

## Stimulation of Erythropoiesis by Pyridine Nucleotides<sup>1</sup> (36715)

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Recent studies [(1-5), Cohen and Keighley, unpublished data] have shown that 3, 5-cyclic adenosine monophosphate (cAMP) stimulates erythropoiesis in polycythemic mice. This effect is claimed to be mediated primarily (3) or in part [(2, 5), Cohen and Keighley, unpublished data] through an increased production of erythropoietin (Ep). A direct stimulating effect of cAMP on heme synthesis by marrow cells *in vitro* has also been reported (6, 7). In addition to cAMP, other adenine nucleotides have been found to stimulate erythropoiesis in polycythemic mice (7). The present studies were undertaken to investigate possible actions of nicotinamide adenine nucleotides on erythropoiesis.

**Materials and Methods.** Female CF-1 mice weighing 22-25 g were made polycythemic by exposure to discontinuous hypobaric hypoxia (0.4 atm 219 hr) (8). On day 3 post-hypoxia the mice were injected ip with the test material. Each mouse received iv 0.5  $\mu$ Ci of <sup>59</sup>FeCl<sub>3</sub> on day 5 posthypoxia. Two days later, the mice were bled by cardiac puncture and the % RBC-<sup>59</sup>Fe incorporation was determined. The blood volume of these mice was assumed to be 8% of the body weight. Mice with hematocrits of less than 58% at the time of bleeding were discarded. Each experimental group contained 5 assay mice. Each single experiment was performed at least three times with essentially similar re-

sults and the data of typical experiments are presented in the tables.

Nicotinamide adenine dinucleotide (NAD<sup>+</sup>), nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>), and their reduced derivatives NADH and NADPH (Sigma Chemical Co.) were dissolved in 0.9% saline. Polycythemic mice were injected ip with 1 mg (1.2  $\mu$ moles) or 3 mg (3.5  $\mu$ moles) of these compounds in 1 ml of saline.

Ep antiserum (anti-Ep) was obtained from rabbits immunized with human urinary Ep (obtained from a patient with hypoplastic anemia) which had been concentrated with benzoic acid and partially purified (9). Induction of the immune response entailed the use of the concentrated heat-denatured Ep in conjunction with complete Freund's adjuvant. Rabbits were injected (im) with 100

TABLE I. Erythropoietic Response of Posthypoxic Polycythemic Mice to Pyridine Nucleotides.

Treatment	% RBC- <sup>59</sup> Fe incorporation <sup>a</sup>	<i>p</i> value <sup>b</sup>
1 mg NADP <sup>+</sup>	4.83 $\pm$ 1.35	.02
3 mg NADP <sup>+</sup>	11.75 $\pm$ 2.34	.01
3 mg NADPH	12.26 $\pm$ 2.05	.001
1 mg NAD <sup>+</sup>	1.06 $\pm$ 0.14	NS <sup>c</sup>
3 mg NAD <sup>+</sup>	6.73 $\pm$ 0.55	.001
3 mg NADH	8.34 $\pm$ 1.99	.01
Saline	0.83 $\pm$ 0.16	
0.05 IU Ep	5.15 $\pm$ 0.75	
0.20 IU Ep	16.92 $\pm$ 1.38	

<sup>a</sup> Mean  $\pm$  standard error of mean.

<sup>b</sup> Indicates the *p* value when compared with saline.

<sup>c</sup> Not significantly different from saline.

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TABLE II. Effect of Anti-Ep on the Erythropoietic Activity of Pyridine Nucleotides in Posthypoxic Polycythemic Mice.

Treatment	% RBC- <sup>59</sup> Fe incorporation <sup>a</sup>	<i>p</i> value <sup>b</sup>
3 mg NAD <sup>+</sup>	9.38 ± 2.59	.02
3 mg NAD <sup>+</sup> ; 0.2 ml anti-Ep	1.22 ± 0.29	NS <sup>c</sup>
3 mg NADP <sup>+</sup>	12.99 ± 4.93	.05
3 mg NADP <sup>+</sup> ; 0.2 ml anti-Ep	0.30 ± 0.01	.01
0.2 IU Ep	18.35 ± 3.06	.001
0.2 IU Ep; 0.2 ml anti-Ep	1.39 ± 0.50	NS <sup>c</sup>
Saline	1.26 ± 0.21	

<sup>a</sup> Mean ± standard error of mean.

<sup>b</sup> Indicates the *p* value when compared with saline.

<sup>c</sup> Not significantly different from saline.

IU Ep over a period of 1 month; 10 days after the last injection serum containing anti-Ep was harvested. Anti-Ep was administered ip at a different site but simultaneously with either NAD<sup>+</sup> or NADP. One ml of this particular immune serum was capable of neutralizing the erythropoietic activity of 10 IRP units of human Ep.

*Results.* Table I shows that NAD<sup>+</sup> and NADP<sup>+</sup> stimulated red blood cell formation in polycythemic mice as judged by significant elevations in RBC-radioiron values. This effect exhibits a tendency toward a dose-response relation and at the 1 mg dose level is more pronounced with NADP<sup>+</sup> than with NAD<sup>+</sup> (*p* < .05). On the other hand there was no significant difference in the activities of these compounds at the higher dose level. The reduced forms (NADH and NADPH) were also erythropoietically active; however, the phosphorylated derivative was not significantly more effective than the nonphosphorylated form at the dose level employed.

The results presented in Table II demonstrate that anti-Ep completely abolishes the erythropoiesis stimulating activity of either NAD<sup>+</sup> or NADP<sup>+</sup> when administered simultaneously to the assay mice.

*Discussion.* These experiments show that the pyridine nucleotides NAD<sup>+</sup>, NADP<sup>+</sup>, and their reduced derivatives enhance erythropoiesis in polycythemic mice. This activity appears to be Ep dependent since the stimulatory action of both NAD<sup>+</sup> and NADP<sup>+</sup> was completely abolished by a simultaneous injection of anti-Ep. It has like-

wise been shown that the stimulation of erythropoiesis by prostaglandins of the E series [(10, 11), Cohen and Keighley, unpublished data] stems from their ability to stimulate Ep production. In this regard, it seems possible that prostaglandins and pyridine nucleotides may alter renal blood flow in a way to reduce the oxygen supply to the kidney thereby engendering the production of increased amounts of Ep. On the other hand, one cannot preclude other possible effects. Thus, these compounds may affect the primitive erythroid precursor cells directly rendering them more sensitive to the action of endogenous Ep.

Because of the widespread occurrence of pyridine nucleotides and other erythropoietic substances (7, 10) in biological materials, the effects of incompletely defined preparations must be cautiously evaluated and their possible contamination by erythropoietically active substances other than Ep precluded before attributing the observed activity to Ep in the injected material.

*Summary.* The pyridine nucleotides NAD<sup>+</sup>, NADP<sup>+</sup>, and their reduced derivatives stimulated erythropoiesis in posthypoxic polycythemic mice. The erythropoietic stimulation appeared to be Ep-dependent since it was completely abolished by anti-Ep.

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