

CONTENT OF BASIC PROTEINS IN RETICULUM CELLS  
OF TRANSPLANTABLE LINES SENSITIVE  
AND RESISTANT TO ENTEROVIRUSES

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The content of basic proteins in strains of primate reticulum cells sensitive (MIO) and specifically resistant (MIO-45) to poliovirus was compared. The cytochemical reaction was assessed visually and cytophotometrically. A higher content of basic proteins was found in the cytoplasm and, in particular, in the nuclei of the resistant than of the sensitive cells.

Lines of primate reticulum cells specifically resistant to the cytopathic action of certain viruses have been obtained in Solov'ev's laboratory [4-8]. Special investigations have shown that the resistant strains did not produce substances of the antibody or interferon type, so that the biological nature of the resistance of these cells to viruses was unexplained. Cytological and cytochemical investigations led to the formation of the hypothesis that this resistance to viruses is based on suppression of synthesis by these cells of certain substances specifically essential for the adsorption and deproteinization of the particular virus, but not essential to the life of the cells themselves [2, 3, 8, 10, 11]; this phenomenon has been described by the authors as "metabolic immunity" of the cells.

The state of metabolic immunity is inherited by the cells over many generations (more than 600), and it may then disappear spontaneously with reversion of all the properties of the original virus-sensitive cells. It was accordingly postulated that the changes in metabolism in the cells during the development of metabolic immunity are based on repression of certain cistrons of the cell by basic proteins of the histone type [8].

It therefore became necessary to compare the content of basic proteins in sensitive and resistant cells, and the present investigation was carried out for this purpose.

EXPERIMENTAL METHOD

The investigation was carried out on cells of transplantable strain MIO obtained by N. E. Gulevich in 1960 from the tonsils of a healthy rhesus monkey, and on cells of strain MIO-45, obtained after prolonged contact of MIO cells with poliomyelitis virus, which possessed marked resistance to the homologous virus. The cells were grown on cover slips in Leyton's tubes in medium No. 199 with 10% heated bovine serum (200,000 cells per ml).

For morphological investigation cultures were fixed in Shabadash's mixture and stained with hematoxylin and eosin. Basic proteins were detected histochemically by the method of Alfert and Geschwind, and arginine by Baker's method (a modification of the Sakaguchi I reaction) from the formulas described in Pearse's textbook [13]. Specimens stained for basic proteins were investigated cytophotometrically on a type MTsF-1 apparatus with MIU-2 integrator and EPP-09M3 potentiometer with monochromator ( $\lambda = 365$  nm).

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## EXPERIMENTAL RESULTS

Both the initial MIO cells and the MIO-45 cells resistant to poliovirus contain basic proteins in high concentrations. As a rule, both the nuclei and cytoplasm of the monolayer were stained with equal intensity. Only in some cells, mostly young, were the nuclei a deeper green color.

Basic proteins were detected in the nuclear membrane, nucleoli, and granules of the karyoplasm. Sometimes a paler central part and a darker peripheral ring could be distinguished in the nucleoli. In the cytoplasm, the reaction product was localized as fine, dust-like particles or as distinct, uniformly distributed granules. In a few cells these granules were concentrated in the perinuclear zone.

Visual comparison of the MIO and MIO-45 strains showed on the whole a darker staining of the MIO-45 monolayer. The difference was because in the MIO-45 culture many of the cells contained large quantities of reaction product, especially in the nuclear structures.

In the test for arginine, structures containing this amino acid were stained pinkish-orange in color. The nucleolus, nuclear sap, and nuclear membrane were homogeneously stained; sometimes the cytoplasm was homogeneous, at others very fine granules were visible in it. Proteins containing arginine were found in highest concentrations in the nucleolus, chromatin granules, and nuclear membrane, and in lowest concentrations in the proteins of the cytoplasm. All these structures in resistant MIO-45 cells gave a stronger reaction than in the original MIO cells.

Preliminary determination of the extinction on the MTsF-1 apparatus (3 times, through the nucleus-containing zone of each cell, along parallel lines) gave mean values of 0.046 conventional unit for the MIO cells and 0.114 conventional unit for the MIO-45 cells.

The results of a more detailed investigation, conducted separately on the nucleus and cytoplasm of each cell are given in Table 1; typical extinction curves are given in Fig. 1.

The results indicate a higher content of basic proteins both in the nuclei and in the cytoplasm of the resistant cells than of the initial cells sensitive to poliovirus ( $P < 0.001$ ).

It was shown previously in the authors' laboratory that the isoelectric points of the ribonucleoproteins of nucleus and cytoplasm are shifted toward the alkaline side in cells specifically resistant to a particular enterovirus [9, 12]. At that time this phenomenon was provisionally regarded as the result of synthesis of basic proteins in the resistant cells. The present investigation yielded direct evidence of a higher content of basic proteins in the structures of resistant cells than in the cells of the original cultures. The use of two different methods thus gave identical results, and the original discovery of an increase in the content of basic proteins in the nuclei and cytoplasm of cells which have acquired specific antiviral immunity was thus confirmed.

It is interesting to note that the differences in the content of basic proteins are more marked in the nuclear structures than in the cytoplasm. Since, in the modern view, protein synthesis takes place in the cytoplasm, it may be supposed that this process takes place with higher intensity in MIO-45 cells than in MIO cells, and that the newly synthesized basic protein is then concentrated in nuclear structures.

In the authors' opinion, these results give further indirect confirmation of the hypothesis concerning the role of repression of certain cistrons of the cell in the development of specific resistance of the cells to viruses of the metabolic cellular immunity type.

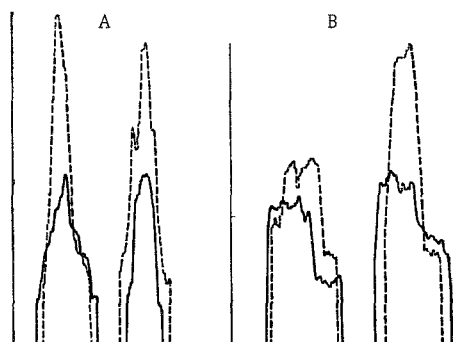


Fig. 1. Typical extinction curves in nuclei (A) and cytoplasm (B) of MIO cells sensitive to poliovirus (continuous lines) and MIO-45 cells resistant to it (broken line) obtained by scanning cytophotometry on the MTsF-1 apparatus. Abscissa, time; ordinate, absorption at 635 nm (in conventional units).

TABLE 1. Extinction ( $\lambda = 635$  nm) in Nuclei and Cytoplasm of Original MIO Cells and of MIO-45 Cells Resistant to Poliovirus (in conventional units)

Strain	Nucleus		Cytoplasm	
	$\bar{x} \pm \sigma_{\bar{x}}$	$t$	$\bar{x} \pm \sigma_{\bar{x}}$	$t$
MIO	$0,094 \pm 0,0056$	11,0	$0,051 \pm 0,0006$	8,7
MIO-45	$0,171 \pm 0,0046$		$0,086 \pm 0,0038$	

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