THE BIOSYNTHESIS OF NAD IN THE LIVER OF IRRADIATED MICE

CHRISTIAN STREFFER

*Radiologisches Institut der Universität Freiburg, 78 Freiburg
in Breisgan, Albertstrasse 23, Germany*

Some days after whole body irradiation of mammals by X-rays there was found a decreased content of NAD in the liver (1,2). In our experiments the mice got a radiation dose of 690 R (LD 80/30) or 810 R (LD 99/30) (Fig. 1). With the higher X-ray dose the reduction of the NAD content was observed about two days earlier. The reduced pyridine nucleotide NADH_2 was diminished as well. Seven days after irradiation with 810 R there were found $0.24 \pm 0.05 \mu\text{moles NADH}_2$ per g liver in comparison to $0.31 \pm 0.04 \mu\text{moles NADH}_2$ per g tissue in the unirradiated controls.

FIG. 1 Content of NAD in the Liver of Mice after Whole Body Irradiation with 810 R (—) or 690 R (---) X-rays, 150 kV, 20 mA, 0.43 mm Cu.

In further experiments the reason for such a radiation effect should be elucidated. Hilz and coworkers (3-5) showed that the radiation-induced decrease of NAD was caused by an increase of the activity of NAD glycohydrolase (EC 3.2.2.5) in Ehrlich ascites tumor cells. So the breakdown of NAD was enhanced in these cells. The investigation of this enzymatic activity resulted in no significant changes in the liver of mice after irradiation, however. Therefore it was concluded that the reason for the reduced NAD content was an impaired biosynthesis of NAD in the liver of irradiated mice.

It is well known that nicotinic acid, nicotinamide and tryptophan are the precursors for NAD in mammalian systems and it is not ruled out which of these

three metabolites is the most important one for the biosynthesis of NAD in liver tissue (6). When nicotinic acid was injected intraperitoneally in a dose of 25 μ moles per mouse (weight 25 g), it turned out that there was no difference in the formation of NAD in the liver tissue between unirradiated and irradiated animals (Fig. 2). The measurements were undertaken 7 days after irradiation with 810 R. The same result was observed with nicotinamide as the precursor for NAD. So the formation of NAD seemed not to be affected from nicotinic acid or nicotinamide by irradiation.

However, quite another picture appeared when tryptophan was used. In these experiments the formed amount of NAD from the amino acid seemed to be less after irradiation (Fig. 3). 100 μ moles L-tryptophan were injected i. p. per mouse and the measured increase of NAD was reduced in the liver of the irradiated animals (Fig. 3).

FIG. 2 Formation of NAD from Nicotinic Acid in the Liver of Mice (25 μ moles nicotinic acid per mouse i. p.)

○, unirradiated controls; △, 7 days after irradiation with 810 R.

FIG. 3 Formation of NAD from L-Tryptophan in the Liver of Mice Receiving 100 μ moles L-Tryptophan per Mouse i. p.

○, unirradiated controls; □, 7 days after irradiation with 810 R; △, 10 days after irradiation with 810 R.

To investigate this problem under better physiological conditions, *i. e.*, to avoid the large amounts of precursors, the biosynthesis of NAD was tested by the incorporation of ^{14}C -labelled nicotinic acid and tryptophan. In the experiments with tryptophan the amino acid was uniformly labelled in the benzene ring. 62 μg of this compound in the DL-form were injected i. p. per mouse. The nicotinic acid was labelled in the carboxyl group; 26 μg were injected i. p. per mouse. Nicotinic acid (NA), deamido-NAD (dNAD), nicotinamide ribonucleotide (NMN) and NAD were added to the liver homogenate as carrier. The organ extracts were

chromatographed on Dowex 1 with formic acid (7). For further separation a thin layer electrophoresis was performed (8) and the radioactivity of the metabolites was measured in a liquid scintillation spectrometer. As is shown in Table 1, under these conditions the biosynthesis of NAD is also impaired by irradiation only with tryptophan as metabolic precursor and not with nicotinic acid.

TABLE 1

Incorporation of Tryptophan-¹⁴C (62 μ g DL-Try per mouse, spec. act. 19.2 mCi/mmole) and Nicotinic Acid-¹⁴C (26 μ g NA per mouse, spec. act. 29.9 mCi/mmole) into NAD and Its Metabolites in Liver Tissue of Mice.

The animals were killed 60 minutes p.i. (Try) and 20 minutes p.i. (NA). The values are given as counts/minute/g tissue.

Animals	NA	dNAD	NMN	NAD
Controls, Try	370	3,700	520	20,400
8 days after 810 R, Try	340	2,480	480	12,800
Controls, NA	13,600	75,200	10,800	565,000
8 days after 810 R, NA	12,700	70,900	11,300	585,000

TABLE 2

The Activity of Tryptophan Pyrrolase (μ moles kynurenine/hr/g liver, 37°), NAD Content (μ moles/g liver) and the Increase of the NAD Content (μ moles/g liver) 6 hours after Injection of 100 μ moles L-Tryptophan i.p. per Mouse in the Liver Tissue of Mice.

The animals were fed *ad libitum*, starved for 24 hours, adrenalectomized 6 days before being killed or injected with 1 mg/mouse hydrocortisol acetate 9 hours before being killed.

	Tryptoph. pyrrol.	NAD content	NAD increase
Fed animals	1.54 $\pm$ 0.30	1.02 $\pm$ 0.05	1.18 $\pm$ 0.15
Starved animals	2.94 $\pm$ 0.64	1.05 $\pm$ 0.06	1.21 $\pm$ 0.12
Adrenalectomy	0.98 $\pm$ 0.22	1.07 $\pm$ 0.08	1.24 $\pm$ 0.14
9 hr after hydrocort.	7.02 $\pm$ 0.53	1.07 $\pm$ 0.09	1.29 $\pm$ 0.16

On the basis of these results it was tried to find out which enzymatic step could participate in the regulation of the metabolic pathway from tryptophan to NAD. As the biosynthesis of NAD from nicotinic acid was not reduced, it was assumed that in the liver of irradiated mice the damage might be situated somewhere between tryptophan and quinolinic acid. As the activity of the tryptophan pyrrolase (L-tryptophan: O₂ oxidoreductase, EC 1.13.1.12) is regulated by substrate (9) hormones (10, 11) and is inhibited by reduced pyridine nucleotides (12), it seemed predestinated to be the regulatory step. As one can see from Table 2, this is not the case. The tryptophan pyrrolase activity induced by starvation or hydrocortisol had no influence on the formation of NAD from tryptophan (Table 2).

In further experiments the enzymatic activities of the kynureninase (L-kynurenine hydrolase, EC 3.7.1.3), kynurenine transaminase (L-kynurenine: oxoglutarate aminotransferase, EC 2.6.1.7) and kynurenine hydroxylase (L-kynurenine: reduced NAD(P): O₂ oxidoreductase, hydroxylating, EC 1.14.1.2) were measured in the liver of mice after irradiation (13, 14). Only the kynureninase activity was

diminished at the time when the NAD content in liver and the NAD formation from tryptophan was reduced (Table 3). In Fig. 4 the NAD content and the kynureninase activity in relative values (values of unirradiated animals equal 1.0) are plotted against time after irradiation. There results a very similar time course for both metabolic measurements.

FIG. 4 The Kynureninase Activity (---) and NAD Content (—) in the Liver Tissue of Mice After Whole Body Irradiation with 810 R.

TABLE 3

The Enzymatic Activities of the Degradation of Tryptophan to 3-Hydroxyanthranilic Acid (μ moles turnover/hr/g liver, 37°), NAD Content (μ moles/g liver) and Incorporation of Tryptophan- 14 C (relative values) in the Liver of Mice Before and 7 days After Whole Body Irradiation with 810 R.

The animals were starved 24 hours before being killed. The standard deviation and the *P* values were calculated by the Student-*t*-test.

	Unirrad. animals	7 days after 810 R	<i>P</i> value
Tryptophan pyrrolase	2.94 ± 0.64	3.08 ± 0.30	0.9 -0.8
Kynurenine hydroxylase	1.18 ± 0.15	1.05 ± 0.16	0.7 -0.6
Kynurenine transaminase	1.29 ± 0.20	1.67 ± 0.25	0.01 -0.05
Kynureninase	1.29 ± 0.10	0.45 ± 0.04	<0.001
Ratio kyn. transaminase/ kynureninase	1.00 ± 0.16	0.27 ± 0.10	<0.001
NAD content	1.02 ± 0.04	0.67 ± 0.05	0.01 -0.005
Incorpor. of Try to NAD	1.00	0.63	0.01 -0.005

From the reported experiments there may be drawn the following conclusions: In the liver tissue of irradiated mice the reduced NAD content seems to be caused by an impaired biosynthesis of NAD from tryptophan. At least under these conditions the metabolic pathway from tryptophan to NAD is apparently limited by the decreased kynureninase activity. It has to be worked out which part the amino acid tryptophan plays for the NAD formation under normal physiological conditions.

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