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pH Conditions under Which Pyrazinamide Works in Humans

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We read with much interest the recent study by Kempker et al. (1). They measured the penetration of pyrazinamide into tuberculosis (TB) cavities and the pH inside the cavities. Although the authors do not dwell much on it, they solved an academic riddle that has been the subject of debate for decades. Sixty years ago, McDermott and Tompsett demonstrated that pyrazinamide activation was achieved by low pH and that pyrazinamide worked principally under acidic conditions (2). This, and their work on persisters, laid the foundations for the model hypothesis of three *Mycobacterium tuberculosis* metabolic populations (3, 4).

Yet, where is this acidic milieu that activates the pyrazinamide? Almost 30 years ago, Crowle et al. demonstrated that *M. tuberculosis*-infected human macrophage vesicles containing *M. tuberculosis* were not acidic and that changing vesicle pH did not change pyrazinamide killing of the intracellular *M. tuberculosis* (5). Our work with simulations of exposure versus effect derived from the hollow-fiber model almost 10 years ago suggested that the most likely scenario in humans was that the acidic milieu was extracellular (6). However, this was recently contradicted by direct measurement of pH in necrotic TB cavities of C3HeB/FeJ mice; pHs were 7.39 (range, 7.19 to 7.54) in mouse lesions and 7.23 (range, 6.99 to 7.52) in guinea pig lesions, which meant that the low pH under which pyrazinamide works must be inside mammalian cells (7). So, what gives? Last year, we identified RNA sequencing signatures and measured the pH inside *M. tuberculosis*-infected human-derived versus mouse-derived activated macrophages, as well as pyrazinamide concentration responses (8). We found that (i) the pH inside *M. tuberculosis*-infected vesicles converted to neutral pH within 7 days, (ii) there was neither a mammalian nor an *M. tuberculosis* RNA sequencing signature of low-pH exposures by day 7, and (iii) there was no pyrazinamide dose response by *M. tuberculosis* inside human-derived macrophages, as opposed to mouse-derived macrophages, in which pH remained low, and there was a good *M. tuberculosis* pyrazinamide dose response (8). Moreover, when we utilized agnostic machine learning algorithms to identify factors predictive of outcome in Indian children with TB, pyrazinamide concentration was the main driver in older children with adult-type cavitary disease but disappeared and was replaced by isoniazid and rifampin in children younger than 3 years in whom TB was caused by intracellular *M. tuberculosis* (8, 9).

Kempker et al. have measured both the pH and the pyrazinamide concentrations in human cavities in surgically explanted lungs of adult TB patients (1). In all tissue samples with necrosis from acellular parts of the cavity, the pH was ≤5.5. This settles the argument once and for all: in human TB cavities, pH is often 5.5 but is neutral in murine TB cavities. This is a caution for TB studies that seek to disprove one preclinical model using another. Rather, the true gold standard is comparison of each preclinical model to human pathology itself; "the proper study of mankind is man" (10). Each model should be proven for accuracy against what is seen in clinics, not

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what is seen with other preclinical models. After all, as George Box famously said, "all models are wrong, but some are useful."

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