ON THE STATE OF DEOXYRIBONUCLEOPROTEIN ISOLATED FROM MOUSE SPLEEN TISSUE FOLLOWING THE ACTION OF γ RADIATION IN DOSES WITH A HIGH LEUKEMIGENIC EFFECT

(UDC 616.001.28-07:616.411-018.3-074+616.411-018.13-02:616-001.28 -074)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 8, pp. 106-109, August, 1965
Original article submitted April 23, 1964

The appearance of malignant neoplasms in the irradiated organism is one of the most dangerous aftereffects of radiation. More and more data are being accumulated, indicating that the appearance of tumoral elements is due to a change in the native properties of the genetic apparatus of the cellular chromosomes, persisting for long periods after irradiation [2].

In their physicochemical structure, the chromosomes represent a supermolecular structure of deoxyribonucleoproteins (DNP) [6]. There is little information on the nature of the changes in DNP during the short-term and long-term periods after irradiation [2]. In most of the investigations devoted to this question, DNP was irradiated after isolation from the organism, and the radiation doses used were extremely large [3].

In this work we studied the nature of the molecular and supermolecular structure (DNP) obtained from spleen cells of mice subjected to the action of γ radiation in doses exerting the greatest leukemigenic action.

EXPERIMENTAL PROCEDURE

The experiments were conducted on noninbred white mice (males) from the nursery of the Academy of Medical Sciences, USSR, among which spontaneous leukemias are encountered extremely rarely [4]. Two series of experiments were conducted on 200 animals. In series I, the mice were irradiated on the cobalt γ setup in a dose of 400 R at a rate of 77 R/min. In series II, the dose was 600 R. A single irradiation of mice in such γ radiation doses gives the greatest percentage of leukemias [1, 5].

The DNP needed for the study was obtained from spleen tissue. It is known that in mice the spleen functions during the entire lifetime as an organ of hematogenesis. DNP was isolated during various periods after irradiation: immediately, after 24 h, and after 5, 10, 20, and 30 days. The mice were killed by decapitation. The spleen was extracted and chilled at 0°. The material taken from 5 animals was combined for one experiment. DNP was extracted in the cold according to the prescription of Hagen [10]. In order to avoid loss and degradation of the material, the DNP extract was not reprecipitated from 1M sodium chloride solution. The cytoplasmic proteins were removed by 7-10 washings of the homogenate with physiological saline.

The DNP content in the prepared extracts was 0.8-3 mg/ml. The molecular weight, determined viscosimetrically [9], lay within the range $2.2\text{-}6 \cdot 10^6$; the N/P ratio was equal to 3.5-5.5.

The state of the molecular and supermolecular structure of DNP was studied by the method proposed by D. M. Spitkovskii [7, 8], observing the dynamics of the formation and relaxation of oriented nucleoprotein strands. The theory of high polymers gives a basis for believing that a disruption of the relationship among the components of the DNP molecules or among the DNP molecules should be reflected in the behavior of the oriented strands of this polymer, changing such parameters as the high elasticity, fluidity, and residual deformation.

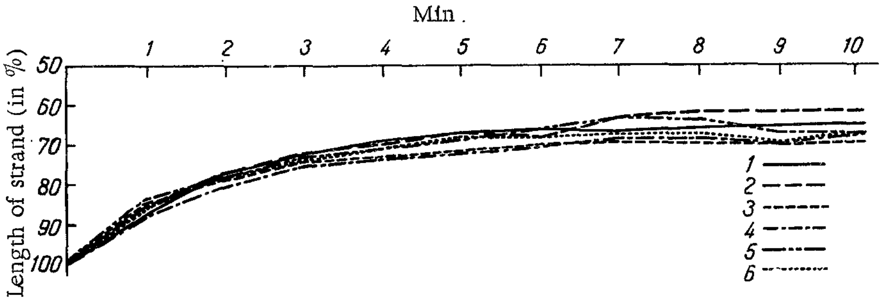

Fig. 1. Relaxation of oriented strands of mouse spleen DNP: healthy (1); killed immediately (2); killed after 24 h (3), 5 days (4), 10 days (5), and 30 days (6) after total γ irradiation in a dose of 400 R. The curves cited were constructed according to the data of observations of the contraction of 10-40 strands.

DNP was always investigated under the same conditions: the concentration of the solution used to obtain oriented strands was 0.03-0.05%, amount of the material 0.02 ml, temperature of the testing medium 33°. The behavior of 10-20 strands was studied in each experiment. Since the dynamics of their relaxation depend on the initial length, only strands 5.8-8.2 cm long were subjected to analysis. The results of the observations were treated statistically according to the Student-Fisher method.

EXPERIMENTAL RESULTS

The question of the influence of the radiation doses used on the conditions of the experimental animals has been rather fully treated in the literature. Hence, we shall not stop for any special discussion of it.

Figure 1 presents curves reflecting the dynamics of the contraction of the oriented strands of DNP in the spleens of healthy mice [1] and mice irradiated in a dose of 500 R. From the figure it is evident that the contraction of oriented DNP strands of the spleens of irradiated and control animals proceeded without any significant differences during the first 6 min of the observation. The strands relaxed most vigorously for the first 3 min. Their length was reduced by an average of 27% during this time. Then the rate of highly elastic deformation decreased. From the 3rd to 10th min, the length of the strands decreased by only 7%. In this case, it may be noted that the DNP strands of the spleens of mice taken for the experiment 24 h after irradiation relaxed worst of all (Fig. 1, 3).

The curves cited in Fig. 1 show that during the last minutes of the observation the relaxation of the spleen DNP strands of the irradiated and intact mice differed. Beginning with the 7th min, a definite tendency in the arrangement of the relaxation curves on the graph is noted. The curve of the contraction of spleen DNP strands from nonirradiated mice occupied a middle position (Fig. 1, 1). By the end of the observation, the length of the strands of this group had decreased by an average of 35%. Next to curve 1 are situated others, reflecting the degree of highly elastic deformation of spleen DNP of mice irradiated 5, 10, and 30 days previously (Fig. 1, 4, 5, 6). The curves of the relaxation of oriented strands of DNP, isolated from spleen tissue of mice immediately and 24 h after irradiation occupied extreme positions on the graph (Fig. 1, 2, 3). This is due to the fact that the contraction of the DNP strands of the spleens of mice killed immediately after irradiation took longer than the contraction of DNP strands of the spleens of nonirradiated animals (see Fig. 1, 2). During this time, the strands of the irradiated polymer were contracted by 38%. Hence, curve 2, reflecting the highly elastic properties of mouse spleen DNP immediately after irradiation, occupied the upper extreme position on the figure.

The contraction of oriented DNP strands of the spleens of mice irradiated 24 h before, just as in the control, took 6 min (see Fig. 1, 3). However, the rate of relaxation of the investigated nucleoprotein was lower. The length of the strands was 30% reduced during this time, and remained the same until the end of the observation. This also explains the fact that this curve occupied the extreme lower position on the graph. Treatment of the data obtained by statistical methods showed that the tendency in the behavior of oriented spleen DNP strands from mice killed immediately and 24 h after irradiation, noted above, actually existed. The value of P proved to be less than 0.05.

The peculiarities of the contraction of DNP strands from the spleens of irradiated animals, described above, appear far more distinctly in series II of the experiments, in which the mice were irradiated in a dose of 600 R. The

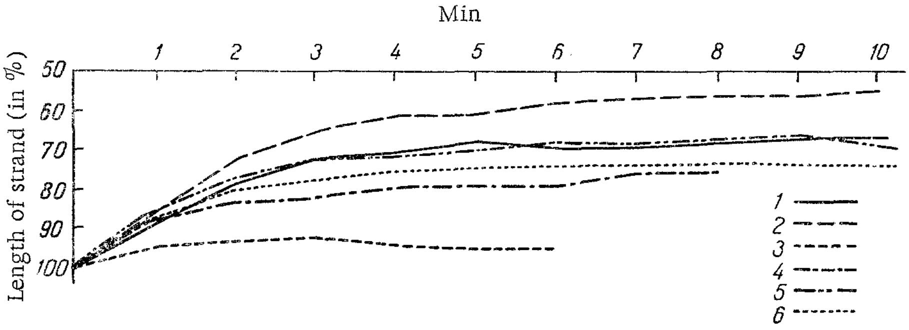

Fig. 2. Relaxation of oriented strands from mouse spleen DNP: healthy (1); killed immediately (2); after 24 h (3); 10 days (4), 20 days (5), and 30 days (6) after total γ irradiation in a dose of 600 R.

curves cited in Fig. 2 proved to be arranged in the same way as in the preceding experiment. The quantitative differences were more sharply revealed. The curve of the contraction of oriented DNP strands from spleens of nonirradiated animals [1] occupied a middle position. The relaxation process took 5 min, occuring most intensively during the first 3 min. During this time, the length of the strands was reduced by 28% of the initial value. From the 3rd to the 5th min, the strands relaxed another 4%. Subsequently, the length of the strands did not change significantly, and as can be seen from Fig. 2, was 33% less than the original length by the end of the observation.

The strands of DNP isolated immediately after irradiation of the animals relaxed more intensively than in the control (Fig. 2, 2). They contracted during the entire time of observation, although this process was the most intensive during the first 7 min. By the end of the experiment, the investigated strands had contracted by 45%, while the length of the control strands had been reduced by only 33% during this time.

The process of formation and relaxation of oriented strands of spleen DNP of mice irradiated 24 h before was sharply disturbed (Fig. 2, 3). In most cases, those strands could be obtained on account of the pronounced process of plastic flow of the polymer. If strands were formed, their length proved greater than normal. The process of highly elastic deformation, after its beginning, turned into irreversible flow, and the strands broke. Cleavage occurred at the boundary of the upper and middle thirds of the strands, most often at the 5th to 6th min of observation. At this time, changes in the structure of the strands, with a configuration resembling the changes that arise during the formation of chromosome rearrangements, could be seen.

The contraction of oriented strands of spleen DNP from mice irradiated 10 and 30 days before did not proceed as well as in the control (Fig. 2, 4, 6). The length of these strands was 26 and 24% less than the initial, respectively, by the end of the observation. The DNP strands from the spleens of mice taken for the experiment 30 days after irradiation bent during the investigation, so that it was impossible to follow their contraction to completion. It is as yet difficult to determine to what the peculiarities of the behavior of these strands are due, all the more in that the relaxation of the spleen DNP strands of mice taken for the experiment 20 days after irradiation proceeded just as in the control (Fig. 2, curve 5).

Thus, the observations outlined above show that γ irradiation of mice in doses with a high leukemigenic activity leads to a change in the native properties of the biochemical substrate of the chromosomes—DNP—in the cells of the hematogenic tissue. These changes are of varied kinds, depending on the time elapsed after the action of irradiation. Directly after irradiation, the magnitude of the highly elastic deformation of the deoxyribonucleoprotein strands increases, while after 24 h or more, it is reduced. During this period, the processes of plastic flow of the polymer are pronounced. The degree of manifestation of the indicated changes increases with increasing radiation dose.

The increase in the highly elastic deformation of DNP strands isolated from mouse spleen immediately after irradiation most likely reflects changes in the molecules of the biopolymer. Probably the number of hydrogen bonds set up between the DNA chains and histone increases, as a result of which the configurational flexibility of the DNP macromolecules, and, consequently, the magnitude of the highly elastic deformation of the strands increase [9]. These changes may be due to the direct action of radiation on the nucleoprotein.

The increase in the residual deformation of DNP strands isolated 24 h and longer after irradiation indicates a disturbance of the supermolecular structure of the latter. Since the organizational details of this structure are largely still obscure [6], it is difficult to judge what links of it are disrupted. It can only be stated that the forces of interaction among the DNP molecules are weakened. The damage to the supermolecular structure of DNP may be secondary in character and may be due to the action of cellular nucleases and proteases, the distribution of which in the cell after irradiation, as is well known, is disturbed [3]. The changes in the DNP described above may possess a definite relationship to the appearance of such effects of radiation as disturbance of the rate of nucleic acid synthesis, and the appearance of pathological deviations in the structure of the nucleus and chromosomes.

I should like to express my deep gratitude to P. I. Tseitlin and D. M. Spitkovskii for suggesting the topic and for their constant consultations, as well as to L. Ya. Kozlova for her direct aid in the work.

SUMMARY

The method suggested by D. M. Spitkovskii was used to study the state of the structure of deoxyribonucleoprotein from the spleen tissue of mice subjected to single total γ irradiation in doses of 400 and 600 R. According to literature data these doses produce the greatest leukemigenic effect. A change was noted in the native properties of spleen deoxyribonucleoprotein in irradiated animals. Immediately after irradiation, the degree of highly elastic deformation of oriented DNP filaments increased whereas after 24 h, 10 and 30 days it proved to be decreased. At the same time, the processes of plastic flow of polymer were more pronounced. Possible mechanisms of the development of the above disorders are discussed in the article.

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