

# MICROSPECTROPHOTOMETRIC STUDY OF THE CONTENT OF PROTEIN SULFHYDRYL GROUPS IN EPITHELIUM OF THE ENDOMETRIAL GLANDS IN HYPERPLASIAS AND CANCER

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A comparative microspectrophotometric study showed that in adenomatous hyperplasia and adenocarcinoma there is a much higher concentration of SH-groups in the cytoplasm of the epithelial cells than in the normal endometrium and in cystadenomatous hyperplasia. The intracellular distribution of the SH-groups is disturbed and the nucleo-cytoplasmic index reduced.

Experiments on primary cultures of a malignant glioma and on Ehrlich's ascites carcinoma cells [2-4] have shown that the thiol mechanism of assembly of the mitotic system is disturbed in tumor cells and these disturbances lead to the formation of pathological mitoses and to the excessive number of metaphases typical of malignant tumor cells.

Cytophotometric studies [5] confirm these experimental findings. In Ehrlich's carcinoma cells, unlike in normal amniotic cells, not only is there no increase in the concentration of thiol groups in prophase, but on the contrary, the content of sulfhydryl groups is slightly reduced. In malignant glioma cells, unlike in normal cells, the relative proportions of SH- and SS-groups are severely disturbed in prophase.

The connection between disturbances of mitotic cell division and carcinogenesis makes the study of protein thiol groups particularly important in the case of pathological hyperplasia and tumors of different tissues.

The few studies which have so far been made of the intracellular content and distribution of sulfhydryl groups in hyperplasia and tumors in man [7] have been based on visual assessment of the intensity of the histochemical reaction. They showed that during precancerous hyperplasia and carcinoma of the larynx, by contrast with normal stratified squamous epithelial cells, there is a decrease in the intensity of the Barrnett and Seligman reaction in the cytoplasm but an increase in the nucleoli.

This paper describes a microspectrophotometric study of the content and distribution of SH-groups in interphase epithelial cells of the endometrial glands under normal conditions, in pathological hyperplasia, including adenomatous (atypical), and in carcinoma.

## EXPERIMENTAL METHOD

The material examined consisted of 20 endometrial scrapings obtained during diagnostic curettage (5 cases of cystadenomatous hyperplasia, 5 of adenomatous hyperplasia, 5 of adenocarcinoma). In 5 cases, normal endometrium obtained from healthy women on the 9th-10th day of the menstrual cycle, as a suction scraping during insertion of intrauterine contraceptive devices, was investigated. The scraping was fixed in a mixture of glacial acetic acid and 96° alcohol (1:6) and embedded in paraffin wax. The Barrnett and

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TABLE 1. Concentration of Sulfhydryl Groups in Endometrial Epithelium (results of comparative microspectrophotometry)

Intracellular localization	Normal endometrium		Cystadenomatous hyperplasia		Adenomatous hyperplasia		Adenocarcinoma	
	D $\pm$ m	$\pm\sigma$	D $\pm$ m	$\pm\sigma$	D $\pm$ m	$\pm\sigma$	D $\pm$ m	$\pm\sigma$
Cytoplasm	0,142 $\pm$ 0,004	0,022	0,166 $\pm$ 0,005	0,022	0,214 $\pm$ 0,006	0,027	0,278 $\pm$ 0,010	0,050
Nucleus	0,141 $\pm$ 0,006	0,025	0,146 $\pm$ 0,006	0,025	0,136 $\pm$ 0,005	0,021	0,521 $\pm$ 0,022	0,107
Nucleolus	0,198 $\pm$ 0,004	0,022	0,198 $\pm$ 0,005	0,022	0,192 $\pm$ 0,004	0,019	0,207 $\pm$ 0,008	0,042
							0,328 $\pm$ 0,016	0,082

Note. 1) Mean optical density per unit area of section in areas of proliferation of tumor cells.

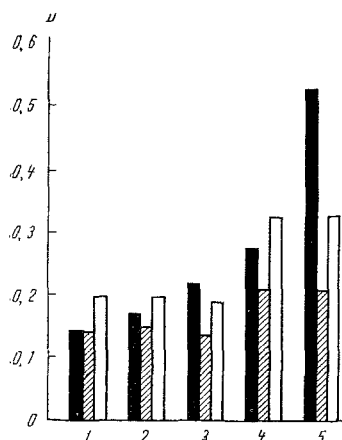


Fig. 1. Changes in concentration of SH-groups in endometrial epithelium. Abscissa, mean optical density per unit area of section, in relative units [D;  $\log(\phi_0/\phi)$ ]. 1) Normal endometrium; 2) cystadenomatous hyperplasia; 3) adenomatous hyperplasia; 4) adenocarcinoma; 5) proliferating areas of adenocarcinoma. Black columns represent cytoplasm, shaded columns nucleus, unshaded columns nucleolus.

Seligman reaction (with DDD and staining with diazotized orthodianisidine) was carried out in sections 5  $\mu$  in thickness, all treated at the same time. Corresponding to the intensity of the reaction, the color of the tissue structures varied from pale pink to reddish-purple, and was homogeneous (nucleolus, less commonly the cytoplasm) or finely granular (nucleus, cytoplasm) in character.

To determine whether the diazotized orthodianisidine was suitable for microspectrophotometry, absorption curves were plotted for different concentrations of the dye in the 450–600 nm waveband.

Comparison of these curves showed that the absorption of diazotized orthodianisidine reaches a maximum between 525 and 550 nm, but at these wavelengths, in the presence of high concentrations of the dye, the possibility of error (overestimation of the results) may reach 20% because of the metachromatic properties of the dye due to its containing two azo groups.

The value of 510 nm was therefore chosen as the working wavelength, for the absorption of the dye is about 95% and the possible error, even with a sharp increase in concentration, does not exceed 8–10%.

The microspectrophotometry was carried out in an integrating scanning microspectrophotometer [1]. The concentration of dye was determined as the mean optical density (D) per unit area of the section, in the cytoplasm, nuclei, and nucleoli of the cells. A statistical analysis was made of the numerical results of microspectrophotometry.

## EXPERIMENTAL RESULTS

The results of comparative microspectrophotometric investigation of the content of sulfhydryl groups (Table 1, Fig. 1) showed an approximately equal level of SH-groups in the cytoplasm and nucleus of the epithelium of normal endometrial glands, with a slightly higher concentration in the nucleolus. In cystadenomatous hyperplasia the concentration and distribution of SH-groups in the cytoplasm, nucleus, and nucleolus of the epithelial cells were unchanged; the slight increase in concentration of SH-groups in the cytoplasm is not statistically significant.

In areas of adenomatous hyperplasia there was a marked increase in the concentration of SH-groups in the cytoplasm of the epithelium of atypical glands ( $P < 0.01$ ), whereas in the nucleus and nucleolus it was only very slightly different from normal and the concentration in cases of cystadenomatous hyperplasia (difference not statistically significant).

An even more marked increase in the concentration of SH-groups in the cytoplasm of the epithelium was observed in adenocarcinomas of the endometrium, especially in areas of active proliferation of tumor cells (zones of growth), where the concentration of SH-groups was more than 3 times higher than in the

normal endometrium or in cases of cystadenomatous hyperplasia ( $P < 0.01$ ). The concentration of SH-groups was also increased in the nucleus and nucleoli of the cancer cells ( $P < 0.05$ ), but this tendency was less marked than in the cytoplasm.

During malignant transformation, there is thus not only an increase in the total concentration of sulfhydryl groups in the epithelial cells of the endometrial glands, but also a disturbance of their quantitative intracellular distribution. For example, whereas under normal conditions the mean nucleo-cytoplasmic index is 0.98, and in cystadenomatous hyperplasia it was 0.88 ( $P > 0.1$ ), in adenomatous hyperplasia and carcinoma it was sharply reduced to 0.63 and 0.64 respectively ( $P < 0.01$ ). Consequently, the results show that in precancer and cancer there is a shift in the distribution of SH-groups between proteins of the nucleus and cytoplasm. This agrees in some measure with the experimental findings of Ikeda [10], who considers that there are two independent cycles of changes in SH-groups, a nuclear and a cytoplasmic cycle, and that synchronization of the two cycles is essential for the normal course of mitosis.

The considerable disturbance of the normal relationship between sulfhydryl groups in the nucleus and cytoplasm of the epithelial cells in precancerous hyperplasia and in carcinoma of the endometrium may be one of the causes of the disturbances of mitosis in these pathological states [8], which are characterized by an increase in the number of metaphases and a sharp increase in the number of pathological mitoses.

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