

## Mechanism of isoniazid uptake in *Mycobacterium tuberculosis*

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**Initial transport kinetics of isoniazid (INH) and its uptake at the plateau were studied in *Mycobacterium tuberculosis* H37Rv under various experimental conditions. The initial uptake velocity increased linearly with INH concentration from  $2 \times 10^{-6}$  M to  $10^{-2}$  M. It was modified neither by addition of a protonophore that abolished proline transport, nor following ATP depletion by arsenate, which inhibited glycerol uptake, two transport processes taken as controls for secondary active transport and facilitated diffusion, respectively. Microaerobiosis or low temperature (4 °C) were without effect on initial uptake. It is thus likely that INH transport in *M. tuberculosis* proceeds by a passive diffusion mechanism, and that catalase–peroxidase (KatG) is not involved in the actual transport. However, conditions inhibiting KatG activity (high INH concentration, microaerobiosis, low temperature) decrease cell radioactivity at the uptake plateau. It is proposed that INH transport occurs by passive diffusion. KatG is involved only in the intracellular accumulation of oxidized derivatives of INH, especially of isonicotinic acid, which is trapped inside cells in its ionized form. This model explains observed and previously known characteristics of the accumulation of radioactivity in the presence of [ $^{14}$ C]INH for various species and strains of mycobacteria.**

Keywords: isoniazid, *Mycobacterium tuberculosis*, isoniazid transport, isoniazid metabolism

### INTRODUCTION

Isoniazid (isonicotinic acid hydrazide or INH) has been used since 1952 against tuberculosis, and is still the basis of antituberculous treatment. However, neither the mode of action of this drug nor the origin of the high sensitivity of *Mycobacterium tuberculosis* towards it is well understood. Most of the current models of the uptake and action of INH in *M. tuberculosis* are based on its chemical transformation by a catalase–peroxidase, now named KatG (for a review see Zhang & Young, 1993), a model first introduced by E. Krüger-Thiemer and colleagues (Krüger-Thiemer, 1957). It is currently postulated that INH is a prodrug which is active after transformation by the peroxidase into radicals that can either react with vital targets in mycobacterial cells, or ultimately produce isonicotinic acid (Johnsson & Schultz, 1994; Marcinkeviciene *et al.*,

1995; Shoeb *et al.*, 1985a), explaining INH resistance of KatG-defective mutants. The only clearly defined target of INH is the biosynthesis of mycolic acids (Takayama *et al.*, 1972), which are specific and major compounds of all mycobacterial cell walls. A cell-free system of mycolic acid synthesis is inhibited by the drug (Quémard *et al.*, 1991), and an enoyl-[acyl-carrier-protein] reductase which may participate in mycolic acid synthesis was identified and characterized (Quémard *et al.*, 1995). The enzyme was found to bind INH only after oxidation of the drug in the presence of KatG (Quémard *et al.*, 1996). It has recently been shown that INH reacts with, and is covalently bound to, the nicotinamide ring of NADH in the active site of the enzyme (Rozwarski *et al.*, 1998).

INH uptake was studied in the late 1960s and early 1970s. It was postulated that KatG was involved in INH transport, since the loss of the catalase–peroxidase activities strongly reduced accumulation of radioactivity in the presence of [ $^{14}$ C]INH. However, labelling of *M. tuberculosis* was followed for incubation times between 0.5 h and 10 d (Beggs & Jenne, 1970; Wang &

**Abbreviations:** CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; INH, isonicotinic acid hydrazide (isoniazid).

Takayama, 1972; Youatt & Tham, 1969b), and conclusions about INH transport were in fact drawn from data on the radioactivity accumulated at the plateau. They did not correspond to data on the transport itself, but to the overall process of transport followed by metabolism, since it was shown that, at the plateau, at least 50% of the radioactivity is borne by isonicotinic acid, while free INH was not detected (Youatt & Tham, 1969b; see also Youatt, 1969).

To study the transport mechanism, INH uptake kinetics were followed within minutes after addition of the drug, under experimental conditions that allowed discrimination between passive and active transport: the influence of substrate concentration, temperature, low air concentration, uncoupler or arsenate on INH transport kinetics was examined. INH uptake in *M. tuberculosis* H37Rv was compared to transport and accumulation of proline and glycerol, shown here to be dependent on an ionic gradient and ATP, respectively. Involvement of KatG in the accumulation of labelled molecules was examined and is discussed.

## METHODS

**Chemicals.** [ $^{14}\text{C}$ ]INH (0.48 GBq mmol $^{-1}$  or 1.92 GBq mmol $^{-1}$ ) was a generous gift of the National Institute of Health (USA), NIH AIDS Research and Reference Program. [ $^3\text{H}$ ]Uracil (1.85 TBq mmol $^{-1}$ ), [ $^{14}\text{C}$ ]proline (8.51 GBq mmol $^{-1}$ ) and [ $^{14}\text{C}$ ]glycerol (5.66 GBq mmol $^{-1}$ ) were from Amersham.

Silicone oil DC550 was from SERVA ( $d_{25^\circ\text{C}} = 1.07$ ; Boehringer Mannheim), and codex paraffin oil was from Gifrer. All other chemicals were from Sigma.

**Bacterial strains and cultures.** *Mycobacterium aurum*, strain A $^+$ , was obtained from the Institut Pasteur collection, and grown overnight in 0.45% Middlebrook 7H9 medium with 0.5% casitone, 1% glucose, with shaking at 37 °C.

*Mycobacterium tuberculosis* H37Rv (ATCC 27294) was grown for 2 weeks at the surface of Sauton medium (100 ml) at 37 °C. For harvesting, the medium was poured off, the cells were dispersed by gentle shaking with glass beads and then resuspended in fresh medium for overnight growth in the presence of [ $^3\text{H}$ ]uracil (0.37 MBq ml $^{-1}$ ).  $^3\text{H}$ -labelled cells were collected by centrifugation (3000 g, 10 min), washed and suspended in the transport buffer. In some experiments, aliquots of the cell suspension were dried, weighed and counted to correlate  $^3\text{H}$ -labelling with dry cell weight.

**Transport.** Transport measurement was performed in a 1 ml assay in HEPES buffer (25 mM, pH 7.3) at room temperature (approximately 23 °C) under continuous shaking. Assays contained 30–40 mg dry cell weight of bacteria and  $1.4 \times 10^{-4}$  M [ $^{14}\text{C}$ ]INH, except when indicated. Aliquots of 0.1 ml were taken up at different times and added on the top of an Eppendorf centrifuge tube containing 0.25 ml oil (silicone oil/paraffin oil 1:0.2, v/v) covered by a layer of 0.1 ml 20 mg non-labelled INH ml $^{-1}$ . Cells were rapidly sedimented through the oil layer by centrifugation (13000 g, 1 min). Centrifuge tubes were frozen on dry ice, and the cell pellets were dropped into counting flasks by cutting off the conical tip of the tube. Then, the scintillation solution (Aqualuma, Lumac) was added and the flasks were sonicated for 30 min in a bath to disperse the cells into the scintillation solution. Uptake determinations were repeated in at least three independent experiments.

Counting was performed on a Packard Tricarb 1900TR, using a  $^3\text{H}/^{14}\text{C}$  program.

Transport activities are expressed as the ratio of  $^{14}\text{C}$  d.p.m./ $^3\text{H}$  d.p.m. in aliquots when comparing various conditions of transport. INH uptake (mg cell dry wt) $^{-1}$  was determined only to calculate its concentration at the uptake plateau (Fig. 1) and to compare initial transport kinetics in the presence of INH concentrations in the medium from  $2 \times 10^{-6}$  M to  $10^{-2}$  M.

When used, arsenate (50 mM final concentration) and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP;  $10^{-5}$  M final concentration) were added 15 min before starting the uptake experiments. Experiments at 4 °C were performed with cells preincubated for 1 h in melting ice.

Transport of proline ( $4.2 \times 10^{-6}$  M final concentration) and of glycerol ( $6.5 \times 10^{-6}$  M final concentration) was followed under the conditions used for INH transport, with the same culture batches, to compare the influence of the various conditions on the transport of these metabolites and of INH.

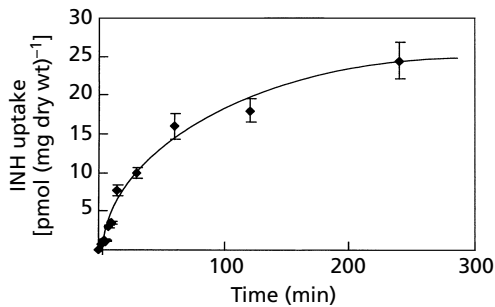
## RESULTS AND DISCUSSION

### Setting up the uptake experiments

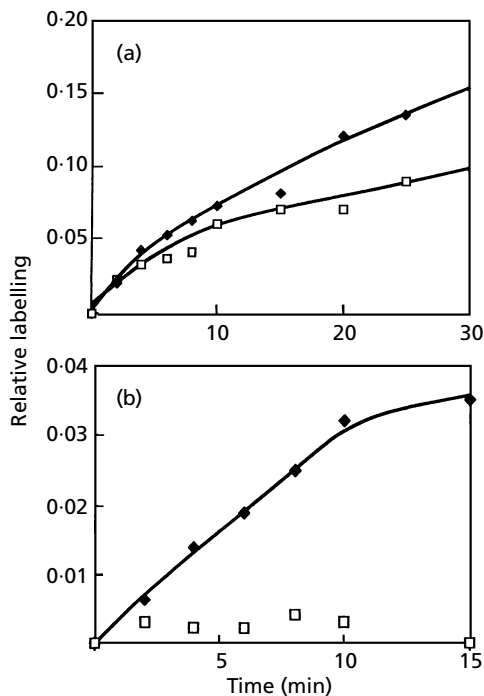
Before studying the transport of INH, we addressed two obvious experimental difficulties with mycobacteria. (i) Most species are known to form aggregates, rendering quantification of the biomass in aliquots difficult. This was solved by using background labelling of cells by [ $^3\text{H}$ ]uracil; then the counts due to  $^{14}\text{C}$ -labelled INH, proline or glycerol were related to the tritium labelling. The validity of the method was tested with proline transport in *M. aurum* A $^+$ . Growth of this strain can be quantified spectrophotometrically because cells are naturally well dispersed. Exactly the same results were obtained for proline uptake with dry cell weights calculated from either optical density measurements or [ $^3\text{H}$ ]uracil counting (data not shown). (ii) *M. tuberculosis* H37Rv is a virulent strain. To minimize hazard, we chose to separate the labelled bacteria from the transport medium by centrifuging cells through silicone oil instead of the more common filtration method. A third problem was encountered: a large amount of INH was adsorbed on the cells of the two mycobacterial species tested (*M. aurum*, *M. tuberculosis*), even after short contact times between bacteria and INH, i.e. for the time (<30 s) needed to start the centrifugation corresponding to the time-zero used as a blank for uptake determinations. Approximately half of the time-zero radioactivity was eliminated by adding cell suspension aliquots to centrifugation tubes previously layered with solutions of non-labelled INH, isonicotinamide, isonicotinic acid or nicotinamide, without changing the initial uptake kinetics. Uptake values were corrected for the residual radioactivity at time-zero.

### Initial uptake kinetics and radioactivity accumulation at the plateau in cells of *M. tuberculosis*

Labelling was linear for about 10 min. Afterwards it increased non-linearly, and a plateau was reached approximately 1 h later. Fig. 1 presents the mean of three uptake assays with *M. tuberculosis* with the lowest



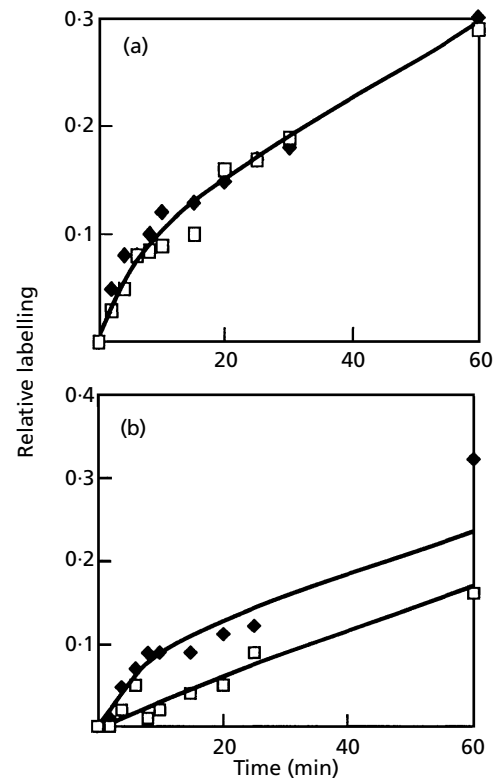
**Fig. 1.** INH uptake kinetics in *M. tuberculosis* H37Rv.



**Fig. 2.** Effects of a protonophore on INH and proline initial uptake. The ordinate corresponds to the ratio of [ $^{14}\text{C}$ ]INH labelling to [ $^3\text{H}$ ]uracil labelling.  $^3\text{H}$ -labelling is used to quantify bacterial cells in uptake assays.  $\blacklozenge$ , Control assay;  $\square$ , uptake in the presence of  $10^{-5}$  M CCCP. (a) INH uptake. (b) Proline uptake.

INH concentration used ( $2 \times 10^{-6}$  M). The accumulation of INH in cells was between four- and fivefold, if it is assumed that the cell volume is between 3 and  $2.4 \mu\text{l}$  ( $\text{mg dry wt}^{-1}$ ) (Wang & Takayama, 1972; Youatt & Tham, 1969b). Labelling at the plateau was dependent upon experimental conditions, as discussed below.

Initial uptake velocities were determined for INH concentrations ranging from  $2 \times 10^{-6}$  M to  $10^{-2}$  M. A linear relationship was observed between INH concentrations and initial labelling rates, with a slope equal to  $9(\pm 1) \times 10^{-8}$  mol ( $\text{mg dry cell wt}^{-1} \text{min}^{-1} \text{M}^{-1}$ ). This can be the experimental consequence of two different



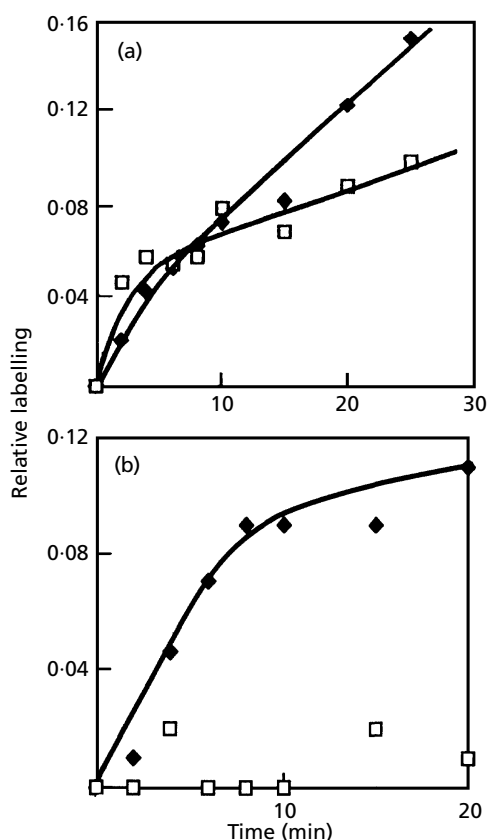
**Fig. 3.** Effects of ATP depletion on INH and glycerol initial uptake. Ordinate as in Fig. 2. ATP depletion was obtained by incubating cells in the presence of 50 mM arsenate.  $\blacklozenge$ , Control assay;  $\square$ , uptake in the presence of  $10^{-5}$  M CCCP. (a) INH uptake. (b) Glycerol uptake.

situations: either the transport is due to a passive diffusion mechanism since its rate is proportional to the gradient (Fick's law), or it is performed by a permease with a  $K_{m(\text{app})}$  constant significantly higher than  $10^{-2}$  M, which would be unusually high for transport in bacteria.

#### Absence of effect of a protonophore (CCCP), arsenate or low temperature on INH initial uptake

It has been shown that  $4 \times 10^{-4}$  M dinitrophenol induces a 50% inhibition of labelling by [ $^{14}\text{C}$ ]INH in *Mycobacterium bovis* BCG (Wimpenny, 1967), suggesting that uptake could result from active secondary transport. However, this experiment again used a long uptake time (40 h). Thus we examined the effect on initial kinetics of the more potent protonophore CCCP, used at a concentration ( $10^{-5}$  M) inhibiting uptake of proline, an amino acid generally transported in bacteria by an active secondary system. The protonophore was ineffective on initial INH uptake (Fig. 2a), at a concentration completely inhibiting proline transport (Fig. 2b), but an effect was observed at the end of the experiment (25 min; Fig. 2a), which is discussed below.

Additional arguments against active secondary transport of INH are provided by uptake experiments



**Fig. 4.** Effects of low temperature on INH and glycerol initial uptake. Ordinate as in Fig. 2.  $\blacklozenge$ , Control assay;  $\square$ , uptake at melting-ice temperature. (a) INH uptake. (b) Glycerol uptake.

performed under conditions known to strongly inhibit this type of transport: initial INH uptake kinetics were modified neither in the presence of valinomycin and 50 mM KCl (which affects transmembrane potential), nor under microaerobiosis (which alters proton electrochemical potential), obtained by bubbling argon through the uptake medium (data not shown). The above data clearly indicate that INH transport is not performed by an active secondary system.

It was checked that the INH transport was not ATP-dependent by treating cells with arsenate prior to the uptake experiments. A concentration of 50 mM arsenate was chosen since it completely depleted ATP in *Mycobacterium phlei* (Prasad *et al.*, 1976). In addition, a control experiment showed that it inhibited the uptake of glycerol (Fig. 3b), a molecule that enters most bacteria by facilitated diffusion, followed directly or indirectly by phosphorylation (Lin, 1976). Arsenate did not inhibit initial INH transport (Fig. 3a), or uptake at the plateau, thus INH transport is not ATP-dependent.

It can be concluded that INH transport is not energy-dependent. However, it could enter bacterial cells by facilitated diffusion. But, in this uptake mechanism, proteins are involved both in entry and accumulation of

metabolites. To inhibit protein-linked transport, uptake experiments were performed in melting ice. Glycerol transport was abolished (Fig. 4b). In contrast, the initial uptake kinetics of INH in *M. tuberculosis* at room temperature were not significantly different from those at low temperature (Fig. 4a). Thus it is quite likely that no protein is involved in the transport of INH.

The best remaining hypothesis is that the transport of INH proceeds via a passive diffusion mechanism, followed by a metabolic processing leading to an accumulation of radioactive metabolites within the cell.

#### KatG is involved in radioactivity accumulation at the uptake plateau

As concluded above, no protein seems to participate in the transport of INH, but it is well-documented that the absence of the catalase–peroxidase (KatG) results in decreased labelling of *M. tuberculosis* cells by [ $^{14}\text{C}$ ]INH (see Youatt, 1969). As indicated above, it is known that INH is a substrate of KatG, and oxidation derivatives of INH accumulate in *M. tuberculosis* cells. Thus it is proposed that [ $^{14}\text{C}$ ]INH enters cells by passive diffusion through the bacterial envelope, and that its oxidation by KatG then leads to the accumulation of radioactive derivatives inside the cells. A consequence of this model is that the initial transport velocity of INH is independent of KatG, but labelling at the uptake plateau depends on the peroxidase activity.

In the above experiments, we did observe effects that are very probably due to low KatG activity. They did not occur at the beginning of the uptake experiments, but only after about 20 min accumulation, and they were quite significant at the uptake plateau under the following conditions. (i) Under microaerobiosis conditions, a 30% inhibition of labelling at the plateau was observed (data not shown). This may result from a low KatG activity due to the low oxygen concentration in the medium. (ii) Uptake at 4 °C resulted in a plateau reached after only about 10 min, the labelling being 40% lower than in the control (Fig. 4a). Inhibition of KatG at 4 °C was due to the known inhibitory effect of low temperature on enzyme activity. (iii) KatG inhibition by hydrazides like INH (Johnsson & Schultz, 1994; Marcinkeviciene *et al.*, 1995) explains the fact that the maximum accumulation of radioactivity decreases with increasing INH concentrations in the medium (Youatt & Tham, 1969b; Beggs & Jenne, 1970). We observed this effect at the uptake plateau in the presence of high INH concentrations (data not shown), and we checked by a semi-quantitative test (Kubica *et al.*, 1966) that the catalytic activity was significantly inhibited for the highest INH concentration tested ( $10^{-2}$  M).

It can be concluded that KatG is involved in the accumulation of radioactivity in the presence of [ $^{14}\text{C}$ ]INH. Thus it may not be fortuitous that the same  $K_{m(\text{app})}$  was obtained for 30 min uptakes by cells (Youatt & Tham, 1969a) and INH oxidation by KatG (Johnsson & Schultz, 1994) (200  $\mu\text{M}$  and 198  $\mu\text{M}$ , respectively).

### The INH uptake mechanism explains how labelling of mycobacteria by [<sup>14</sup>C]INH can differ

It is proposed here that INH enters *M. tuberculosis* cells by passive diffusion. INH is a small, water-soluble molecule, not ionized between pH 6 and 9 (Krüger-Thiemer, 1956), three properties which lead to its high diffusibility through cell barriers. It was postulated early on that peroxidase can transform INH into a radical (Winder, 1960), now proposed to be stabilized by electron resonance (Shoeb *et al.*, 1985a). This radical can react with either targets in the cells or oxygen to produce active derivatives of intracellular molecules (Shoeb *et al.*, 1985b). It has been shown recently that *M. tuberculosis* is naturally deficient in the oxidative stress response present in most mycobacterial species (Deretic *et al.*, 1995; Sherman *et al.*, 1995; Zhang *et al.*, 1996; Dhandayuthapani *et al.*, 1996); thus it is likely that *M. tuberculosis* cannot efficiently eliminate these toxic oxygen derivatives.

Ultimately, INH can give isonicotinic acid (Johnsson & Schultz, 1994), which is trapped within cells in its ionized form. As INH is a neutral molecule that enters by passive diffusion, its inside concentration cannot be higher than its outside concentration, but isonicotinic acid can be accumulated in the cells, provided that the intracellular pH is higher than the pH in the medium.

Any event that significantly lowers peroxidase activity should reduce INH oxidation and consequently isonicotinic acid accumulation. This offers a good explanation for the observed inhibition of labelling either under anaerobiosis or in the presence of haem-enzyme inhibitors like hydrazides (including INH), cyanide or iron chelators (Ortiz de Montellano *et al.*, 1983; Wimpenny, 1967; Gayathri Devi, 1975). Even the inhibition of the labelling observed after 40 h accumulation in the presence of dinitrophenol (Wimpenny, 1967) or, as in Fig. 2(a), after 25 min in the presence of CCCP, can be explained by two effects: (i) oxygen depletion inside cells due to the known activating effect of uncouplers on respiration; (ii) dissipation of the transmembrane pH difference which should increase the protonated (neutral) form of isonicotinic acid within the cells, a form which can diffuse passively through membranes, allowing an outward diffusion of this acid.

The model of passive INH diffusion and trapping of its oxidized derivatives explains why *M. tuberculosis* mutants with a low KatG activity do not accumulate label from [<sup>14</sup>C]INH. It also explains the absence of accumulation of INH derivatives and the resistance of most mycobacterial species, since they have several systems for scavenging active oxygen derivatives.

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### REFERENCES

- Beggs, W. H. & Jenne, J. W. (1970). Capacity of tubercle bacilli for isoniazid accumulation. *Am Rev Respir Dis* **102**, 94–96.
- Deretic, V., Phillip, W., Dhandayuthapani, S., Mudd, M. H., Curcic, R., Garbe, T., Heym, B., Via, L. E. & Cole, S. T. (1995). *Mycobacterium tuberculosis* is a natural mutant with an inactivated oxidative-stress regulatory gene: implications for sensitivity to isoniazid. *Mol Microbiol* **17**, 889–900.
- Dhandayuthapani, S., Zhang, Y., Mudd, M. H. & Deretic, V. (1996). Oxidative stress response and its role in sensitivity to isoniazid in mycobacteria: characterization and inducibility of *ahpC* by peroxides in *Mycobacterium smegmatis* and lack of expression in *M. aurum* and *M. tuberculosis*. *J Bacteriol* **178**, 3641–3649.
- Gayathri Devi, B., Shaila, M. S., Ramakrishnan, T. & Gopinathan, K. P. (1975). The purification and properties of peroxidase in *Mycobacterium tuberculosis* H37Rv and its possible role in the mechanism of action of isonicotinic acid hydrazide. *Biochem J* **149**, 187–197.
- Johnsson, K. & Schultz, P. G. (1994). Mechanistic studies of the oxidation of isoniazid by the catalase-peroxidase from *Mycobacterium tuberculosis*. *J Am Chem Soc* **116**, 7425–7426 (and supplementary material).
- Krüger-Thiemer, E. (1956). Chemie des Isoniazids. *Jahresbericht Borstel* 1954/55, pp. 192–424. Berlin: Springer.
- Krüger-Thiemer, E. (1957). Biochemie des Isoniazids. *Jahresbericht Borstel* 1956/57, pp. 299–509. Berlin: Springer.
- Kubica, G. P., Jones, W. D., Abbott, V. D., Beam, R. E., Kilburn, J. O. & Cater, J. C. (1966). Differential identification of mycobacteria. I. Test on catalase activity. *Am Rev Respir Dis* **94**, 400–405.
- Lin, E. C. C. (1976). Glycerol dissimilation and its regulation in bacteria. *Annu Rev Microbiol* **30**, 535–578.
- Marcinkeviciene, J. A., Magliozzo, R. S. & Blanchard, J. S. (1995). Purification and characterization of the *Mycobacterium smegmatis* catalase-peroxidase involved in isoniazid activation. *J Biol Chem* **270**, 22290–22295.
- Ortiz de Montellano, P. R., Augusto, O., Viola, F. & Kunze, K. L. (1983). Carbon radicals in the metabolism of alkyl hydrazines. *J Biol Chem* **258**, 8623–8629.
- Prasad, R., Kalra, V. K. & Brodie, A. F. (1976). Different mechanisms of energy coupling for transport of various amino acids in cells of *Mycobacterium phlei*. *J Biol Chem* **251**, 2493–2498.
- Quémar, A., Lacave, C. & Lanéelle, G. (1991). Isoniazid inhibition of mycolic acid synthesis by cell extracts of sensitive and resistant strains of *Mycobacterium aurum*. *Antimicrob Agents Chemother* **35**, 1035–1039.
- Quémar, A., Sacchetti, J. C., Dessen, A., Vilcheze, C., Bittman, R., Jacobs, W. R. & Blanchard, J. S. (1995). Enzymatic characterization of the target for isoniazid in *Mycobacterium tuberculosis*. *Biochemistry* **34**, 8235–8241.
- Quémar, A., Dessen, A., Sugantino, M., Jacobs, W. R., Sacchetti, J. C. & Blanchard, J. S. (1996). Binding of catalase-peroxidase-activated isoniazid to wild-type and mutant *Myco-*

- bacterium tuberculosis* enoyl-ACP reductases. *J Am Chem Soc* **118**, 1561–1562.
- Rozwarski, D. A., Grant, G. A., Barton, D. H. R., Jacobs, W. R. & Sacchettini, J. C. (1998)**. Modification of the NADH of the isoniazid target (InhA) from *Mycobacterium tuberculosis*. *Science* **279**, 98–102.
- Sauton, B. (1912)**. Sur la nutrition minérale du bacille tuberculeux. *CR Acad Sci (Paris)* **155**, 860–863.
- Sherman, D. R., Sabo, P. J., Hickey, M. J., Arain, T. M., Mahairas, G. G., Yuan, Y., Barry, C. E. & Stover, C. K. (1995)**. Disparate responses to oxidative stress in saprophytic and pathogenic mycobacteria. *Proc Natl Acad Sci USA* **92**, 6625–6629.
- Shoeb, H. A., Bowman, B. U., Ottolenghi, A. C. & Merola, A. J. (1985a)**. Peroxidase-mediated oxidation of isoniazid. *Antimicrob Agents Chemother* **27**, 399–403.
- Shoeb, H. A., Bowman, B. U., Ottolenghi, A. C. & Merola, A. J. (1985b)**. Evidence for the generation of active oxygen by isoniazid treatment of extracts of *Mycobacterium tuberculosis* H37Ra. *Antimicrob Agents Chemother* **27**, 404–407.
- Takayama, K., Wang, L. & David, H. L. (1972)**. Effect of isoniazid on the *in-vivo* mycolic acid synthesis, cell growth and viability of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* **2**, 29–35.
- Wang, L. & Takayama, K. (1972)**. Relationship between the uptake of isoniazid and its action on *in vivo* mycolic acid synthesis in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* **2**, 438–441.
- Wimpenny, J. W. T. (1967)**. The uptake and fate of isoniazid in *Mycobacterium tuberculosis* var. *bovis* BCG. *J Gen Microbiol* **47**, 389–403.
- Winder, F. (1960)**. Catalase and peroxidase in mycobacteria. A possible relationship to the mode of action of isoniazid. *Am Rev Respir Dis* **81**, 68–78.
- Youatt, J. (1969)**. A review on the action of isoniazid. *Am Rev Respir Dis* **99**, 729–749.
- Youatt, J. & Tham, S. H. (1969a)**. An enzyme system of *Mycobacterium tuberculosis* that reacts specifically with isoniazid. *Am Rev Respir Dis* **100**, 31–37.
- Youatt, J. & Tham, S. H. (1969b)**. Radioactive content of *Mycobacterium tuberculosis* after exposure to <sup>14</sup>C-isoniazid. *Am Rev Respir Dis* **100**, 77–78.
- Zhang, Y. & Young, D. B. (1993)**. Molecular mechanisms of isoniazid: a drug at the front line of tuberculosis control. *Trends Microbiol* **1**, 109–113.
- Zhang, Y., Dhandayuthapani, S. & Deretic, V. (1996)**. Molecular basis for the exquisite sensitivity of *Mycobacterium tuberculosis* to isoniazid. *Proc Natl Acad Sci USA* **93**, 13212–13216.

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