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Wells Revisited: Infectious Particles vs. Quanta of *Mycobacterium tuberculosis* Infection–Don't Get Them Confused

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Introduction

Perhaps the clearest thinker on the subject of airborne infections to date was William Firth Wells, a sanitary engineer born in Boston in 1887. Wells trained in the military and later conducted seminal experiments at Harvard University between 1930-1937, and at the University of Pennsylvania 1937-1944, producing clear evidence for the airborne spread of infection. Charles Chapin, the influential health officer of Providence, Rhode Island, had dispelled the importance of airborne infection in his 1910 monograph, "Sources and Modes of Infection" [1]. Well's challenge to that belief is summarized in his still relevant 1955 masterwork, "Airborne Contagion and Air Hygiene: An Ecological Study of Droplet Infections" published by Harvard University Press [2]. In the preface Wells attributes the crucial distinction between droplet nuclei (airborne inhalable dried residua of larger respiratory droplets) and germ-laden dust (non-inhalable larger respiratory droplets) to Richard L. Riley, a medical student working with him at Harvard at the time. Riley became Wells' lifelong protégé, and between 1957-1962 conducted the classic human-to-guinea pigs studies in Baltimore that Wells had long before envisioned to prove beyond doubt that contact with contaminated air was sufficient to result in *Mycobacterium tuberculosis* transmission[3-6]. That brilliant experiment quantified infection, showing great patient to patient variability in infectious source strength, and also, importantly, that effective chemotherapy almost immediately stopped human to guinea pig transmission.

Less well understood and appreciated is the importance of Wells' introduction of the term "quantum" to represent the minimum dose of *M. tuberculosis* necessary to cause infection in the host. Quantum is the Latin word for amount and, in modern understanding, means the smallest possible discrete unit of any physical property, such as energy or matter, and in this case, unit of contagion. Not knowing for sure how many airborne infectious particles (conceivably containing more than one infectious microorganism), Wells used quantum or quanta (q) to describe whatever that unknown number was. For example, in the vulnerable guinea pig, good evidence showed that infectious could be caused by inhaling, on average, just one culturable airborne particle, so q=1. However, in a more resistant host (a BCG vaccinated or previously infected man, for example), many inhaled infectious droplet nuclei might not result in sustained infection (measurable by tuberculin skin test or risk of disease) because of enhanced innate, adaptive, or even "learned" immunity – a recently described immunological response between innate and adaptive immunity. With greater host response, many more inhalations might be necessary for transmission and sustained infection, so q=1+X, the exact number rarely known and likely to be highly variable by host, immune status, organism virulence, and even region of the lung. Finally, Wells understood that inhalation and infection was an inherently statistical process involving low probabilities due to dilution and other factors, and he introduced the Poisson distribution in his definition of quanta:

"The response induced by infective droplet nuclei is quantal; the probability that an airborne particle, drifting at random indoors, will be breathed before it is vented is governed by chance. The number of occupants who become infected bears a Poisson relation to the number of infective particles which they breathe; 63.2 per cent of the occupants will be infected when, on the average, each occupant breathed 1 infective particle. Hence, by definition, 36.8% of the occupants homogenously exposed to quanta of infection will not respond. Thus, in our experiments with rabbits 1 tubercle of Ravenel strain constitutes a quantum of infection when breathed as a fine droplet nucleus" [2]. (pp 140-141 Wells)

This definition of infectious dose, being circular, is dissatisfying to those investigators who insist on a definite number, or even an average number, to apply to human infection, which is certain to be much more variable than guinea pig infection due to variable genetic, perhaps epigenetic, and adaptive immunity, in addition to varying microbial virulence. However, the subsequent human-to-guinea pig studies by Escombe and colleagues in Lima, and by Nardell and colleagues in South Africa, have continued to report infectious quanta, not particles, because what the guinea pig transmission model measures is infections, sometimes transient, sometimes sustained and sometimes leading to disease - not infectious particles[7-11]. Those successful, sustained infections represent the end result of a cascade of much larger numbers of potentially infectious particles. From the host perspective, not all inhaled infectious particles are virulent or settle into alveoli, and not all virulent *M. tuberculosis* reaching the alveoli overcomes local innate or adaptive immune responses to initiate or sustain infection within a macrophage – an essential target cell. Not well-studied, but it is believed that only a fraction of airborne (preferentially intercellular) organisms remains viable during the stress of aerosolization, dehydration, and airborne transport, and even smaller fraction are both viable and remain virulent enough to initiate sustained infection in an immunocompetent host.

M. tuberculosis has not been cultured successfully from ambient room air by microbial air sampling due to overgrowth by much more numerous and rapidly growing environmental organisms, especially fungi and rapidly growing environmental mycobacteria. With the advent of nucleic acid amplification, however, some investigators have attempted to detect and quantify organisms concentrations close to the source, or in special test chambers with little ventilation [12]. The result has been an apparent disparity between the very large concentration of nucleic acid copies (viable and non-viable) detected and the relatively low number of infections reported by the three human-to-guinea pig investigations.

In a recent paper in this journal Issarow and colleagues attempted to explain the apparent disparity between their findings of large numbers of nucleic acid copies (that they call "infectious particles") and few infections reported in the guinea pig model [13]. Their hypothesis is that the human-to-guinea pig model must be wrong, far too insensitive to detect the large numbers of infectious particles that they document. They further suggest that the reason is likely due to excessive room ventilation in the human to guinea pig studies, diluting infectious particles, in addition to a failure to account for the large number of particles that impinge on the upper airways. They propose a variation of the steady-state Wells-Riley mass balance equation in which the alveolar settling faction is included. Using their model, with two key assumptions (first, every infectious droplet reaching the alveolus results in sustained infection; and second, the average infectious dose in the guinea pig is 10-50 infectious particles), they conclude that their hypothesis is correct, that the human-to-guinea pig models of Riley, Escombe, and Nardell are insensitive and do not accurately reflect real world transmission, especially in epidemic regions like Cape Town, South Africa [13].

Unfortunately, the authors have confused infectious quanta with infectious particles. None of the 3 studies have reported concentrations of infectious particles. They did not measure particles. Based on the number of resulting guinea pig infections, they have reported concentrations of infectious quanta, which by definition already incorporates unknown, but potentially very substantial losses along the cascade from great numbers of released organisms (dead and alive), further die off in air, dilution losses due to room volume and ventilation, chance inhalation, failure to reach alveoli, and importantly, failure to initiate sustained infection in a host for a variety of possible reasons. To compare infectious particles to quanta is far worse than comparing apples and oranges. Quanta measurement in the guinea pig model reflect the end result, sustained infection of a more or less uniformly vulnerable animal host, whereas quantifying (potentially) infectious particles begins at the infectious source, or the immediate room environment, and uses the detection of microbial DNA, infectious or not. These are totally different concepts – both potentially useful – but not comparable even with a mathematical model because of the number of unknown variables, such as microbial virulence, host immunity, and environmental stress on microbes airborne microbes, to name a few.

Finally, in using their model, the authors made two serious errors in their assumptions. Clearly not every droplet nucleus reaching the alveolus results in sustained infection [9,14]. Preventing initial infection is the role of innate immunity, and there is evidence that it can be enhanced by prior exposures, so called "learned immunity" and early infections can be aborted by adaptive immunity without detection. As noted by the authors, we devote an entire paper to describing the phenomenon of transient TB infection in naturally infected guinea pigs – also observed by Riley and now by natural transmission studies at Porton Down, UK (personal communication, Ann Rawkins, 2016), and confirmed by in vitro gamma interferon release studies. Reversion of human *M. tuberculosis* infection is also well described among human contacts in outbreak situations [15]. Secondly, the authors use 10 – 50 cfu as the infectious dose for the guinea pig and attribute the value to chamber studies by David McMurray and colleagues [16,17]. However, that is the standard, so called, "low dose" exposure protocol selected for experimental, not natural infection, where the goal is to infect all 6 to 8 exposed guinea pigs with certainty during 20 m exposure to standard, virulent laboratory strains of *M. tuberculosis*. Natural exposures require months to achieve high rates of transmission. There are many differences between human-to-guinea pig natural transmission of clinical strains and experimental guinea pig infections with aerosolized, cultured laboratory strains. In McMurray's and all chamber exposure studies, the infecting dose was selected for efficiency, not measured as the lowest effective dose (quanta). The assumption of 10-50 cfu has nothing to do with natural transmission.

There is good evidence of mostly single foci of infection in human children and in naturally infected guinea pigs [18,19]. How many infectious droplet nuclei were actually inhaled to result in one sustained infection in humans is never known, and must vary from infection to infection. They need not be contemporaneous inhalations. Smith suggested that exogenous reinfection may be a critical alternative pathway to apical lung cavitation in regions of high exposure and heightened acquired immunity from either BCG or repeated natural infection [20]. Recently, animal work suggests that immune exhaustion from repeated exposure may lead to sustained infections that would otherwise not have occurred. Repeated exposures over time leading to immune exhaustion and sustained infection can be considered another form of dose in tuberculosis transmission [21].

Finally, the effects of dilution on the calculation of quanta in human-to-guinea pig exposure studies is accounted for by using the Wells-Riley equation, which incorporates the room ventilation rate, Q [22]. Due to experimental differences, and differences in source strength (infectiousness) between TB patients in Baltimore in the mid-twentieth Century, and South African now, validated infections were rare events in Riley's longer exposure studies compared to shorter exposures in South Africa where 75% of 360 animals were infected over 4 months exposure to patients[18]. However, Riley calculated that the reported rates of student nurse skin test conversion in hospitals in the pre-chemotherapy era could be explained by the low concentrations estimated for his human-to-guinea pig exposure ward [23]. Tuberculosis epidemiology suggests that only 1 in 3 patients with smear positive pulmonary tuberculosis infects any contacts. Moreover, cough air sampling has also found that only about one in 3 patients with smear positive TB is positive by aerosol culture [24]. Finally, where human contact testing has been used to estimate q in transmission situations, the results are in range with those reported by human-to-guinea pig transmission studies, especially since almost all patients in human-to-guinea pig studies have been on treatment, and only unsuspected drug resistant cases have transmitted [11,25].

In conclusion, thanks in part to the remarkable insights of William Firth Wells and his extremely useful human-to-guinea pig model, we do understand much about the fundamentals of human-to-human transmission. Of course, there is still much to learn, especially about the aerobiology of *M. tuberculosis* – how this intracellular organism adapts to the stresses of aerosolization, dehydration, and airborne transport, and how relatively few surviving organisms manage to overcome host defenses, perhaps through repeated exposures, to achieve sustained infection leading to disease. Studying source strength by detection of microbial nucleic acid may well contribute to a greater understanding of aerobiology, but it in no way discredits 70 years of human-to-guinea pig transmission studies. Most important, of course, is to understand how we can better interrupt transmission at the source, in the air, and in the host, preventing sustained infection and progression to disease.

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