CYTOFLUOROMETRIC STUDY OF THE DNA CONTENT IN SURFACE EPITHELIAL CELLS OF THE GASTRIC MUCOUS MEMBRANE IN ULCER AND CARCINOMA

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The DNA content in surface epithelial cells of the gastric mucous membrane was studied by a cytofluorometric method in 38 patients undergoing operations for duodenal and gastric ulcer and for carcinoma of the stomach. A clear difference in DNA distribution was found between cells from the ulcer margin and tumor cells. It is considered that the results may prove useful in the future for the differential cytodiagnosis of gastric ulcer and carcinoma.

Quantitative investigations of DNA in tumors of various organs have shown that a characteristic feature of tumor cells is a high level of and wide variation in their DNA content compared with normal tissues [3, 4, 7, 10-12, 15, 17, 18, 21, 23, 24]. The suggestion has been made that a study of the DNA content could be of diagnostic value [10, 13, 16, 22]. However, the authors are aware of only a few investigations [14, 19] into the DNA content in the gastric mucous membrane, and then only in the presence of malignant tumors.

In clinical practice the greatest difficulties in the differential diagnosis of benign and malignant disease of the stomach are presented by carcinoma and ulcer. Ulcerative carcinoma of the stomach, for example, is very common, accounting for about 54% of all cases of carcinoma [9]. Its clinical and radiological manifestations are frequently indistinguishable from those observed in peptic ulcer.

Cytomorphological investigations of biopsy specimens of the gastric mucous membrane have also shown that the differential diagnosis between carcinoma and ulcer can give rise to great difficulty, for in gastric ulcer the features of atypia characteristic of cancer cells are frequently discovered.

The object of the present investigation was to compare the DNA content in the surface epithelium of the gastric mucous membrane in gastric ulcer and carcinoma.

EXPERIMENTAL METHOD

The material of this investigation consisted of cytological specimens (films) obtained from the mucous membrane of stomachs resected from 38 patients undergoing operative treatment of duodenal (four cases) and gastric ulcer (11 cases) and carcinoma of the stomach (23 cases).

The films were fixed in a mixture of alcohol and glacial acetic acid (3 : 1) for 5 min and stained with Schiff's luminescent reagent. The quantity of fixed dye in the interphase nuclei was determined by means of a cytofluorometer constructed from a type ML-2 luminescence microscope and FÉU-79 photoelectronic multiplier. Details of the method of staining the specimens and making the measurements were described previously [6]. In each case, to determine the DNA from 170 to 200 nuclei were measured. The relative DNA content was assessed from the intensity of fluorescence of the nuclei, measured in conventional units.

The standard for the diploid DNA content per nucleus used in the investigation was the DNA content in 50 circulating blood lymphocytes or in 50 leukocytes present in the test films. The results were subjected to statistical analysis.

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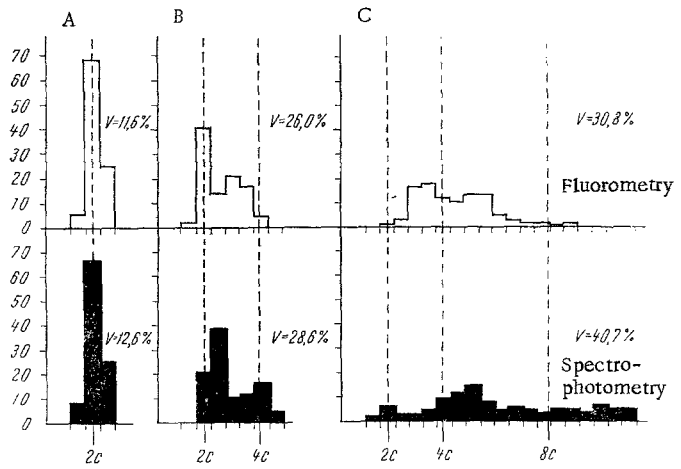

Fig. 1. DNA content in surface epithelial cells of normal mucous membrane of the stomach (A), from the margin of an ulcer (B) and in cancer cells (C) determined by fluorometric and single-wave cytospectrophotometric methods. Here and in Fig. 3: ordinate, number of nuclei (in percent); abscissa, DNA content (in conventional units).

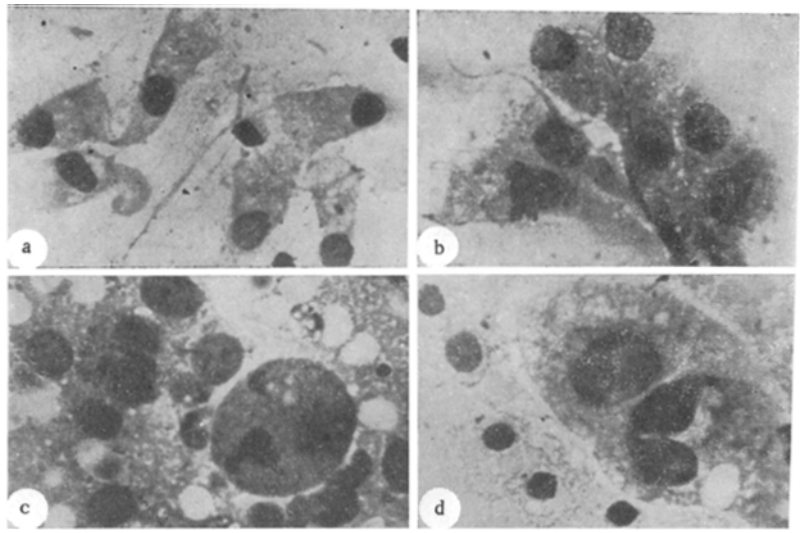

Fig. 2. Cells of normal cylindrical epithelium of the stomach (a), from the margin of an ulcer (b), and cancer cells (c and d). Pappenheim's stain, objective 100 ×, ocular Homal 2.4 ×.

A parallel cytomorphological investigation was made of material in films stained by Pappenheim's method. The morphological changes in the cells were assessed by the classification suggested by Papanicolaou and Cooper [20]. A histological control was used.

EXPERIMENTAL RESULTS

The surface epithelium of the gastric mucous membrane in patients with duodenal ulcer was taken as the normal, for in this disease the gastric mucous membrane has the normal histological picture [5, 8].

The stability of the cytofluorometric method for investigations such as these has been described previously [6]. However, work has recently been published in which the results of cytofluorometric investigations differed from those obtained by cytospectrophotometry, such as is usually used to determine the DNA content in cells [1, 2].

TABLE 1. Results of Cytofluorometric Investigations of the DNA Content in Surface Epithelial Cells of the Normal Gastric Mucous Membrane, Cells from the Ulcer Margin, and Cancer Cells

	Test No.	Results of histological investigation	Number of nuclei measured	Coefficient of variation	Ploidy of cells	Percent of cells containing DNA >4c ±0.5c
Normal mucous membrane	P* 10	Mucous membrane of normal structure	100	8,4	2c	
	F*	The same	100	6,0	2c	
	P 31	» »	100	12,4	2c	
	F	» »	100	15,0	2c	
	P 62	» »	100	7,0	2c	
	F	» »	100	7,7	2c	
	P 66	» »	100	12,1	2c	
	F	» »	100	11,6	2c	
Gastric ulcer	16	Chronic, in stage of exacerbation	200	24,1	2c — 4c	
	17	The same	200	22,0	2c — 4c	
	43	» »	200	24,6	2c — 4c	
	28	Chronic, in stage of scar formation	180	26,6	2c — 4c	
	45	Chronic	190	26,6	2c — 4c	
	37	The same	200	25,5	2, — 4,	
	64m	Chronic, in stage of exacerbation	200	26,0	2c — 4,	
	64 b	The same	200	25,1	2c — 4c	
	61	» »	200	26,9	2c — 4c	
	63	» »	200	25,6	2c — 4c	
	65	Chronic	170	25,1	2c — 4c	
	79	The same	200	24,6	2c — 4c	
Carcinoma of the stomach	8	Adenocarcinoma, undifferentiated in places	200	30,9	2c — 6c	5,0
	2	Solid	200	37,7	2c — 8c	24,5
	11	Undifferentiated	200	33,0	2c — 6c	6,0
	13	The same	200	30,1	2c — 6c	26,0
	41	Adenocarcinoma	200	32,4	2c — 6c	8,0
	32	Undifferentiated, scirrhus in places	200	40,3	2c — 8c	16,0
	20	Adenocarcinoma	170	36,4	2c — 7c	8,5
	22	Mucous, adenocarcinoma in places	200	40,5	2c — 10c	48,5
	39	Undifferentiated, adenocarcinoma in places	200	42,7	2c — 10c	29,5
	44	Adenocarcinoma	180	30,6	2c — 6c	15,0
	60	Adenocarcinoma, solid in places	200	44,9	2c — 8c	16,5
	71	Adenocarcinoma, scirrhus in places	200	30,8	2c — 9c	46,5
	78	Solid	192	40,7	2c — 9c	23,9
	74	Adenocarcinoma	200	43,8	2c — 10c	28,5
	76	Adenocarcinoma, solid in places	200	44,7	2c — 10c	22,5
	82	Solid, adenocarcinoma in places	200	57,3	2c — 11c	31,5
	84	Adenocarcinoma, scirrhus in places	200	31,5	2c — 8c	52,0
	85	Solid	170	38,9	2c — 8c	36,0
	91	Adenocarcinoma, scirrhus in places	200	35,3	2c — 11c	60,5
	88	Mucous	170	38,9	2c — 8c	35,3
	92	Adenocarcinoma	200	36,5	2c — 10c	65,0
	81	The same	200	45,2	2c — 11c	46,0
	59	Mucous	200	37,5	2c — 7c	7,5

*P) pyloric portion; F) fundal portion.

For this reason a comparative study was made of material taken from the same patients and using cytofluorometric and single-wave cytospectrophotometric (λ = 498 nm) methods; the films for cytospectrophotometry were stained by Feulgen's method. The DNA content was determined in the surface epithelium of the normal gastric mucous membrane and in patients with ulcer and carcinoma (one case of each).

Despite the differences in coefficients of variation (Fig. 1) and the higher percentage of polyploid and aneuploid cells detected by the cytospectrophotometric method in carcinoma, the results confirmed the reliability of the fluorometric measurements and gave good agreement with those obtained by other workers by the cytophotometric study of DNA in various normal and malignant tissues [4, 15, 16, 23].

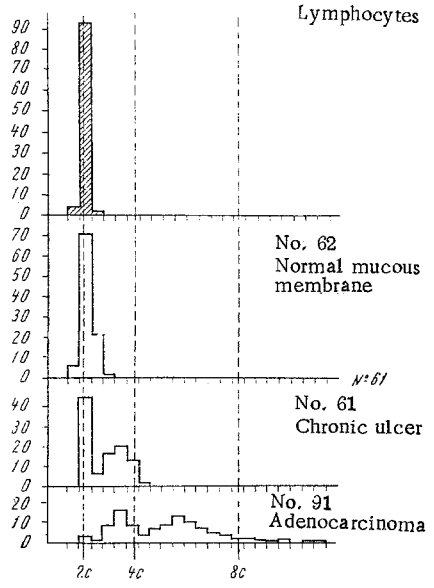

Fig. 3. Histograms of distribution of nuclei in circulating blood lymphocytes, normal surface epithelial cells, cells from the ulcer margin, and cancer cells by DNA content.

It was therefore decided to use the cytofluorometric method as the most convenient and the quickest for the purpose of the investigation.

In patients with duodenal ulcer, material was taken in each case from two regions of the stomach (pyloric and fundal) along the lesser curvature. In all cases the gastric mucous membrane on histological examination was normal in structure. On cytomorphological investigation of the cells no changes could be found (Fig. 2a). The cells had a well preserved cytoplasm, clearly defined cell boundaries, a round or oval nucleus about 7 μ in diameter and a delicate network of chromatin.

It will be clear from Table 1 and the histogram given in Fig. 3 as an example that the distribution of nuclei by DNA content in the surface epithelium of the normal gastric mucous membrane has one maximum, indicating that this population consists of cells homogeneous as regards their DNA content. Most cells contain the diploid (2c) amount of DNA. The coefficients of variation, which do not exceed 15%, also indicate homogeneity of these cell populations as regards their DNA content.

The distribution of cell nuclei obtained from the margin of the gastric ulcer by DNA content differed appreciably from the distribution of nuclei of the normal mucous membrane (Table 1, Fig. 3). The coefficients of variation were very significantly higher than those for homogeneous cell populations. A high percentage of nuclei contained more than the diploid amount of DNA, but not more than 4c, i.e., the characteristic amount for diploid cells which have completed DNA synthesis. This increase in the DNA content can be explained by the high proliferative activity of the cells at the ulcer margin in connection with regeneration.

On cytomorphological investigation of cells from the ulcer margin some degree of cellular atypia was observed (Fig. 2b). The cells had well-preserved cytoplasm with distinct cellular boundaries, but their nuclei were slightly enlarged and contained a coarse chromatin network. Often the nucleoli were enlarged and increased in number.

Cellular polymorphism was particularly marked in the malignant tumors. On cytomorphological investigation the cells showed well-marked atypia (Fig. 2c, d). The cells were enlarged, with highly vacuolated cytoplasm, and with an abnormal nucleo-cytoplasmic ratio. The nuclei measured up to 36-38 μ in diameter and considerable anisokaryosis was observed. The nucleoli were enlarged, increased in number, and irregular in shape.

In their DNA content the cancer cells also differed sharply from normal cells and cells from the ulcer margin. The table and Fig. 3 indicate an even wider variation among the cells than in gastric ulcer. In many cases the DNA content per nucleus was 4-5 times above the diploid level. The percentage of diploid cells was greatly reduced and the percentage of cells with a polyploid and aneuploid DNA content increased. A high percentage (up to 65) of tumor cells contained definitely more than the tetraploid (4c) amount of DNA. The presence of such a high percentage of cells with a genetically unbalanced DNA content can be explained not only by the presence of nuclei in the stage of DNA synthesis, but also by various chromosomal disturbances.

This investigation thus revealed a marked difference in the distribution of cells from the ulcer margin from cancer cells by their DNA content.

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