BONE NICOTINAMIDE ADENINE DINUCLEOTIDE LEVELS IN RESPONSE TO NICOTINAMIDE AND PARATHYROID EXTRACT INJECTIONS*

Robert Van Reen and Marilee Quesada

Naval Medical Research Institute
National Naval Medical Center
Bethesda, Maryland

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There are considerable data in the literature which suggest that the bone resorption occurring in response to the administration of parathyroid extracts (PTE) is related to citric acid metabolism (Alwall, 1944; L'Heureux and Roth, 1953; Neuman et al., 1956). The exact mechanism by which PTE increases the concentration of citric acid in bone and serum is not known; however, recently attention has been directed at the influence of PTE on the levels of NAD and NADP in skeletal tissues (Van Reen, 1965; Hekkelman, 1965; Herrmann-Erlee, 1966). The present investigation was undertaken to determine whether PTE has any effect on the increased concentrations of the nicotinamide coenzymes which occur in response to nicotinamide injections. Kaplan et al. (1956) have shown that there is approximately a 10-fold increase in the concentration of liver NAD following the injection of nicotinamide in mice. No data are available in the literature concerning the effect on bone levels.

Materials and methods: Female rabbits of the New Zealand White strain, 6 to 8 weeks of age, were used in these experiments. PTE (Lilly) at 1000 Units/kg of body weight was administered 18 hours previous to sacrifice.

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This dosage has been demonstrated to result in bone resorption and increased serum calcium levels. Nicotinamide in physiological saline solution was administered at 500 mg/kg of body weight either 4 or 18 hours previous to sacrifice. Control animals were injected with saline in place of the nicotinamide solution and with a phenol-glycerin mixture (0.2% phenol in 1.6% glycerin) identical to the vehicle of the PTE. At appropriate times the animals were anaesthetized, exsanguinated, and the distal head of the femur removed. The bone was freed of extraneous tissue, the epiphyseal-metaphyseal area crushed, and immediately placed in cold 5% trichloroacetic acid solution. NAD and NADP were determined by the procedure of Jacobson and Astrachan (1957) which depends on the measurement of a fluorescent complex formed by the oxidized coenzymes with methyl ethyl ketone. A similar procedure was employed when liver was assayed.

TABLE I

NAD in Bone and Liver in Response to Nicotinamide and PTE Injections*

Treatment before sacrifice, hours		Tissue NAD levels, μg/g	
PTE	Nicotinamide	Liver	Bone
0	0	273 ± 7 (16)	23.3 ± 0.7 (15)
0	-4	1350 ± 215 (10)	51.7 ± 2.5 (18)
-18	-4	1708 ± 143 (6)	53.4 ± 3.2 (12)
0	0		21.7 ± 1.0 (10)
0	-18		57.1 ± 6.9 (7)
-18	-18		49.1 ± 2.2 (14)

*Value in parentheses indicate the number of assays. Mean values are followed by the standard error of the mean.

Results and discussion: The levels of bone and liver NAD in response to nicotinamide and PTE injections are shown in Table I. It can be seen that when nicotinamide was injected 4 hours prior to sacrifice the level of NAD in the calcified tissue rose from 23.3 μg/g to 51.7 μg/g. The administration of PTE 18 hours before sacrifice had no significant influence on the ability of nicotinamide to increase the NAD concentration. While there was about a twofold increase in bone NAD there was about a five- to sixfold increase in liver NAD concentration.

It seemed possible that the above pattern of nicotinamide and PTE administration might miss showing an effect if the PTE were eliminated or inactivated before the nicotinamide was administered. Therefore, a second series of rabbits were studied in which the nicotinamide and PTE were given simultaneously, 18 hours before sacrifice. The results were essentially the same as in the previous series, therefore it appears that PTE does not interfere with the increase in tissue NAD in response to nicotinamide.

The influence of nicotinamide and PTE injections on NADP concentrations in bone was also investigated. It can be seen from the data presented in Table II that nicotinamide administration 18 hours prior to sacrifice resulted in a higher level of bone NADP than normally found in control animals. Again PTE administration had no influence on the nicotinamide effect. Kaplan et al. (1956) observed that the increased liver nicotinamide adenine dinucleotides resulting from nicotinamide injections into mice were predominately due to NAD synthesis. Bone appears to give a similar response but a little more NADP results (about 15% of the total increase in oxidized coenzymes).

The administration of PTE alone resulted in reduced bone NADP concentrations. Previously, in preliminary experiments no reduction in NADP was observed. The reason for this apparent discrepancy is being investigated.

The above observations may be of considerable importance in unravelling the mechanism of PTE action. If PTE acts by reducing the available NADP in

TABLE II

NADP in Bone in Response to Nicotinamide and PTE Injections*

Treatment before sacrifice, hours		Tissue NADP levels, μg/g
PTE	Nicotinamide	Bone
0	0	14.9 ± 0.9 (9)
0	-18	20.7 ± 3.8 (9)
-18	-18	20.2 ± 5.2 (7)
-18	0	8.4 ± 2.0 (8)

*Values in parentheses indicate the number of assays. Mean values are followed by the standard error of the mean.

bone, the increased citric acid concentration is understandable. It would be anticipated from the above that the injection of nicotinamide previous to, or simultaneous with PTE would mitigate the effect of the hormone. Studies are under way to investigate this in the intact animal. There are some indications from *in vitro* studies with cultivated radius rudiments (Voogd van der Straaten, 1963) and from studies on oxidative phosphorylation in kidney mitochondria (Costello and Darago, 1964) that NAD or NADP can protect against some of the effects of PTE. The administration of nicotinamide to the intact animal should provide a means of increasing tissue levels of NAD and NADP and thus avoid the difficulties in interpretation when the preformed coenzymes are administered.

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