EFFECT OF METHYLURACIL ON THE PROTEIN AND NUCLEIC ACID CONTENT IN HEp-2 CELLS

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Methyluracil in doses of 1 to 50 μg/ml increased the total nucleic acid content in the nulcei and protein content in the cytoplasm of HEp-2 cells. A dose of 20 μg/ml gave a gradual increase in the nucleic acid concentration in the cells from 122 to 197%. A relatively stable increase in protein with all durations of exposure was produced by a dose of 30 μg/ml (132-145%). The results suggest an increase in the intensity of cell metabolism under the influence of methyluracil.

KEY WORDS: methyluracil; nucleic acid and protein content; HEp-2 cells.

The ability of pyrimidine derivatives (methyluracil and pentoxyl) to reduce the intensity of inflammation [3, 4, 10], to accelerate wound healing [1, 7, 11, 12], and to increase the resistance of the organism to adverse factors [2, 5, 6, 8, 9] suggests that these substances could have a useful role in clinical practice. However, the inadequate attention paid to the study of the mechanism of action of the pyrimidine is a definite handicap to the wider use of these substances.

The object of this investigation was to study the effect of methyluracil on metabolism in a monolayer tissue culture.

EXPERIMENTAL METHOD

Cells of line HEp-2 were used. A culture was grown in the usual way. On the third day of growth, when a good monolayer had formed and the culture fluid was changed methyluracil was added in doses of 1, 5, 10, 20, 30, and 50 μg/ml, with exposures of 3, 6, 12, 24, and 48 h. The cells were fixed in ethanol–formalin. The total nucleic acids were determined in the nuclei [15] and protein in the cytoplasm [14], and the concentrations of substances in the cells were estimated by probe cytophotometry. With each exposure 30 cells were photographed under standard conditions with the aid of a microcamera, with highly stabilized constant illumination and a combination of filters SZS-7 and ZhS-17. The film was developed simultaneously. Correlation between the degree of blackening of the film and the intensity of the light flux passing through the object undergoing photometry was determined with the aid of a characteristic curve in which the intensity of blackening of the film was plotted against duration of exposure. Measurements were taken with the MF-2 microphotometer. With each exposure of the preparation photometry was carried out at 30 points of the nucleus to determine nucleic acids and 50 points of the cytoplasm to determine the protein content. The area of the object in projections of the negatives obtained with constant magnification was measured with a planimeter and values for the concentration of the test substance were calculated by the equation

$$I = \frac{(I_O - I_S) \cdot S}{\gamma},$$

where I is the concentration of the substance in the structure, S the area of the organoid, I_0 the intensity of light passing through the structure not containing the test substance, I_S the intensity of light passing through the structure with the substance, and γ the coefficient of contrast.

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The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

As a result of the action of methyluracil in a dose of 1 μg/ml for 24 h the concentration of nucleic acids in the cells was unchanged, but after 48 h it had risen to 120% ($P < 0.05$). The protein content in the cells rose gradually to 145% ($P < 0.01$) after 12 h, and returned to normal ($P < 0.01$) after 48 h. When methyluracil was given in a dose of 5 μg/ml no change in the nucleic acid level was observed for 6 h ($P < 0.001$). After 12 h their content had increased to 171% ($P < 0.001$), falling to 147% ($P < 0.001$) after 48 h. The protein content was increased by this dose of methyluracil to 137% ($P < 0.001$) after 3 h, and to 152% ($P < 0.001$) after 12 h; the increase in the protein content reached its highest level (218%) after 48 h. Methyluracil in a dose of 10 μg/ml increased the nucleic acid concentration to 201% ($P < 0.001$) after 3 h, their content fell a little (190%) after 12 h, and returned to its initial level, which was the same as in the control series ($P < 0.05$), in the course of 24-48 h. The protein concentration was increased by this dose to 131% ($P < 0.02$) after 3 h, to 159% after 6 h, and to 196% ($P < 0.001$) after 12 h; it fell to 168% ($P < 0.001$) after 48 h. Methyluracil in a dose of 20 μg/ml caused a gradual increase in the nucleic acid content from 122 to 197% ($P < 0.001$). The protein concentration was increased to 220% ($P < 0.001$) after 3 h by this dose, it fell to 128% ($P < 0.02$) after 12 h, rose to 157% ($P < 0.001$) after 24 h, and to 231% ($P < 0.001$) after 48 h. Methyluracil in doses of 30 and 50 μg/ml caused an increase in the concentration of nucleic acids after 3 h to 158-171% ($P < 0.001$), with slight fluctuations during observation for 48 h. The protein content after a dose of methyluracil of 30 μg/ml was 132-145% ($P < 0.001$) relative to the control, compared with 150-200% ($P < 0.001$) after a dose of 50 μg/ml.

The results thus show that methyluracil, in doses of 1 to 50 μg/ml, stimulates protein and nucleic acid synthesis in cells; in a dose of 20 μg/ml a gradual increase in the concentration of nucleic acids in the cells was observed from 122 to 197%, whereas a dose of 30 μg/ml caused a relatively stable increase in the protein content in the cells after all exposures (132-145%). The results indicate an increase in the intensity of metabolism in the cells under the influence of methyluracil, evidently on account of the anabolic action of this compound.

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