The first line TB drug pyrazinamide is a phoenix

Jacques Grosset MD
Johns Hopkins University
School of Medicine
Structure of nicotinamide and related compounds

Nicotinic Acid
(Niacin, vitamin B₃, vitamin PP)

Nicotinamide
Other names: pyridine-3-carboxamide; niacinamide, nicotinic acid amide, Vitamin PP

Pyrazinamide
(pyrazine-2-carboxamide)

Isoniazid (isonicotinohydrazide)

Ethionamide (2-ethylpyridine-4-carbothioamide)
Main properties of nicotinic acid (niacin) and nicotinamide

• Niacin cannot be directly converted to nicotinamide
• Both niacin and nicotinamide could be converted to the coenzymes NAD and NADP *in vivo*
• Niacin and nicotinamide are identical in their vitamin activity
• In contrast to niacin, nicotinamide does not reduce cholesterol or cause flushing, although nicotinamide may be toxic to the liver at doses exceeding 3 g/day for adults
• Nicotinamide has demonstrated anti-inflammatory action, especially in patients with inflammatory skin conditions
• Nicotinamide is considered safe as a food additive, and as a component in cosmetics and medication
• This explains the followings:
(Pre)-History of pyrazinamide: the pyridine carboxylic acids

At the present time there exist, among cyclic amides, various amido derivatives of nicotinic acid or, more generally, of the pyridine carboxylic acids, which derivatives have therapeutic properties.
This invention relates to a new and improved process for the manufacture of pyridine carboxylic acids, especially quinolinic and nicotinic acids. More particularly, it relates to an improved method for preparing nicotinic acid from salts, particularly the heavy metal salts, of pyridine monocarboxylic and pyridine dicarboxylic acids.
Pre-history of pyrazinamide I.

- Pyrazinamide was synthesized (ammonolysis of methyl pyrazinoate) by Dalmer and Walter in 1936 (German patent 632257).

Two serendipitous findings in 1945 from treatment with high doses of Vit PP:
- V. Chorine*: High doses (300-500 mg/kg) of nicotinamide or nicotinic acid cure *M. lepraemurium* infection in the rat model and slow down TB infection in guinea pigs; In vitro, the MIC of nicotinamide for *M.tuberculosis* is ~300 µg/ml while nicotinic acid is inactive.
- E.Huant**: in patients with cancer + tuberculosis receiving IM 300 mg + oral 500-1000 mg of nicotinamide, clear-cut improvement of TB disease occurred!

Pre-history of pyrazinamide II.

1948. McKenzie et al.* Out of 2,000-3,000 chemicals screened against the tubercle bacillus on the chorioallantoic membrane of the chick embryo:
- 15% were inhibitory, especially pyridine carboxylic acids.
- These 15% were tested using the mouse model: Nicotinic acid and nicotinamide were the most active at a dose of 0.5 to 0.75% in the diet (800-1000 mg/kg) and as active as 200 mg/kg streptomycin S/C.
- Kushner and coworkers** synthesized 30 derivatives of nicotinamide but they were less active than nicotinamide!
- Drug resistance developed in mice treated with nicotinamide alone but not in combination with streptomycin
- Their belief was that the role of nicotinamide in the treatment of experimentally infected mice is that of a vitamin!

Mouse model: Swiss mice IV infected with $10^6-7$ Mtb CFU and treated the day after infection, drug mixed in the diet. Untreated controls died in 3-4 weeks

1952. Discovery of pyrazinamide’s antituberculosis activity

Kushner*: pyrazinamide = nicotinamide + nitrogen atom substituted in the ring

• In the mouse**, 0.1% (vs 0.75% for nicotinamide) in the diet or 125 mg/kg is 7 times > nicotinamide
• In the guinea pig***, limited activity with daily doses of 50-75 mg
• In humans****, evaluation on 43 patients between 1949 and 1951 showed it to be clinically active (Reduction of fever and diminution of cough and sputum), at doses of 350 mgx4 on day 1 and to 700 mgx4/per day, i.e., 2800mg/day or 23-46 mg/kg daily for 42 days. Two patients had hepatitis and 10 had joint pain.

Improvement of the mouse model**: “use as indices of activity the survival time of 50% of mice (T_{50}) as compared with 50% of nontreated controls, together with the weight of the mice”.

In addition mice and guinea pigs infected with *M. bovis* strains do not respond to pyrazinamide treatment.
W. McDermott and R. Tompsett  
Amer. Rev. Tuberc. 1954; 70: 748-754

**TABLE 3**

**Activity of Pyrazinamide Against H37Rv Tubercle Bacilli According to Environmental Acidity**  
(Inoculum of standard size; medium, oleic-albumin; activity measured by macroscopically demonstrated bacillary growth at three weeks)

<table>
<thead>
<tr>
<th>pH</th>
<th>450</th>
<th>200</th>
<th>100</th>
<th>50</th>
<th>25</th>
<th>12.5</th>
<th>6.25</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>7.5</td>
<td>1+</td>
<td>2+</td>
<td>3+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>7.0</td>
<td>2+</td>
<td>2+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>6.5</td>
<td>2+</td>
<td>2+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>6.0</td>
<td>0</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>5.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Conclusion:** Even at high concentrations, pyrazinamide is inactive or poorly active at basic and neutral pH.
W. McDermott and R. Tompsett
Amer Rev Tuberc 1954; 70: 748-754

**TABLE 5**

Activity of Streptomycin Against H37Rv Tubercle Bacilli According to Environmental Acidity

*(Inoculum, standard size; medium, Tween®-albumin; activity measured by macroscopically visible bacillary growth at two weeks)*

<table>
<thead>
<tr>
<th>pH</th>
<th>10</th>
<th>5</th>
<th>2.5</th>
<th>1.25</th>
<th>0.63</th>
<th>0.32</th>
<th>0.16</th>
<th>0.08</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1+</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>7.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>7.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1+</td>
<td>3+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>6.5</td>
<td>0</td>
<td>0</td>
<td>1+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>6.0</td>
<td>0</td>
<td>1+</td>
<td>3+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>5.5</td>
<td>0</td>
<td>1+</td>
<td>2+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Conclusion:** Even at high concentrations, streptomycin is inactive or poorly active at acid pH. It is the opposite of pyrazinamide
Pyrazinamide-isoniazid in tuberculosis

- 55 patients with pulmonary tuberculosis
  Treatment for 3 months with
  - PZA 50 mg/kg (3 g per day), twice 25 mg/kg a day
  - INH 5 mg/kg (300 mg per day), twice 2.5 mg/kg a day

Results
- Culture conversion in 90%
- X-ray improvement in 75%
- Hepatitis in 6 patients (10%), one of which was fatal

Authors’ conclusions:
1. “PZA+INH exerts a high degree of antituberculous action in vivo of a sort superior and qualitatively different from that of current antituberculous drugs used either singly or together”
2. “The high incidence of hepatitis during the administration of INH+PZA makes their use together in present dosage an unadvisable procedure in the treatment of tuberculosis”

It was the death penalty for pyrazinamide!
The intracellular activation of pyrazinamide

Intracellular *M. tuberculosis* present in monocyte cultures

<table>
<thead>
<tr>
<th></th>
<th>After 24h of incubation</th>
<th>After 4 days of incubation</th>
<th>µg/ml of pyrazinamide (Z)</th>
<th>H*</th>
<th>Z/H</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>10</td>
<td>12.5/10</td>
</tr>
<tr>
<td>Bacilli per monocyte</td>
<td>1.1</td>
<td></td>
<td>4.1</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>% of infected monocytes</td>
<td>37.0</td>
<td></td>
<td>55</td>
<td>27</td>
<td>25.0</td>
</tr>
<tr>
<td>Coefficient of increase</td>
<td>-</td>
<td></td>
<td>3.7</td>
<td>0.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*H= isoniazid
1953 -1956 -1959. K. Konno: the niacin test to identify *M. tuberculosis*

*M. tuberculosis* produces a great amount of niacin and has a pyrazinamidase/nicotinamidase

![Pyrazinamide](pyrazine-2-carboxamide)

Pyrazinamide

![Pyrazinoic acid](pyrazine-2-carboxylic acid)

Pyrazinoic acid

![Nicotinamide](pyrazine-2-carboxamide)

Nicotinamide

![Nicotinic acid](pyrazine-2-carboxylic acid)

Nicotinic acid

The same enzyme
Pyrazinamide, a phoenix?

The phoenix depicted in the Aberdeen Bestiary (ca. 12thC)

---

The phoenix depicted in the Aberdeen Bestiary (ca. 12thC)
Pyrazinamide, a phoenix?

Pyrazinamide, a phoenix?

The phoenix on top of Kinkaku-ji temple, Kyoto, Japan
Where is or who is the phoenix?
The phoenix properties

(Ancient Greek: Φοίνιξ, phoínix)

• A phoenix is a mythical bird that is a fire spirit with a colorful plumage and a tail of gold and scarlet.
• It has a 500 to 1000 year life-cycle, then burns fiercely and reduces to ashes, from which a new, young phoenix reborn anew to live again.
• The bird's cry is that of a beautiful song.
• The Phoenix's ability to be reborn from its own ashes implies that it is immortal.
The rebirth of the phoenix pyrazinamide

“It has been argued that streptomycin plus pyrazinamide together form a single bactericidal drug, with streptomycin active in tissues with alkaline pH and pyrazinamide, in those with an acid pH*; pyrazinamide is much cheaper than rifampin”

References:
Controlled Clinical Trial of daily 6-month regimens for pulmonary tuberculosis  

<table>
<thead>
<tr>
<th>Drug regimens</th>
<th>Total patients with favorable results at 6 mo.</th>
<th>Relapses between 6 and 30 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>6SHR</td>
<td>152</td>
<td>4 (3%)*</td>
</tr>
<tr>
<td>6SHZ</td>
<td>153</td>
<td>13 (8%)*</td>
</tr>
<tr>
<td>2STH/16TH</td>
<td>133</td>
<td>5 (3%)</td>
</tr>
</tbody>
</table>

S=streptomycin 1 g; H=isoniazid 300 mg daily; R, rifampin 450 (<50 kg b.w.) -600 mg; Z=pyrazinamide 2 g; T, thiacetazone 100 mg  
*p=0.05
Controlled Clinical Trial of daily 6-month and 9-month SHZ regimens for pulmonary tuberculosis in Hong Kong (Am. Rev. Respir. Dis. 1977; 115: 727-735)

<table>
<thead>
<tr>
<th>Drug regimens</th>
<th>Total patients with drug-susceptible strains</th>
<th>Relapse rate at 30 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>6SHZ</td>
<td>167</td>
<td>35 (21%)</td>
</tr>
<tr>
<td>9SHZ</td>
<td>179</td>
<td>10 (6%)</td>
</tr>
</tbody>
</table>

S = streptomycin 1 g (< 40 y.o.) - 0.75; H = isoniazid 300 mg daily; Z = pyrazinamide 2 g (≥ 110 lb) - 1.5 g.
1972. Short-course chemotherapy in pulmonary tuberculosis with rifampin (R) and isoniazid (H) + S or E for 2 months

<table>
<thead>
<tr>
<th>Treatment duration (months)</th>
<th>Total patients</th>
<th>Relapse after follow-up (in months) of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Up to 18</td>
</tr>
<tr>
<td>6</td>
<td>182</td>
<td>5 (2.7%)</td>
</tr>
<tr>
<td>9</td>
<td>153</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>181</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>149</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>665</td>
<td>5</td>
</tr>
</tbody>
</table>

*2 patients relapsed 4-5 years after stopping treatment R, 600 mg (450 if <50 kg); H, 300 mg; E, 25 mg/kg daily; S, 0.75 g IM, six days a week.

Conclusion (1976); “Treatment with 9 months is acceptable as standard chemotherapy for tuberculosis in Britain”.

The 6-month short-course chemotherapy for tuberculosis with 2RHZE/4RH


<table>
<thead>
<tr>
<th>Duration</th>
<th>Regimen</th>
<th>Total patients</th>
<th>Total (%)</th>
<th>Bact.</th>
<th>Radio. ± Histo.</th>
<th>Month of relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 months</td>
<td>2RHZS/4RH</td>
<td>119</td>
<td>2 (1.7)</td>
<td>1</td>
<td>1</td>
<td>5, 45</td>
</tr>
<tr>
<td></td>
<td>2RHZE/4RH</td>
<td>127</td>
<td>4 (3.1)</td>
<td>3</td>
<td>1</td>
<td>2,4,6,33</td>
</tr>
<tr>
<td>9 months</td>
<td>2RHE/7RH</td>
<td>127</td>
<td>2 (1.6)</td>
<td>2</td>
<td>0</td>
<td>6,15</td>
</tr>
</tbody>
</table>

Authors’ conclusion: The Research Committee of the British Thoracic Society now recommends the use of either of the two 6-month regimens as an alternative to the currently recommended 9-month regimen.
Conclusion
from the TB viewpoint

- 1952: Birth of Z
- 1954: Death of Z because of hepatitis when given at high dose (3 g / day) in combination with H
- 1970: Rebirth of Z (D. Mitchison)
- 1972-1977: The 9-month regimens with SHZ and SHR
- 1984: The 6-month short-course treatment of TB with R + Z + H + E (versus 18 mo. without R and Z)
- Being active only on *M. tuberculosis*, Z is “the” TB sterilizing drug … and, when present, somewhat mysteriously, is the critical component for the success of nearly all experimental and clinical drug regimens.