Incubation of the L-8 cells for 24 h with glutamin(asparagin)ase from *Ps. fluorescens* in a concentration of 1×10^{-1} I.U./ml led to complete suppression of DNA synthesis in the experimental samples compared with the control. Inhibition of [^{3}H]thymidine incorporation in the cells by the preparations in a concentration of 1×10^{-5} I.U./ml amounted to 50.2%. When the concentration of the preparation was low (1×10^{-8} I.U./ml), the degree of inhibition was 17.4% (Fig. 1).

Inhibition of DNA synthesis in CaOv cells in the presence of enzymes from *Ps. fluorescens* was independent of concentration (10, 1, and 0.1 I.U./ml) and was 36.8%-38.3% (Fig. 2, 2).

The study of the effect of glutamin(asparagin)ase from *Ps. boreopolis* 526 on incorporation of [^{3}H]thymidine in CaOv and L-8 cells showed that exposure of the cells with the preparation in concentrations of 10, 1, and 0.1 I.U./ml led to complete suppression of DNA synthesis in the second case but did not affect incorporation of [^{3}H]thymidine into the CaOv cells (Fig. 2, 3).

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WEAKENING OF DNA—PROTEIN INTERACTIONS IN MOUSE LEUKEMIC LYMPHOBLASTS UNDER THE INFLUENCE OF SOME ANTITUMOR AGENTS

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A method of testing DNA—protein interactions in eukaryote cells with the aid of nucleoprotein-celite chromatography (NPCC) was suggested previously [6, 7]. Sharp differences in the strength of DNA—protein interaction in resting and proliferating cells have been detected by the NPCC method: In the first case the cell DNA is eluted from the column under relatively mild conditions (1.5 M LiCl, 8 M urea, 4°C), whereas in the second case much more rigorous conditions are required (4 M LiCl, 8 M urea, 95°C). Actions leading to withdrawal of the cells from the division cycle cause transition of DNA from the form firmly bound with protein (form II) into a form relatively weakly bound with protein (form I). Exciting cells to divide causes the opposite transition. The use of the NPCC method to study changes in DNA—protein interactions which may arise in the deoxyribonucleoprotein complex (DNA complex) of eukaryotes after exposure of the cell to biologically active substances and, in particular, after the action of the well-known mutagen, carcinogen, and carcinolytic agent 1-nitroso-1-methylurea

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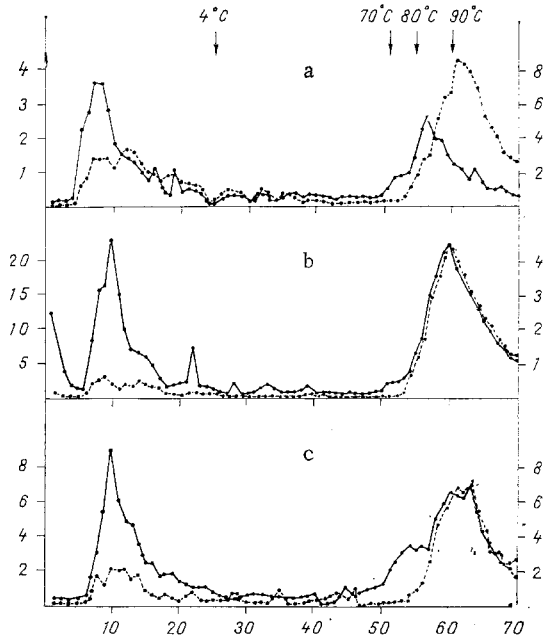

Fig. 1. Nucleoprotein-celite-chromatography of LL cells after treatment with NMU (a), potassium cyanate (b), and prospidin (c). Abscissa, fractions; 1-25) elution with lithium chloride concentration gradient and urea at 4°C, 26-75) elution by temperature gradient; ordinate: on left — radioactivity of fractions measured as ^{3}H-label (in cpm $\times 10^{-3}$, continuous line); on right — radioactivity of fractions as ^{14}C-label (in cpm $\times 10^{-2}$, broken line). Cells treated with noxious agent labeled with ^{3}H; control cells labeled with ^{14}C.

(NMU), is very interesting. It was postulated previously, on the basis of a study of structural injuries induced in the DNP complex by NMU *in vitro*, that exposure to NMU *in vitro* ought to lead to the development of a special type of lesions in the genome, namely a change in its supramolecular structures and (or) the appearance of DNA regions partly or completely free from protein [5]. However, experimental proof of the validity of this hypothesis has not hitherto been obtained.

In the present investigation the NPCC method was used to study the state of DNA—protein interactions in eukaryote cells after exposure to NMU, through prospidin, a new Soviet antitumor drug whose method of action has so far received only little study, and also of the NMU breakdown product potassium cyanate, which reproduces the carbamoylating action of NMU and, like the latter, can induce supramolecular injuries in the DNP complex *in vitro* [2]. Cells of a monolayer culture of leukemic lymphoblasts from LL mice, cultured by the method described previously [4], were used as test object. The cells were grown in a balanced medium up to the logarithmic stage of growth (3 days). [^{14}C]- or [^{3}H]thymidine was added to the medium 24 h before the experiment up to a concentration of 1 and 5 μCi/ml respectively. After the end of cell culture the medium together with the label was poured off and fresh medium containing 0.2 mg/ml NMU (or prospidin, or potassium cyanate) was added to the cells labeled with [^{3}H]-thymidine. After 1 h the medium was poured off and the cells were washed with fresh medium and removed from the substrate with versene. Cells labeled with [^{3}H]thymidine and treated with the antitumor agent were mixed with control intact cells labeled with [^{14}C]thymidine. Subsequent lysis of the cells in NPCC was carried out as described in [7].

EXPERIMENTAL RESULTS

The results of NPCC of DNA from cells treated with NMU, prospidin and potassium cyanate, are given in Fig. 1. Treatment of the cells with these agents led to significant changes in the elution profile of DNA from the column: In all cases as a result of treatment the DNA fraction weakly bound with protein was increased two to threefold. A significant difference also was observed as a result of the action of these agents: Under the influence of NMU the peak of firmly bound DNA changed its position on the elution profile and was shifted through

7-8°C toward lower temperatures; hence it follows that the strength of binding of this form of DNA with protein also was changed after the action of NMU. Potassium cyanate did not change interaction of this DNA fraction with protein. Under the influence of prospidin interaction of the main quantity of DNA of this fraction with protein was unchanged, but a DNA subfraction was revealed by the presence of a shoulder on the elution profile in the 70-80°C range.

On the one hand, the results revealed for the first time some hitherto unknown features of the mechanisms of action of two antitumor agents — NMU and prospidin, namely their ability to induce relaxation of the supramolecular structure of the cell chromatin. They agree with previous hypotheses on the ability of these compounds to induce supramolecular injuries to the genome [1, 5], although the concrete nature of these lesions has not yet been established. The role of each of the two reactions characteristic of NMU, namely the alkylation reaction and the carbamoylation reaction, in the creation of the cytotoxic effect has been discussed for a long time in the literature [3], but it still remains far from solution. Comparison of the results of NPCC of cells treated with NMU and cells treated with potassium cyanate, which has no alkylating properties but which fully reproduces the carbamoylating action of NMU, showed that the carbamoylation reaction was responsible for weakening of the DNA—protein bond and the appearance of form I of DNA under the influence of NMU, whereas the alkylating action of NMU was responsible for the temperature shift in the elution profile (weakening of DNA—protein interaction of form II). Each reaction thus leads to its own characteristic change in the supramolecular structure of the cell. There is no doubt that the ability of NMU to change DNA—protein interactions in the cell, which we have demonstrated, is relevant to the explanation of the mechanism of the carcinogenic action of NMU.

On the other hand, the results are important for the development of the actual technique of NPCC, for they suggest (admittedly, only very provisionally) the primary changes that are responsible for the changes observed in DNA—protein interaction. In particular, we know that potassium cyanate does not cause breaks of DNA strands [2]. NMU, on the other hand, is active in this respect [3], and the breaks which arise are the result of alkylation of DNA. Prospidin is weakly active in the formation of single-stranded breaks in DNA [1]. It can be tentatively suggested, therefore, that the temperature shift observed in the DNA elution profile is connected with relaxation of the chromatin structure because of breaks in DNA.

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