RADIATION EFFECTS ON NAD- AND DNA-METABOLISM IN MOUSE SPLEEN

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1. Introduction

It is well known that the NAD pyrophosphorylase (EC 2.7.7.1) is localized in the cell nucleus [1]. Green and Dobrjansky reported [2] that two enzyme activities of the NAD glycohydrolase (EC 3.2.2.5) are also found in this cell compartment of Ehrlich ascites tumor cells. One of the enzyme activities is bound to the nuclear membrane, the second is accompanied by the chromatin. This latter enzyme activity is probably identical with the polymerase which forms poly-adenosine-diphosphate ribose (poly-ADPR). In the course of these studies we were able to make the same observations regarding mouse spleen. Experimental evidence has been published that a relationship exists between NAD- and DNA-metabolism [3–5] in such a way that the DNA synthesis can be inhibited when the NAD turnover is changed by NAD precursors [5].

During these investigations it was found that especially the NAD pyrophosphorylase and the chromatin-bound NAD glycohydrolase decrease considerably in mouse spleen after low X-irradiation. The dose dependence of the radiation effects on NAD metabolism and on DNA synthesis, which has also been investigated and is known as a radiosensitive metabolic process [6], shows close parallels. These changes also occur at the same time after X-irradiation.

2. Materials and methods

[^{14}C]NAD (labelled in the nicotinamide moiety, 59 mCi/mmole) and [^{3}H]thymidine (5000 mCi/mmole) were purchased from Amersham, England; biochemicals from Boehringer, Mannheim. Male inbred mice weighing about 25 g were used throughout the present study. The animals were killed and the spleen removed. The nuclei were isolated as described by Blobel and Potter for rat liver [7]. The preparation was examined and the cell nuclei counted under the microscope. The nuclear membranes and the chromatin were prepared according to the method of Green et al. [2].

The NAD pyrophosphorylase was determined in the whole nuclei [5]. For the assay of the NAD glycohydrolase 10 μl of the enzyme preparation were incubated at 37°C with 10 μl 0.1 M Tris buffer, pH 6.8, and 10 μl [^{14}C]NAD (59 mCi/mmole, 5 μCi/ml). The reaction was stopped by heating the incubation mixture in a boiling water bath for 2 min; 10 μl with NAD and nicotinamide (10 mg/ml each) were added before. The liberated nicotinamide was separated by thin layer electrophoresis [8] and the radioactivity measured in a liquid scintillation spectrometer (Packard, Tricarb 3375). The incubation time for the enzymatic assay was 5 and 10 min for each assay.

The determination of DNA and of DNA synthesis by incorporation of [^{3}H]thymidine has been described [9]. Protein was measured by the method of Lowry et al. [10].

The mice were whole body X-irradiated (150 kV, 0.43 mm Cu) with a dose rate of about 100 R per min [9]. The radiation dose was measured in each experiment. A minimum of 10 mice were used for each experiment.

3. Results and discussion

Several metabolic processes in the nuclei of lymphatic cells have been reported to be very radiosensitive (see [6,11]). Also, it has been observed that the NAD content and its biosynthesis decrease in thymocytes after a radiation dose of several hundred R [12]. We were especially interested whether nuclear enzyme activities of NAD metabolism are changed after low radiation doses as soon as the decrease of the DNA synthesis can be observed. Fig. 1 shows the NAD pyrophosphorylase activity in the nuclei of spleen 24 hr after a whole body X-irradiation of mice with radiation doses from 50–510 R.

From unirradiated mice 5.3×10^8 cell nuclei per g spleen were isolated. This is in agreement with the number of cell nuclei determined by histological methods [13]. After irradiation the number of isolated cell nuclei decreases with increasing radiation doses (fig. 1). 24 hr after irradiation with 100 R the number of cell nuclei isolated was only 65% of the normal value. However, the DNA content of these nuclei falls even more rapidly than does the number of isolated nuclei. When the DNA content was measured in the whole homogenate it was reduced to only about 75% of the controls 24 hr after 100 R. Comparing the mentioned reduction of the isolated nuclei after irradiation it must be assumed that there are highly damaged nuclei in the irradiated tissue not isolated by the preparation procedure. This means

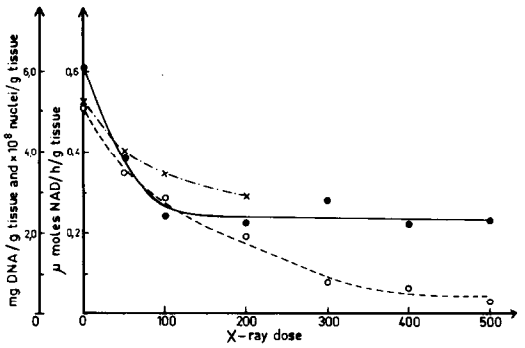

Fig. 1. The NAD pyrophosphorylase activity (•, μmoles NAD formed hr/g tissue at 37°C), the number of isolated cell nuclei per g tissue (×) and the DNA content in the isolated nuclei (○, mg/g tissue) in mouse spleen 24 hr after whole body X-irradiation.

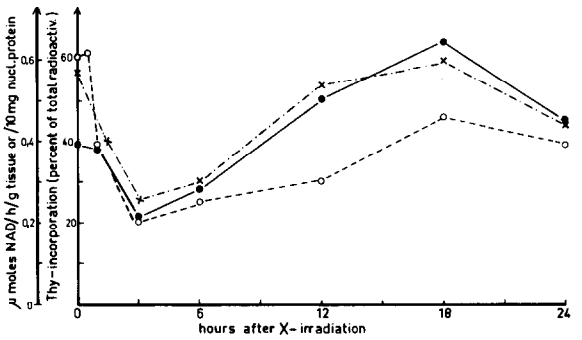

Fig. 2. The NAD pyrophosphorylase activity (○, μmoles NAD formed hr/g tissue at 37°C) and (×), μmoles formed NADH/hr/mg nuclear protein at 37°C) and [^{3}H]thymidine incorporation into DNA (•) in mouse spleen after whole body X-irradiation with 50 R.

that a selected population of nuclei is used for the enzymatic assays in which the most damaged nuclei remain to be considered. This selection may – at least to some extent – contribute to the observation that the enzyme activity of the NAD pyrophosphorylase per g tissue decreases remarkably after whole body X-irradiation. However, after radiation doses below and up to 100 R this radiation effect is higher than the reduction of the DNA content (fig. 1) in the protein content of the isolated nuclei, so that the decrease of the enzyme activity is a specific process and not only due to a loss of nuclei or nuclear protein.

The time course of this radiation effect was studied after a radiation dose of 50 R (fig. 2). While no significant change was observed 30 min after irradiation, an appreciable reduction appeared one hr and especially three hr after irradiation. At later periods the enzyme activity increased again. For comparison, DNA synthesis in spleen, measured by thymidine incorporation into DNA, is also demonstrated after a whole body X-irradiation with 50 R in fig. 2. The labelled thymidine incorporated into the DNA 20 min after the injection was calculated as a percentage of a total radioactivity found in the spleen at the time (for details see [9]). Fig. 2 shows that the radiation effect and its time course are very similar as in the case of the DNA synthesis and that of the NAD pyrophosphorylase activity. It appears very remarkable that the enzyme activity increase coincides with the recovery of the DNA synthesis. This

finding is in agreement with the observation made by Haines et al. [1] that a correlation exists between DNA synthesis and NAD pyrophosphorylase in different classes of liver cell nuclei.

During these studies we found two NAD glycohydrolase activities in the cell nuclei isolated from mouse spleen as Green et al. [2] did for Ehrlich ascites cells. When nuclear membranes were prepared the specific NAD glycohydrolase activity rose from 7.8×10^7 dpm (radioactivity of the released nicotinamide) per mg protein in the intact nuclei to 2.6×10^8 dpm per mg protein in the isolated membranes. The specific enzyme activity which accompanied the chromatin was much lower. The radiosensitivity of both enzyme activities was very different. The NAD glycohydrolase of the chromatin was already decreased considerably after a radiation dose of 50 R and was no longer measurable after higher doses. The period of six hr after irradiation was chosen since the recovery of the DNA synthesis starts at this time with low radiation doses (fig. 2). In contrast, the enzyme activity bound to the nuclear membrane even increased after an irradiation with 50 R and was found to decrease only after relatively high radiation doses (table 1). Under these conditions the turnover of NAD in the nuclei, which is very high under normal conditions through the pyridine nucleotide cycle [14], is reduced without any change of the NAD level (unpublished data).

As yet, the role of these NAD glycohydrolases in cell metabolism is unknown. Yamada et al. [15] found that the fragmentation of DNA was reduced by incubation of nuclei with NAD. These authors assumed that poly-ADPR, formed in cell nuclei, inhibits endonucleases resulting in a reduction of the template capacity for DNA synthesis and in DNA polymerase activity. However, Hilz and Kittler found no correlation between poly-ADPR formation and DNA synthesis [16].

Nevertheless, the parallel time courses of NAD pyrophosphorylase activity and of DNA synthesis after irradiation, the enormous decrease of the chromatin-bound NAD glycohydrolase activity coinciding with the inhibition of DNA synthesis, and the increase of the NAD glycohydrolase activity bound to the nuclear membrane when DNA synthesis starts to recover, emphasize the idea that a correlation exists between these metabolic processes and their radiation induced changes. This assumption is supported by the observation that the recovery of the DNA synthesis after the radiation induced inhibition starts earlier when mice were injected with $NADH_2$ 1 hr prior to irradiation [9].

Table 1
The NAD glycohydrolase activities in whole cell nuclei (Nucl.), nuclear membranes (Nucl. M.) and bound to the chromatin (Chrom.) in mouse spleen 6 hr after whole body X-irradiation

Radiat. dose	dpm [^{14}C]nicotinamide/hr/g tissue		
R	Nucl.	Nucl. M	Chrom.
0	8.3×10^8	1.11×10^8	4.5×10^7
50	10.6×10^8	2.5×10^8	2.3×10^7
100	6.5×10^8	1.5×10^8	1.9×10^7
150	6.1×10^8	1.2×10^8	–
510	3.0×10^8	0.6×10^8	–

The data are expressed as dpm released [^{14}C]nicotinamide per hr per g tissue at 37°C.

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