# INVESTIGATION OF THE STRUCTURE OF DESOXYRIBONUCLEOPROTEIN IN THE SPLEEN AND LUNGS OF MICE SOON AFTER EXPOSURE TO IRRADIATION AND URETHANE

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Recent investigations have shown that histones in the nucleoprotein molecule regulate the activity of DNA as the genetic template [2, 7]. It has been discovered that one of the characteristic properties of malignant tumor cells is disturbance of the links between DNA and histone in the chromatin apparatus of the nucleus [6, 8]. Little is known of the state of the bond between DNA and protein in the nucleoprotein molecule, as also of the state of the supramolecular structure of these complex proteins in the initial period of tumor development.

The object of the present investigation was to study the tertiary and quaternary structure of desoxyribonucleoprotein (DNP) isolated from the spleen and lungs of mice in the early period after exposure to oncogenic factors such as radiation and urethane.

## EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino mice, which very rarely develop spontaneous leukemia or adenoma of the lungs. Two groups of experiments were carried out. The animals of group 1 were irradiated 5 times from a $\gamma$-ray source in a dose of 400 R at a dose rate of 78 R/min. The intervals between irradiation were 30 days. The total dose of radiation received by the mice surviving 5 months after the beginning of the experiment (32 of the 125 animals) was thus 2000 R. Under these conditions of irradiation, a myeloid form of leukemia develops most frequently among noninbred mice [1]. Groups of 5-6 mice were sacrificed immediately after the end of irradiation, and after intervals of 24 h and 10 and 20 days, and DNP was extracted from their spleen tissue.

The animals of group 2 (125 mice) received a subcutaneous injection of urethane in a dose of 1 mg/g body weight every 3 days. These doses of urethane caused the development of adenoma of the lungs in mice 2-3 months after the beginning of administration. The macromolecular structure of DNP from the mouse lung tissue was investigated immediately and 24 h and 7, 14, and 30 days after the beginning of the experiment. Material from 4 or 5 animals was pooled for one investigation. Morphological changes in the lungs were studied at the same time. Pieces of lung tissue taken at the times indicated above were fixed in formalin and embedded in paraffin wax, and sections were stained with hematoxylin-eosin.

The macromolecular structure of the DNP was investigated by D. M. Spitkovskii's method, noting the formation and relaxation of regularly arranged desoxyribonucleoprotein fibrils [4]. The character of the highly elastic deformation of these fibrils gives indirect evidence of the state of the bonds between DNA and protein in the nucleoprotein molecule [5]. The dynamics of formation of regularly arranged fibrils and the magnitude of their residual deformation reflect changes in the macromolecular structure of DNP [4].

DNP was extracted from the tissue homogenate with 1 M sodium chloride solution. The cytoplasmic proteins were removed by washing the material in the cold 5-7 times with physiological saline containing 0.1 M sodium citrate. The value of $E_p$ for the isolated preparations was 6400-6800. The N/P ratio was 4.5-5 for DNP from the spleen and 11-14 for DNP from the lung tissues. The DNP preparations isolated

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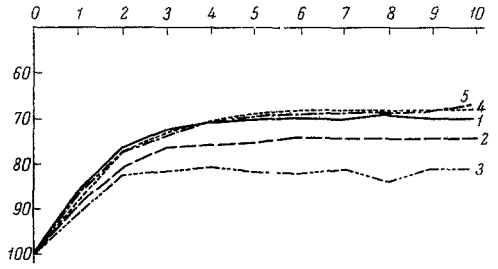


Fig. 1. Curves of relaxation of regular fibrils from spleen DNP of healthy mice (1) and of mice receiving fractionated $\gamma$-irradiation, immediately after the end of irradiation (2) and 24 h (3) 10 days (4) and 20 (5) thereafter. Each curve plotted from results of observations on contraction of 20-30 fibrils. Abscissa) time (in min), ordinate) length of fibrils (in percent).

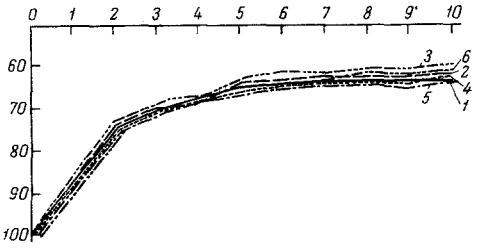


Fig. 2. Curves of relaxation of regular fibrils from DNP extracted from lung tissue of healthy mice (1) and mice receiving urethane, 24 h (3), and 7 (4), 15 (5), and 30 (6) days after beginning of administration. Each curve represents the mean of 40-60 observations.

from each organ were essentially indistinguishable in molecular weight, which varied from $19 \times 10^6$ to $22 \times 10^6$. The results of the observations were subjected to statistical analysis.

## EXPERIMENTAL RESULTS

The results are given in Figs. 1 and 2. Relaxation of the regularly arranged DNA fibrils from the spleen of mice sacrificed at once and 24 h after the end of irradiation was disturbed (Fig. 1). The fibrils at this period were less able to undergo highly elastic deformation, and the phenomenon of plastic flow of the polymer was observed Fig. 1, 2 and 3). Whereas the regular DNA fibrils from the spleen of healthy mice relaxed in 5 min, when their length was reduced by 30%, DNP fibrils from the spleen of mice examined immediately after the end of irradiation or after an interval of 24 h contracted by only 25 and 18% respectively (Fig. 1, 1-3). The ability of the regular DNP fibrils from the spleen of mice 24 h after the last irradiation to relax was particularly severely disturbed. Relaxation of these fibrils ceased after 4 min, whereupon their length actually increased slightly because of commencing polymer flow (Fig. 1, 3). The differences in behavior of regular DNP fibrils from the spleen of healthy and irradiated animals described above are statistically significant ($P < 0.05$).

The course of relaxation of regular fibrils from DNP extracted from the spleen of mice sacrificed 10 and 20 days after the end of irradiation was the same as that of DNP fibrils from the spleen of healthy animals (Fig. 1, 1, 4, 5). The slight differences in the dynamics of contraction of these fibrils after the 5th minute of observation were not statistically significant. Regeneration is known to be a prominent feature in the spleen of irradiated animals at this time. Probably the macromolecular structure of DNP in the newly formed cells was not significantly different from normal.

The results described above show that fractionated $\gamma$-ray irradiation of mice causes a transient disturbance of the macromolecular structure of the DNP in hemopoietic tissue cells. The decrease in highly elastic properties of the regular DNP fibrils and the intensity of polymer flow indicate changes in the tertiary and quaternary structure of these complex proteins.

Regular fibrils from DNP extracted from the lung tissue of mice sacrificed immediately and at intervals of 7, 15, and 30 days after the beginning of urethane administration relaxed just like fibrils from DNP of the lung tissue of healthy animals (Fig. 2, 1, 2, 4-6). The slight differences in behavior of these structures were not statistically significant. The fibrils contracted for 6-7 min, shortening on the average by 37% of their initial length. Starting from the 7th minute, relaxation ceased and no further change in the length of the fibrils took place before the end of the observations.

DNP fibrils from the lung tissue of mice sacrificed 24 h after injection of urethane behaved rather differently (Fig. 2, 3). Starting from the 5th minute of observation, they contracted more intensively than the others. At the 6th minute after the experiment began they had shortened by 39%, and at the 10th minute by 41% of their initial length. Although the difference in the degree of contraction of regular DNP fibrils from the lung tissue of mice used in the experiment 24 h after injection of urethane and of the control animals was small (Fig. 2), nevertheless it was statistically significant, ($P < 0.05$). These changes in behavior of the regular fibrils are evidence of increased flexibility of the DNP macromolecules at this period.

Parallel with the study of the macromolecular structure of the DNP, the lungs of the mice receiving urethane were investigated histologically. This showed that urethane possesses a marked oncogenic action. By the 15th day most animals investigated showed a clear proliferation of the alveolar epithelium with thickening of the interalveolar septa. In the lungs of the mice investigated one month after the beginning of urethane administration, besides the picture described above, clusters of proliferating cells could be seen under the pleura and near the bronchi. These cells differed from the alveolar epithelium in their narrower cytoplasm, often appearing as a crescent displacing the hyperchromic nucleus toward the edge. Changes of this type in the lung tissue of mice represent an early stage of development of adenoma [3]. This group of experiments showed that the development of the neoplastic process in the mouse lungs under the influence of urethane was not accompanied by prolonged changes in the macromolecular structure of the DNP in the lung tissue cells.

It may be concluded from the results described above that in the early period after exposure of animals to carcinogenic agents such as $\gamma$-rays and urethane (under conditions giving maximal tumor production), transient changes are observed in the tertiary and quaternary DNP structure. Changes in DNP structure caused by these carcionogens differ in character. At the end of fractionated $\gamma$-ray irradiation of mice a temporary disturbance of the tertiary and quaternary structure of DNP in the hemopoietic tissue was observed. The decrease in highly elastic deformation of the regular DNP fibrils, and the development of a process of polymer flow are evidence of weakening of the bonds between DNA and protein in the nucleoprotein molecule, and of a weakening of the forces of intermolecular interaction in the macromolecular structure [4, 5]. Urethane caused ill-defined changes in the tertiary structure of DNP from the lung tissue 24 h after administration. The increase in highly elastic properties of the regular DNP fibrils at this period denotes an increase in the strength of the bond between DNA and protein in the nucleoprotein molecule [5].

Although the changes described above are transient in character, they may be biologically important, being responsible for disturbance of the activity of DNA as the genetic template. Deviations in the transcription of genetic information may be induced both by disturbance of interaction between DNA and histone in the nucleoprotein molecule and by changes in its macromolecular structure [7, 9, 10].*

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