Effect of Hyperphysiological Levels of Hexestrol on the Hepatic Metabolism of Nicotinate in the Mouse[1] (34430)

L. S. DIETRICH, L. FRANKLIN, AND B. FARINAS

Department of Biochemistry, University of Miami School of Medicine, Miami, Florida 33136

Nicotinate is readily converted to NAD in animal tissues. The pathway involves nicotinate mononucleotide and nicotinate adenine dinucleotide as intermediates (1, 2). Animal tissue also contains enzyme(s) having NAD glycohydrolase activity that breaks NAD down to nicotinamide and adenosine diphosphoribose (3). The administration of low levels of nicotinate to mice results in a marked increase in hepatic nicotinamide levels (4) presumably from NAD via NAD glycohydrolase. This observation that nicotinate appears to be a more efficient precursor of hepatic nicotinamide than nicotinamide itself motivated us to explore the matter further. The results presented here indicate that hyperphysiological levels of hexestrol cause a marked alteration in the means whereby nicotinate is converted to nicotinamide in mice.

Materials and Methods. The C57 black male mice (25–35 g) received hexestrol or sesame oil intramuscularly as described in the tables. The ^{14}C-nicotinate was administered by intravenous injection at the level indicated. The animals were sacrificed by cervical rupture at the time indicated, the livers immediately removed and dropped into a dry ice–acetone freezing mixture. Individual frozen livers were dropped into 3 ml of boiling 0.04 *M* potassium phosphate buffer, pH 5.4. After 1 min in a boiling water bath the samples were homogenized for 30 sec, cooled in an ice bath and centrifuged at 21,000*g* for 15 min. Carrier NAD, nicotinamide, and nicotinate where then added to the supernatant fluid and aliquots were applied to sheets of Whatman 3MM paper. The sheets were developed in either the isobutyrate–NH_3 solvent of Magasanik *et al.* (4), or in butanol saturated with water containing solid NH_4HCO_3 (5). The spots corresponding to NAD, nicotinamide, and nicotinate were cut out and quantitated for radioactivity (6).

Results and Discussion. The intravenous administration of tracer amounts of ^{14}C-nicotinate (1.5–7.5 μg) (Table I, Fig. 1) results in the following events: (i) The intrahepatic levels of labeled nicotinate decreases rapidly during the first few minutes after intravenous injection. This is evident in the case of intraportal injection. The level of nicotinate then gradually increases at a linear rate during the experimental period (60 min). The rapid disappearance of labeled nicotinate from the liver under these conditions is in keeping with the findings of Ijichi *et al.* (7).

(ii) Hepatic NAD and nicotinamide are rapidly labeled, significant amounts of these labeled compounds appearing in less than 0.2 or 1 min in the cases of intraportal or tail vein administration, respectively. These latter data are illustrated in Fig. 1 where the percentage change in specific activity is plotted against time, employing the values obtained at 1 min as 100%. One-min values of 4497 cpm/μmole of NAD and 575 cpm/μmole of nicotinamide were obtained upon administration of nicotinate-^{14}C via the tail vein. Since the specific activity of the NAD increased much faster than the specific activity of the nicotinamide during the first minute after injection, the percentage increases observed, based on these 1-min values are relatively small for NAD as compared with the relative increases in nicotinamide. These relative changes in the specific activity of NAD and

[1] This study was supported by Grants CA 04868 and AM 03049 and a Career Development Award (K3-GM-15, 1986) from the United States Public Health Service.

TABLE I. Effect of Intravenous Injection of ^{14}C-Nicotinate on the Specific Activity of NAD, Nicotinate, and Nicotinamide in Mouse Liver.[a]

Expt. no.	Route of injection and amount	Time after injection (min)	(cpm/μmole ± SE)		
			Nicotinate	NAD	Nicotinamide
1	Tail vein	1 (6)	3214 ± 400	4497 ± 1604	575 ± 107
	0.2 μCi, 3 μg	2 (5)	3057 ± 1871	7280 ± 233	1606 ± 202
		7 (4)	2871 ± 1757	25,472 ± 3217	4644 ± 450
		15 (6)	5595 ± 1743	26,705 ± 2917	8067 ± 570
2	Tail vein	15 (6)	17,985 ± 2271	80,050 ± 7014	21,159 ±1603
	0.5 μCi, 7.5 μg	30 (6)	24,100 ± 1814	85,063 ± 5926	23,704 ±1684
		60 (6)	41,343 ± 3886	85,590 ±10,035	22,698 ± 575
3	Intraportal	0.17 (4)	687,314 ±139,714	454 ± 92	61 ± 10
	0.1 μCi, 1.5 μg	1.50 (4)	31,528 ± 10,957	4982 ± 503	1634 ± 273
		2.0 (4)	17,171 ± 2085	16,328 ± 2340	5265 ± 314

[a] Values are the average of the number of male C57 black mice shown in parentheses. The concentrations in μmoles/g of wet weight of NAD, nicotinate, and nicotinamide in mouse liver tissue are 0.72 ± 0.025, 0.07 ± 0.01, and 2.03 ± 0.13, respectively. SE = standard error.

nicotinamide are shown by the data for control mice plotted in Fig. 2, where the ratio of the specific activity for NAD to the specific activity of nicotinamide is plotted against time.

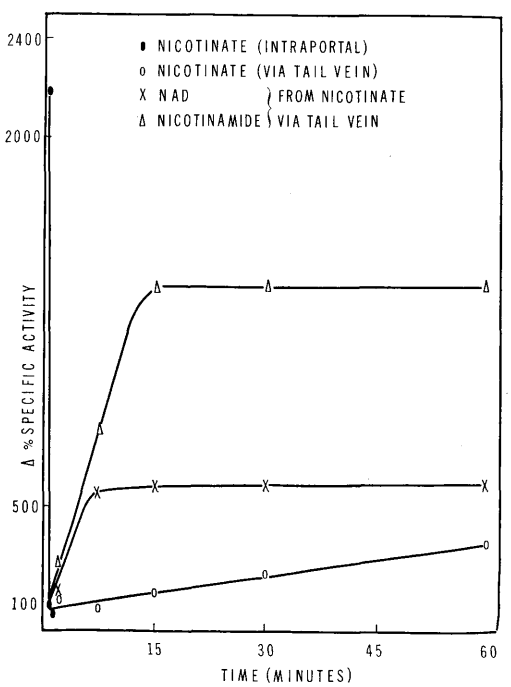

FIG. 1. Specific activity changes observed in normal mice after the administration of ^{14}C-nicotinate. Data are a composite summary of Expts. 1 and 2 taken from Table I.

Of interest and without explanation are the observations that this initial rapid increase in specific activity of NAD from ca. 4500 cpm/μmole at 1 min to ca. 25,000 cpm/μmole at 7 min occurs at a time when the specific activity of the precursor nicotinate varies between 3214 and 2871 cpm/μmole. The specific activity of the nicotinate administered was 5 μCi/μmole (2.22×10^6 cpm/μmole).

These data indicate that the problem of NAD metabolism in liver tissue is a complex affair, compartmentalization with resulting localized pools being one possible explanation of the increase in NAD specific activity to ca. 5 times that of the nicotinate specific activity. Other evidence that this is the case is indicated by the constant ratio of NAD specific activity to nicotinamide specific activity seen between 5-min to 60-min period after injection (Fig. 2).

In the case of hexestrol treatment, marked differences with respect to nicotinamide formation are observed. Although the pools of NAD and nicotinate are unaltered by hexestrol treatment (the apparent lowering of the nicotinate levels upon hexestrol treatment is not significant), the hepatic pools of nicotinamide are more than halved (Table II). Since the changes in pool sizes are not consistent for the various pyridine derivatives, it is doubtful that the change observed with nico-

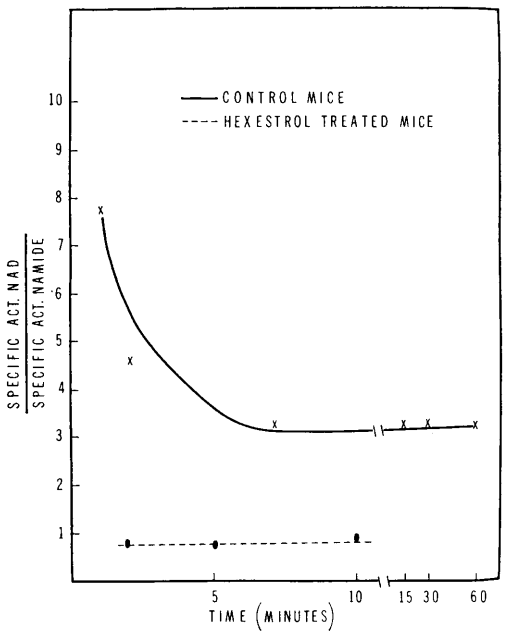

FIG. 2. Ratio of NAD specific activity to nicotinamide specific activity in nontreated and hexestrol-treated mice obtained after the intravenous administration of ^{14}C-nicotinate. Data for control mice were taken from Expts. 1 and 2 of Table I. Data for hexestrol-treated mice were taken from Expt. 1, Table II.

tinamide can be solely explained by increased liver size, water content of liver, or other nonspecific effects of hexestrol. In addition, the changes in specific activity of nicotinamide and the changes in the NAD:nicotinamide specific activity ratios as a result of hexestrol treatment are very striking. The specific activity of the nicotinamide in the hexestrol-treated mice is consistently higher than in nontreated animals. Regardless of whether the analyses were made 2 or 10 min after ^{14}C-nicotinate administration, the specific activity of the nicotinamide is equal to or slightly higher than the specific activity of the NAD in spite of the fact that hexestrol treatment has no effect on the level of NAD or its specific activity. This is in marked contrast to the ratios of NAD specific activity to nicotinamide specific activity obtained under identical conditions employing nontreated mice (Fig. 2).

The nature of the effect of hyperphysiolog-

TABLE II. Effect of Hyperphysiological Levels of Hexestrol on the Metabolism of ^{14}C-Nicotinate in Mouse Livers.[a]

Expt. no.	Time after injection (min)	(cpm/μmole ± SE)					
		Nicotinate		NAD		Nicotinamide	
		Control	Hexestrol	Control	Hexestrol	Control	Hexestrol
1	2	—	10,739 ± 703	—	5410 ± 335	—	8046 ± 675
	5	—	5410 ± 2289	—	8921 ± 1475	—	12,479 ± 1715
	10	—	7358 ± 3691	—	18,212 ± 1194	—	20,142 ± 2391
2	2	4532 ± 1130	15,108 ± 3538	8982 ± 1371	7450 ± 920	2382 ± 946	10,154 ± 684
	10	4748 ± 930	7637 ± 2193	20,345 ± 4219	18,637 ± 2559	5551 ± 762	22,687 ± 2152

[a] C57 male mice treated with hexestrol in sesame oil (10 mg/kg) for 6 consecutive days. Control animals received sesame oil. Vehicle and estrogen were administered intramuscularly. The day following the last injection of hexestrol, animals were injected with 0.2 μCi of ^{14}C-nicotinate (3 μg) via the tail vein. In all cases, the values are the average of duplicate determination carried out on six different mice. The concentrations (μmoles/g of wet wt) of NAD, nicotinate and nicotinamide in mouse liver tissue are 0.72 ± 0.025, 0.07 ± 0.01, and 2.03 ± 0.13, respectively, in the case of untreated animals; and 0.65 ± 0.03, 0.040 ± 0.01, and 0.97 ± 0.10, respectively for hexestrol-treated animals. SE = standard error.

ical levels of hexestrol on pyridine nucleotide metabolism is unknown. Earlier studies (8) involving neoplastic growth and the niacin antagonist 6-aminonicotinamide indicate that the effect of hyperphysiological levels of estrogen was indeed endocrine-related as opposed to a nonhormonal pharmacological effect. Whether this is the case in the present studies remains to be resolved.

The decrease in hepatic pools of nicotinamide in hexestrol-treated mice, together with the shift in relative specific activities resulting in the nicotinamide specific activity being equal to or greater than that of the NAD, indicate that the metabolism of nicotinate to nicotinamide is strikingly different in hexestrol-related animals as opposed to nontreated animals.

1. Preiss, J. and Handler, P., J. Biol. Chem. **233**, 488 (1958).

2. Preiss, J. and Handler, P., J. Biol. Chem. **233**, 493 (1958).

3. Dietrich, L. S., Martinez, L., and Franklin, L., Naturwissenschaften **55**, 231 (1968).

4. Magasanik, B., Vischer, E., Doniger, R., Elson, D., and Chargaff, E., J. Biol. Chem. **186**, 37 (1950).

5. Tsuboi, K. K. and Price, T. D., Arch. Biochem. Biophys. **81**, 223 (1959).

6. Dietrich, L. S. and Fuller, L., J. Biol. Chem. **239**, 1653 (1964).

7. Ijichi, A., Ichiyama, A., and Hayaishi, O., J. Biol. Chem. **241**, 3701 (1966).

8. Dietrich, L. S., Cancer Res. **24**, 61 (1964).

Received June 25, 1969. P.S.E.B.M., 1970, Vol. 133.