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PYRIDINE NUCLEOTIDE COENZYME BIOSYNTHESIS: A CELLULAR SITE OF OXYGEN TOXICITY

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SUMMARY: Hyperbaric O_2 caused a decline in pyridine nucleotide coenzymes and impaired growth of Escherichia coli which were prevented and reduced, respectively, by niacin but not quinolinate. These data support previous evidence that hyperbaric O_2 poisons quinolinate phosphoribosyl transferase, an enzyme required in both prokaryotes and eukaryotes for de novo NAD biosynthesis. Thus, there is a biochemical basis that niacin may be beneficial for humans requiring therapeutic O_2 at potentially toxic concentrations.

INTRODUCTION: Humans are detrimentally affected by breathing oxygen at tensions only 2 to 2-1/2 times that of air (1). This toxicity limits the concentration and duration of oxygen therapy for various dysfunctions including prematurity, aeroembolism, gas gangrene, and cardiovascular and respiratory disorders.

Most other life forms, including prokaryotes, are susceptible to oxygen poisoning (2) and we have used Escherichia coli to study fundamental cellular and enzymatic damage sites (3-6). Inhibition of biosynthesis of specific amino acids was found to be an early effect of oxygen toxicity (7,8). By providing the amino acids whose biosyntheses are impaired in hyperbaric oxygen, poisoning of other processes whose effects require longer for expression, was detected. Thus, preliminary evidence was published that niacin protected against growth inhibition in hyperoxia and that quinolinate phosphoribosyl transferase was poisoned (3). The predicted biological consequence is impaired synthesis of pyridine nucleotide coenzymes. In the present study, we report a decrease in cellular pyridine nucleotide coenzymes during hyperbaric oxygen exposure, and that this decrease is prevented by niacin.

MATERIALS AND METHODS: Escherichia coli strain K-12 obtained from the Genetic Stock Center of Yale University was grown and exposed to 4.2 atm of oxygen as previously described (8). Growth was monitored by measuring absorbance at 500 nm and by plate colony counts (7). The cells were harvested through a cooling coil

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which reduced the temperatures of the culture from 37°C to 4°C within approximately 15 sec. [method C, Brunker and Brown, 1971 (9)]. The reliability of this method for preserving the oxidized/reduced ratios of the conezymes was documented (9). Cells were extracted in 0.5 N perchloric acid to preserve the oxidized coenzymes and in 0.5 N KOH in ethanol:water (1:1 v/v) to preserve the reduced coenzymes (10). The coenzymes were assayed by a sensitive polarographic recycling method (11).

RESULTS AND DISCUSSION: The concentration of NAD and NADH decreased dramatically during exposure to hyperbaric oxygen in medium without niacin or quinolinate (Table 1). NADP and NADPH, which were present initially in much smaller amounts, did not change significantly or increased transiently in hyperoxia (Table 1). The data suggest that the processes which convert NAD (the biosynthesized form of the coenzymes) into NADH, NADP, and NADPH are comparatively resistant to hyperbaric oxygen.

Table 1. Decreases in coenzyme concentration during poisoning of *Escherichia coli* in minimal medium by hyperbaric oxygen[a].

Min in HPO	Generation time (min)	NAD	NADH	NADP	NADPH	TOTAL
		(Thousands of molecules per cell)				
0	26.8±0.9	918±161	665±92	44±9	65±9	1726±257
0-5	-	605±71[b]	731±251	58±15	111±13[c]	1505±341
0-15	-	464±111[c]	589±69	94±22[c]	99±11[c]	1228±63[c]
0-30	-	482±105[c]	593±115	62±16	85±16	1204±221[b]
0-60	65.0±3.2[c]	228±42[d]	335±75[c]	32±2	60±11	637±154[c]
0-120	134±99[a]	58±57[d]	113±71[d]	25±15	52±28	260±227[d]

[a] *Escherichia coli* strain K-12 was grown at 37°C in glucose basal salts medium (7) supplemented with 20 amino acids, each at 0.65 mM, thiamine, 0.1 mM and niacin (0.2 mM) or quinolinate (0.1 mM), where indicated. Cultures were pressurized with 4 atm of oxygen plus 1 atm of air (HPO) at an absorbance of approximately 2 at 500 nm wavelength where there are 1.56×10^8 cells per 1 absorbance in each of these media as determined by standard plate colony counting techniques. The relationship between colony counts and absorbance is linear up to 0.8; all measurements were made below this value by dilution as necessary. Cultures were stirred with a bar magnet via an external, coupled magnetic stirrer. The data are averages ± S.D. for 3 to 5 experiments with triplicate determinations of coenzymes and single determinations of culture absorbance for each experiment.

[b,c,d] Significantly different, respectively, at: $(0.01 < p \leq 0.05)$, $(0.001 < p \leq 0.01)$, $(p \leq 0.001)$ compared to the air control in medium with the same supplement.

Table 2. Effects of niacin and quinolinate on coenzyme concentrations during poisoning of Escherichia by hyperbaric oxygen[a].

Supplement	Min in HPO	Generation time (min)	NAD	NADH	NADP	NADPH	TOTAL
			(Thousands of molecules per cell)				
Niacin	0	26.3±0.9	1826±161	1300±131	79±16	122±5	3327±298
"	0-60	70±13[d]	1589±175	1630±178	114±7[b]	239±32[c]	3573±118
"	0-120	72.5±7.7[d]	2059±449	2065±132[c]	122±29	324±82[b]	4474±646[b]
Quinolinate	0	26.5±1.4	950±30	831±46	52±19	68±6	1900±26
"	0-60	64.9±4.8[d]	235±105[c]	319±70[c]	37±9	59±7	650±176[d]
"	0-120	84.5±2.9[d]	70±21[d]	121±29[d]	27±5	47±2	265±49[d]

[a,b,c,d]See footnotes to Table 1.

Addition of 0.2 mM niacin to the culture medium (Table 2) caused an approximate doubling of each coenzyme without affecting the oxidized/reduced ratios in cells growing with air as the gas phase (Table 3). Niacin prevented the

TABLE 3. Oxidized to reduced ratios of coenzymes calculated from the data of Tables 1 and 2[a].

Supplement	Min in HPO	Ratio NAD/NADH	Ratio NADP/NADPH
None	0	1.43±0.09	0.70±0.26
None	0-5	0.87±0.19[c]	0.52±0.08
None	0-15	0.83±0.26[c]	0.95±0.22
None	0-30	0.81±0.07[d]	0.76±0.28
None	0-60	0.68±0.03[d]	0.53±0.06
None	0-120	0.44±0.18[d]	0.49±0.15
Niacin	0	1.41±0.07	0.65±0.13
Niacin	0-60	0.99±0.19[b]	0.48±0.06
Niacin	0-120	0.99±0.17[b]	0.38±0.05[b]
Quinolinate	0	1.15±0.09	0.78±0.30
Quinolinate	0-60	0.71±0.18	0.64±0.22
Quinilinate	0-120	0.58±0.03[c]	0.58±0.14

[a,b,c,d]See footnotes to Table 1.

decrease in coenzymes in hyperoxia; quinolinate did not (Table 2). It should be noted that the medium contained amino acids and thiamine, previously found to protect in hyperoxia (3,7). Amino acids must be present in order to observe the protective effects of naicin for growth in hyperoxia.

Without niacin or quinolinate, the oxidized to reduced ratios of NAD in hyperbaric oxygen at all time intervals were changed significantly toward a more reduced state and the ratio steadily decreased (Table 3). A similar pattern was seen in cells grown in medium supplemented with quinolinate but the oxidized to reduced ratios changed less during the first hr and not at all thereafter in medium with niacin (Table 3).

Even when the NAD concentration, without niacin in the medium (Table 1), fell to 25% of normal at 1 hr and 7% at 2 hr, growth continued although the generation times were increased about 6-fold and 7-fold, respectively (Table 4). With niacin, the growth rate still declined in hyperoxia but was increased less than 3-fold at 1 hr and was stabilized at about a 3 1/2-fold increase from 90 min onward (Table 4).

The decrease in coenzyme concentrations which occurred in Escherichia coli exposed to hyperbaric oxygen theoretically might result from inhibition of syn-

Table 4. Comparative increases in generation time during exposure of Escherichia coli to hyperbaric (4.2 atm) oxygen with and without niacin.

Time Interval in Hyperoxia (min)[a]	Generation Time (min)	
	With Niacin	Without Niacin
60-70	73	158
70-80	73	161
80-90	91	167
90-100	98	174
100-110	96	182
110-120	99	189

[a]The conditions were as described in Table 1 except that absorbance was continuously recorded using a Brinkman PC 1000 spectrophotometer with a probe sealed into the chamber.

thesis or increased breakdown. However, inhibition of synthesis is consistent with published observations that quinolinate phosphoribosyl transferase, required for de novo synthesis of NAD is poisoned in hyperoxia (3). The failure of quinolinic acid (an intermediate in the biosynthesis of NAD before the oxygen-sensitive enzyme) to protect against growth inhibition (Table 4) or coenzyme decrease (Table 2) while niacin (which is after the oxygen-sensitive enzyme in the pathway) protected (Tables 2 and 4) is also consistent with inhibition of coenzyme synthesis at this step.

Quinolinate phosphoribosyl transferase is present in higher plants (12), microorganisms (13-15), and in mammalian liver and kidney (16,17). The observed decrease in pyridine nucleotide coenzymes associated with decreased growth rate reported in this paper, provides documentation that the inhibition of quinolinate phosphoribosyl transferase is biologically significant during oxygen poisoning of Escherichia coli.

Since man, E. coli and many other life forms synthesize pyridine nucleotide coenzymes from quinolinate by the same pathway, involving the oxygen-sensitive quinolinate phosphoribosyl transferase, it appears that the herein reported decreases in NAD and in NADH, and the decreases in oxidized/reduced ratios may have general significance as a mechanism of cellular oxygen toxicity from exposures of 1 hr and more. Relevant to this point is the fact that NAD, in addition to its coenzyme function, is consumed for the synthesis of polyadenosine diphosphoribose [poly(ADP-ribose)] in mammalian cells. The half-life of NAD is reported to be approximately two hours in E. coli and in animals (18-20), and approximately one hour in cultured human cells (21,22). However, the nicotinamide moiety is not consumed in the process of poly(ADP-ribose) synthesis and is efficienty reutilized for NAD biosynthesis by the salvage pathway which does not involve quinolinate phosphoribosyl transferase (23). Thus, impairment of quinolinate phosphoribosyl transfersase would appear to have more significance for rapidly multiplying cells.

The decrease in coenzyme in bacteria in hyperoxia may be relevant to mammalian lung oxygen toxicity but probably is not relevant to the central

nervous system effects, including convulsions, which can be produced within minutes. Chance et al. have elegantly examined changes in oxidized/reduced pyridine nucleotide coenyzmes in animals during brief exposures to convulsive pressures of oxygen (24). They did not report decreases in the total coenzyme concentration, which is not contradictatory to the data reported herein because of the brief period of hyperoxia used (24). The protection afforded E. coli by niacin, an intermediate in the salvage pathway below the poisoned site (10), provides a potential which remains to be evaluated for amelioration of oxygen toxicity for human patients.

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