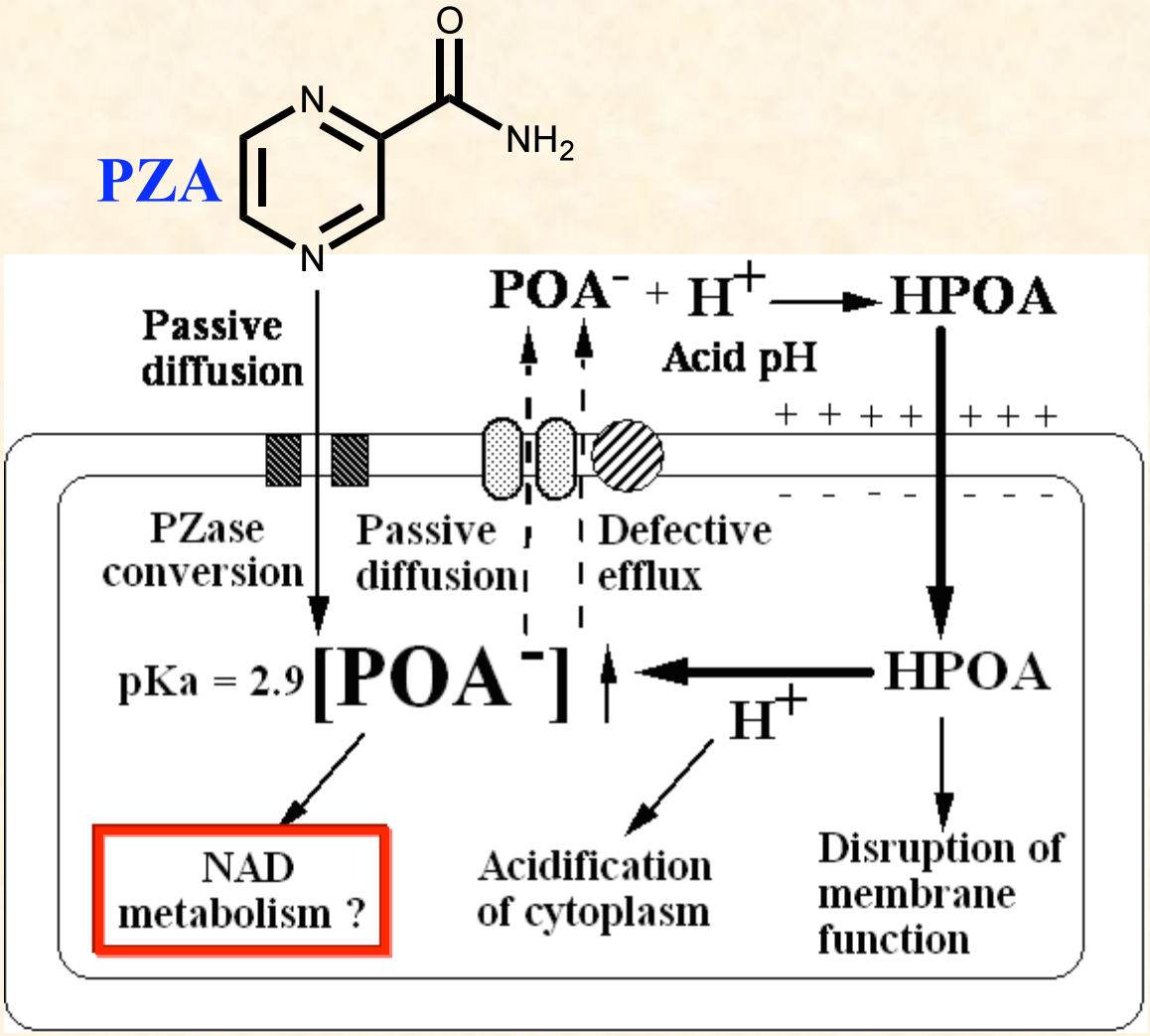
nad biosynthetic mutants of *Mycobacterium tuberculosis*: a tool to elucidate PZA action?

Essentiality of PZA Workshop May31-June1

Mechanisms of Action of PZA



Y. Zhang, D. Mitchison. 2003. Int. J. Tuberc. Lung Dis. 7(1):6–21

PZA increases NAD^+ content in rats

➢Effects of dietary pyrazinamide, an antituberculosis agent, on the metabolism of tryptophan to niacin and of tryptophan to serotonin in rats.

Shibata K, Fukuwatari T, Sugimoto E. Biosci Biotechnol Biochem. 2001, 65:1339-46.

The administration of pyrazinamide significantly increased the metabolites, 3-hydroxyanthranilic acid and beyond, especially quinolinic acid, nicotinamide, N'-methylnicotinamide, and N1-methyl-4-pyridone-3-carboxamide. However, no difference in the upper metabolites of the tryptophan to niacin pathway. It is suggested from these results that the **action of pyrazinamide against tuberculosis is linked to the increase in turnover of NAD and to the increased content of NAD in the host cells.**

➢Pyridine nucleotide levels in liver of rats fed clofibrate- or pyrazinamide-containing diets.

Shin M, Iwamoto N, Yamashita M, Sano K, Umezawa C. Biochem Pharmacol. 1998, 55:367-71.

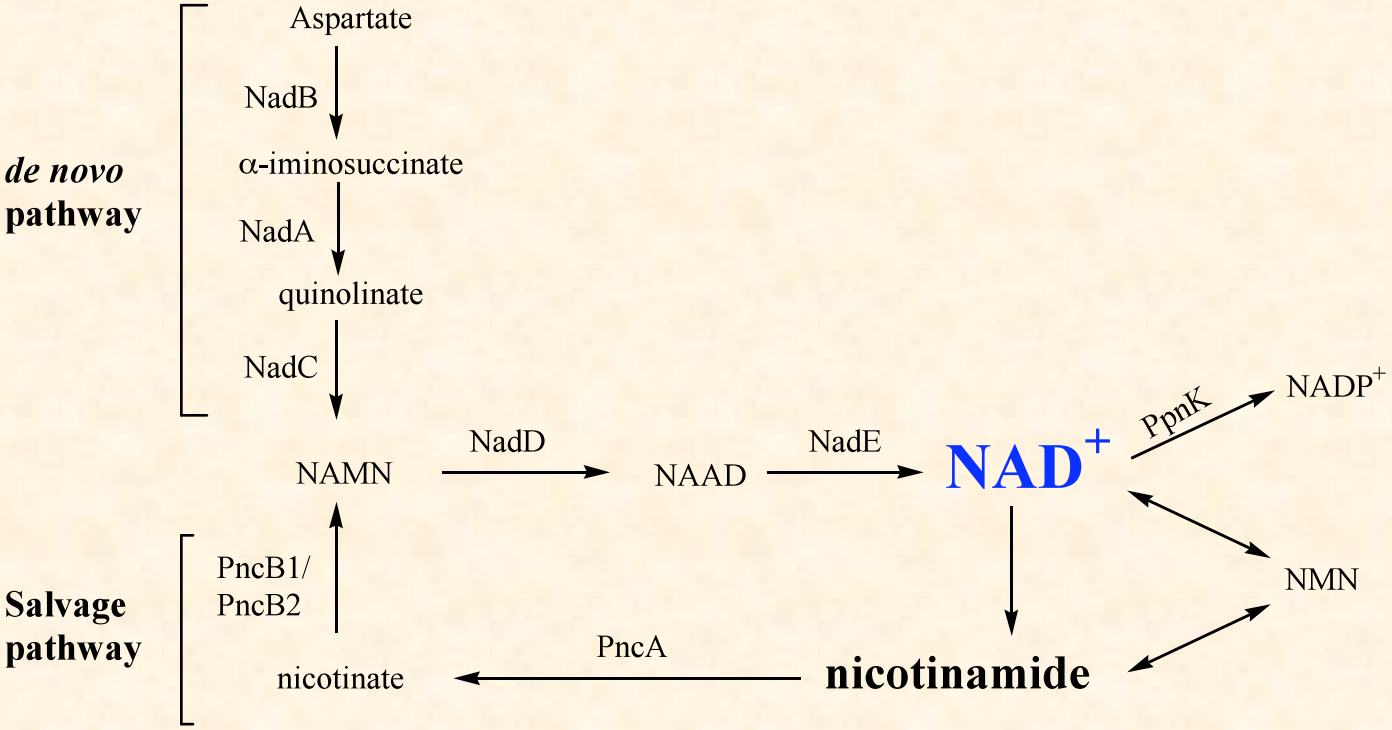
The NAD^+/NADH ratio remained unchanged with a 1% pyrazinamide diet. Hepatic quinolinate phosphoribosyltransferase (QAPRTase) activity was increased to 1.3 times that of the control animals in the pyrazinamide-fed rats, respectively, while hepatic aminocarboxymuconate-semialdehyde decarboxylase (ACMSDase) activity was decreased to 19% of that of the control animals. We conclude that **these changes of enzyme activities may contribute to the increase of pyridine nucleotides in the liver of rats fed pyrazinamide.**

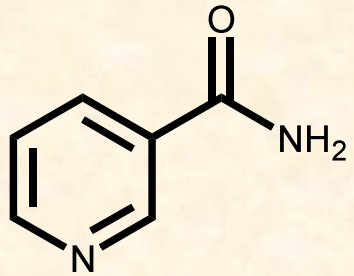
➢The effect of pyrazines on the metabolism of tryptophan and nicotinamide adenine dinucleotide in the rat. Evidence of the formation of a potent inhibitor of aminocarboxy-muconate-semialdehyde decarboxylase from pyrazinamide.

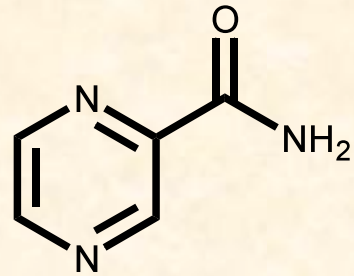
Nasu S, Yamaguchi K, Sakakibara S, Imai H, Ueda I. Biochim Biophys Acta. 1981, 677:109-19.

The intraperitoneal or oral administration of pyrazinamide and pyrazinoic acid resulted in a marked increase of the NAD content in rat liver. The unknown inhibitor of aminocarboxymuconate-semialdehyde decarboxylase was dialysable and heat-stable, and mostly excreted in urine by 6 and 12 h after injected of pyrazinoic acid and pyrazinamide, respectively. **These results suggest that the glutarate pathway of L-tryptophan was strongly inhibited by the inhibitor produced after the administration of pyrazinoic acid and pyrazinamide.**

NAD^+ biosynthetic pathway





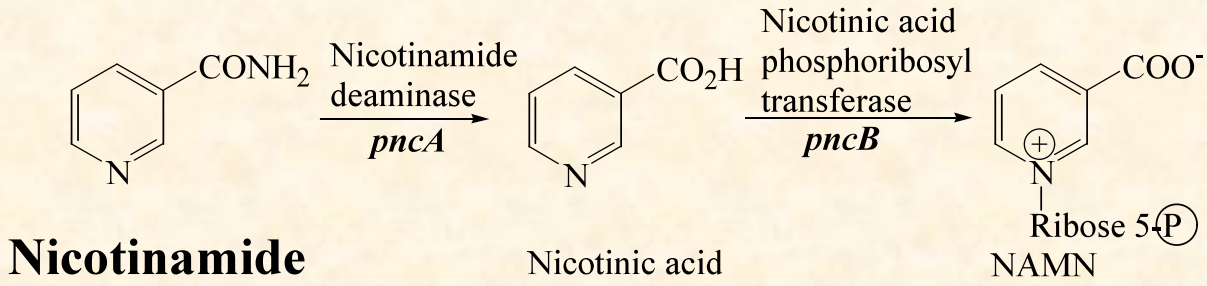


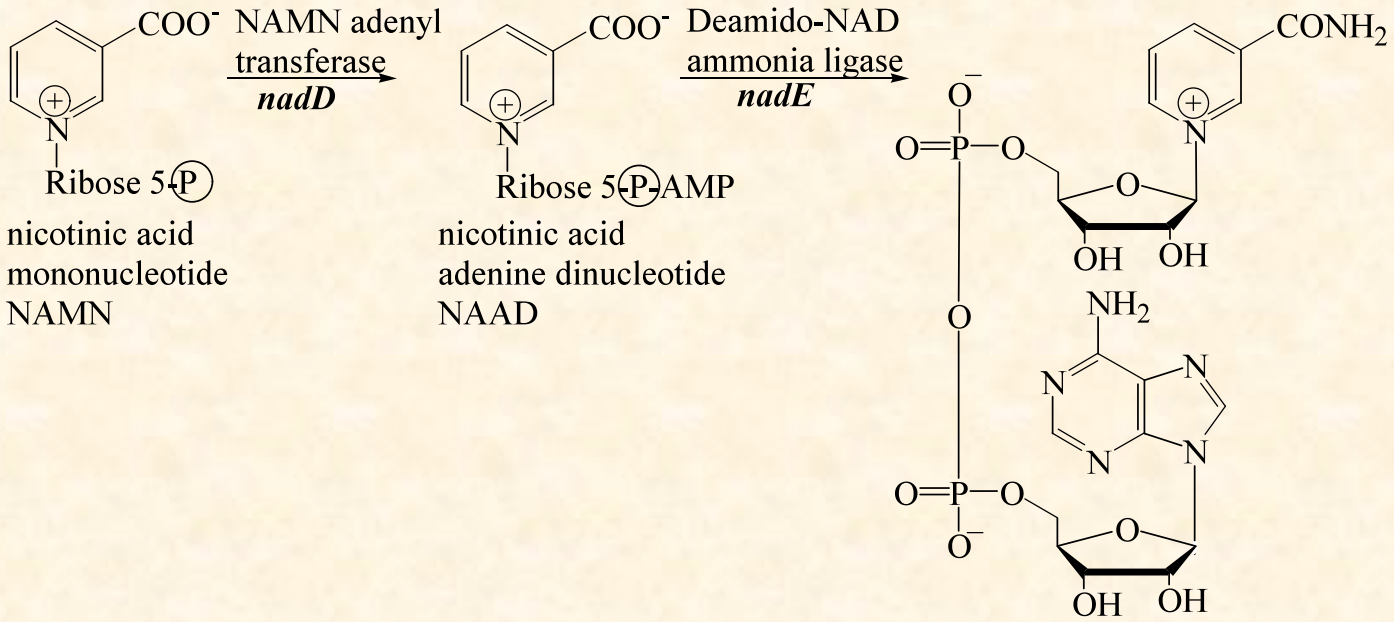
PZA

Hypothesis

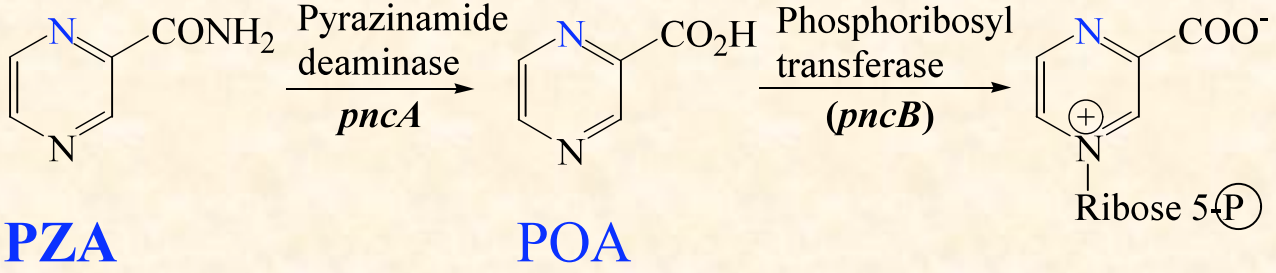
PZA can enter the NAD^+ salvage pathway as an analog of nicotinamide and alter the NAD^+ biosynthesis.

NAD^+ salvage pathway

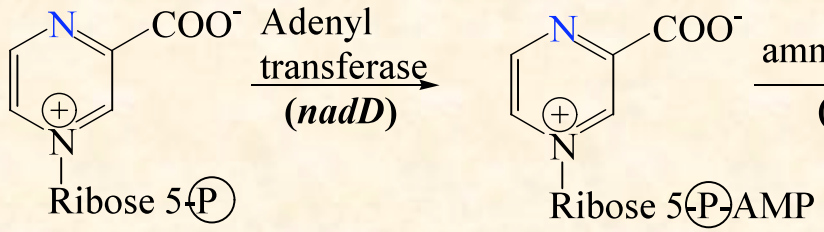




PZA in the NAD$^+$ salvage pathway

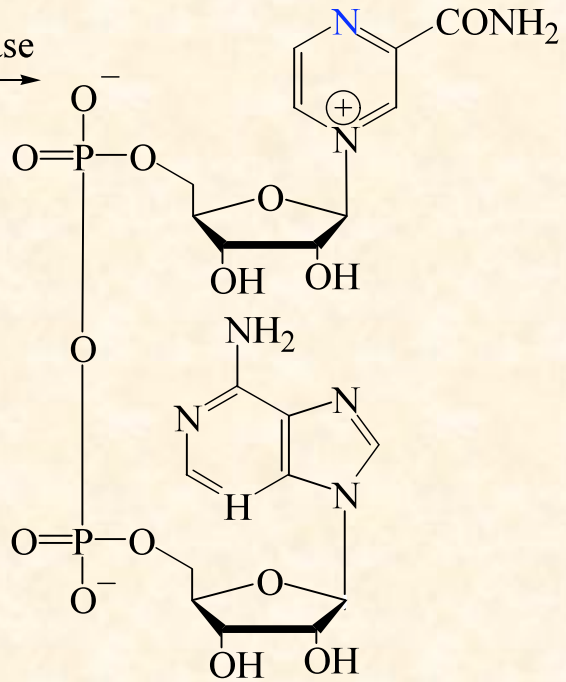


PZA POA



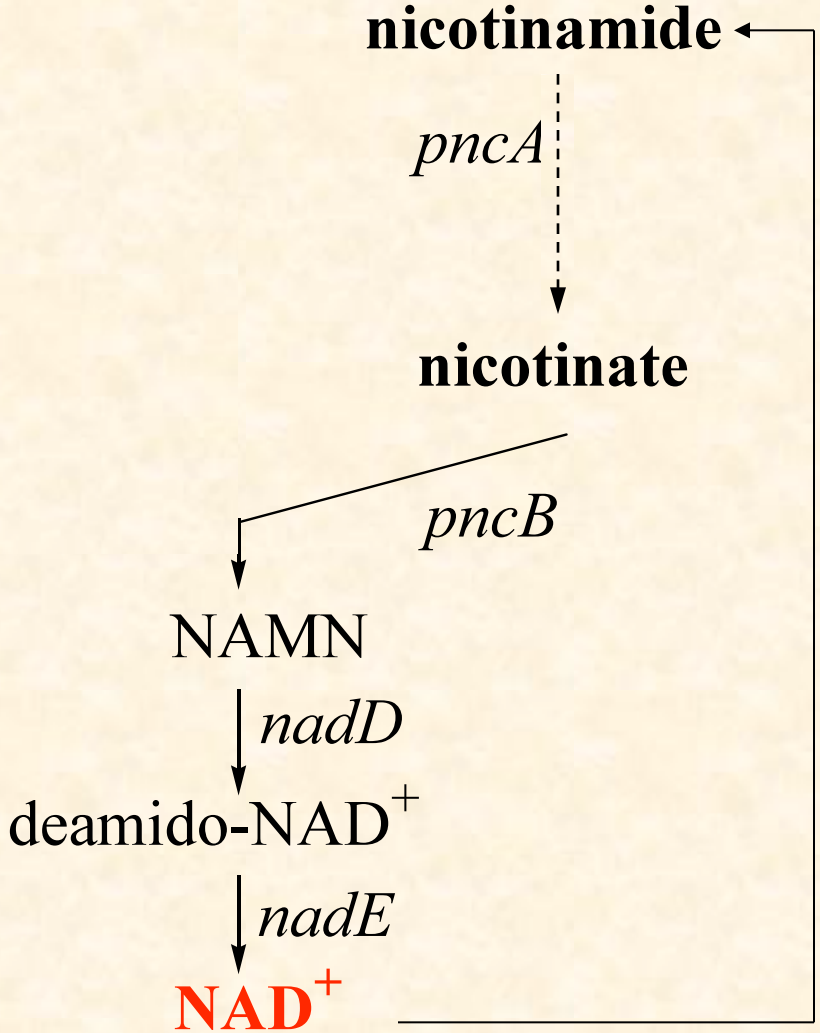
pyrazinoic acid
mononucleotide
POAMN

pyrazinoic acid
adenine dinucleotide
POAAD



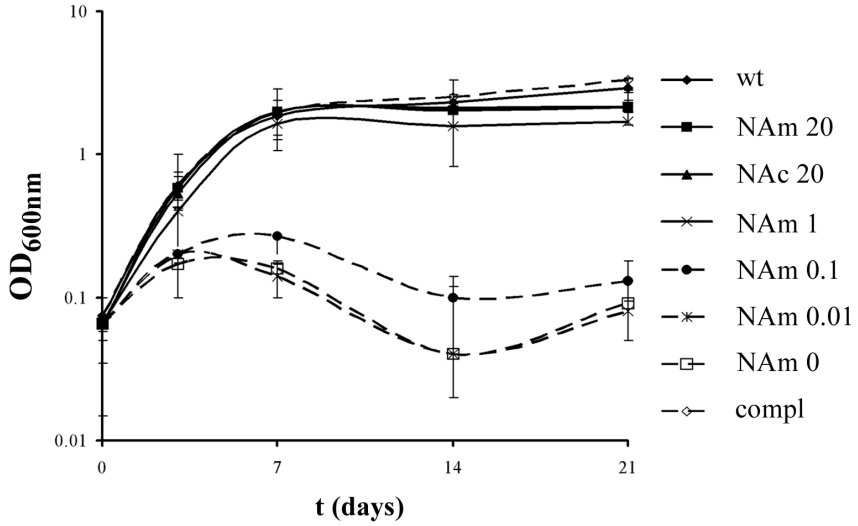
pyrazinamide
adenine dinucleotide **PAD$^+$**

NAD^+ biosynthesis

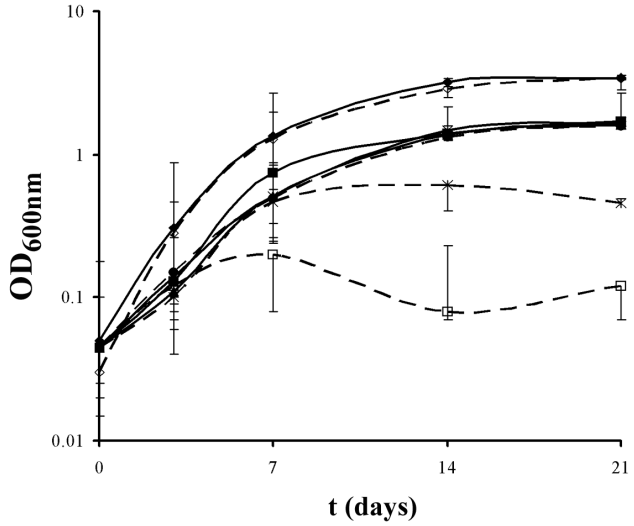


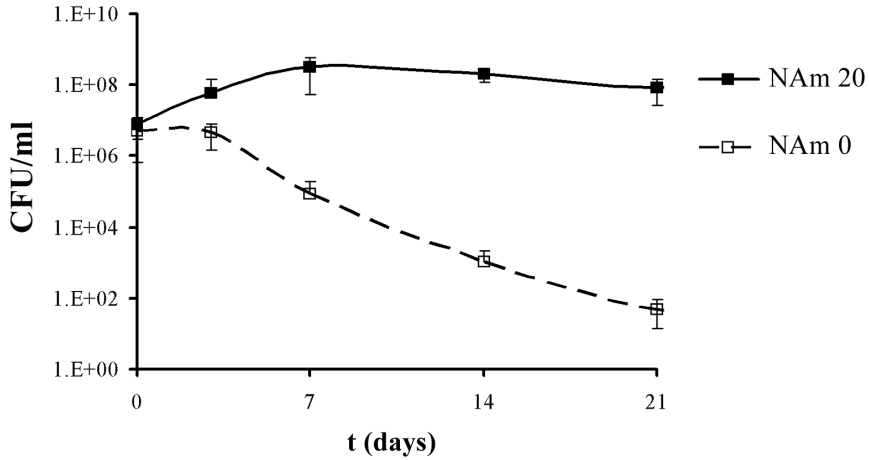
Growth of Δ*nadABC* mutants in vitro

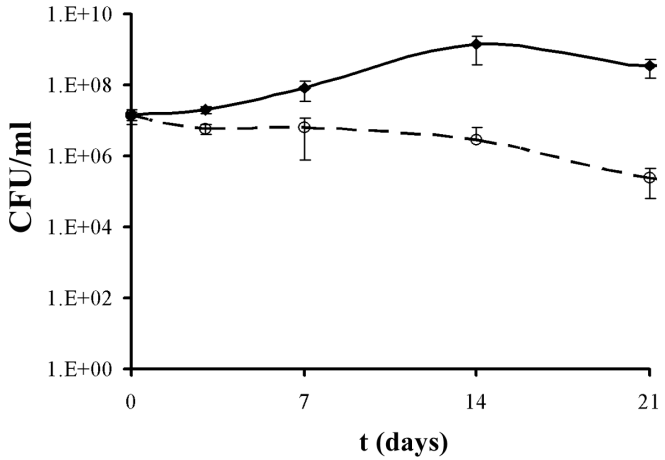
***M. bovis* ΔnadABC**



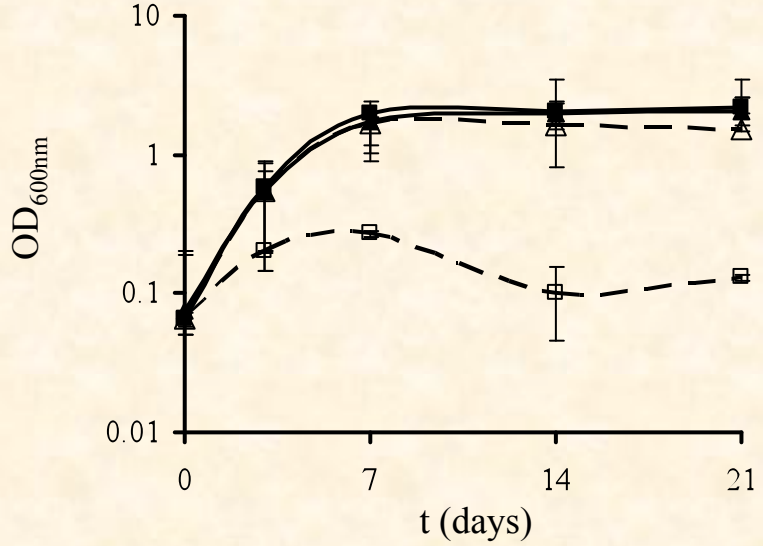
***M. tuberculosis* ΔnadABC**







M. bovis Δ*nadABC* pMV261::*pncA*^{TB} growth in vitro

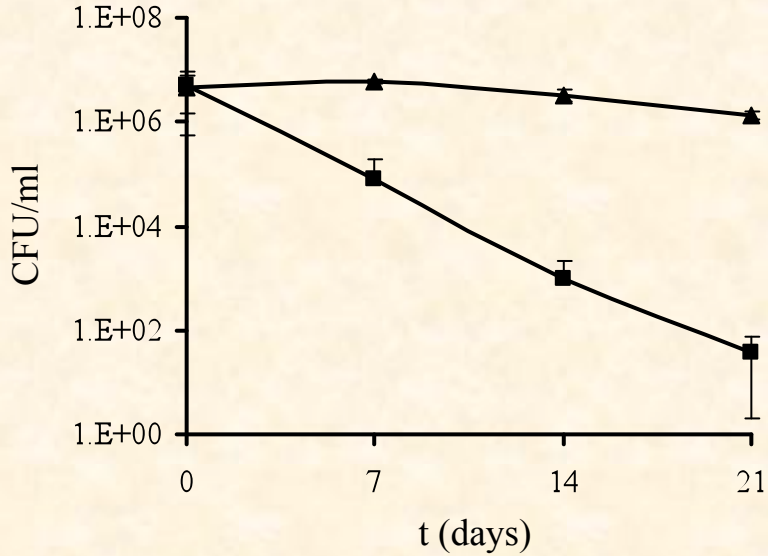


M. bovis Δ*nadABC* NAm^{20}

M. bovis Δ*nadABC* pMV261::*pncA*^{TB} NAm^{20}

M. bovis Δ*nadABC* NAm$^{0.1}$

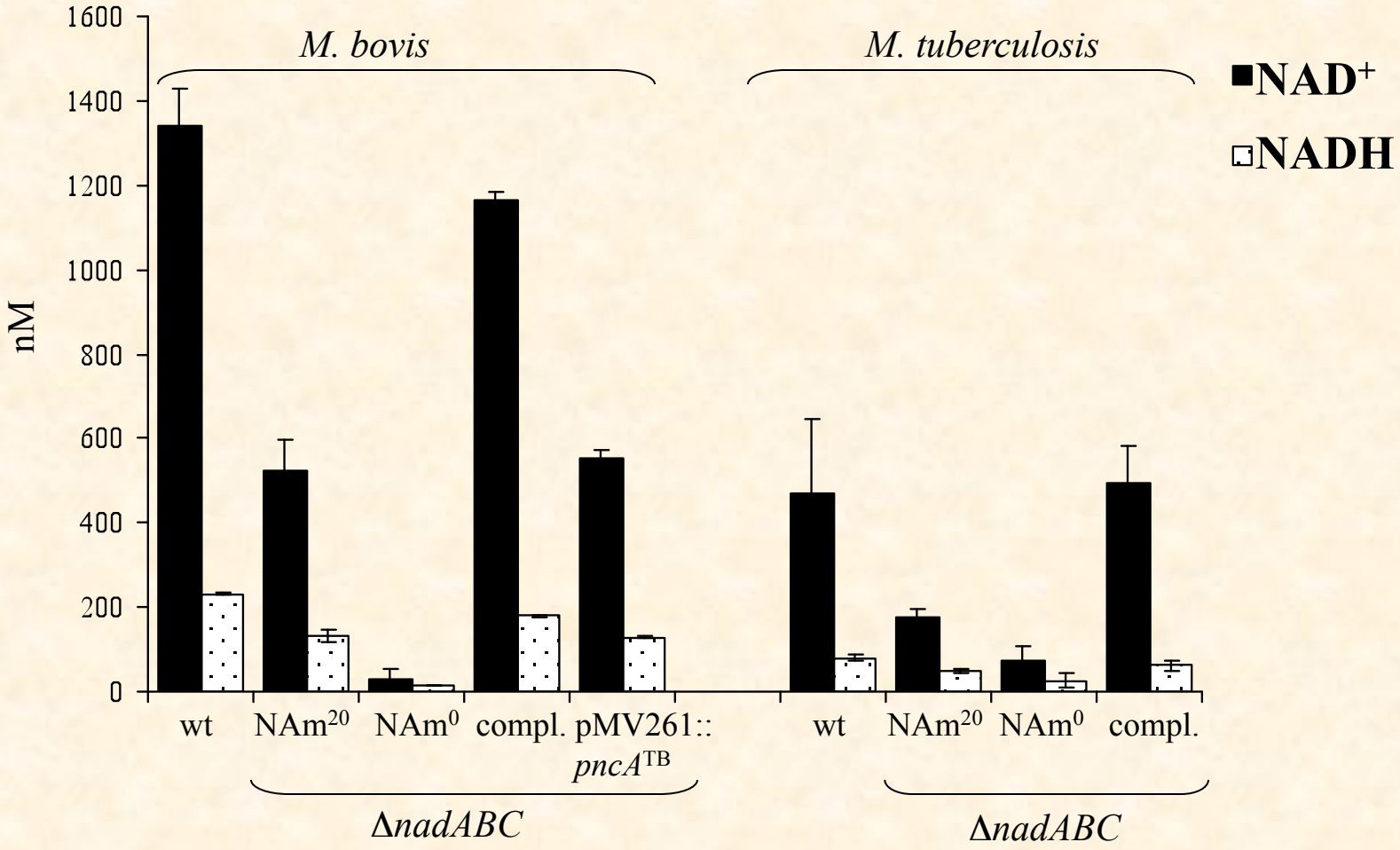
M. bovis Δ*nadABC* pMV261::*pncA*^{TB} NAm$^{0.1}$



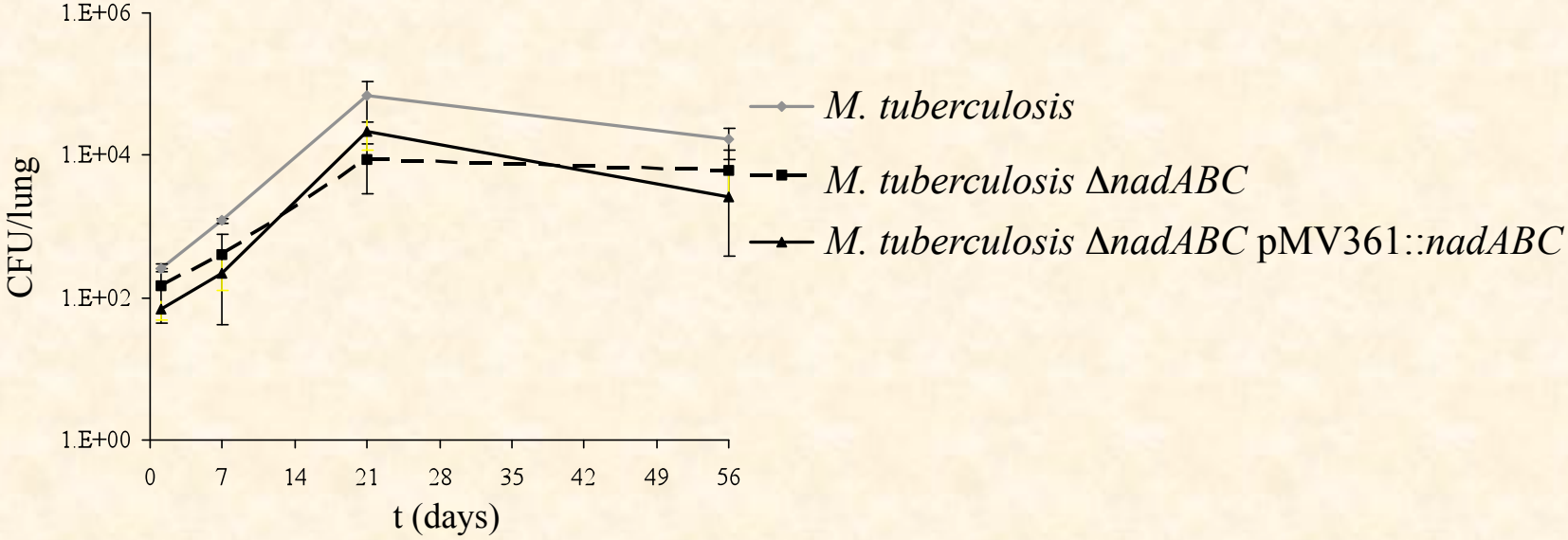
M. bovis Δ*nadABC* pMV261::*pncA*^{TB}

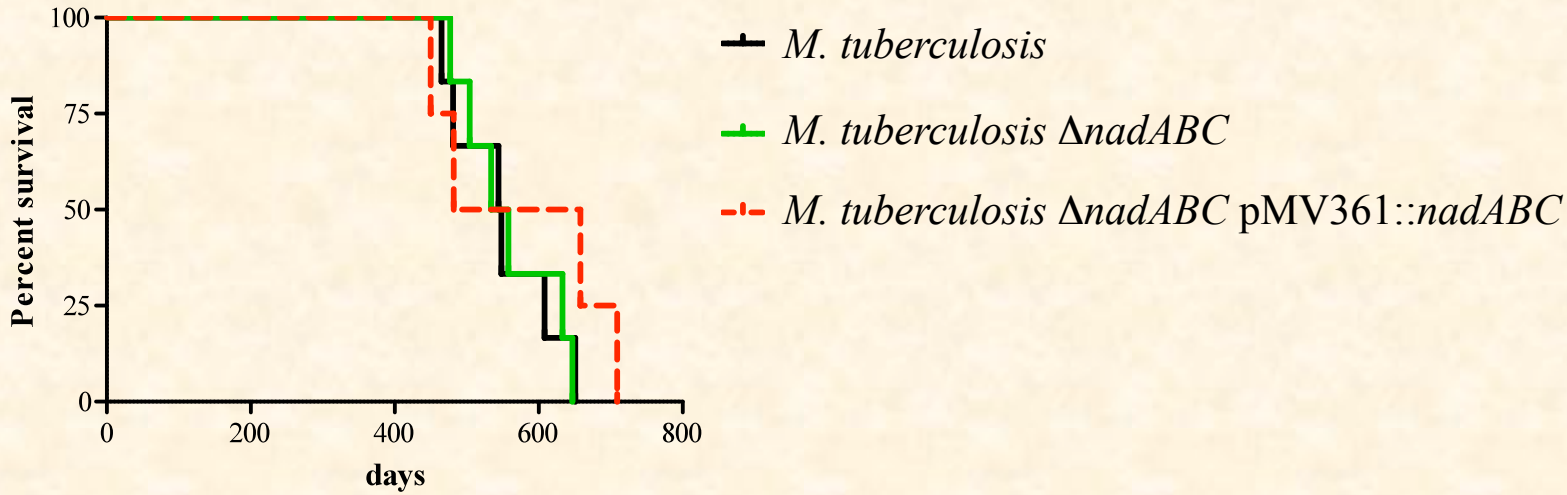
M. bovis Δ*nadABC*

NAD$^+$ and NADH concentrations in Δ*nadABC* mutants

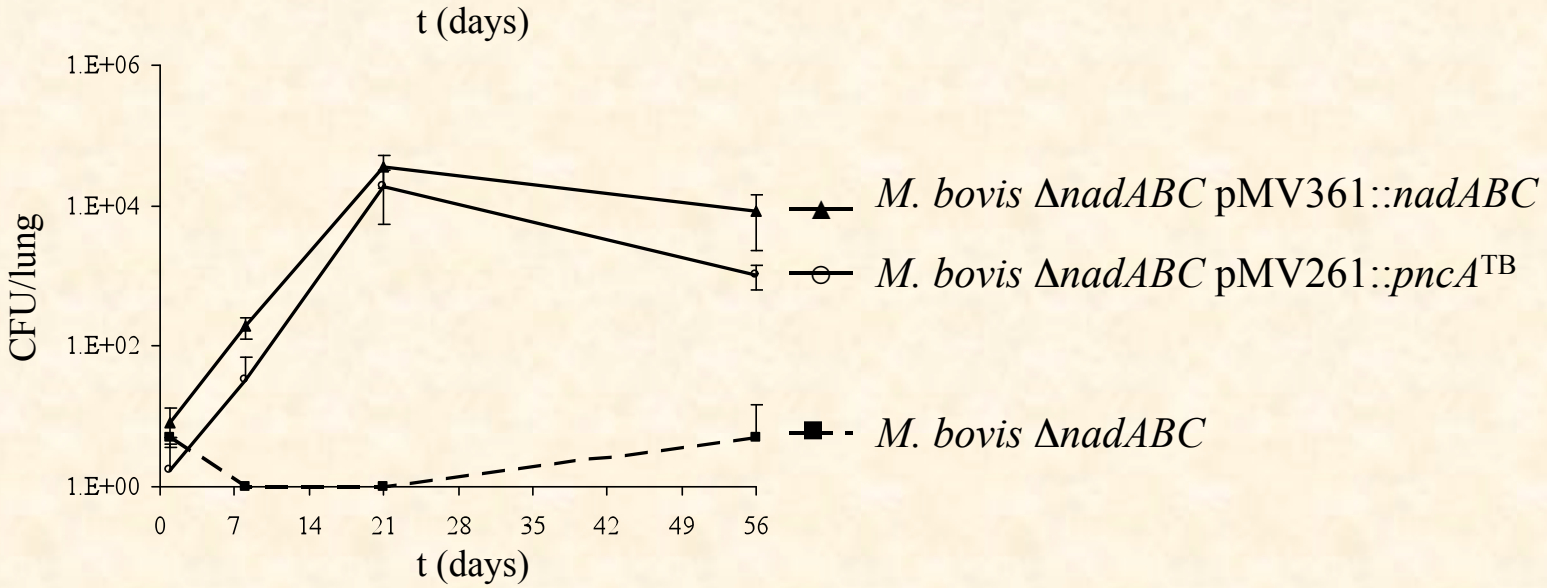


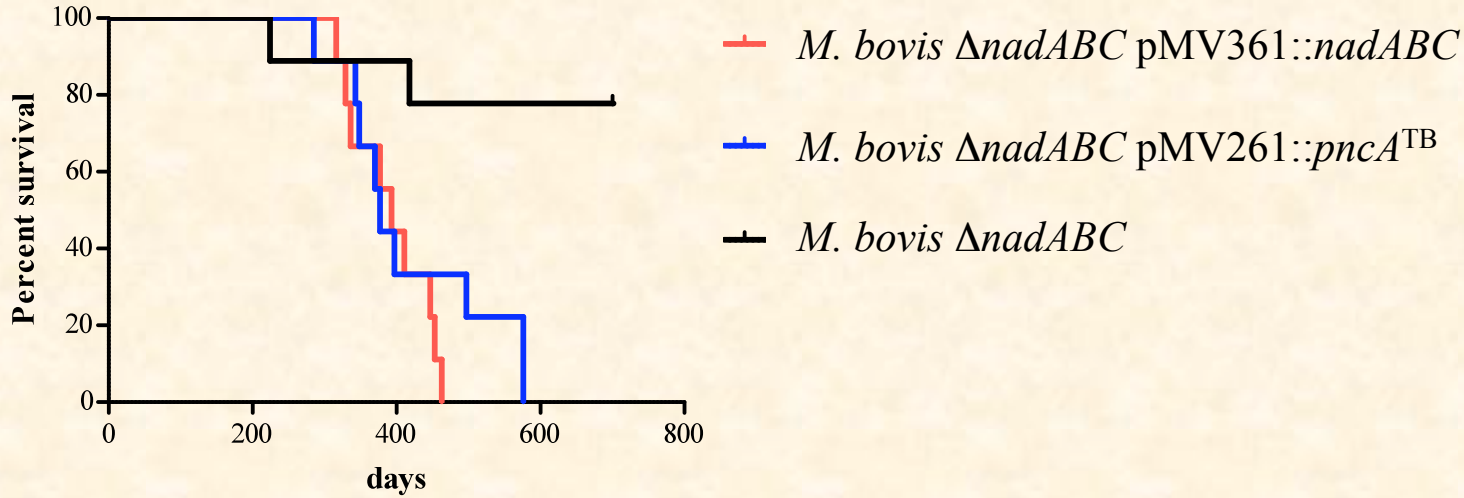
CDC1551 Δ*nadABC* mutant in C57Bl/6 mice



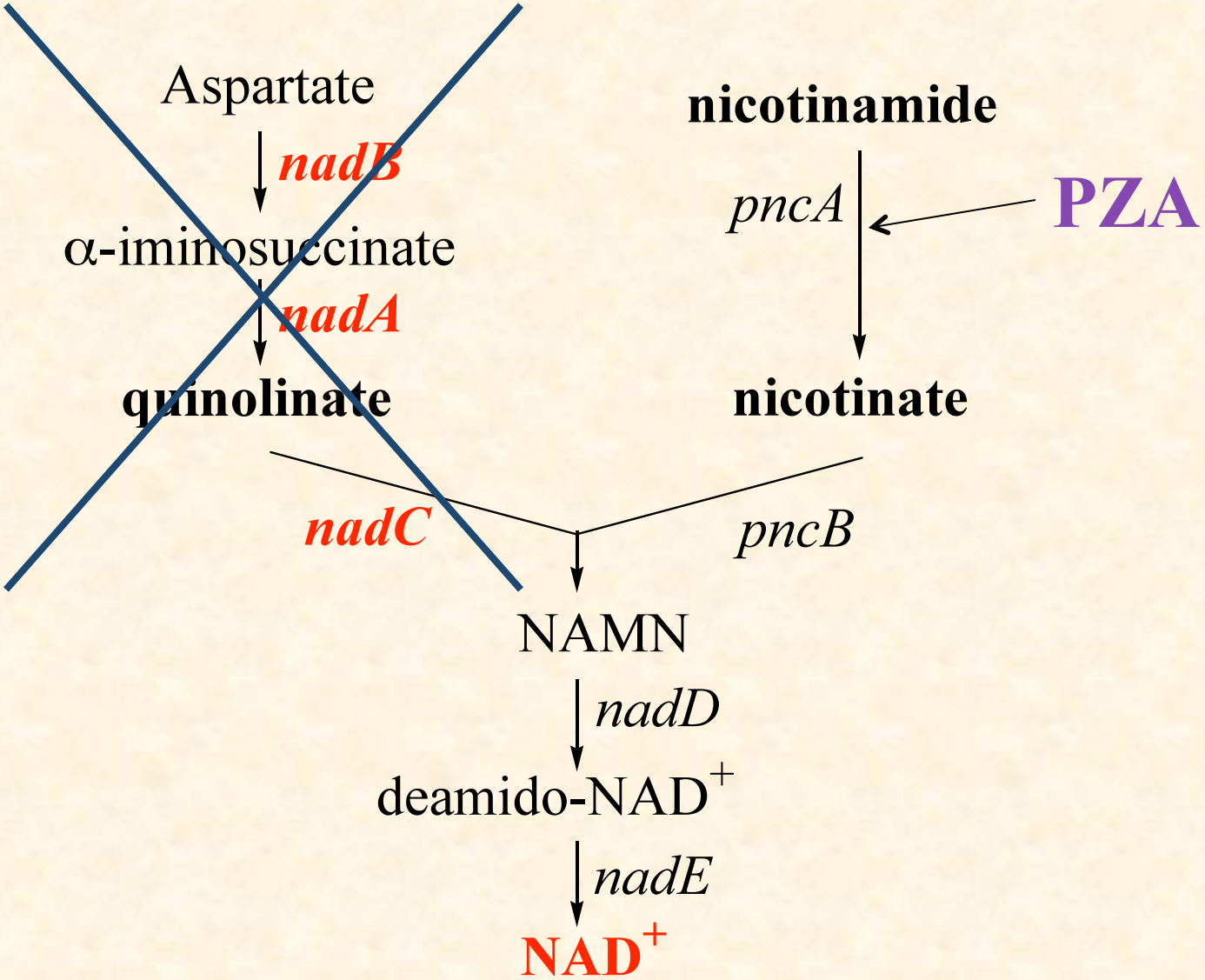


Ravenel Δ*nadABC* mutant in C57Bl/6 mice

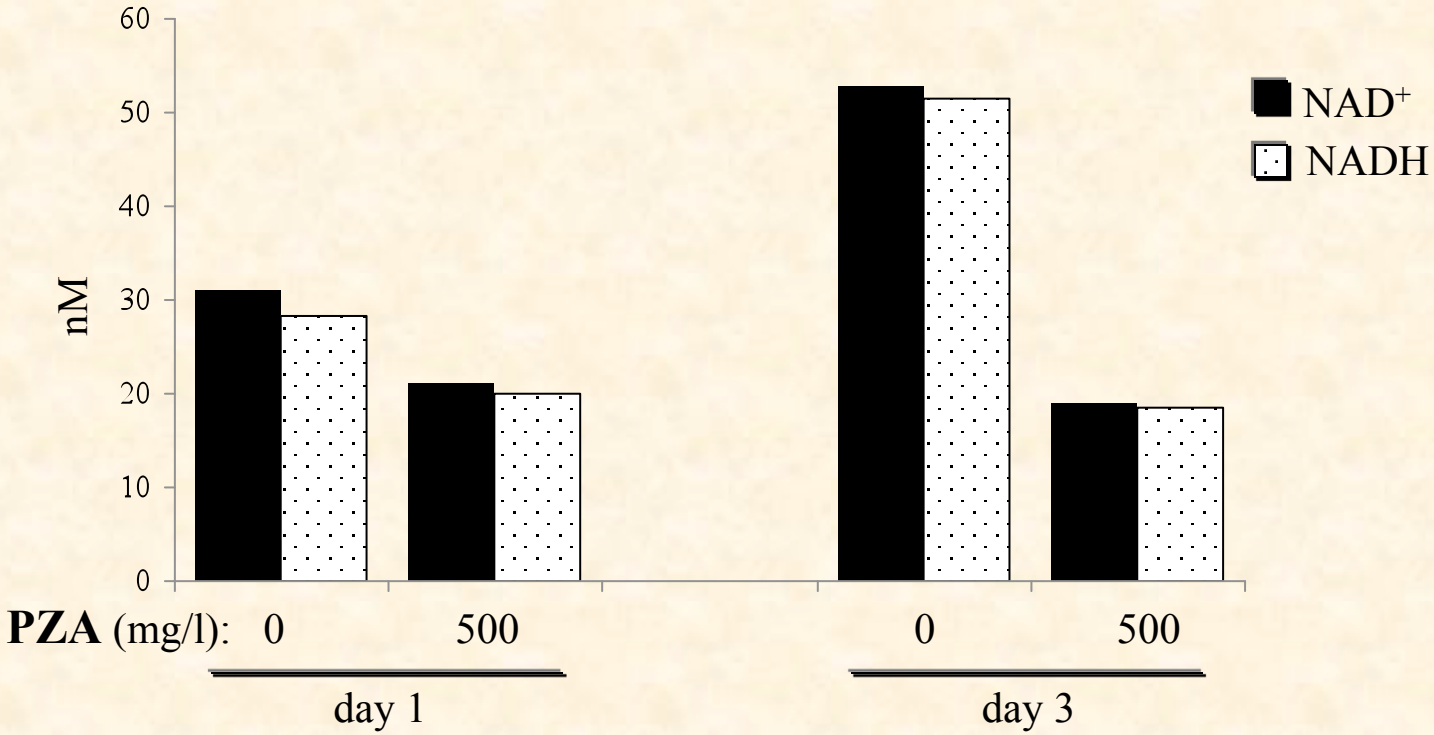




NAD$^+$ biosynthesis in *M. tuberculosis* Δ*nadABC*



NAD^+ and NADH concentrations in PZA-treated *M. tuberculosis*



M. tuberculosis H37Rv, grown in Middlebrook 7H9-OADC-Tylo (pH 6.0), was treated with PZA (500 mg/l) prior to NAD^+ and NADH extraction.

Possible experiments to test if PZA disrupts NAD^+ biosynthesis

- Treat Nam-starved *M. tuberculosis ΔnadABC* with PZA and measure NAD^+ and NADH concentrations.
- Treat Nam-starved *M. tuberculosis ΔnadABC* with PZA and identified PZA metabolites by MS.
- Treat Nam-starved *M. tuberculosis ΔnadABC* with labeled PZA and identified PZA metabolites.
- PZA and INH.