QUANTITATIVE INVESTIGATION OF CHANGES IN THE CHIEF CELLS OF THE GASTRIC MUCOSA AFTER THE BILLROTH I SUBTOTAL GASTRECTOMY

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UDC 616.33-089.87-07:616.33-007.286-018.
73-008.939.633.2

The RNA content in the chief cells of the fundal glands of the stomach was determined cytophotometrically in male Wistar rats after subtotal gastrectomy by the Billroth I technique, 1 and 3 months after the operation. A significant decrease in the number of chief cells was found in the fundal glands after 3 months (by 53%), together with a sharp decrease in the RNA content of these cells. Data for the RNA content and number of the chief cells in the late period after operation (3 months) show that, despite adaptive phenomena, the function of pepsinogen formation in the stomach is disturbed.

Investigations [21, 20, 19, 10] have shown that the RNA of the chief cells of the stomach plays a direct part in synthesis of the specific protein pepsinogen. To investigate the cause of the atrophic changes which appear in the residual part of the stomach after subtotal gastrectomy [2, 4-8, 11, 13, 14] and also to obtain a more objective assessment of the functional capacity of the organ and, in particular, the functional activity of its pepsinogen-forming cells, the RNA content was determined in the chief cells of the fundal glands of intact and gastrectomized animals and the number of cells present in each gland also was counted.

EXPERIMENTAL METHOD

Subtotal gastrectomy by the Billroth I technique was performed on male Wistar rats weighing 140-170 g. The animals were decapitated 1 and 3 months after the operation. Pieces of the fundal part of the stomach, taken 3-4 mm away from the forestomach, were fixed in Brodskii's fixative. Sections 5 μ in thickness, were selected with the aid of the MIS-11 microscope [1], stained with gallocyanin and chrome alum, and examined and photographed with the MUF-6 UV-microscope at λ-579 nm. The negatives were processed

TABLE 1. RNA Content, Optical Density, Area of Cytoplasm, and Number of Chief Cells Per Gland in Gastric Mucosa of Rats under Normal Conditions and after Gastrectomy

Time of investigation	Optical density	Area of cytoplasm	RNA content (in conventional units)	Number of chief cells
Control	0,261±0,006	14,21±0,43	3,63±0,10	37,8±0,67
1 month after gastrectomy.....	0,281±0,007 <0,05	12,25±0,37 <0,001	3,46±0,14 <0,4	—
3 months after gastrectomy.....	0,225±0,005 <0,001	13,75±0,43 <0,4	3,08±0,11 <0,001	18,0±0,65 <0,001

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Laboratory for Biophysical Problems, Department of Histology, Tashkent Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 7, pp. 37-40, July, 1972. Original article submitted February 10, 1972.

TABLE 2. Distribution of Chief Cells of Gastric Mucosa by Groups Based on RNA Content, Optical Density, and Area of Cytoplasm after Subtotal Gastrectomy

Time of investigation	Group of cells	Optical density	Area of cytoplasm	RNA content (in conventional units)
Control	1	0,220±0,018	8,9±0,66	1,86±0,08
	2	0,250±0,004	13,6±0,36	3,25±0,07
	3	0,300±0,014	17,4±0,89	5,49±0,13
1 month after gastrectomy	1	0,220±0,030	9,9±1,36	1,96±0,09
	P		<0,6	<0,4
	2	0,270±0,009	11,7±0,31	3,11±0,084
	P	<0,05	<0,001	<0,02
	3	0,350±0,006	14,9±0,92	52,3±0,192
	P	<0,001	<0,001	<0,05
3 months after gastrectomy	1	0,190±0,010	9,83±0,43	1,82±0,108
	P	0,2	<0,3	<0,8
	2	0,230±0,007	13,9±0,45	3,13±0,083
	P	0,02	<0,8	<0,3
	3	0,258±0,014	19,0±1,11	5,00±0,09
	P	0,05	<0,3	<0,005

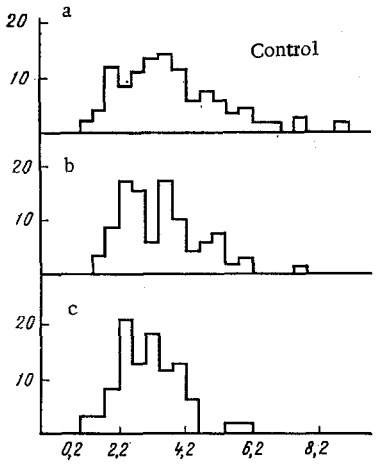

Fig. 1. Histograms of quantitative distribution of RNA in chief cells of fundal glands of the stomach after Billroth I subtotal gastrectomy: a) control; b) 1 month after operation; c) 3 months after operation. Abscissa, RNA content (in conventional units); ordinate, number of cells (in present).

by the scanning method on an MF-4 recording microphotometer. From 70 to 100 cells were measured at each time. Altogether 12 animals were used. Not less than 3 curves of optical densities were recorded for each cell. The mean height of each curve was measured by integration and converted into units of blackening [3]. From the blackening values thus obtained the optical density and RNA concentration were calculated using data for determination of the coefficient of contrast of the negatives. The areas of the cells and their nuclei were determined by means of the PP-2K planimeter. The RNA content was calculated by the equation:

$$Q = D \times S,$$

where Q is the RNA content, D is the optical density of the cytoplasm, and S is the area of the cytoplasm.

The number of chief cells was counted in 50 strictly longitudinally sectioned glands [16]. The numerical results were subjected to statistical analysis by the Fisher-Student method [17]. Besides analysis of the mean values of the RNA content, optical densities, and areas of the cytoplasm of the chief cells, an attempt was made to establish correlation between the area of the cytoplasma and optical density of the chief cells, subdivided into groups on the basis of their quantitative RNA content.

EXPERIMENTAL RESULTS

The histogram of the quantitative distribution of RNA in the chief cells of the control animals is a flat curve with 3 peaks (Fig. 1). This is evidence of the very varied functional state of the chief cells and of fluctuations in the RNA content. These effects depend on different stages of secretary activity of the cells, which are highly characteristic of the fundal glands of the stomach in healthy rats [9]. Accordingly, the whole population of chief cells to be investigated, under both normal and experimental conditions, could advantageously be subdivided into groups corresponding to the main peaks of the histograms. To analyze the degree of dependence of changes in the RNA content on the area of the cytoplasm and the optical density, the areas of the chief cells studied and the optical density were distributed in accordance with the RNA content.

Cells with the lowest RNA content (from 0.2 to 2.2 conventional units) were included in group 1. These were the smaller cells with lower optical density. Cells in which the RNA content varied between 2.2 and 4.2 conventional units were placed in group 2. This was a more numerous group. The RNA content per cell and also the optical density and the area of the cytoplasm in this group were almost indistinguishable from the general mean values of these indices (Tables 1 and 2). The cells of group 3 had the highest RNA content (4.2 conventional units or over). The high RNA content in these cells was associated with high optical density and also with a marked increase in the area of their cytoplasm (Table 2).

A significant increase in the optical density and a marked decrease in the cytoplasm were observed 1 month after subtotal gastrectomy. Although no significant change in the mean RNA content in the chief cells compared with the control occurred (Table 1), the ratio between the number cells with particular RNA contents compared with the control showed changes.

The histogram of the quantitative distribution of RNA was now bimodal, both peaks corresponding to the most numerous second group of cells. Compared with the control, a higher content of RNA and a smaller range of variations could be seen on the histogram. By comparison with the corresponding groups of cells of the control animals there was a slight decrease in the number of cells in groups 1 and 3 and an increase in the number of cells in group 2 (Fig. 1). Although no significant changes in the RNA content could be found 1 month after operation, the decrease in the number of cells of groups 1 and 3 and also the decrease in the RNA content in the cells of groups 2 and 3 led to a more homogeneous distribution of the population of chief cells investigated (Tables 1 and 2, Fig. 1).

The mean area and also the optical density of the cells of group 1 showed no significant change 1 month after the operation. In the cells of group 2, despite the increase in optical density, the marked decrease in area of the cytoplasm led to a decrease in their RNA content. In the cells of group 3 the decrease in area compared with the control also led to a lower RNA content (Table 2).

By the end of the 3rd month after subtotal gastrectomy the RNA content was reduced (Table 1) and the ratio between the number of cells in the individual groups showed considerable changes. The histogram of the quantitative distribution of RNA (Fig. 1) had 3 peaks, and the peaks were higher than in the control. No significant change in the number of cells or in the RNA content were found in group 1 compared with the control. In group 2 the number of cells was increased by 22% and although the mean areas of the cytoplasm in the cells of this group and their RNA content were unchanged (Table 2), the optical density was rather lower than the mean of the control measurements. In the cells of the smallest group 3 the RNA content was significantly reduced. The decrease in the RNA content in the cells of this group 3 months after the operation led to a more homogeneous distribution of its content by comparison with the control.

A large number of small chief cells with high optical density was very characteristic of the picture 3 months after gastrectomy.

The RNA content 1 month after the operation was thus not significantly changed, but 3 months after the operation it was reduced.

The presence of a large number of small cells with high nucleo-cytoplasmic ratios and with a reduced RNA content, a marked decrease in the number of cells in group 3 and an average RNA content in them 3 months after gastrectomy are evidence of a decrease in the intensity of functional activity of the population of chief cells studied.

The decrease in number of chief cells after 3 months by 53% (Table 1), the inactivation of an extensive secretory surface as a result of the operative removal of the pyloric and a large segment of the fundal part of the stomach, and also the decrease in the RNA content in the chief cells are all evidence of considerable depression of the pepsinogen-forming function of the residual part of the stomach, in agreement with the results of investigations by physiologists [12, 18] and pathologists [11, 13, 14, 4-8, 15, 2], who have demonstrated various types of changes in the fundal glands of the residual portion of the resected stomach (shortening of the glands, nucoid degeneration of the chief cells, an increase in their mitotic activity, an increase in the nucleo-cytoplasmic ratios, and a decrease in the activity of oxidoreductases), leading in the late stages after operation to the development of atrophic gastritis with disturbance of the function of the fundal glands.

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