral cavity, their resistance to an acidic environment explains their predominance in the stomach.

In the stomach contents of intact rats, fungi of the genus *Candida* and others occupy quantitatively an intermediate position between the coccal and intestinal groups. In the early stages (7 - 15 - 30 days) after total colon resection, the number of all types of aerobic microflora increases by 2 times with the predominance of the coccal group over the intestinal. By the end of the period after total colon resection (day 90), an increased number of aerobic microbes persists.

There is an increase in the number of fungi of the genus *Candida* and others in all periods of observation compared with the control.

In recent years, the role of fungi of the genus *Candida* and *Bacteroids* in the development of pathological processes in chronic and surgical diseases has been reported.

With total colon resection on the 15 - 30th day of observation, there is a rapid increase in the number of *Bacteroids* in the stomach contents, and in subsequent periods, their number gradually decreases.

Thus, in all periods after the operation, there was a sharp change in the composition of the microflora of the stomach contents. These changes were characterized by a significant decrease in anaerobic non-spore rods, lactic acid bacteria and *Staphylococci* and an increase in yeast and yeast-like fungi of the genus *Candida*, pathogenic *Enterococci*, *Escherichia coli* and *Proteus* sticks.

Conclusion

1. In the stomach contents of intact rats, *lactobacilli*, *Bifidobacteria* and *Bacteroids* prevail over aerobic microflora.

2. In the adaptive restructuring of the gastric microflora after total colon resection, a distinct staging can be traced, where two periods can be distinguished:

a. The period of early after operational changes (7 - 30 days).

b. Adaptation period (60 - 90 days).

3. In the case of total colon resection, dysbacteriosis develops in the early stages, characterized by a decrease in the number of *lactobacilli*, asporogenic anaerobes and an increase in the number of all studied types of aerobic microorganisms; by the end of the observation period of *Candida* fungi and *Bacteroids*, an increased content of aerobic autoflora remains in the stomach contents.

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