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Comparative Microspectrophotometric Study of the DNA Content in the Diagnosis of Pre-Tumorous Processes and Cancer*

G. G. Avtandilov and I. A. Kazantseva

Central Pathoanatomical Laboratory (Head: Prof. G. G. Avtandilov), Institute of Human Morphology, USSR Academy of Medical Sciences, Moscow, USSR

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Summary. A comparative microspectrophotometric study of the Nuclear Feulgen DNA content of various types of epithelial tissue (multi-layer flat epithelium of the larynx, glandular epithelium of the large intestine and endometrium) in different forms of dysplasia, hyperplasia and tumorous growth was made, using histological slices of biopsy material (66 biopsies from 62 patients).

The study showed that different forms of pretumorous and tumorous transformation of epithelial tissues are marked by definite shifts in the Nuclear Feulgen DNA content and revealed different levels of their cellular polyploidy and genetic heterogeneity.

These levels attest to the beginning of tissue malignization and can be used as an objective diagnostic test.

Concepts of the morphogenesis of various tapes of epithelial tissue malignization and of the related classification and therapy of precancerous conditions and cancerous processes are discrepant and require new methodological approaches to the estimation of the biological state of proliferating tissues. It is known that cell ploidy changes are one of the essential features of blastomogenesis. The correlation between the DNA content and the number of chromosomes allows the results of microspectrophotometric investigation of DNA content to be used as an objective test for the evaluation of ploidy and degree of cell-population heterogeneity in pretumor conditions as well as during the malignization processes. A histopathologist is now faced with difficulties both in the diagnosis of the initial cancerous stage and in the prognosis and interpretation of the hyperplastic process directly connected with the method of treatment. These difficulties may be obviated by determination of the above-mentioned indices. It is difficult to conceive of the dynamics of DNA-content changes at different developmental stages of hyperplastic and tumorous processes of general histogenesis because the data available in the literature on the quantitative determination of DNA content in human tumorous cells were rather scanty and often based on the examination of isolated cells (Seidel and Sandritter, 1963; Caspersson, 1964; Sandritter, 1965; Wied *et al.*, 1966; Böhm and Sprenger, 1968; Filkuka, 1968; Lawrence and Sven, 1968; Tavares, 1968; Sprenger *et al.*, 1971; Neishtadt and Shalumovich, 1971).

The main object of the present work was a comparative microspectrophotometric study of the content of DNA in nuclei of cells in histological slices of various

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types of epithelial tissue (multi-layer flat epithelium of the larynx, glandular epithelium of the large intestine and endometrium) in different forms of hyperplasia and tumorous growth.

The study was performed on biopsy material (66 biopsies from 62 patients). The pathomorphological changes and clinical distinctions of the process made it possible to divide all these cases into five groups, reflecting possible stages of blastomogenesis·

I. Normal epithelium.

II. Benign tumour (juvenile papilloma of the larynx, adenomatous polyps of the large intestine).

III. Hyperplastic and dysplastic processes without signs of atypical epithelium (leukoplakia, parakeratosis with basal cellular proliferation in the larynx; glandular dyshormonal hyperplasia in the endometrium).

IV. Hyperplastic and tumorous processes with focal atypical epithelium (hyperplasia of the epithelium of the larynx with partial decompensation of the layers and atypical cells in the basal and other layers; proliferating sections of the filiform polyps and hyperplasified mucous membrane of the large intestine, close to the proliferation of an adenocarcinoma; adenomatous (atypical) hyperplasia and adenomatous polyps of the endometrium).

V. Cancerous tumor (flat cellular cancer of the larynx, adenocarcinoma of the large intestine and the endometrium).

Methods

The Feulgen reaction was performed simultaneously in a series of 20–25 preparations (slices 5 microns thick, time of hydrolysis 7 minutes). For the quantitative determination of DNA we used the method of adsorptive cytophotometry on an integrating scanning microspectrophotometer (G. G. Avtandilov and co-authors) in a light with a wavelength of 5750 Å The most typical sections in each preparation were studied, in which we determined the concentration of DNA, the specific optic density per square micron of the slice in 25–30 nuclei of epithelial cells and in 30 lymphocytes. In these sections, too, we determined in the slice, through the nucleolus, the area of the 25–30 nuclei of the epithelial cells and 30 lymphocytes and subsequently calculated the DNA content (multiplying the specific optic density by the area of the slice of the nucleus). We accepted the average content in the nucleus of lymphocyte of the same slice as the quantity of DNA which corresponds to a diploid set of chromosomes (2C). The possibilities of variability of the results determined by different levels of the slice of the nucleus in the "equatorial" zone (near the nucleolus) are excluded by the same variations in the levels of the slice of the nucleus of the lymphocyte. The quantity of DNA in nuclei of epithelial cells was expressed in conventional units of ploidy (C). The weighted mean of the DNA content in one cell was calculated for every process.

Results and Discussion

The study showed (Figs. 1–3) that in a normal epithelium the modal class is diploid and paradiploid cells, the proportion of these ranging from 50 to 85 percent in different types of the epithelium.

Benign tumors are also characterized by a distinct prevalence of diploid and paradiploid cells (85 percent in juvenile papillomas of the larynx and 56.7 percent in polyps of the large intestine) and their histogram hardly differs from that of the initial epithelium.

In contrast to benign tumors "transitional" local-diffusive and hyper-dysplastic processes are characterized by more pronounced heterogeneity of the cells,

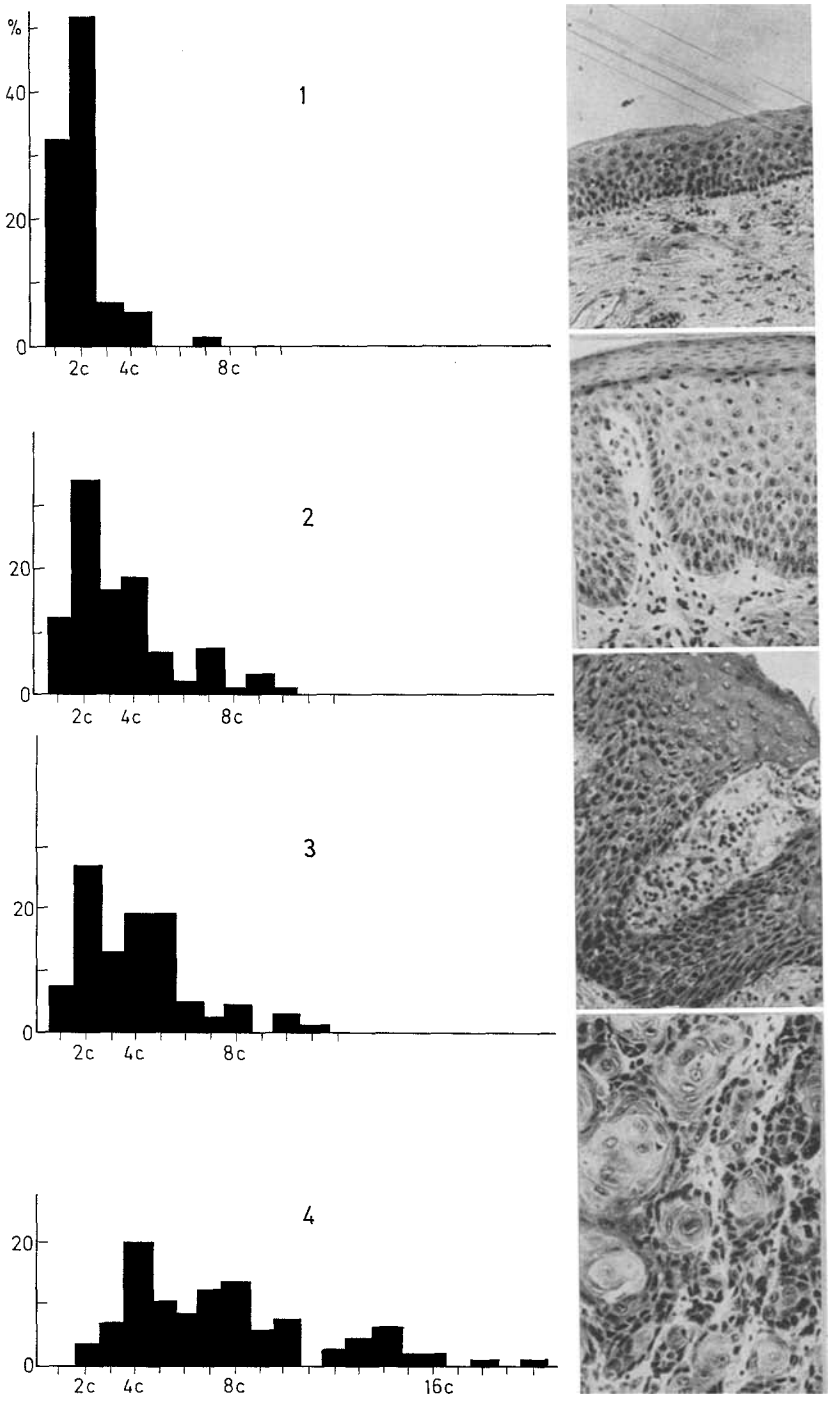
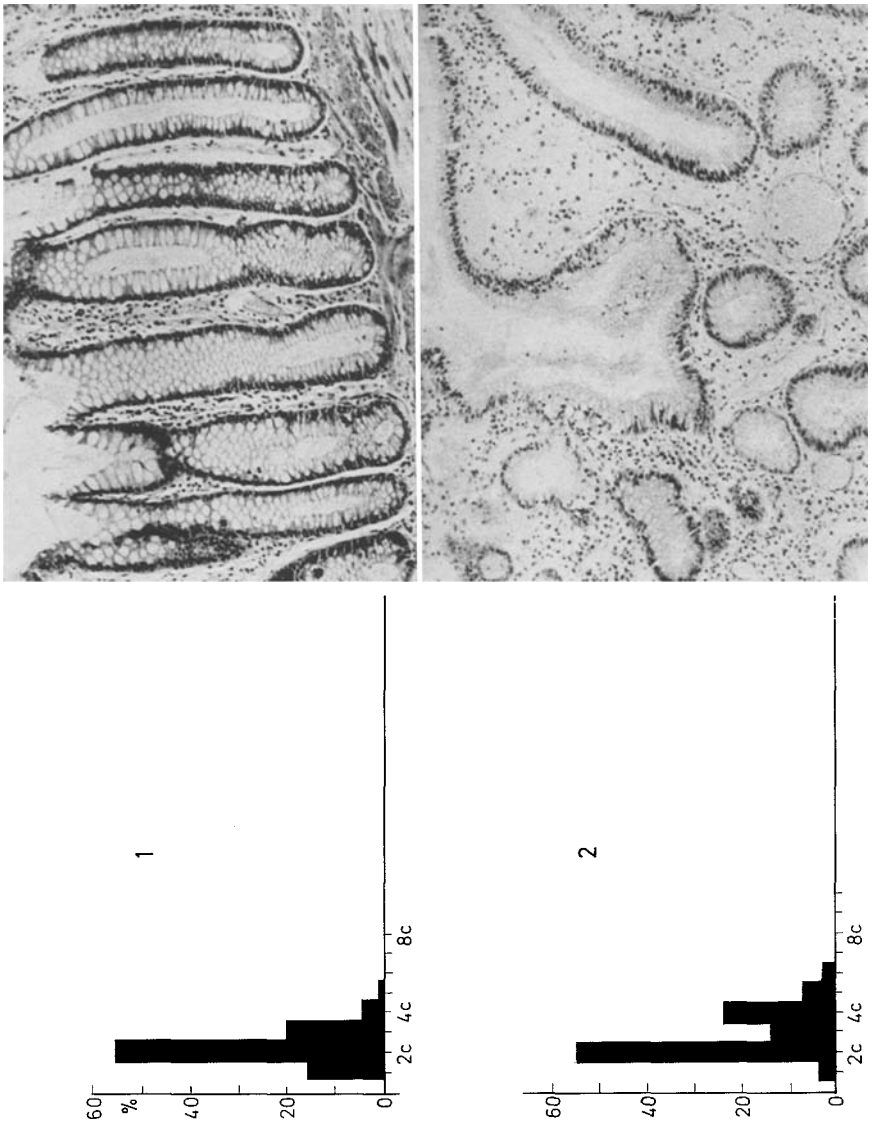


Fig. 1. Histogram of the distribution of DNA in the laryngeal epithelium. *1* Normal epithelium, *2* displasia, *3* hyperplasia atypical, *4* flat cellular cancer



with a substantial increase in the number of paratetraploid and paraoctoploid cells. During hyperplastic and tumorous processes with an atypical epithelium the number of polyploid and especially paraoctoploid, cells continues to increase The proportion of paraoctoploid cells in all the studied types of the epithelium with atypical hyperplasia rises to 30 percent or more (Fig. 4).

The cancerous tumors studied are characterized by the prevalence of paraoctoploid cells, the proportion of which exceeds 40 percent, as well as by increasing genetic heterogeneity. In cancerous tumours the number with a ploidy exceeding 8n is also high and reaches 30 percent or more.

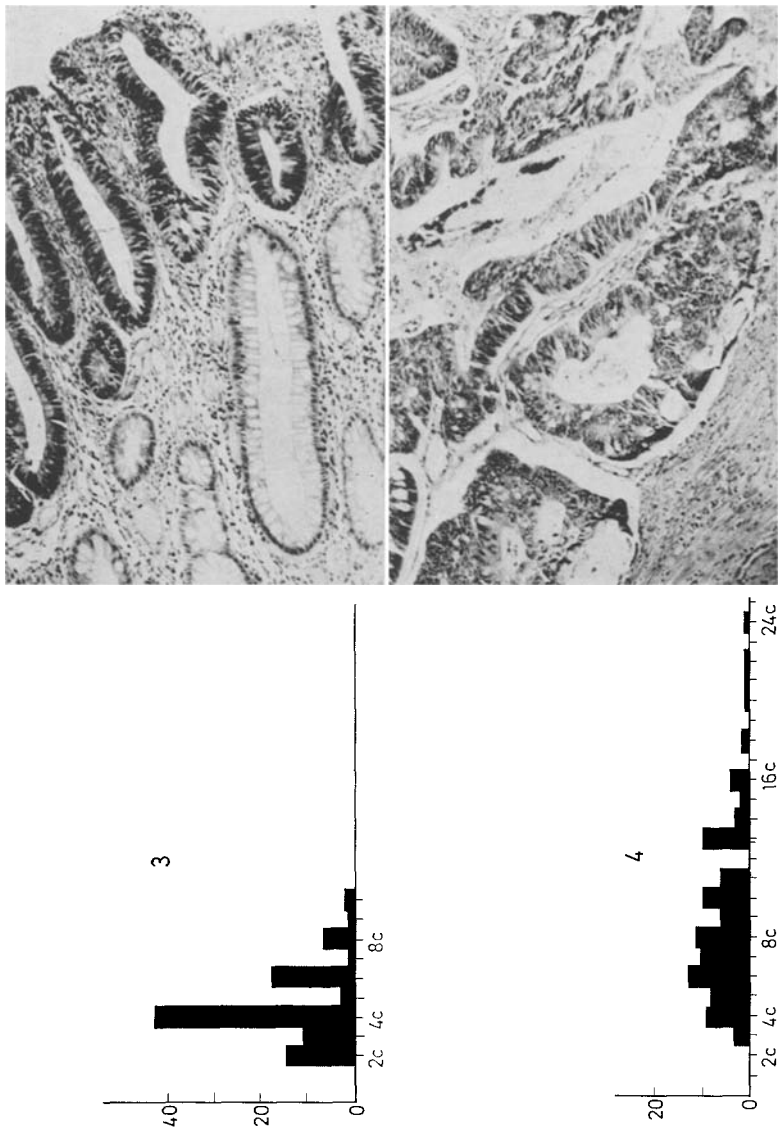
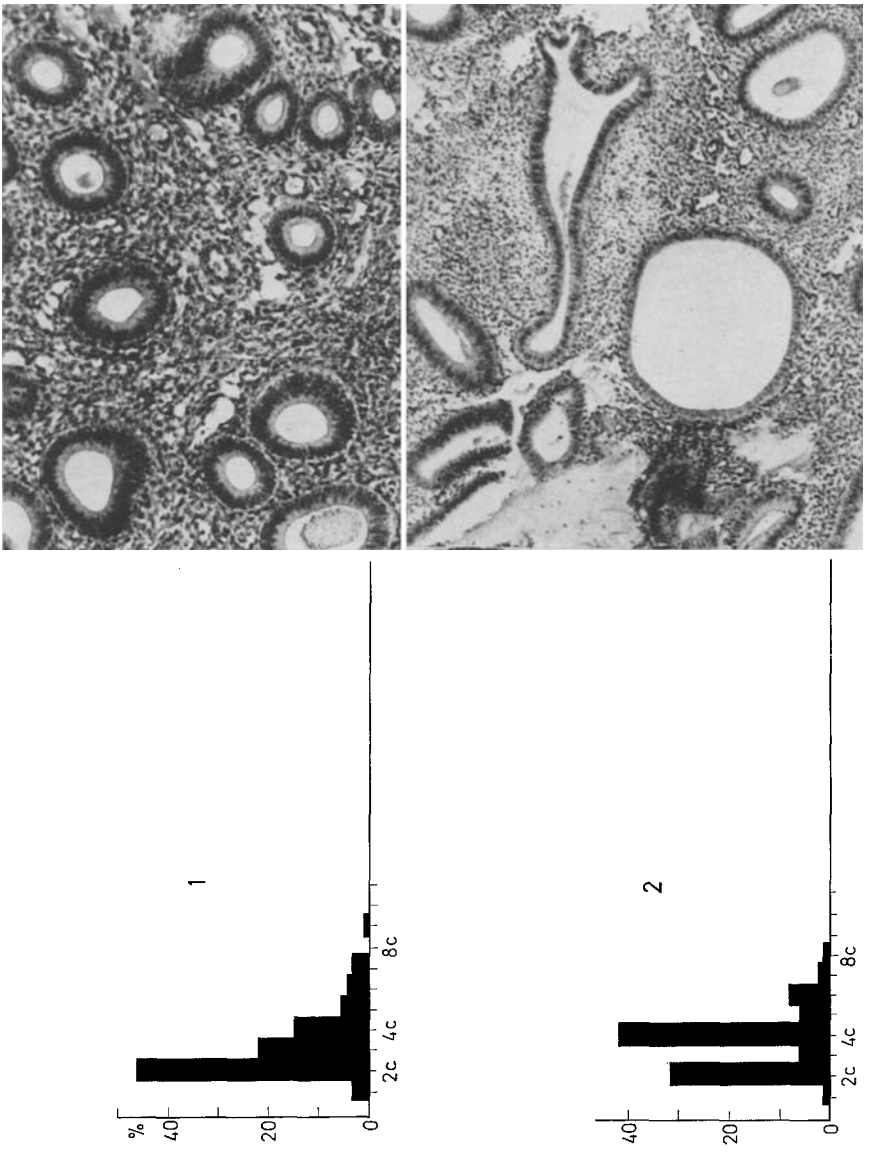


Fig. 2. Histogram of the distribution of DNA in the large intestinal epithelium. *1* Normal epithelium, *2* polype, *3* hyperplasia atypical, *4* adenocarcinoma

Thus, different forms of pretumours and tumorous transformation of epithelial tissues are marked by definite shifts in the DNA content in the nuclei of epithelial cells and different levels of polyploidy. These levels, notwithstanding certain differences determined by the morpho-functional distinctions of the initial tissue, are on the whole correlated with the tendency of the process towards malignization and the stage of blastomogenesis. Transitional dysplastic and hyperplastic conditions of the epithelium, characterized by an increased degree of heterogeneity of cell populations and increased polyploidy, represent a much greater probability of further malignization.



But only the achievement of a definite level of polyploidy of the epithelial tissue (according to our data, this level is an increase in the number of paraoctoploid cells to approximately 30 percent or more) is critical for the epithelium of the larynx and the large intestine and attests to the inevitability of further malignization, although, of course, it is not always possible to draw a precise boundary between background and obligatory precancerous changes. It should also be borne in mind that the rate of increase of polyploidy in various epithelial tissues differs. In the objects we studied the fastet rate was observed in the epithelium of the endometruim, in which the level of paraoctoploid cells rises almost to 15 percent even during ordinary hyperplasia, while in atypical hyperplasia paraoctoploid cells become a modal class and their number reaches almost 40 percent. The epithelium of the endometrium in general is characterized by a higher level of polyploidy, probably determined by its functional and plastic distinctions as a constantly and swiftly reacting structure.

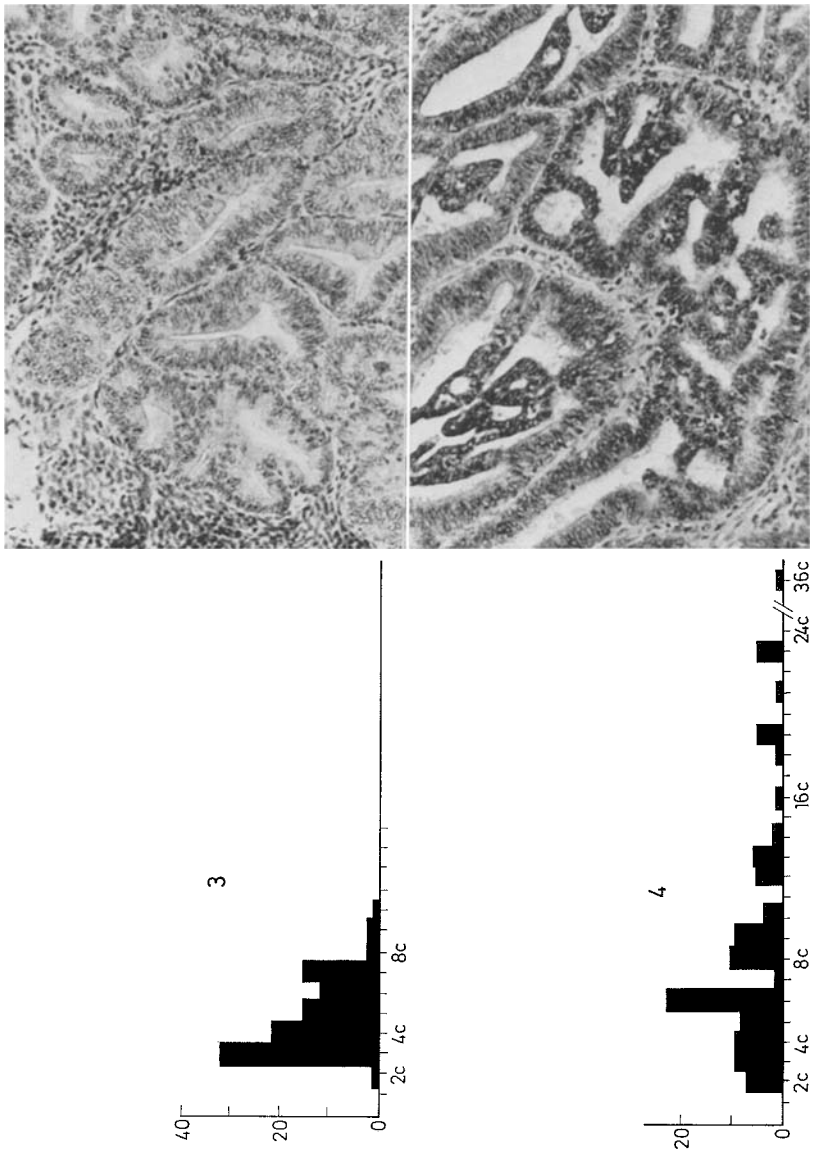


Fig. 3. Histogram of the distribution of DNA in the endometrical epithelium. *1* Normal epithelium, *2* hyperplasia simplex, *3* hyperplasia atypical, *4* adenocarcinoma

Thus, a microspectrophotometric study of the content of DNA makes it possible to reveal the upsetting of the balanced "clonic constitution" of the initial tissue, in pretumorous processes, which ensures its physiological regeneration and maintains definite rates of tissue growth regulated by the organism's systems. It is possible to give a rough outline of the level of growth of heterogeneity and polyploidy of the cell elements which attests to the beginning of tissue malignization and, can thus be used as an objective diagnostic test. Comparison of the weighted means of the DNA content in epithelial cell nuclei revealed the important sequence: the precancerous modification characterized by the first replication

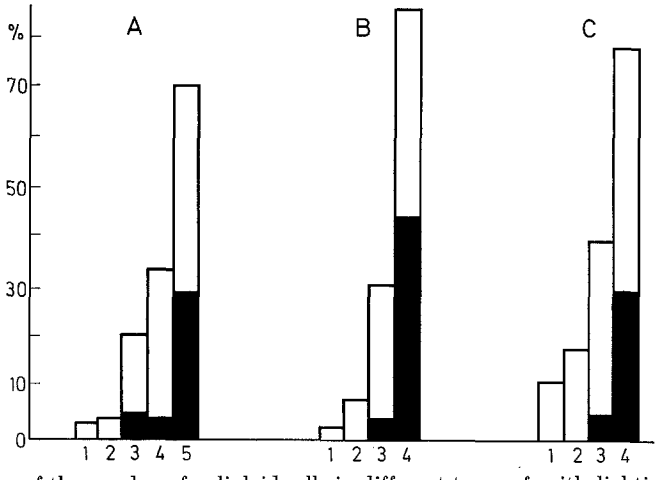


Fig. 4. Increase of the number of poliploid cells in different types of epithelial tissue. *A* Multilayer flat epithelium of the larynx (1-normal epithelium, 2-papilloma, 3-displasia, 4-hyperplasia atypical, 5-flat cellular cancer). *B* Glandular epithelium of the large intestine (1-normal epithelium, 2-polype, 3-hyperplasia atypical, 4-adenocarcinoma). *C* Glandular epithelium of the endometry (1-normal epithelium, 2-hyperplasia simplex, 3-hyperplasia atypical, 4-adenocarcinoma). — White columns: the number of cells with a level of ploidy above 8c. Black columns: number of cells with a level of ploidy higher than 8c

Table 1. The distribution of DNA in epithelial cell nuclei

The process	The tissue				
	Para-diploid cells (2c) (%)	Para-tetraploid cells (4c) (%)	Para-octoploid cells (8c) (%)	More than 8c (%)	The weihte mean of the DNA content (in relative units)
Epithelium of the larynx					
Normal epithelium	85.0 ± 3.2	13.3 ± 3.1	1.7 ± 1.2	—	2.3
Papilloma	84.0 ± 3.6	13.0 ± 3.4	3.0 ± 1.7	—	2.4
Displasia	46.0 ± 4.1	34.0 ± 3.9	16.0 ± 3.0	4.0 ± 1.6	3.5
Hyperplasia atypical	34.2 ± 4.3	32.5 ± 4.3	30.0 ± 4.2	3.3 ± 1.5	4.9
Flat cellular cancer	3.3 ± 1.2	26.1 ± 3.0	42.4 ± 3.4	28.2 ± 3.1	9.6
Epithelium of the large intestine					
Normal epithelium	63.9 ± 3.0	35.3 ± 3.3	0.8 ± 0.6	—	2.8
Polype	56.7 ± 4.5	35.8 ± 4.4	7.5 ± 2.5	—	3.2
Hyperplasia atypical	15.3 ± 2.9	53.9 ± 4.1	28.1 ± 3.7	2.7 ± 1.4	5.2
Adenocarcinoma	—	12.3 ± 2.2	42.4 ± 3.4	45.3 ± 3.4	10.6
Epithelium of the endometry					
Normal epythelium	50.0 ± 4.1	37.3 ± 3.9	12.0 ± 2.6	0.7 ± 0.6	2.6
Hyperplasia simplex	32.3 ± 5.0	50.0 ± 4.5	17.7 ± 3.8	—	3.1
Hyperplasia atypical	1.7 ± 0.3	53.4 ± 4.0	41.6 ± 3.8	3.3 ± 0.6	5.8
Adenocarcinoma	5.2 ± 1.1	14.3 ± 2.0	47.5 ± 3.3	33.0 ± 3.0	10.8

of the weighted mean of the DNA content, and the cancerous tumor characterized by the second replication of this index.

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Prof. Dr. G. G. Avtandilov
Central Pathoanatomical Laboratory
Institute of Human Morphology
USSR Academy of Medical Sciences
Moscow, USSR