Metabolism of Labeled Nicotinamide Coenzyme in Different Organs of Mice and Rats (34861)

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Although synthesis of nicotinamide coenzymes has been frequently studied, these investigations were usually restricted to certain organs, *e.g.*, liver, lymphoid organs, tumor cells (1, 2), and little is known concerning the metabolic replacement of NAD in organs other than liver. In the course of studies dealing with the metabolism of NAD in irradiated mice and rats (3, 4), we noted that labeled NAD is conserved in some organs for a considerable length of time and that differences exist between organs with respect to incorporation of precursors into NAD. Since it is still uncertain by which pathways nicotinamide is converted to NAD *in vivo* and since degradation of NAD is an important factor in metabolic disturbances of the cell, incorporation of various precursors into NAD and replacement of labeled NAD in different organs have now been investigated in more detail.

Methods. Nicotinic acid-7-^{14}C or nicotinamide-7-^{14}C (obtained from Amersham at a sp. act. of 59 mCi/mmole) was injected intraperitoneally into adult male Wistar rats (5 μCi = 30 mμmoles/250 g) or into adult male BALB/c mice (2 μCi = 12 mμmoles/30 g). The animals received food and water *ad libitum* and were sacrificed at various times after injection as indicated in the tables and figures. In one experiment, the rats were giv-

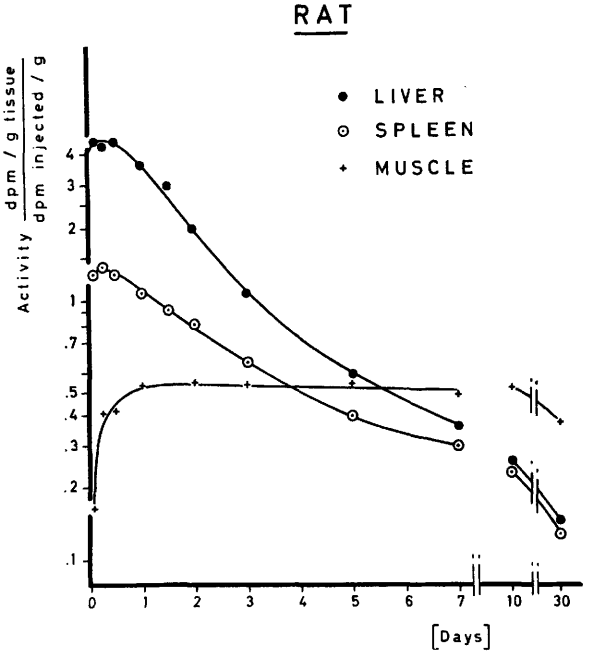

Fig. 1. Plots of activity *vs* time after injection of 5 μCi of ^{14}C nicotinic acid in different organs (liver, spleen, muscle) of adult rats. From 4 to 8 animals were used per point. The standard deviation of the mean is in the order of 10%

en 1 mg/ml of nicotinamide in the drinking water, *i.e.*, about 20 mg/day. After sacrifice, liver, kidney, small intestine, spleen, testis, brain, and a sample from the abdominal muscle were removed, frozen immediately in acetone–Dry Ice and homogenized in 4 vol of ice-cold 0.6 *N* perchloric acid. Radioactivity in 100 μl of the protein-free supernatant was measured by liquid scintillation counting and the quantity of NAD was determined by fluorometric assay with ethyl methyl ketone (5). In one experiment, the concentration of nicotinic acid derivatives in tissues was assayed by means of the König reaction (6). NAD was also separated from other metabolites by high voltage electrophoresis in pyridin acetate buffer, pH 4.4, at 3 kV for 20 min and by paper chromatography in 1 *M* ammonium acetate–ethanol (3:7) in the isobutyric acid system and in isopropanol–H_2O–conc NH_4OH (20:2:1) (7–9). After electrophoresis NAD was eluted and its specific radioactivity was determined.

Results and Discussion. Plots of radioactivity in tissues at various times after injection of labeled nicotinic acid for different organs of rats and mice are shown in Figs. 1–4.

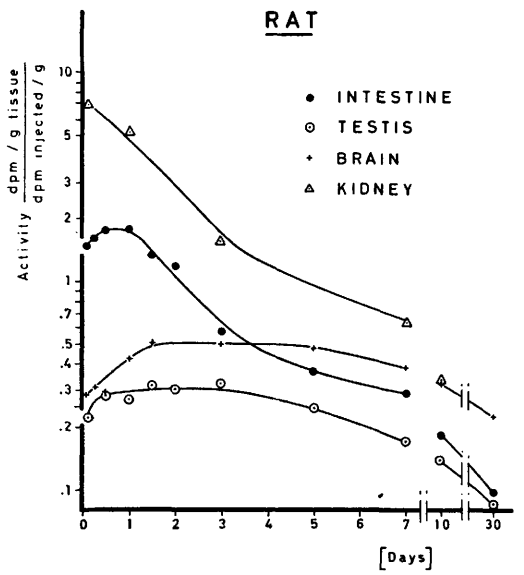

FIG. 2. Plots of activity *vs* time after injection of 5 μCi of ^{14}C nicotinic acid in different organs (intestine, testis, kidney, brain) of adult rats. From 4 to 8 animals were used per point. The standard deviation of the mean is in the order of 10%.

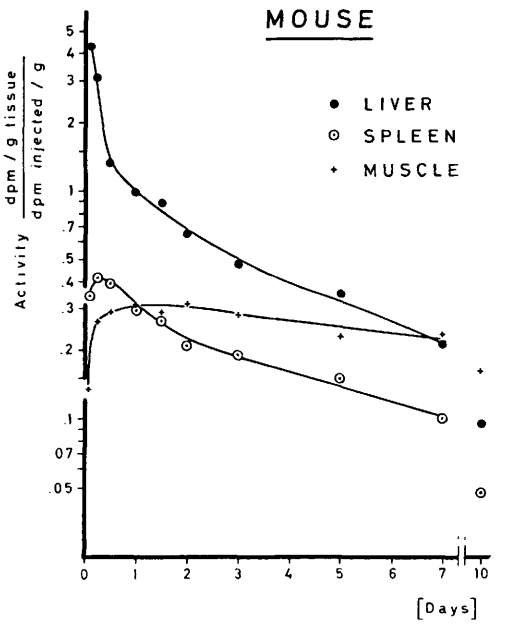

FIG. 3. Plots of activity *vs* time after injection of 2 μCi of ^{14}C nicotinic acid for different organs (liver, spleen, muscle) of adult mice. From 2 to 4 animals were used per point. The standard deviation of the mean is in the order of 10%.

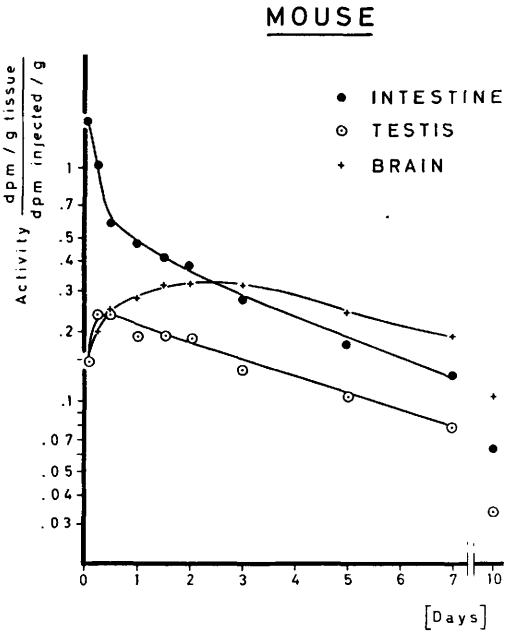

FIG. 4. Plots of activity vs time after injection of 2 μCi of ^{14}C nicotinic acid for different organs (intestine, testis, brain) of adult mice. From 2 to 4 animals were used per point. The standard deviation of the mean is in the order of 10%.

These curves parallel and approach closely those of activity of NAD in tissues. The curves of specific activity of NAD were, however, subject to greater variations than those of total activity and are thus not shown. Indeed, electrophoresis and chromatography demonstrate that at all times later than 3 hr after injection of nicotinic acid, NAD represents 70–80% of the radioactivity in the tissues and that the only other important radioactive metabolite present is nicotinamide.

Nicotinamide mononucleotide, nicotinic acid mononucleotide, NADP and deamido NAD contain no significant activity. Nicotinamide is, in part, derived from NAD degraded during the procedures of isolation and homogenization. Nevertheless, even tissues frozen *in situ* contain from 5 to 15% of the activity as nicotinamide. It is evident that NADH is destroyed under the acid conditions of isolation, but this does not affect the interpretation of our results since the ratio NAD to NADH in an organ remains constant and the activity time curves of NAD parallel those of total tissue. Moreover, identical results were obtained when homogenization and subsequent protein precipitation were carried out at a neutral pH conserving NADH although more nucleotide is hydrolyzed to nicotinamide under these conditions.

Figures 1 to 4 show that radioactive NAD is replaced most rapidly, and activity shortly after injection is highest in kidney, followed by liver and intestine. Incorporation is small and replacement slow in brain, muscle, and testis. The value found for replacement of liver NAD is in good agreement with the half-life of 10 hr reported by Ijichi *et al.* (10) for mouse liver NAD.

The activity time curves do not, however, exhibit an exponential decline and show even an increase with time for brain, muscle, and testis during the first 2 days after injection. Since free radioactive nicotinic acid disappears rapidly from all tissues, this observation indicates that extensive reutilization of breakdown products of NAD, *i.e.*, nicotinamide, takes place, and that this effect may differ from one organ to another. A comparison between incorporation of nicotinic acid and that of nicotinamide should indicate to what extent organs are capable of utilizing nicotinamide for synthesis of NAD. Moreover, if the labeled nicotinamide is injected together with a large amount of nonlabeled nicotinic acid, one might deduce whether nicotinamide is incorporated directly or only after the prior deamidation to nicotinic

TABLE I. Incorporation of Labeled Nicotinic Acid and Nicotinamide into Different Organs of Rats (15 and 2 hr) After Injection.

	Activity [(dpm/g of tissue)/(dpm injected/g of body wt)]				
		^{14}C Nicotinamide[a]		^{14}C Nicotinamide + 20 mg of nicotinic acid	
Organ	^{14}C Nicotinic acid	15 hr	2 hr	15 hr	2 hr
Kidney	10.81 ± 0.29	1.80 ± 0.11	1.95 ± 0.28	1.04 ± 0.14	1.49 ± 0.11
Liver	3.89 ± 0.04	2.34 ± 0.12	2.10 ± 0.30	1.36 ± 0.25	1.81 ± 0.17
Spleen	1.41 ± 0.12	0.810 ± 0.074	0.92 ± 0.092	0.555 ± 0.041	0.677 ± 0.125
Intestine	1.53 ± 0.05	1.11 ± 0.09	2.08 ± 0.21	0.646 ± 0.069	1.06 ± 0.17
Testis	0.281 ± 0.046	0.232 ± 0.011	0.231 ± 0.015	0.225 ± 0.013	0.205 ± 0.024
Brain	0.255 ± 0.014	0.369 ± 0.023	0.312 ± 0.028	0.355 ± 0.044	0.243 ± 0.014
Muscle	0.329 ± 0.032	0.548 ± 0.120	0.432 ± 0.033	0.497 ± 0.036	0.352 ± 0.021

[a] At 2 hr, NAD content had not yet increased appreciably, but incorporation is not complete and considerable activity is found in nicotinamide, NMN, nicotinic acid, and catabolites of nicotinamide. At 15 hr, NAD levels in all organs are no longer affected by the previous treatment with 20 mg of nicotinic acid and total activity differs but little from activity of NAD. Only the former is, therefore, shown.

acid (11) followed by the Preiss–Handler pathway (1, 9). In the latter, but not in the former, case one would expect a dilution of the radioactivity in NAD (provided that there is no large change in the pool size of NAD).

The results of these experiments, shown in Table I, demonstrate that in most organs nicotinic acid is a better precursor for NAD synthesis than nicotinamide, whereas muscle, brain, and to a lesser extent, testis utilize nicotinamide very effectively. The slow turnover and the early increase in activity in these organs can, therefore, be explained as a result of reutilization of nicotinamide. Since cold nicotinic acid does not markedly depress incorporation of nicotinamide, deamidation is apparently not the principal factor in the salvage pathway of these organs. In contrast, liver, intestine, kidney, *etc.*, incorporate less nicotinamide in the presence of nicotinic acid. Since application of large amounts of nicotinic acid causes a transient raise in hepatic NAD content (2), the radioactivity could have been diluted also by changes in the pool of NAD and not only by those in the pool of nicotinic acid. This seems less likely, however, since such a dilution effect in liver, intestine, and kidney is seen at 2 hr as well as at 15 hr after injection, at a time, when NAD content had not yet increased. Nevertheless, further studies using isolated perfused organs are desirable in order to decide whether nicotinamide amidohydrolase is responsible for reutilization of nicotinamide in liver.

In an attempt to determine "true" turnover of NAD in different organs, rats were given nicotinamide in the drinking water during the experimental period. This treatment causes a sizeable increase in metabolic replacement of radioactive NAD in all organs [liver, kidney, intestine (about twofold), testis, brain, and muscle (about threefold)] and abolishes the early raise in activity in brain, testis, and muscle. In addition, concentration of nicotinic acid derivatives (mostly nicotinamide) increases under this treatment in most tissues, particularly in kidney; whereas, content of NAD remains unaltered (Table II). Nevertheless, reutilization is not entirely impeded. It can, therefore, be concluded that nicotinamide is reutilized to a considerable extent in all tissues; its exact contribution to total NAD synthesis must still be determined.

TABLE II. Content of NAD and Nicotinamide in Different Organs of Normal Rats and of Rats Receiving 1 mg/ml Nicotinamide in the Drinking Water.

	NAD (μg/g)		Nicotinamide (μg/g)[a]	
Organ	Control	N-amide treated	Control	N-amide treated
Liver	587	675	7.5	10.5
Spleen	90	125	6.2	8.3
Intestine	245	275	11.5	12.0
Kidney	350	400	25	50
Testis	97	90	3.5	6.8
Muscle	287	310	5.6	5.0
Brain	138	185	7.5	12

[a] Assayed by the BrCN procedure; includes also small amounts of other free nicotinic acid derivatives. Standard deviation of the mean is about 10% for NAD, about 15% for nicotinic acid derivatives.

Summary. Replacement of radioactive NAD in different organs of rats and mice was found to be most rapid in kidney, liver, and intestine and slowest in brain, muscle, and testis. Experiments with nicotinamide as precursor indicate that nicotinamide derived from NAD degradation is extensively reutilized in the latter organs. Injection of labeled nicotinamide together with a large amount of cold nicotinic acid suggests that this reutilization in brain and muscle probably does not involve deamidation of nicotinamide to nicotinic acid.

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