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Please Forget to Floss: Developing an Assay for Identifying Tuberculosis in Dental Calculus from the Smithsonian's Huntington Collection (1893-1921)

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Abstract

Tuberculosis is one of the most ubiquitous diseases in human history. Despite the long history of human interactions with the disease, tracking it retroactively is difficult because of its low rate of associated, diagnostic bony changes. Ancient DNA, also called aDNA, is a novel method for examining the presence of disease in the past. Currently, the only way to isolate tuberculosis aDNA is to drill and section bone, a method that is both invasive and expensive, limiting its use in the archaeological record. This capstone examines new ways of tracking and understanding tuberculosis in the past, utilizing the DNA trapped in calcified dental plaque, or dental calculus.

Tuberculosis DNA has never been isolated from dental calculus before. In this paper, I report the first-ever successful isolation of tuberculosis aDNA from the dental calculus of 13 individuals from the Smithsonian's Huntington Collection (1893-1921). Calculus from two unaccessioned mandibles, as well as calculus from four Irish immigrants tested positive for tuberculosis, illustrating that dental calculus is a good reservoir for tuberculosis aDNA. Two of the Irish immigrants had no other indicators of tuberculosis, either skeletal or archival, and their identification as tubercular enriches our understandings of their lives, illustrating the fruitfulness of this technique.

Executive Summary

Tuberculosis is a bacterial disease caused by a group of closely related bacterium known as the *Mycobacterium tuberculosis* complex (MTBC). Appearing in the skeletal record as far back as 9,000 years ago, some researchers estimate that tuberculosis has been evolving alongside humans since we left Africa some 60,000 years ago. Tuberculosis primarily attacks the alveolar region of the lung, or the area of the lung where gas is exchanged. In 2015, 1.8 million people died of tuberculosis, making it the leading infectious disease killer. As tuberculosis strains resistant to antibiotics begin to emerge, it is more critical than ever that we gain a wider comprehension of the history of tuberculosis's interactions with humans, in order to better understand of the evolutionary and population dynamics of tuberculosis.

During the 19th century, tuberculosis was called consumption, and was thought to be caused by poor constitution. Associated with white upper-class femininity, consumption was considered a fashionable way to die. During the end of the 19th century, with the discovery of "germs" and rising middle-class fears about immigration, it was transformed to an infectious disease of moral and intellectual failing caused by uncleanliness. These social meanings have been understood as separate from the "biological" understanding of the disease, an approach that has limited the study of tuberculosis. The result is a refusal to confront the ways in which the "doings" of tuberculosis is shaped by a variety of forces, as well as how these forces act upon the body to affect bodily understandings of, and the very character of, tuberculosis.

The primary method of studying tuberculosis in past populations is to look for changes to the bone caused by prolonged infection. Unfortunately, diagnostic bony changes associated with tuberculosis are rare, making it difficult to identify skeletons that

had the disease. In recent years, a new method has emerged: the isolation and sequencing of ancient DNA (aDNA). Bone is composed of a calcium phosphate matrix. DNA gets trapped in the matrix, where it is protected from degradation. Researchers can extract aDNA from bone and sequence it. Because tuberculosis DNA has been isolated from bone, researchers have a new method for studying the disease in the past. However, drilling and extracting bone samples is invasive and expensive, limiting the use of this method. In recent years, researchers have begun to isolate aDNA from dental calculus, or calcified dental plaque. Sampling dental calculus is less invasive, making it a more attractive method for studying disease in the past. However, no study until now has successfully isolated and amplified tuberculosis from dental calculus.

Dental calculus from 13 individuals from the Smithsonian's Huntington Collection (1893-1921) was collected using a dental scalar. These individuals included seven mandibles unassociated with bodies, and six Irish immigrants with known histories and cause of death. Prior to collecting the calculus samples, the mandibles and maxillae were photographed and a dental inventory was taken. In order to release aDNA from the calcium phosphate matrix in which it was persevered, the calculus was decalcified, releasing the DNA into an aqueous solution. A phenol-chloroform extraction was then utilized, to separate the DNA from proteins. Using this purified extract, which contained small, damaged fragments of the DNA trapped in the calculus, I ran a nested polymerase chain reaction (PCR), which amplifies specific sequences of DNA so that they can be sequenced. A 123 base-pair fragment of an insertion sequence in the 16s rRNA gene of MTBC was targeted. The *IS6110* sequence is specific to MTBC and present in multiple copies throughout the genome, thus serving as a good diagnostic sequence. The products

of the PCR reactions were run on an agarose gel, which separates DNA sequences by size, allowing the identification of the presence of specific DNA fragments with known lengths. The reactions that showed bands at 123 base-pairs were cloned into *E. coli* and subsequently sequenced to confirm the presence of MTBC DNA, as they were too small to sequence as they were.

Two of the unassociated mandibles and four of the Irish immigrants tested positive for tuberculosis, demonstrating that tuberculosis aDNA can be isolated from dental calculus. Although future work needs to be done to understand the rate of efficacy of this method, as well as see if full genome sequences of tuberculosis strains can be generated from the DNA trapped in calculus, this result is promising and shows the utility of dental calculus for studying tuberculosis in past populations. Hopefully this study will pave the way for more molecular studies of past tuberculosis strains and human-bacterium interactions.

Importantly, the individuals in this study would have had tuberculosis during the second half of the 19th century. This was a time when public health efforts were being advanced in Europe and America, partially as a response to the "polluting" effects of immigrants. Tuberculosis became inexplicably tied up with middle class fears over degeneration of the population, and was transformed into a disease of poor hygiene and overcrowding rather than constitution. The middle-class demanded state-level public health programs to deal with the dirt and filth of the lower class, particularly for the "mass treatment and *isolation* of the tubercular. The six tubercular individuals in this study likely understood tuberculosis, and their own disease and manifestations in this context. Perhaps this caused emotional distress, or shame as their bodies came to embody

dirt and degeneracy. Certainly the inadequate conditions in which these individuals lived, their limited access to the fresh food, and the general stress they were under exacerbated the course of the illness, perhaps leading to more severe symptoms. By combining historical, skeletal, and molecular data, a more nuanced picture of the lives of these individuals is formed, as the ways in which their (tubercular) bodies were made becomes clearer.

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Preface

This work comprises my thesis in biology and anthropology, two related but disparate fields. Throughout this work, I have strived to incorporate their methods, theory, and perspectives. I hope I have succeeded, for, following in the steps of Bruno Latour and Annemarie Mol, I believe it is impossible to separate nature (biology) and culture (anthropology). By taking an approach that views the body as a material reality constructed in relation to other bodies, other things, other species, and the wider environment, I have tried to shed new light and a new perspective on tuberculosis and the tubercular.

Although I feel that I have, by my standards, succeeded in this regard, I am still slightly nervous. Biology and the "hard" sciences are not always welcoming to social theory, just as anthropology and the "soft" sciences are not always welcoming to scientific interrogations of the body. If you are reading this from a position firmly in one of these fields, or another place altogether, I ask that you keep an open mind during discussions of concepts not in your field. I like to think that research methods and epistemologies fall on a scale ranging from an ultra focus on "nature" to an ultra focus on "culture." There is room for, and indeed we need, a variety of perspectives along this continuum. Darwin saw speciation as a process in which "differences blend into each other in an insensible series" (Darwin 2009 [1859], 51); I choose to see academic disciplines as an insensible series, a space for blending. I hope this has, at least somewhat, come across.

Acknowledgments

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CHAPTER 1

Purity, Dirt, and the Tubercular Body: Toward a Multiple Ontology of Tuberculosis

"Tuberculosis is a fascinating disease which challenges our ability to unravel the multitude of factors which enable it to flourish"(Roberts and Buikstra 2008, 44)

One of science's biggest goals has always been to "triumph" over disease and suffering. The annihilation of tuberculosis, the leading infectious disease killer worldwide, is considered a goal of great scientific achievement. But what tuberculosis *is* is not as simple as it seems. Today, it is commonly accepted to be a painful disease caused most commonly by an infection of the bacterium *Mycobacterium tuberculosis*, and in rarer cases by another species in the *Mycobacterium tuberculosis* bacterium complex (MTBC), a group of closely related bacteria responsible for tuberculosis. But in the 19th century, it was a romantic way to die, a disease caused by inherent bodily weakness and not foreign agents—at least for a certain *class* of people. In this chapter, I will trace the history of the infection associated with the bacterium *Mycobacterium tuberculosis* from the romantic, non-infectious disease of consumption, to the dirty and dangerous disease of tuberculosis, a switch that occurred during the second half of the 19th century.

Although this ontological change has been widely examined in the social sciences, it has always been treated as a social transformation, with no examination of the biology or etiology of the disease. This treatment is not uncommon in medical anthropology, where researchers focus on the social aspects of a disease, or "illness," while leaving the biology of it unquestioned. Using a theoretical framework of disease developed by Annemarie Mol (Mol 2002), a "multiple ontology" framework, it becomes clear that the shift from consumption to tuberculosis was also accompanied by a shift in

how tuberculosis, as a "biological" disease, was experienced and conceived, allowing us to envision multiple ontologies of tuberculosis.

Mycobacterium tuberculosis

Mycobacterium tuberculosis is a pathogenic, obligate aerobic bacterium (Cole et. al., 1998). *M. tuberculosis* primarily infects the alveolar region of the lungs, the area of the lungs where gases are exchanged. The bacterium often spreads systemically, causing the disease that is currently referred to as tuberculosis. Each time an infected person coughs or sneezes, thousands of *M.* tuberculosis cells are expelled in aerosolized droplets. These droplets are then breathed into the lungs of someone close by, where the bacterium starts its life cycle in a new host (Roberts and Buikstra 2008). Although host macrophages and T cells (immune cells) will try to wall off the bacteria or trigger apoptosis, or programmed cell death, *M. tuberculosis* has developed ways to prevent host immune cells from killing it (Yuk and Jo 2014). As its name suggests, *M. tuberculosis* is in the mycobacterium genus and, as such, has a waxy coating made of mycolic acids (a specific type of long chain fatty acid) around its cell wall. These mycolic acids help protect the bacterium from degradation by the lysosome of alveolar macrophages (Cole et. al., 1998). Instead of fusion of *M. tuberculosis*'s cell wall with the macrophage's lysosome, *M. tuberculosis*'s cell wall fuses with nutrient vesicles within the macrophage (Yuk and Jo 2014). This is *M. tuberculosis*'s main way of obtaining nutrients. Inside the macrophage, *M. tuberculosis* cells multiply, eventually killing it.

When initially infected, only the alveolar area of the lungs is affected. This is called primary tuberculosis. However, *M. tuberculosis* can travel to other areas of the body, including the cranium and acetabulum joint, causing secondary, or post-primary

tuberculosis (Roberts and Buikstra 2014, 19). Many symptoms are associated with primary tuberculosis, most notably the frequent coughing of blood or sputum. However, difficulty breathing, weakness, lethargy, loss of appetite and corresponding weight loss, chills, night sweats, irritability, pallor, female amenorrhea (cessation of menstruation), male impotence, chest pain, and high fevers are also common (Roberts and Buikstra 2014, 20). Left untreated, tuberculosis leads to death almost 50% of the time (Gagneux 2012).

From Phthisis, to Consumption, to Tuberculosis

One of the oldest and most ubiquitous infectious diseases in human history, dating back at least 6,000 years, tuberculosis has gone by many names (Zimmer 2014). The ancient Greek philosopher Hippocrates (460-375 B.C.E) referred to pulmonary tuberculosis as phthisis. Phthisis comes from the Greek word *phthien*, which means "to decay or waste away" (Roberts and Buikstra 2008, 8). The symptoms most commonly associated with phthisis were "wasting, coughing of blood, and the formation of empyema" (Frith 2014, 30). Most physicians thought phthisis was a heritable disease, and related to weak disposition. Hippocrates, a 5th century B.C.E Greek physician, believed that having pale, smooth skin, blonde hair, blue eyes, pallor, and winged shoulder blades (an abnormal angle of protrusion of the scapula) predisposed individuals to phthisis (Roberts and Buikstra 2008, 52). Physicians encouraged moderate exercise, "plentiful and good food," sea voyages, and long walks in cypress groves to strengthen "those 'weak in the lungs'" (Frith 2014, 30).

By the 18th century, tuberculosis had reached epidemic proportions in Europe and North America (Daniel 2006), and was most commonly referred to as "consumption" or

"the white plague/death," although it was also sometimes called "the robber of youth" (Frith 2014, 32). As in the ancient world, consumption was widely believed to be hereditary or caused by internal weakness, in large part because whole families would die of the disease. Starting in the 18th century, most Italian physicians believed that phthisis was infectious, but this view was not widely shared by American and British physicians, who thought consumption was caused by "constitutional weakness," weakness that was often hereditary (Frith 2014, 33). Even the build of the body was seen to put a body at risk, as 18th-century physician Frederic Hoffman believed that tall people with long necks were predisposed to the disease (Roberts and Buikstra 2008, 52).

Importantly, consumption was a romantic disease of the white upper-class woman. In many ways, it stood for innocence, fragility, and beauty. The term "consumption" refers to the extreme weight loss that often accompanies the disease, as people literally "eat themselves" (Roberts and Buikstra 2008, 9). The phrases "white plague" and "white death" reference the pale, anemic pallor of the consumptive, while also evoking ideas of "childhood and innocence" (Roberts and Buikstra 2008, 10). To have consumption was to embody fragility and whiteness, two concepts inextricably linked with upper-class femininity, and to be fashionable and desirable, especially to men. Women without consumption "drank lemon juice and vinegar to kill their appetite and dressed in white" to emulate the aesthetic of consumption (Roberts and Buikstra 2008, 220). Although coughing up blood and sputum was recognized as a symptom of consumption, the most privileged manifestations were wasting and paleness.

Because of consumption's associations with upper-class femininity, it was often used as a poetic device in literature and art. Edgar Allen Poe described his young wife

dying of consumption as “delicately, morbidly angelic” and “ethereal” (Poe in Frith 2014, 32). The poet George Lord Byron once remarked that he “should like, I think, to die of consumption” because “all the women would say ‘See that poor Byron— how interesting he looks in dying’” (Byron in Frith 2014, 32). The protagonist of *Wuthering Heights*, Catherine Earnshaw, had consumption, and the literary tradition of vampirism often invoked the “imagery of the consumptive” in descriptions of both vampires and their victims (Frith 2014, 32). Images of beautiful, sad-looking young women with consumption were common in the art of the 18th century (Roberts and Buikstra 2008, 222). One particularly popular artistic trope was that of a young, beautiful white woman lying limp in bed with the specter of death hovering over her (**Figure 1**).

Figure 1: A young white (presumably) upper class woman lounges elegantly on her deathbed, as the specter of death looms over her (Richard Tennant Cooper, 1912, *The Wellcome Library)*.

However, during the second half of the 19th century, a dramatic shift occurred. Consumption became tuberculosis and "quickly transformed from a disease of the upper class to a disease associated with poverty and morally questionable immigrant masses" (Pearlstein 2015, 97). Social historians, anthropologists, and sociologists attribute this shift to the new medical philosophy of "germs" as causative agents of disease and bad health that occurred during the second half of the 19th century. For tuberculosis, this shift was facilitated by the discovery of "the *Tubercle bacillus*" (which would later be renamed *M. tuberculosis*) in 1882 by Robert Koch (Frith 2014, 33), as the causative agent of the disease. Acceptance of germ theory accompanied, or may have been spurred by, increasing attempts to eradicate "dirt" and "filth," often synonymous with the poor, rural, and "backward" (Barnes 2006, 217; Craddock 2001). Marc Arnold, author of *Disease, Class, and Social Change,* argues in the introduction to his book that not only does "scientific knowledge impact(s) upon society by affecting cultural perceptions and social policy" but that "scientific accounts of *natural* phenomena are themselves continually reconstructed in accordance with prevailing cultural beliefs" (2012, 1).

The discovery of *M. tuberculosis* spurred public health initiatives and a new focus on tuberculosis as a disease of poor hygiene. Yet it was only because of an already existing public health focus on hygiene and cleanliness that *M. tuberculosis* was accepted as the new "causative factor" of tuberculosis. The second half of the 19th century also saw the rise of social Darwinism and political fears about immigrants and population degeneration (Arnold 2012, 4). It was within this social context that tuberculosis became a disease of poor hygiene and overcrowding, and cries from the middle class for "the mass treatment and isolation of the tubercular" arose (Arnold 2012, 7). To have

tuberculosis was to be dirty. Coughing, an act that traverses the "boundaries" of the human body, suddenly became dangerous to everyone around the sick individual.

Purity and Disease: The Dirty, Tubercular Body

Although there may have been truth to the association of tuberculosis with poor, often immigrant populations (due to overcrowding and a weakened immune system caused by limited access to food, clean water, and other resources), tuberculosis as a poor, "foreign" disease is best approached through understandings of purity and impurity. Dame Mary Douglas, a well-known English anthropologist, argues that Western practices of hygiene and contamination through germs and other external agents must be understood as pollution beliefs. Dirt and germs are not natural medical categories. Dirt is "matter out of place," and is created through a system of ordering and classification, "insofar as ordering involves rejecting inappropriate elements" (Douglas 2002 [1966], 44). Dirt is always dangerous, because it threatens the structure and continuity of society. Pollution beliefs often involve the margins of the body: the mouth, anus, vagina, penis, etc. "The body is a model which can stand for any bounded system. Its boundaries can represent any boundaries which are threatened or precarious" (Douglas 2002 [1966], 142). This is why the coughing of a tubercular patient is so dangerous. It is a transgression of the boundaries of the body with a foreign agent. Bacterium and germs are dirt, in other words, dangerous matter out of place.

The association of dirt, disease, and social boundaries is geographically and temporally widespread, even in "western, scientific" temporalities. For example, Jacques Botrel, a French doctor during the second half of the 19th century, was convinced that typhoid fever was widespread in poor, rural French towns because of the townspeople's

"insufficiently civilized way of life" (Barnes 2006, 194). Botrel's campaign against diseases like typhoid fever was really a crusade against "rural backwardness." Botrel had already been involved in a campaign against "filth" and "miasmas" before germ theory gained prominence. But Botrel's crusade against 'filth" was easily merged with germ theory as "the causal role of filth was refined, and bacteria could actually be found in certain disgusting substances" (Barnes 2006, 203).

While the rural poor were long considered backward and dangerous, "science" could now be used to confirm up their status as dangerous contaminates to the French population. Indeed, it was their proximity to urban, "civilized" French society that made them particularly dangerous: "filthy Frenchmen stood closer to the bourgeois ideal on the scale of civilization than did their colonial counterparts and because of their spatial proximity, represented a more immediate threat to the sensibilities and health of their compatriots. Less exotic, they were therefore even more disgusting" (Barnes 2006, 222). They were not exotic, but their habits were also distinctly "not French." They were close categorically, and spatially, to pure, urban Frenchmen, but they did not neatly fit into this category. They were anomalous, and, as Douglas argued, that made them impure and dangerous. Pollution and pollution behaviors effected and transformed understandings of sick, French bodies.

In the same way that typhoid fever became emblematic of impure, uncivilized Frenchmen, consumption was also transformed to a disease of impurity— tuberculosis. Consumption was not viewed as infectious and polluting, both because of its association with upper-class white women's bodies and because it was viewed as bodily weakness rather than an infectious foreign agent. "Tuberculosis" became something polluting and

dangerous through its association with both “germs” and the lower class, often immigrant bodies, who were already threatening the boundaries of the “middle-class” and “respectable” society (**Figure 2).**

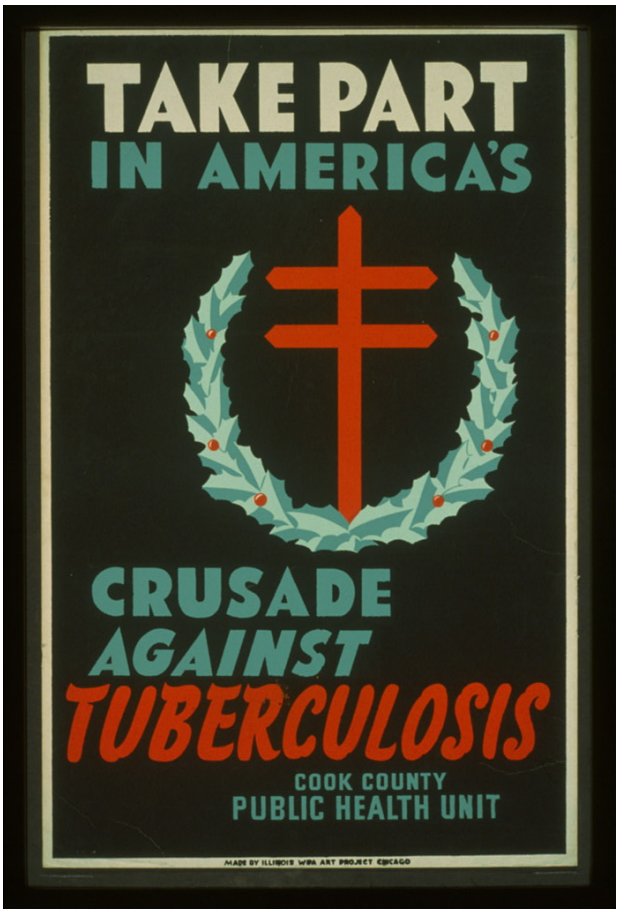

Figure 2: Example of early 20th century public health propaganda link the nation’s well-being with germs, specifically TB (retrieved from Barely a Historian).

The advent of germ theory served to establish a “new kind of truth in which the existence of a particular microbe in a tested sample was enough to identify a person, place, or thing as a health hazard, regardless of whether actual cases of disease were associated with that person, place, or thing” (Barnes 2006, 9). Bodies and substances labeled as containing the “tuberculosis germ” were also labeled dangerous, and thus subjected to large programs of hygiene that were meant “to keep *all* people healthy… *the*

people, the population" (Mol 2002, 131). One key focus of this new public health campaign against tuberculosis was the lower class, often immigrant woman. Once tuberculosis became associated with filth and dirt, women became "gatekeepers of health because they were responsible for keeping the home clean and bacillus free" (Craddock 2001, 338). If a family had tuberculosis, it was because the wife had not done a good enough job keeping the home "pure" and protected from the invading pathogens. Isolated institutions called sanatoriums grew in popularity.

Sanatoriums served as places for women to "recover" from their tuberculosis, but also as institutions through which "civilized," middle-class behavior could be taught. Strict supervision of food, leisure, and social interactions, coupled with mandated daily labor therapy for those women not on bed-rest, was supposed to transform their impure, uncivilized, tubercular bodies into healthy ones (Craddock 2001. 348). At one sanatorium in California, the Arequipa asylum, awards for the "best smile" and "sweetest nature" were given out during "Arequipa's perfect girl" contest (Craddock 2001, 349). A focus on improving the home, both through "work" (i.e., cleaning and raising moral children) and decorum served to bring lower-class white women closer to ideals of white femininity and the "cult of true womanhood" **(Figure 3)** [Craddock 2001, 340]. Consumption was emblematic of upper class white femininity; tuberculosis was emblematic of a lack of it.

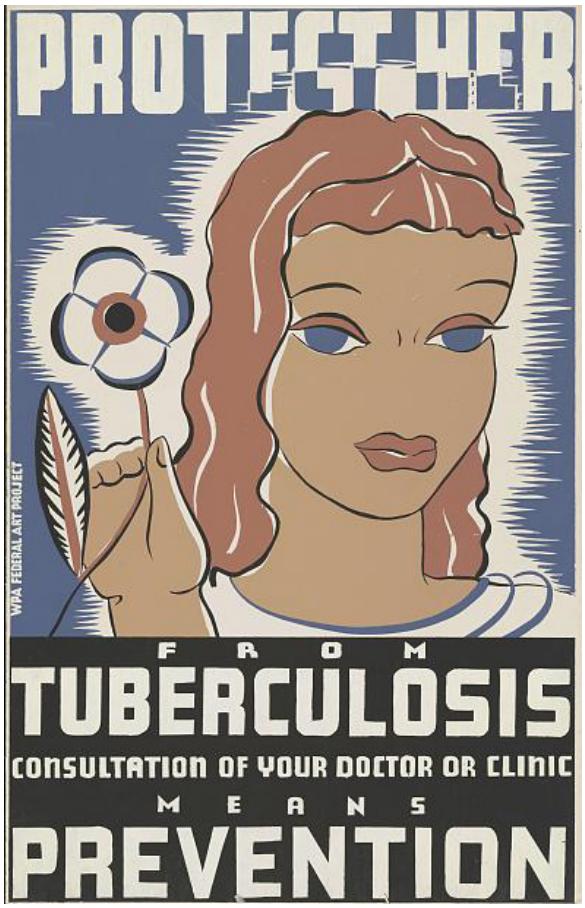

Figure 3: Propaganda from themed-20th century linking the health of white children with prevention of tuberculosis (retrieved from Barely a Historian)

Breaking Down Illness/Disease Dichotomies: Toward a Multiple Ontology of Tuberculosis

Although historians, anthropologists, and sociologists like Barnes and Arnold who studied the transformation of consumption into tuberculosis use illness and disease interchangeably, what they are really examining is the changing nature of tuberculosis as an *illness*. Annemarie Mol, in her book *The Body Multiple: Ontology in Medical Practice* (2002), argues disease and illness have been treated as two distinct categories in research. Talcott Parsons, the founder of medical sociology, defined illness as "a state of disturbance in the 'normal' functioning of the human individual, including both the state

of the organism as a biological system and of his personal and social adjustments" (Parsons in Mol 2002, 10). The social adjustments, to Parsons, were in the domain of the social scientist, while the biologists and medical doctors studied the "biological" truth of the disease (Mol 2002, 10). Social scientists have long argued that there exists a multiplicity of meanings surrounding "illness," but maintain that disease is a single, unchanging, and biologically grounded entity. By relegating disease to the field of the "hard" sciences, diseases like tuberculosis remain a single biological entity, an entity that is unquestioned and unexamined.

Mol rejects this distinction. To her disease and illness must be understood as one and the same, and the biological reality of disease is just as multiple as the "social reality" of illness. A disease can be understood in medical practice as a single disease, but even in the same time period is in fact enacted and understood as many, "slightly different one(s)" (Mol 2002, vii). Each enactment does "the body different" (Mol 2002, 176). Differing medical practices and specialties understand and treat bodies differently based on what their definition of "disease" is or what their object of inquiry is. This multiplicity means that the "*body* (singular) is *multiple* (many)" (Mol 2002, 84). "In this ontological genre, a sentence that tells us "what a disease is, is to be supplemented with another that reveals *where* this is the case" (Mol 2002, 54).

In her examination of the disease arthrosclerosis, Mol reveals how the disease is defined differently to different people in the hospital. To the pathologist, it is a thickening of a vessel wall that can cause death. To the surgeon, it is a stenosis to be scraped away. To the clinical practitioner, it is pain that interferes with someone's ability to walk. Sometimes the disease is considered severe by one medical specialist or standard but

mild by another. For example, a patient may complain of little pain on walking, but the lab results may indicate severe impairment of blood flow to a limb, which is supposed to correlate with pain on walking (Mol 2002, 66). Medical professionals will try to find a flaw in one of the diagnoses; either the lab results were faulty or the patient underreported pain. This way, they can resolve the inconsistency and make the patient's atherosclerosis a single entity again. Mol argues that instead of trying to reconcile the two, we should instead complicate the picture by accepting that it is "possible to understand the objects of two different techniques as indeed being different objects… troubles that have a relation, but not necessarily one that is linear" (Mol 2002, 66). If instead we "foreground practicalities, materialities, events," then "disease becomes part of what is done in practice" (Mol 2002, 12-13).

Through differing practices, the atherosclerotic body becomes multiple. Atherosclerosis is enacted differently, and each enactment entails a different ontology of atherosclerosis which, in turn, constructs the body differently (Mol 2002, 176). And yet all these enactments of atherosclerosis "hang together," a single disease with multiple ontologies— the body multiple. Although the body may seem like a "singular" object, different enactments of the body and of atherosclerosis produce slightly different bodies every time. And just as arteriosclerosis can be a single, multiple disease, so too, I argue, can tuberculosis. The tubercular body is multiple. There is one "causative" agent (*M. tuberculosis*), but there are multiple biological realities of having tuberculosis.

Perhaps the most obvious way the tubercular body is multiple is the fact that, as with atherosclerosis, different medical professionals studying tuberculosis define it in different ways. To microbiologists, "tuberculosis is characterized by the microbes

causing it; internists chart and treat a problem of the lungs; and since it is the task of those who work in social medicine to take care of the health of the population, they see tuberculosis as an infectious disease" (Mol 2002, 110). A single disease enacted in multiple ways. But this is not the only mechanism through which the tubercular body becomes multiple. Bodily experiences of the disease also differ.

As discussed earlier, there were two distinct understandings of tuberculosis in the 19th century. There was the romanticized, feminine, and "pure" consumption of the first half of the 19th century, and the infectious, filthy, "impure" tuberculosis of the second half. What it meant "socially" (pretending for a second that the social sphere can be separated from the biological sphere) to have tuberculosis was different in these two time periods. And yet the question of what this difference meant to the tubercular individual, how they understood their symptoms, has been left unexamined. Weight loss, pallor, coughing of blood and sputum, male impotence, female amenorrhea, high fevers, night sweats, and chest pain are all presumed to be universal symptoms, experienced the same by everyone. To have a fever is to have a heightened body temperature; to cough is to forcibly expel mucus and irritants from your lungs and throat. These are understood as biological events symptomatic of a pathological process. But to treat these symptoms as such leaves out the bodily experiences and understandings of the tubercular.

Let us examine a single symptom: the cough. The cough today is considered the most common and distinctive symptom associated with tuberculosis. A cough is considered medically dangerous for its ability to infect others with "foreign" pathogens, and for its transgression of internal bodily boundaries (Douglas 2002 [1966], 142). Speaking from personal experience, coughing can be painful, and often feels "gross."

Pain from coughing is viewed in a negative light, as coughing is indicative of bodily malfunction and, in the second half of the 19th century, suggestive of infection with a highly stigmatized illness. But does every patient with consumption experience coughing in the same way? Is it such a negative experience? Is it understood as a dangerous, dirty act caused by their disease?

The term *tuberculosis* derives from the word *tubercle*. Tubercles are round projections, and refer to a number of anatomical structures, but are also the word used to describe the nodules that form in the lungs of patients with tuberculosis (Frith 2014, 33). The name tuberculosis emphasizes coughing and lung pain. In contrast, consumption, and the contemporaneous phrase "white death," emphasizes the symptoms of weight loss and pallor instead of coughing. During the era of consumption, the bodily experience of coughing might be different from coughing during the era of tuberculosis. For one, coughing would have been viewed as an associated symptom rather than essential, ontological part of the disease. Secondly, coughing might not have been experienced in the same unpleasant way. It would not have been understood as infectious, and thus dirty and impure. The pain caused by it may have also been experienced differently, as a sort of "positive pain." Describing a sudden coughing fit of his consumptive wife, Edgar Allen Poe remarked, "suddenly she stopped, clutched her throat, and a wave of crimson blood ran down her breast… It rendered her even more *ethereal*" (Poe in Frith 2014, 32). Coughing often conjures up images of forcible expulsion. Poe's description seems to insinuate the blood flowed easily from his wife's mouth, in a less "violent" manner. Her coughing and the blood it produced only served to enhance her feminine beauty.

What is it like to experience coughing that renders you more beautiful in comparison to coughing that renders you filthier? I seek not to necessarily answer this question (at least not here), but simply to bring to light the fact that bodily experiences of tuberculosis and its associated symptoms cannot be understood as universal. There are differences in experiences between purity and impurity, filth and innocence, desirable feminine bodies and dangerous, degenerative bodies. The biological reality of tuberculosis is not fixed, but changes, just as the "social reality" of tuberculosis changes. There are multiple ontologies of tuberculosis, produced through multiple enactments and experiences of the disease, although there is connection and overlap between them. There is a single tuberculoses (I use the plural here purposefully). And the lived experiences of the tubercular body must be privileged and emphasized to a greater extent in anthropological writing on tuberculosis.

The body has traditionally been viewed as universal, and static in medicine and the biological sciences (Sofaer 2006). Bioarchaeologists, who study human remains, are traditionally thought of as studying "nature," as they use the bones left behind to elucidate the "biology" of past people. As more recent osteological research, and Joanna Sofaer in her book *The Body as Material Culture: A Theoretical Osteoarchaeology* (2006), demonstrate, the body is never static. The human skeleton is plastic; it changes in response to biosocial contexts, and is never pre-social or experienced out of context (Sofaer 2006, 74). One of the biocultural contexts that shape the body, and that the body responds to, is disease— diseases like tuberculosis. It is to the osteological study of tuberculosis that we turn now.

CHAPTER 2

Tracking Tuberculosis: Skeletal Evidence of Tuberculosis in Past Populations

Although tuberculosis, or consumption as it was known then, reached epidemic proportions in the 18th and 19th centuries (Daniel 2006), it has been around for much longer, albeit at much lower rates. Still largely an evolutionary puzzle, tuberculosis has captured the interest and imaginations of poets, artists, sociologists, biologists, and bioarchaeologists. In this chapter, a clinical overview of tuberculosis is presented, followed by a discussion of the primary method of identifying tuberculosis in past peoples: osteological examination. As I will show, skeletal studies have yielded evidence of tuberculosis in the past in places ranging from Korea to Peru. Despite this, the study of tuberculosis is still largely plagued by "absence of evidence," as tuberculosis only causes skeletal changes in a small minority of individuals. This limitation, therefore, requires the development of new techniques for studying "the bioarchaeology of tuberculosis" in the past.

Currently, more than a third of the world's population is infected with tuberculosis (although infection is latent in most of these cases). In 2015, 1.8 million people worldwide died from tuberculosis. Most of these deaths occurred in low- and middle-income countries like Pakistan, India, and China. Tuberculosis is the leading infectious disease killer worldwide, as well as the leading cause of death of HIV-positive people (WHO 2016). Despite epidemic rates of tuberculosis worldwide, both rates and mortality have fallen sharply in recent years, thanks to public health efforts and the development of isoniazid, the first oral tuberculosis drug, in 1952 (Daniel 2006). WHO

reports that 49 million tuberculosis patients were saved between 2000 and 2015, resulting in a 22% fall in mortality rates from the previous decade.

Most strains of tuberculosis can be treated with antibiotics, usually a combination of isoniazid and rifampin. However, multidrug-resistant tuberculosis (MDR-TB) is on the rise. The Centers for Disease Control (CDC) currently lists MDR-TB as a "serious threat" (CDC 2013). This threat level includes drug-resistant Shigella and Camplyobacter. These drug resistant strains are not currently the most common strain of their disease, but if not dealt with, will become serious threats in upcoming years. MDR-TB is resistant to isoniazid, rifampin, and at least one other anti-tuberculosis drug. In recent years, extremely multidrug-resistant tuberculosis (XDR-TB) has also emerged. XDR-TB is resistant to all first-line drugs, and at least one of the second-line injectable drugs (CDC 2013). The drugs used to treat XDR-TB can lead to hearing loss, psychosis, and liver and kidney damage and treatment costs for a full course of treatment can range up to $490,000 (CDC 2013). It's clear that despite many recent gains, there is still a long way to go before the WHO's goal of eliminating tuberculosis is a reality (WHO 2016). Although many in the United States think of tuberculosis as a "disease of the past," the threat of tuberculosis very much continues today.

Tuberculosis may be caused by a variety of complex closely related bacterium, referred to as the *Mycobacterium tuberculosis* complex (MTBC). The complex consists of *M. tuberculosis*, *M. bovis*, *M. microti*, *M. africanum*, *M. pinnipedii*, and *M. caprae* (Gutierrez et al. 2005). In humans, the most common causative agent of tuberculosis is *M. tuberculosis* (Muller, Roberts, and Brown 2014). *M. africanum* is the only other MTBC bacterium limited to humans besides *M. tuberculosis*, but is found only in West

Africa, while *M. tuberculosis* is found worldwide (Brites and Gagneux 2015). *M. microti* is restricted to voles, shrews, and mice, and *M. caprae* to goats and deer, but neither seem to be able to jump to humans (Brites and Gagneux 2015, Roberts and Buikstra 2004, 76). *M. pinnipedii*, found in seals and sea lions, and *M. bovis*, found in cows, can both infect humans through zoonotic transmission, usually through consumption of milk or meat from an infected animal (Brites and Gagneux 2015). Indeed, *M. bovis* was once a major problem in Britain and other Western nations, but is relatively rare today thanks to the implementation of pasteurization and strict livestock testing protocols (Gagneux 2012), although it remains a problem in areas of the world where raw milk is frequently consumed (Golden and Vikram 2005).

The question of when tuberculosis emerged or, in other words, how long ago it began its association with anatomically modern humans, is hotly contested. Estimates tend to cluster into two groups: researchers who claim that the most recent common ancestor (MRCA) is very old, between 40,000 and 70,000 years (Comas et al. 2013; Gutierrez et al. 2005; Luo et al. 2015), and researchers who claim that the MRCA is very young, or 7,000 to 6,000 years old (Bos et al. 2014; Kay et al. 2015). Differences in estimates largely result from differences in methods. Bos et al. and Kay et al. both used sequences of ancient strains of MTBC to calculate mutation rates and extrapolate back based on sequence divergence in modern strains.

Researchers who believe that the MRCA of tuberculosis is older rely less on calculated mutation rates, focusing instead on calibrated divergence times and tuberculosis population demographics. Mutation rates have been shown to decrease on deep time scales as deleterious mutations are removed from the population and alleles

become fixed, making it difficult to estimate divergence on deep time scales (Comas et al. 2013). While this issue is still being debated, it is likely that the MRCA of MTBC is on the older side, especially since evidence has been found of tuberculosis' existence 9000 years ago. What has been definitely shown is that humans gave cows and other animals tuberculosis, and not the other way around as was originally thought. Tuberculosis does not undergo recombination between strains, and so the presence of deletions in *M. bovis* and *M. pinnipedii* that are not present in *M. tuberculosis* and *M. africanum* indicates that these zoonotic strains are derived from human specialized ones (Brites and Gagneux 2015).

Tuberculosis starts in either the pulmonary tract, in the case of *M. tuberculosis*, or in the gastrointestinal tract, in the case of *M. bovis*, although the former shows a higher infectivity rate (Roberts and Buikstra 2004, 5). Once an infection is established, individuals develop primary tuberculosis. After an active infection subsides—if it does—individuals can still develop active tuberculosis again later in life, as the bacterium can lie in a latent phase until re-activation as post-primary tuberculosis. The factors triggering re-activation are complicated and poorly understood, although stress, reinfection, and changes to the immune system have been implicated (Roberts and Buikstra 2004, 5-6).

The majority of tuberculosis infections are pulmonary. About 20% of patients have extra-pulmonary tuberculosis, or tuberculosis that does not affect the lungs (Sheer and Coyle 2003). Tuberculosis that begins in the lungs and spreads is still classified as pulmonary tuberculosis, even though it affects multiple tissues (Lee 2015). The most common form of extra-pulmonary tuberculosis is infection of the lymph nodes, or lymphadenitis. Other common types include pleural tuberculosis, or infection of the

cavity surrounding the lungs, tuberculosis of the central nervous system, leading to meningitis, abdominal tuberculosis, and skeletal tuberculosis. Skeletal tuberculosis accounts for 35% of all extra-pulmonary tuberculosis cases, most commonly in the spine and weight-bearing joints such as the hip and knee (Golden and Vikram 2005). The tuberculosis bacilli establish themselves in red bone marrow, and gradually destroy bone tissue until skeletal elements collapse (Roberts and Buikstra 2004, 89).

Skeletal Involvement of Tuberculosis

Bone is a dynamic and plastic tissue that responds to both the external and internal environment of the body. Diet, chronic illness, and long-term activity all leave markers on the bone (Agarwal 2016; Ellis 2014; Phillips 2003; Roberts and Buikstra 2004). Bone responds to chronic exposure to environmental conditions and stressors, including pathogens, in two ways: by laying down new bone (periostitis) in a process that involves increased activity of osteoblasts, or by breaking down existing bone to form lytic lesions, through the increased activity of osteoclasts. However, these processes take time, meaning whatever stimulus is leading to a change in bone structure must be prolonged. In order for periostitis or lytic lesions to form, the individual must survive long enough to mount an immune response (Roberts and Manchester 2007, 7). Individuals who contracted a disease and died quickly from it will show no skeletal changes. Thus, only chronic illnesses will show up on bone.

Pathogens that kill quickly, like cholera and other viral disease, generally do not leave skeletal lesions (Roberts and Manchester 2007, 167). Chronic diseases, like tuberculosis, still only rarely affect bone. Even with skeletal lesions, it can be difficult to diagnose a specific etiology because most of these pathological changes are non-specific

(Roberts and Manchester 2007, 168). However, certain diseases, including tuberculosis, show specific patterns of lesions differentially across the body (**Figure 4**), which allow the pathological condition to be identified in skeletal remains (Roberts and Manchester 2007, 182).

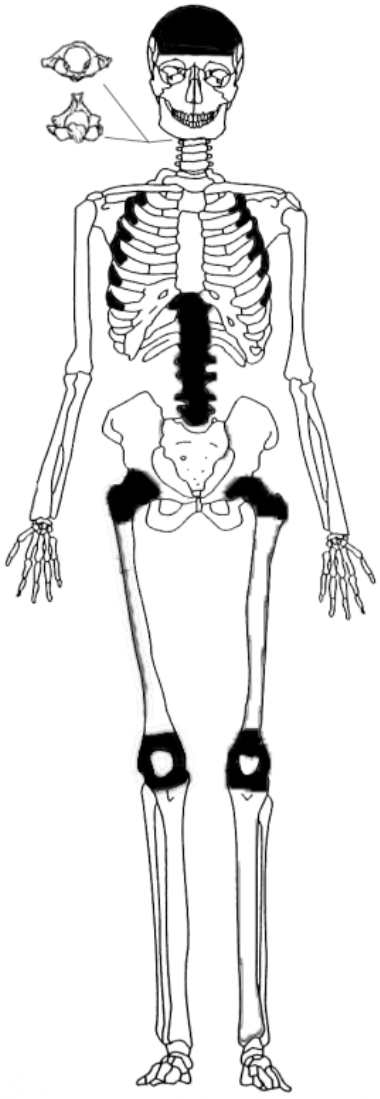

Figure 4: A distribution map indicating the skeletal elements, colored black, most commonly affected by tuberculosis. Figure adapted from Roberts and Buikstra (2004) and Buikstra and Ubelaker (1994). Credit to Matthew Bosworth.

Although rates vary, it is estimated that pulmonary tuberculosis leads to changes in bone only 1 to 5% of the time, and only as post-primary (systemic) tuberculosis. By contrast, rates of skeletal change for extra-pulmonary tuberculosis may be as high as 30% (Roberts and Buikstra 2004, 88). One of the most commonly effected elements is the spine. Destruction of vertebral bodies, beginning as lesions on the centrum of the body and slowly spreading to the outside aspects, leads to spinal collapse. The vertebral bodies re-fuse in an angled position, also referred to as kyphosis (**Figure 5**), leading to a condition called Pott's disease. This deformation is named after Sir Percivall Pott, who first described the condition in the 18th century (Roberts and Buikstra 2004, 89-92). Pott's disease usually affects one to six of the lower thoracic and upper lumbar vertebrae (Roberts and Buikstra 2004, 94-95). The presence of Pott's disease is the most reliable skeletal marker of tuberculosis.

Figure 5: The spinal column of a Neolithic man from Hungary, showing the classic kyphosis (arrow) of tuberculosis-associated Pott's disease. Image from Kohler et al., 2014.

The hip joint is affected about 20% of the time, leading to osteomyelitis of the acetabulum and proximal epiphysis of the femur (Roberts and Buikstra 2004). Osteomyelitis is an infection of the medullary cavity of the bone, and is characterized by a decrease in bone density and pitting and irregularity, as well as plaques of new bone (periostitis), on the external surface. In the most severe cases of hip tuberculosis, also called septic arthritis, the acetabulum and femoral head will be completely destroyed (Roberts and Manchester 2007, 168-169). Septic arthritis caused by tuberculosis generally affects a single element, usually the hip or knee, and tends to be more destructive than formative, whereas arthritis or osteomyelitis caused by other infections tend to be more widespread. However, caution must still be taken when using the presence of septic arthritis to diagnose tuberculosis in skeletal remains, as there is still a possibility that the degenerative processes were caused by other pathogens or general arthritic processes (Roberts and Manchester 2007, 187).

Tuberculosis affects the skull only rarely. It can lead to multiple lytic lesions perforating the cranial tables, although this is also seen in syphilis and generalized osteomyelitis, and so is not diagnostic of tuberculosis (Roberts and Buikstra 2004, 99-100). Tuberculosis of the meninges of the brain can sometimes lead to Serpens Endocrania Symmetrica (SES). These snake-like lesions (**Figure 6**), branch symmetrically late in the development of this condition (Palfi et al. 2012). It is thought that SES is the result of abnormal blood vessels in the dura mater caused by infection of the thoracic cavity, or intercranial arteriovenous malformations (AMVs). AMVs can be genetic or caused by intercranial vascular pathologies. However, like tuberculosis, AMVs are only implicated in SES (Janovic et al. 2015).

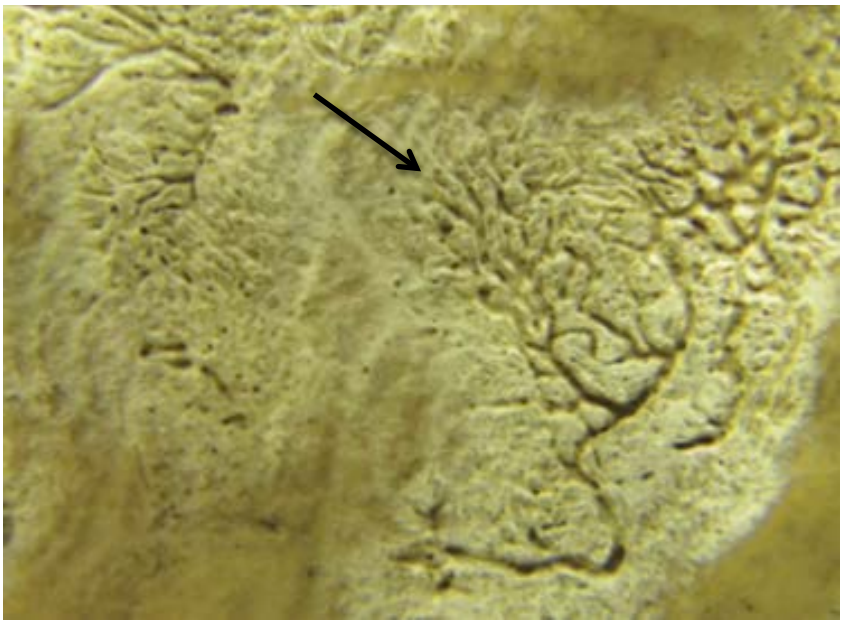

Figure 6: SES (arrow) on the endocranial surface of the frontal bone of a 17-year-old female from the Robert Terry Anatomical Collection. Image from Palfi et al., 2012.

Hershkovitz et al. (2002), who first described the term, found SES in 4% of individuals who had died of tuberculosis in the Hamann-Todd collection (1912-1938), a skeletal collection housed at the Cleveland Museum of Natural History. However, they also found SES in 0.53% of individuals who did not die of tuberculosis. It is possible that these lesions were caused by tuberculosis, and the individuals simply did not die of the disease. This condition, however, cannot be definitively associated with tuberculosis at this point, and should be viewed only as suggestive (Hershkovitz et al. 2008; Palfi et al 2012).

Skeletal tuberculosis infects the ribs about 3% of the time (Roberts and Buikstra 2004, 92). However, pulmonary tuberculosis has been shown to cause periostitis on the visceral heads and middle shaft area of ribs at a fairly high rate. This reaction stems from generalized inflammatory response to pulmonary infection, and not infection of the bone itself (Roberts and Manchester 2007, 190). An interesting study in this regard involved analysis of the Coimbra Identified Skeletal Collection, a series of remains from

Portuguese individuals who died during the first half of the 20th century and who were interred at the Cemitério Municipal da Conchada. From a sample of 171 individuals, the results showed that 85.2% of those who died from tuberculosis had periostitis on the visceral surface of the rib (**Figure 7**). In comparison, 18.8% of individuals who died of a non-pulmonary disease had periostitis, and 15.4% of individuals who died of a pulmonary disease that was not tuberculosis had periostitis (Santos and Roberts 2006).

Figure 7: Periostitis on the head and neck of upper ribs of Vault II, Burial 8 from the Spring Street Presbyterian Church. These lesions are likely caused by pulmonary tuberculosis. Image courtesy of Werner and Novak 2010. Photo by Dana Kollmann

Other studies, including one by Charlotte Roberts in the Terry Collection (1927-1967), have also shown a strong correlation between visceral rib periostitis and tuberculosis (Roberts and Buikstra 2004, 105). Periostitis in tubercular individuals tends to form non-coral like lesions, or lesions that are not wavy (Santos and Roberts 2006). Although these lesions have a much higher association with tuberculosis than other diseases, they are still

sometimes present in non-tubercular individuals and cannot be taken as definitive evidence of tuberculosis. Like with SES, it is possible that the observed periostitis in patients who did not die of tuberculosis was actually caused by another disease.

Because of both the difficulties of diagnosis and the fact that most tubercular individuals exhibit no associated bone changes, bioarchaeologists face a challenge studying tuberculosis in past populations. An additional confounding factor for assessing health and tuberculosis in the past, involves the "osteological paradox" (Wood et al. 1994). Wood and colleagues identified three main problems that affect assessing paleopathological conditions and health in skeletal *populations.* The first issue that they raise is that any skeletal population is, by definition, comprised of those that died and not those who survived. Thus, it is an inherently biased sample that does not represent the living population. The second issue is "demographic nonstationarity." This qualification emphasizes that it is impossible to tell if migration into and out of a population was occurring, or if there were shifts in death and birth rates. Thus the population is in flux, and not a static snapshot of people in place and time.

Finally the third variable, and the one that has received the most attention, is hidden heterogeneity of fragility. Wood et al. argue that because of differing genetic and environmental factors, individuals in a population will have different susceptibilities to disease and other stressors. Importantly, for any pathological lesions associated with ill health to be recorded in the skeleton, one has to survive long enough for the body to mount an immune response. If someone gets a disease, and dies very quickly, their skeleton might not show any pathological changes, and one could assume they were in good health when they died. However, the reality will be the opposite.

This is of particular import in the study of tuberculosis, as individuals who die very quickly from the disease will show no skeletal signs of the infection. Additionally, the condition will be underrepresented in the wider population, making epidemiological estimates difficult to assess. Compounded with the low rate of bone change in tubercular individuals who do survive, tuberculosis is difficult to identify and study in skeletal populations. As Roberts and Buikstra put it, "absence of evidence, of course, is not evidence of absence" (Roberts and Buikstra 2004, 129). Despite these difficulties however, the "bioarchaeology of tuberculosis" is a fairly large specialty, and many studies have been published examining the disease. A brief discussion of these studies will be given below.

The "Bioarchaeology of Tuberculosis"

In their keystone book, *The Bioarchaeology of Tuberculosis* (2004), Charlotte Roberts and Jane Buikstra summarize all existing evidence for the presence of tuberculosis in skeletal populations, up to 2004 that is. They lament that the field has largely comprised case study reports instead of population-based approaches (Roberts and Buikstra 2004, 129-130). They also report that there is no evidence or data for sub-Saharan Africa and many Asian countries. This absence, however, should not be taken as evidence for the lack of tuberculosis in these countries, and instead is likely due to sampling bias for European countries (Roberts and Buikstra 2004, 130). Still, there is evidence, in varying degrees, for tuberculosis in skeletal populations from Northern Europe, the Mediterranean, and Asia in the "Old World," and from North America and South America in the "New World" (Roberts and Buikstra 2004, 131). Table 1 contains a

summary of the data presented in Roberts and Buikstra, and presented in this paper (**Table 1**).

TABLE 1: Probable Tuberculosis in the Human Archaeological Record, Prior To 2003 (adapted from Buisktra and Roberts, 2004)

Area	Number of Individuals	Period of Examination
United Kingdom	11	Roman Period (43 CE-410 CE)
United Kingdom	14	5th to early 14th c. CE
United Kingdom	48	Late 11th c. to 1845 CE
Denmark	11	2500 BCE to 1536 CE
Hungary	15	7th to 8th c. CE
Hungary	5	11th to 13th c. CE
Hungary	11	14th to 17th c. CE
Lithuania	5	2nd to 9th c. CE
Lithuania	6	14th to 19th c. CE
Germany	12 (contested)*	11th to 15th c. CE
The Netherlands	2	1265-1652 CE
Russia	6	100-12th c. CE
Sweden	4	990-1536 CE
Austria	4	2nd c, to 800 CE
the Czech Republic	3	13th to 18th c. CE
Poland	17	5000 BCE-18th c. CE
Egypt	13	4500-3000 BCE
Egypt and Nubia	18	3300-1500 BCE
Italy	6	5800 BCE-79 CE
Japan	8	3rd to 1912 CE
China	1	206 BCE to 7 CE
South America	6	150 BCE to 1000 CE

South America	10	11th to 16th c. CE
North America	44	11th to 18th c. CE

*Cases were diagnosed based on the presence of SES, making the diagnosis controversial

The oldest accepted tubercular skeletal case (so far) dates to two 9,000-year-old individuals, one woman and one infant, from the region of Haifa, Israel. The infant had SES, and the woman had non-specific periosistis. Ancient DNA (aDNA) analysis of the bone tissue confirmed the presence of MTBC DNA in both individuals. aDNA as a mode of analysis will be discussed later, but this case is important for its antiquity (Hershkovitz et al. 2008).

Northern Europe evidence comes from the British Isles. There is a steady increase in the number of reported cases between the Roman period and the medieval periods. Roberts and Buikstra note that the estimated rate for late- and post- medieval cases based on skeletal evidence from before 2003 is lower than would be expected based on documentary and art evidence for the same time period. This is likely due to the low rate of diagnostic bone formation, namely Pott's disease and septic arthritis. A burial at Newcastle dating to 1753-1845 C.E. yielded 2 bodies out of 210 (<1%) bodies with the classic skeletal markers of tuberculosis. However, burial records indicate 27% of this same skeletal population died of tuberculosis, again illustrating the under-representation of tuberculosis cases in the skeletal record. A further molecular study in 1999 indicated that at least a third of the bodies had tuberculosis, a rate much closer to what was expected (Roberts and Buikstra 2004, 142).

There are an abundance of cases of tuberculosis from Denmark, Hungary, Poland and Lithuania. In Denmark, it appears that tuberculosis was well established as an infection by 500-1 B.C. (Roberts and Buikstra 2004, 150). The earliest evidence in

Hungary dates to 7th to 8th century C.E. (Roberts and Buikstra 2004, 151). Lithuania, like Denmark, has a long history of infection. The earliest cases date to the Late Roman period, between 2nd and 3rd century C.E. There are scattered cases of tuberculosis in skeletal remains up until the 15th century, at which point the number of undisputed tuberculosis cases increases. A male skeleton with spinal tuberculosis, dating from 5000 B.C.E., reveals the early presence of tuberculosis in Poland. Like Hungary and Lithuania, most cases date to and after the late medieval period (10th to 13th century). Although this increase could be due to limited preservation of older skeletons, the late medieval period is when tuberculosis seemed to increase in frequency in many European countries (Roberts and Buikstra 2004, 158). There are scattered cases of tuberculosis in skeletal remains from Germany, the Netherlands, Poland, Russia, Sweden, Austria, and the Czech Republic.

There is limited evidence from the Mediterranean region and Africa. The oldest confirmed tubercular skeleton, as mentioned previously, came from the modern Haifa region, dating to 9,000 years ago (Hershkovitz et al. 2008). Although there is relatively little skeletal evidence of tuberculosis outside of Northern Europe, scattered early burials, dating as far back as 5800 B.C.E in Italy, have been found in Egypt, Sudan, China, Japan, and Italy (Roberts and Buikstra 2004, 173). Most evidence from Africa comes from Nubian and Egyptian mummies.

The question of whether tuberculosis was present in the Americas before colonization was controversial in the 1950s. At the time, many scholars believed the pathogen was brought over after "contact," because it was assumed that Native Americans in the past lived in small, mobile groups too small to support tuberculosis

(Roberts and Buikstra 2004, 188). It is now accepted that tuberculosis was present in the Americas prior to Columbus, brought over from the "Old World" by seals (Bos et al. 2014). Although Mesoamerica lacks any skeletal evidence of pre-Columbian tuberculosis, something that Roberts and Buikstra suspect may be due to different mortuary patterns for kyphotic individuals, there are a fair number of identified cases in North and South America. The earliest case of tuberculosis in the Americas belongs to an adult male found in Peru, who died around 160 B.C.E. (Roberts and Buikstra 2004, 199). There are a few more reported cases in South America between 200 C.E. and 1000 C.E., but most identified tubercular individuals died after 1000 C.E., probably due in part to an increase in the number of cases of tuberculosis as the disease spread, as well as the higher rate of preservation of more modern skeletal remains.

Although there are recorded cases of tuberculosis in South America dating back to 150 B.C.E., no bodies with tuberculosis have been discovered in North America before 900 C.E. Most North American cases are clustered in the Midwest and Southwest, areas that were (relatively) crowded pre-Columbus [prior to approximately 1495] (Roberts and Buikstra 2004, 190). Like South America, most identified cases date to after 1000 C.E., indicating that tuberculosis probably reached epidemic or near-epidemic levels after 1000 C.E.

Roberts and Buikstra published *The Bioarchaeology of Tuberculosis* in 2004. Since then, there have been about 426 more reported cases in 32 journal articles (there are likely more in book chapters, dissertations, and grey literature like cultural resource management reports) [**Table 2**]. As expected, the number of countries with skeletal evidence for tuberculosis increased, especially among non-European nations. This

includes the first reported cases from Thailand and Korea, one of a young adult female from 2500-1700 years ago and one of a middle-aged female from 350 years ago (Tayles and Buckley 2004; Kim et al. 2016). Skeletal collections consisting of identified individuals are, as evidenced by Santos and Roberts (2006), Matos et al. (2006), and Palfi et al. (2012), being used more and more to examine patterns of tuberculosis related osteological changes.

TABLE 2: Cases, since 2003, of Probable Tuberculosis in the Human Archaeological Record

Source	Site	Date	#	Elements Affected	Type of Analysis	Diagnosis
Arrieta et al. 2014	Rincon Chico, Argentina	850-1400 CE	4	Ribs, vertebral bodies	Osteological	Possible tuberculosis
Bianucci et al. 2012	Italy	1522-1562 CE	1	N/A	Molecular	Tuberculosis
Bos et al. 2014	Peru	7000-6000 BCE	2	Vertebrae (Pott's disease)	Molecular, Osteological	Tuberculosis
Botha and Steyn 2016	Khoesan, South Africa	Late 19th to early 20th c. CE	1	Ribs, scapulae	Osteological	Probable tuberculosis
Bouwman et al. 2012	Leeds, UK	19th c. CE	1	Ribs	Molecular, Osteological	Tuberculosis
Canci et al. 2005	Nomentana way, Italy	1st to 2nd c. CE	1	Vertebrae	Osteological	Probable tuberculosis
Christensen et al. 2013	Zalavar, Hungary	9th to 13th c. CE	1	Os coxae, sacrum, tibia, fibula, femur, calcaneus	Osteological	Probable tuberculosis
Dabernat and Crubezy 2009	Adaima, Egypt	3200-3100 BCE	1	Vertebrae (Pott's disease), radius, ulna, scapula, clavicle, tarsals, carpals, femur, tibia, fibula	Osteological	Probable tuberculosis
Dawson and Brown 2012	Somerset, UK	1150-1539 CE	1	Parietal, ribs,	Osteological	Possible tuberculosis
Guichon et al. 2015	Tierra del Fuego, Argentina	1445-1504 CE	1	Vertebrae	Osteological	Possible tuberculosis
Hershkovitz et al. 2008	Atlit-Yam, Israel	9250-8150 YBP	2	Endocranium (SES), tibia	Molecular, Osteological	Tuberculosis
Kay et al. 2015	Vac, Hungary	18th c. CE	8	Rib, lung	Molecular, Osteological (mummy)	Tuberculosis
Kim et al. 2016	Mungyeong, South Korea	1650 CE	1	Lungs	Osteological (mummy)	Probable tuberculosis

Kohler et al. 2014	Alsonyek-Bataszek, Hungary	5000-4000 BCE	1	Vertebrae (Pott's disease)	Osteological	Tuberculosis
Lewis 2011	Dorset, UK	1st to 3rd c. CE	7	Vertebrae, radii, ulnae, os coxae, humeri, metatarsals, metacarpals, ribs	Osteological	Possible tuberculosis
Masson et al. 2013	Hodmezovsarhely-Gorzsa, Hungary	4970 BCE-4594 BCE	1	Skull, ribs, femurs, tibiae, fibulae, ulnae, radii, humeri, tarsals	Molecular, osteological	Tuberculosis
Mariotti et al. 2015	Bologna, Italy	1891-1944	93	Vertebrae, ribs, sternum, Femura, tibiae, fibulae, carpals, tarsals, joints	Osteological, archival	Tuberculosis
Matos et al. 2011	Castelo Branco, Portugal	13th to 19th c. CE	1	Vertebrae (Pott's disease)	Osteological	Probable tuberculosis
Matos et al. 2006	Lisbon, Portugal	Late 19th c. to 1980 CE	84	Rib	Osteological, archival	Tuberculosis
Muller et al. 2014	United Kingdom	2nd to 19th c. CE	10	N/A	Molecular	Tuberculosis
Nicklisch et al. 2012	Saxony-Anhalt, Germany	5450-4780 BCE	8 (15*)	Vertebrae (Pott's disease), ribs	Osteological	Tuberculosis (8), possible tuberculosis (7)
Paja et al. 2015	Batmonostor-Pusztafalu, Hungary	12th to 16th c. CE	2	Epiphyses and metaphyses of the tibia and femur	Osteological	Probable tuberculosis
Palfi et al. 2012	Washington, D.C., USA	1920's-40's	2	Vertebrae, ribs, femur, os coxae, endocranium (SES)	Osteological, archival	Tuberculosis
Prates et al. 2015	Egypt	954-712 BCE	1	Kidney	Osteological (mummy)	Probable tuberculosis
Santos and Roberts 2006	Coimbra, Portugal	1904-1936	81	Ribs	Osteological, archival	Tuberculosis
Sparacello et al. 2017	Liguria, Italy	5710-5770 BCE	1	Humerus, scapula, ribs, vertebrae, os coxae	Osteological (mummy)	Possible tuberculosis
Suzuki and Inoue 2007	Japan	454 BCE-124 CE	2 (8**)	Ribs and vertebrae (Pott's Disease)	Osteological	Tuberculosis (2), probable tuberculosis (6)
Suuki et al. 2008	Korea	100 BCE to 0CE	1	Ribs and vertebrae (Pott's Disease)	Osteological	Tuberculosis
Tayles and Buckley 2004	Khorat Plageau, Thailand	300 BCE-500 CE	1	Vertebrae, maxillae	Osteological	Possible tuberculosis
Taylor et al. 2005	Dorset, UK	400 BCE-230 BCE	1	Vertebrae (Pott's disease)	Molecular, Osteological	Tuberculosis
Vare et al. 2016	Kemi, Finland	1560-1629 CE	1	Vertebrae (Pott's disease), scrotum	Osteological	Probable tuberculosis
Zink et al. 2004	Egypt	3500-500 BCE	26	N/A	Molecular	Tuberculosis

Zink et al. 2007	South Germany, Hungary, and Egypt	3500 BCE-1800 CE	70	N/A	Molecular	Tuberculosis

*7 individuals showed periostitis of the ribs, a condition associated with tuberculosis but not currently considered pathognomonic of tuberculosis

**6 individuals showed periostitis of the ribs, a condition associated with tuberculosis but not currently considered pathognomonic of tuberculosis

The number of papers using molecular methods to diagnose tuberculosis has also increased since 2003. While the skeletal evidence available for tuberculosis in the past has been slowly increasing over the years, as I have argued there is still an "abundance" of absence, due to issues of preservation of bodies as well as low rates of tuberculosis associated bone changes. What is clear is that tuberculosis reached its peak in the late medieval period in Europe and after 1000 AD in the Americas. But researchers wanted a way to study tuberculosis in skeletal remains that showed no osteological changes. And in the 1980s, a tool that could allow them to do just that emerged: ancient DNA.

When DNA becomes incorporated into, or trapped in, certain mineral structures like calcium phosphate, it is protected from degradation. Bone, which is made of a calcium phosphate matrix, can thus serve as a reservoir for the DNA of past animal, bacterial, fungal, and viral species. This is a topic of interest we turn to in the following chapter.

CHAPTER 3

Scraping the Teeth, Testing the Body: Dental Calculus as a Reservoir for Tuberculosis in the Smithsonian's Huntington Collection (1893-1921)

Introduction

Ancient DNA (aDNA), or DNA isolated from historical and pre-historic material and organic remains, has become a common tool for bioarchaeologists and paleontologists in recent decades. The first report of successful aDNA isolation came in 1981 from two Chinese scientists who claimed to have isolated aDNA from the liver of a 2,000-year-old corpse (2001). Three years later, in 1984, researchers from Allan Wilson's laboratory at UC-Berkeley cloned two short fragments of mitochondrial DNA from a 140-year old quagga (Shapiro 2013). From here, the field of aDNA work exploded. Much of the early work was fraught with issues of contamination and retracted results. Famously, in 1994, a group of researchers claimed to have isolated dinosaur DNA, although it was later revealed that they had in fact amplified modern human DNA (Shapiro 2013). In order to combat these problems, Svante Pääblo set out a series of rules for aDNA work, including strict contamination control and independent repetition of results which remain the gold standard for the field.

The oldest DNA sequences generated, so far, come from the bones of a 430,000 year-old hominin (Meyer et al. 2013). Typical aDNA studies, however, usually range from 20,000 to 50 years. DNA is best preserved in cold, arid environments with limited sunlight. In contrast, hot, humid environments promote DNA damage and degradation. So far, only organic material with a calcium phosphate structure, i.e. dental calculus and bone, has been found to preserve DNA over long time periods. Despite the widespread view popularized by *Jurassic Park* (1993), that amber preserves DNA for thousands

(even millions) of years, no studies so far have successfully isolated aDNA from amber. It is possible that this is due to both poor preservation and a high rate of contamination by modern, endogenous DNA (Wayne et al. 1999).

In 2010, a full draft sequence for the Neanderthal genome was published (Green et al. 2010), followed by the draft sequence of another extinct hominin, the Denisovans, two years later (Meyer et al. 2012). aDNA isolated from the calcified dental plaque of Neanderthals has provided insights into the their heath and behavior, including demonstrating that they chewed poplar bark, which contains the active ingredient found in aspirin (Weyrich et al. 2017).

Research in paleopathology identified tuberculosis as a good candidate for aDNA studies. The first successful isolation of tuberculosis aDNA came in 1994, when Salo et al. amplified the *IS6110* sequence from a lung lesion of a 1,000-year-old mummy (Salo et al. 1994). Other studies followed, including genotypes of historical *M. tuberculosis* strains (Bathurst and Barta 2004; Bouwman et al. 2012; Zink et al. 2004). All the tuberculosis studies so far have relied on drilling and sectioning bone. Despite a continued reliance on bone, recent aDNA studies have started to use a new source of material: dental calculus.

Formation of Dental Calculus

Dental calculus is a calcified deposit of plaque that builds up on the teeth over time. Biofilms, or communities of bacteria adhered together, form on the surfaces of teeth, digesting sugars from food particles caught in the mouth and from saliva to form a plaque (Radini et al. 2017; Gonchukov et al. 2010). Proteolytic metabolism of trapped food particles produces by-products like ammonia, which raise the pH of the plaque and

the surrounding tooth area (Radini et al. 2017). Saliva containing calcium phosphate salts are absorbed by the plaque, leading to mineralization (White 1997). The mineralization process begins with a hardening of the cell walls of plaque bacteria, and can take as little as two weeks (Radini et al. 2017). Calculus is a multi-layered structure, building up over time as plaque that adheres to the surface of the calculus is mineralized (White 1997).

Oral microorganisms that make up dental plaque thus become "preserved" in dental calculus, as the calcium phosphate structure, protecting the DNA of said bacteria from degradation (De La Fuente et al. 2013). Dental calculus also contains viruses, fungus, upper and lower respiratory tract bacteria, food debris, and other organic material such as pollen that are breathed into the mouth. These materials become incorporated during plaque formation or calcification (Radini et al. 2017; Weyrich et al. 2015).

Dental calculus in aDNA studies

Almost all teeth in historic or prehistoric contexts have dental calculus. This substance has been found on teeth as old as 8 to 12 million years from an extinct ape of the genus *Sivapithecus* (Radini et al. 2017). Although researchers started to examine the biomolecular structure of calculus in the early 1970s (Armitage 1974), ancient DNA (aDNA) was first isolated from dental calculus in 2013 by De La Fuente et al. Prior to the successful isolation of aDNA, microfossils of plant and animal material, including pollen grains and animal hair, that had been trapped in calculus were used to examine the diet and biotic environment of ancient humans and hominids, including in a two million year old *Australopithecus sediba* (Dobney and Brothwell 1986, 1988; Wesolowski et al. 2010; Henry et al. 2010; Piperno and Dillehay 2008; Henry et al. 2012). Proteins from milk have also been identified in dental calculus from several individuals from Russia and

Hungary dating to 3000 to 1500 B.C.E., providing evidence for the adult consumption of milk in these populations (Warriner et al. 2014a).

The majority of genetic studies of calculus following De La Fuente et al. (2013) have focused on reconstructing ancient oral microbiomes, with a special attention to commensal oral bacteria and oral pathogens. Adler et al. (2013) sequenced modern dental calculus and calculus from medieval, Bronze Age, Neolithic, and Mesolithic skeletons to examine shifts in the diversity and composition of dental calculus over time. They found a sharp decline with the onset of the Industrial Revolution, and its subsequent dietary changes. More recently, the oral microbiome of a 48,000-year-old Neanderthal was reconstructed using dental calculus, shedding light on Neanderthal behavior and diet. This work also led the reconstruction of the oldest microbial genome to date, from the commensal bacteria *Methanobrevibacter oralis* (Weyrich et al. 2017). In 2016, 700 year-old mitochondrial DNA was identified and sequenced from six Oneota individuals, demonstrating the potential for calculus to act as a reservoir for human aDNA studies (Ozga et al. 2016).

The examination of disease in aDNA calculus studies has generally been limited to oral pathogens, although there is limited evidence for the presence of gastrointestinal and respiratory tract pathogens. Weyrich et al. 2017 also identified the presence of a gastrointestinal pathogen, *Enterocytozoon bieneusi* in the dental calculus of a 48,000 year-old Neanderthal. Warriner et al. (2014b) identified several respiratory pathogens such as *Streptococcus pneumonia* in calculus samples from four skeletons dating to 950-1200 C.E. However, despite this evidence that respiratory pathogens are present in

ancient calculus, research into the extent to which these bacteria can be isolated has been limited.

No study thus far has attempted to amplify *Mycobacterium tuberculosis* from historical dental calculus. Because current methods for identifying tuberculosis in skeletal remains have limitations (i.e. low rates of bony changes and the destructiveness of sampling), the development of a method using dental calculus to assay the pathogen is ideal. Importantly, it would offer a non-invasive method to study tuberculosis. Because of the destructive and invasive nature of bone drilling, scientists are often prevented from doing this kind of sampling and analysis, both in museum collections and remains from archaeological contexts. Particularly, descendant populations can often be sensitive to analyses that involving destructive sampling of bone. As calculus is often considered external to the body, there is usually less objection to sampling calculus for DNA analyses. This method could be especially helpful when studying commingled or fragmented collections, as elements like the vertebrae and ribs may be missing, preventing diagnosis of disease. The Smithsonian's George S. Huntington collection is an example of just such a fragmentary skeletal population, and one with a suspect high rates of tuberculosis.

Historical Context: The Huntington Collection

The Huntington Collection, housed in the archives at the Smithsonian's Museum of Natural History, is comprised of 3,070 partial skeletons of people who died in New York City between 1893 and 1921 (Hunt and Spatola 2008). These include immigrants and U.S. citizens born between 1798 and 1901; 43% of these individuals are native-born Americans of European and African ancestry, and 52% of which are European

immigrants. Overall, there is a skew in sex distribution, as 73% of the collection are male and 27% are female. Associated with each body is basic demographic information and personal profile, usually including a name, place of birth, sex, age at death, and cause and location of death (Pearlstein 2015).

The ability to amass such large numbers of bodies with known details of their lives and deaths is due, in part, to the passage of the 1854 "Bone Bill." New York passed this law in response to wide-spread cadaver "snatching" used to supply medical schools and other research institutions with a supply of bodies for teaching and study. It authorized the appropriation of unclaimed bodies at almshouses and hospitals for physicians and surgeons. Most unclaimed bodies belonged to poor individuals from marginalized populations, who either had no relatives to claim their bodies or whose relatives could not pay for their burial (Pearlstein 2015; Nystrom 2014).

The prevalence of poor, marginalized people in skeletal collections, and their dissection and fragmentation, and thus disembodiment, can be understood as an act of structural violence as their bodies in death were understood as unworthy of protection, an extension of their "flaws" in life (Nystrom 2014). Thus these people were perceived to be the undeserving poor who could repay their debt to society by "donating" their body to science (Novak 2017). This trend holds true for the demographics of the Huntington collection, something Huntington himself acknowledged (Pearlstein 2015).

Named after Dr. George S. Huntington (1861-1927), the surgeon who assembled the collection, Huntington was the first full-time professor of anatomy in the United States. He is known for pioneering the pedagogical tool of using cadaver demonstrations rather than simply lecturing to teach students (Pearlstein 2015). Collected while Dr.

Huntington was a professor of anatomy at the College of Physicians and Surgeons in New York City, the series was supposed to serve as a robust source of data "on a scale which will render possible a thorough comparative study in reference to racial character, variations and reversions, measurements, etc." (Huntington in Pearlstein 2015, 48-49; Hrdlička 1918). This mirrors the general goals of physical anthropology at the time, which were dominated by race science that attempted to demonstrate the racial superiority of "whites" (Watkins and Muller 2015).

Instead of a reference collection comprised of complete skeletons, Huntington wanted to collect "5,000 of each of the bones of the body" (Huntington in Pearlstein 2015, 50). He therefore sorted bones by element instead of individual, breaking up bodies and resulting in commingling their parts. Aleš Hrdlička (1869-1943), one of the founders of American Physical Anthropology and the first editor of the *American Journal of Physical Anthropology*, wrote glowingly of Huntington's efforts, noting that the collection had, in 1918, secured "an extensive series of data of much value" (Hrdlička 1918, 285).

Despite the demographic and other details that came with each body, Huntington's attempts to create a reference collection of individual bones led to many "floating," unassociated elements becoming detached from these details. Efforts by museum curators to sort the collection back into individual bodies is complicated by this high rate (16%) of comingling (Hunt and Spatola 2008). Moreover, because the bodies were used for dissection, many of the smaller elements, particularly the ribs, have been lost (pers comm. Alanna Warner-Smith). In particular, it is the incomplete nature of the

bodies, particularly the loss of the ribs, which has hindered researcher's attempts to examine diseases like tuberculosis in the collection.

Most bodies in the collection have an associated cause of death. Tuberculosis, a "disease of poverty," at least during the second half of the 19th century and 20th century, is well represented in the series (Hunt and Spatola 2008; Pearlstein 2015). If researchers want to study tuberculosis using the Huntington Collection, they can simply pick bodies with tuberculosis listed as the cause of death. But the incomplete nature of the skeletons makes it difficult to diagnose tuberculosis based on skeletal changes alone, and it is nearly impossible to examine the prevalence of tuberculosis in individuals who died of something else. The utility of a calculus assay for diagnosing tuberculosis in the Huntington collection, coupled with the fact that associated death records allow for the selection of positive controls, makes this collection an ideal place to develop a method using dental calculus to isolate tuberculosis aDNA.

Collection and Sampling

This study was conducted in two stages: (1) a pilot study, and (2) a study of individuals with cause of death. For very good reasons, the Smithsonian does not allow you to simply conduct destructive sampling on their collections. In order to test my hypothesis that tuberculosis DNA could be isolated from dental calculus, a pilot study was conducted using eight unaccesioned mandibles from the Huntington Collection. These were kindly loaned to Syracuse University by Dr. David Hunt. Prior to sampling, the mandibles were photographed and an inventory and pathological analyses was conducted (Owsely et al. 1995). Calculus was scraped using a dental scalar following a procedure from the Warriner lab at the University of Oklahoma **(Figure 8)**.

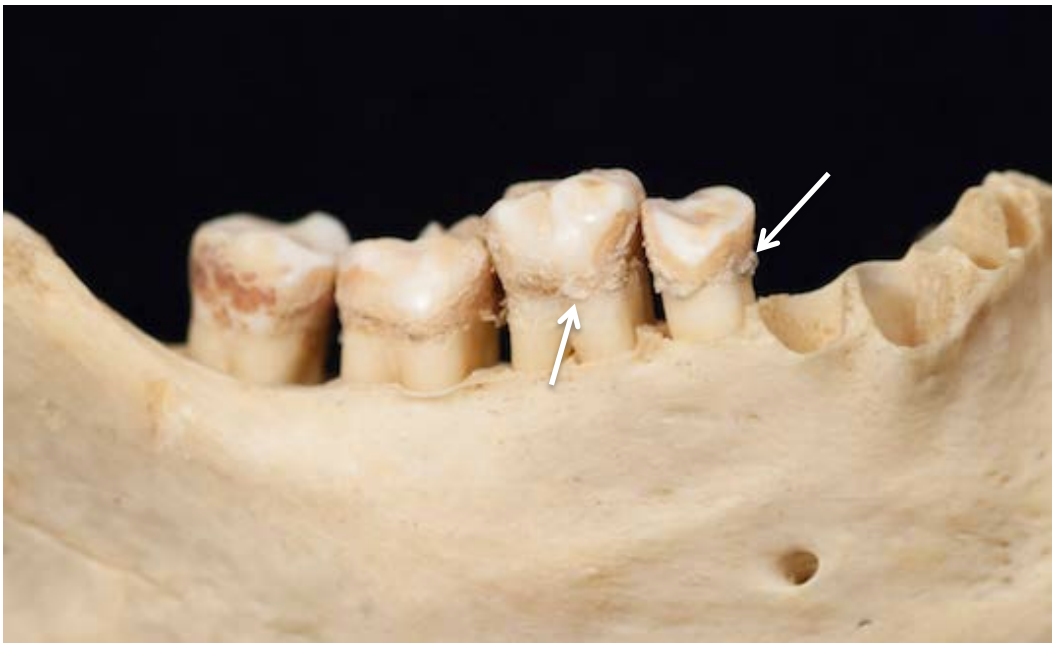

Figure 8: Dental Calculus (arrows) on the molars of individual 174/SY05, an unaccesioned mandible from Stage 1of the study.

No biographical information was available for these samples. Written on all eight mandibles was “white, U.S., ♂," meaning they likely belonged to U.S-born men of Euro-American descent. Only molars were present in any of the mandibles. There was a fairly low rate of caries, and most were pit caries on the occlusal surface. However, analysis was limited because of extensive post-mortem tooth loss. Widespread sub-gingival abscesses had started to form in every mandible. Interestingly, individual 127 had some sort of dental filling, possibly lead, on the occlusal-buccal surface of his $RM2_1$, indicating at least some sort of access to dental care.

Following the first stage of this study (the results of which I will discuss shortly), a sampling proposal was submitted to the Smithsonian, requesting to sample the calculus of six (n=6) known Irish immigrants. The proposal was accepted, and Alanna Warner-Smith, a graduate student in Dr. Novak’s lab, and I, travelled to the Smithsonian to collect the calculus samples. Two of these individuals died of tuberculosis, and served as

positive controls. The four other individuals had different causes of death listed, including Bright's disease, apoplexy, pneumonia, and endocarditis. Although names are known for each of the sampled individuals, they cannot be used in publication. Each body has a six-digit identification number assigned to them, however, with which they will be referred to in this study. As in the pilot study, the maxillae were photographed, and a dental inventory was taken. Overall, the individuals had a low rate of carious lesions, mostly pit caries on the occlusal surfaces. The two exceptions were 318420, a 54-year-old woman, who had had more extensive involvement on the occlusal, lingual, and interproximal surface of her $LM2^{1}$, and 319625, a 68-year-old male, who had complete destruction of the surface of his $RPM1^{1}$ and root involvement.

In contrast, there was a fair amount of antemortem tooth loss and abscessing, especially involving the maxillary molars. This pattern is expected in older populations, as caries have more time to progress to tooth loss (Bruwelheide et al. n.d.). There was minor to mild calculus on all individuals examined, except for individual 318298, a 54-year-old female, who has extensive calculus deposits, including on the occlusal surfaces of her teeth.

Methods and Materials

Washing and Sterilization of Samples

In order to decontaminate the outer surface of the scraped calculus, samples were washed in a 4% bleach solution. 1mL of bleach was added to each tube, which were then agitated slowly on a tabletop vortex for five minutes. The bleach and calculus solution was pipetted onto a clean filter on a vacuum filter. The bleach solution was filtered off, and 3mL of 90% ethanol was then applied to the calculus in order to wash off any

remaining bleach. The ethanol was then filtered off. This step was repeated an additional two times. The dry calculus was then transferred to a new sterile centrifuge tube.

Decalcification and DNA extraction

The sterilized calculus was crushed to a fine powder using the blunt end of a clean spatula. 720μL of 0.45M pH 7 EDTA, to decalcify the calculus matrix, and 80 μL of proteinase K, to denature any surviving proteins, was added to each sample and mixed. The samples were incubated overnight at 55°C, and then transferred to a nutator at room temperature for the next 24 hours. The six samples from Irish immigrants were incubated for an additional 24 hours in order to completely dissolve remaining calculus flecks.

A phenol-chloroform extraction was carried out on the decalcified solution. 1mL of 25:24:1 phenol-chloroform-isoamyl alcohol was added to each sample, and then spun at 13,000 RPM for five minutes. The aqueous phase was removed and transferred to a sterile centrifuge tube. This step was repeated twice, first with 25:24:1 phenol-chloroform-isoamyl alcohol (pCi), and then with pure chloroform. 100 μL of the aqueous phase were purified using a MinElute reaction cleanup kit (Qiagen). The rest of the aqueous phase was saved in a -20°C freezer for future use.

Nested PCR

Nested PCRs involve running two sets of consecutive PCRs with two sets of primers. The first set of primers is located directly upstream and downstream of the sequence of interest. Two frequent problems in aDNA studies are contamination and non-specific priming, both of which can be alleviated by using nested PCR since nested PCR increases the specificity of priming (Warriner et al. 2015; Weyrich et al. 2015).

A 123 base-pair region of the *M. tuberculosis* specific *IS6110* sequence was targeted for amplification. *IS6110,* a 1361 bp insert in the 16s rRNA gene (Thierry et al. 1990), is commonly used in aDNA tuberculosis studies because it is species specific and present multiple times throughout the *M. tuberculosis* genome (Taylor et al. 1996). The primers used in these reactions were borrowed from sequences provided by Taylor et al. (1996). The sequences of the primers for the first PCR reaction, IS-1 and IS-2, were 5'-CCTGCGAGCGTAGGCGTCGG-3' and 5'-CTCGTCCAGCGCCGCTTCGG-3' respectively. The sequences of the primers for the second PCR reaction, IS-3 and IS-4, were 5'-TTCGGACCACCAGCACCTAA-3' and 5'-TCGGTGACAAAGGCCACGTA-3' respectively. These primer pairs should amplify a region of MTBC DNA that is 123 base pairs long.

The first PCR reaction contained of 36 μL nuclease-free water, 10 μL 5X Phusion HF buffer, 1 μL 10mM dNTPs, 0.25 μL IS-1, 0.25 μL IS-2, 2 μL of purified pCi extract, and 0.5 μL of Phusion high-fidelity DNA polymerase. PCR parameters consisted of an initial denaturation at 98°C for 30 seconds, followed by 35 cycles of 98°C for 10 seconds, 65.4°C for 30 seconds, 72°C for 30 seconds, and a final extension for 10 minutes at 72°C. The second PCR reaction contained 37 μL nuclease-free water, 10 μL 5X Phusion HF buffer, 1 μL 10 mM dNTPs, 0.25 μL IS-3, 0.25 μL IS-4, 1 μL of the first PCR reaction, and 0.5 μL of Phusion high-fidelity DNA polymerase. The products of the second PCR reaction were then run on a 2% agarose gel to visualize product size.

Results

Two of the unaccesioned mandibles (110 and 173), which comprised the first stage of the study, tested positive for tuberculosis. These results demonstrated that

tuberculosis DNA can be isolated from dental calculus, and served as a proof of concept. Because of the success of this first stage, I moved onto the second set of samples: the six Irish immigrants with known demographics.

Four of the samples, 318066, 318420, 317896, 319625, tested positive for *M. tuberculosis*. 318420, a 54-year-old woman, and 317896, a 78-year-old man, both had "phthisis," a historical name for tuberculosis (Frith 2014), listed as their cause of death. The amplicons of 317935, a 70-year-old woman who died of endocarditis, 318298, a 54-year-old who died of pneumonia, and the negative control that contained no calculus extract showed no bands when run on a gel, suggesting no presence of tuberculosis in

these three calculus samples **(Figure 9)**. A full breakdown of the results and demographics of the six Irish Immigrants can be found in **Table 3**.

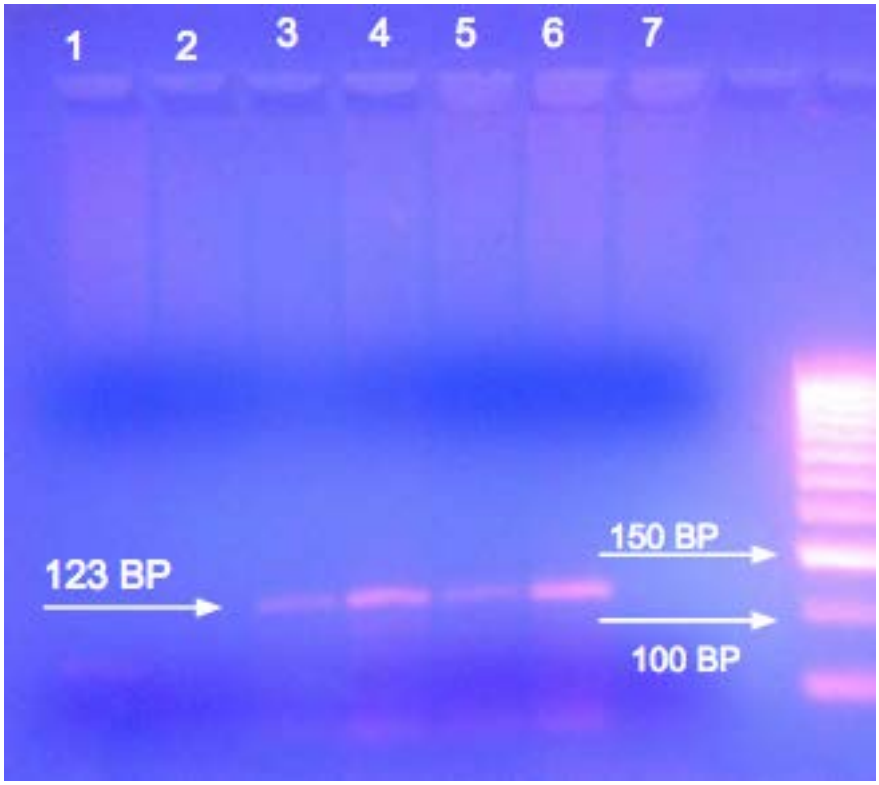

Figure 9: Gel electrophoresis of amplicons from the six known individuals. Ladder is 1kb. A lane guide can be found in Table 3.

Number	**Result**	**Sex**	**Age**	**Year of Death**	**Cause of Death**
318298 (1)	Negative	F	54	1895-96	Pneumonia
318935 (2)	Negative	F	70	1902	Endocarditis
319625 (3)	Positive	M	68	1901	Apoplexy
318420 (4)	Positive	F	54	1895-96	Phthisis (TB)
318066 (5)	Positive	M	37	1894-95	Bright's Disease

317896 (6)	Positive	M	78	1902	Phthisis (TB)

Table 3: Summary of results for the six Irish immigrants tested. Phthisis is an alternative name for tuberculosis sometimes used at the turn of the 20th century

Conclusion

We are currently sequencing the amplicons from these reactions. The pattern of amplification and specificity of the bands, however, make it very likely that the 123 base-pair band in **Figure 9** corresponds to the targeted sequence, and thus indicates the presence of tuberculosis, and full sequences will be needed to be provide confirmation. Despite the promising results of this method, and the expanded ability to diagnose tuberculosis in skeletal collections, there are some limitations. It is impossible to determine whether the samples that were negative never had the disease, or whether they simply did not have an active infection at the time of their death. While the demographic records that accompany the skeletal remains are important, they simply record the cause of death, and not the individual's medical history across the life course. There are many questions still left to answer about what this method can and cannot tell us about tuberculosis in past populations.

The most important next step is to have the results of this study confirmed in an independent laboratory. Independent confirmation is standard for all aDNA studies, in order to make sure contamination was not an issue (Gilbert et al. 2005). The blank controls run with both sets of samples was negative both times, indicating that contamination of the calculus with environmental DNA was unlikely. The laboratory in which the samples were processed has also not previously worked on tuberculosis, and no member of the laboratory has ever had tuberculosis. No laboratory in the Life Sciences

complex, where the laboratory is located, works with tuberculosis (prof. Garza, pers. comm.). Because *IS6110* is specific to the MTBC, it should not be present in the sequences of normal environmental bacterial flora. Still, the confirmation of this method in another lab would not only show the ability to replicate of this method, but also provide independent confirmation that it works.

Further studies with a larger sample size of individuals who died of tuberculosis are needed in order to determine the relative success rate of isolation and amplification. Tuberculosis DNA was isolated from positive controls, 318420 and 317896. This is a (hypothetical) success rate of 100%, but given the extremely low sample size, it should not be taken as a real reflection of the test's efficacy, and merely as a suggestion that dental calculus is a good reservoir of tuberculosis aDNA for individuals who died of tuberculosis. Additional questions that need to be addressed in future studies include the relative rates at which different types of tuberculosis are incorporated into calculus. *M. bovis* is more likely to cause extra-pulmonary infections than *M. tuberculosis*, especially in children (Grange 2001). Tuberculosis cells must be in the mouth in order to be incorporated into dental calculus, and so it seems more likely that pulmonary tuberculosis would be incorporated into calculus at a higher rate than extra-pulmonary tuberculosis, as the tuberculosis bacilli are brought into the mouth after a coughing fit. *M. bovis* and *M. tuberculosis* can both cause Pott's disease and septic arthritis, the two most reliable skeletal markers of tuberculosis, making it almost impossible to distinguish between the two types of infection in skeletal population. If *M. tuberculosis* is represented at a higher rate than *M. bovis* in calculus, its presence, along with other lines of evidence such as

historical context, documentation, and other skeletal markers, may allow researchers to make a clearer distinction between tuberculosis caused by the two bacteria.

Only recently have full genome sequences of ancient strains of tuberculosis been generated from bone and mummified tissue, thanks to advances in next-generation sequencing (Bos et al. 2014; Kay et al. 2014). Full genome sequences should be generated from dental calculus. This will need to be done in a clean, aDNA specialized lab in order to prevent environmental contamination. Endogenous DNA contamination was not a concern in this study because, as discussed, the *IS6110* element is specific to MTBC; the full genome sequence of the MTBC, however, contains many non-specific sequences and elements, and so contamination will be a greater concern when constructing any full genome sequences (Gilbert et al. 2005). Full genome sequence data from calculus would be of great use to researchers studying the evolution of tuberculosis, as it would allow them to understand the dynamics of genetic changes and evolution on deeper time scales than clinical and lab studies allow (Comas et al. 2013). This is especially important as antibiotic resistant tuberculosis is on the rise. Full genome sequence data would also allow researchers to definitively distinguish between tuberculosis caused by *M. bovis* and *M. tuberculosis*.

Lastly, further study should be undertaken to see if it is possible to distinguish between infections active at the time of an individual's time of death, and infections latent at their time of death. Both of the individuals who died of tuberculosis, 318420 and 317896, had bands on the gel electrophoresis twice as bright as the two individuals, 319625 and 318066, who did not die of tuberculosis. Samples of higher nucleotide mass will fluoresce brighter than those with a smaller nucleotide mass. Comparisons of the

fluorescence level of different gel bands allows for the calculation of the relative concentration of DNA in each sample (Tweedie and Stowell 2004). The bands of 318420 and 317896 are twice as bright as 319625 and 318066, and all the samples are the same length, meaning that 318420 and 317896 are twice as concentrated as 319625 and 318066. This raises the possibility that active infections will yield higher concentrations of amplicons than latent infections, simple due to larger starting concentrations. Further studies of calculus from individuals who died of tuberculosis and individuals who had tuberculosis but did not have an active infection when they died are needed to answer this intriguing question.

CHAPTER 4

Conclusions and Beginnings: Untangling the Multifactorial Web of Tuberculosis

Tuberculosis, one of the oldest human diseases, is caused by a family of closely related bacteria, the MTBC. The bacteria most commonly responsible for tuberculosis in humans is *M. tuberculosis*, which most often causes pulmonary tuberculosis, or tuberculosis of the lungs. Throughout history, tuberculosis has had many names: phthisis, consumption, the white plague, and, of course, tuberculosis.

Up until very recently, the study of tuberculosis in skeletal remains has relied on the presence of Pott's disease or septic arthritis. Serpens Endocrania Symmetrica (SES) and periostitis of the head and shaft on the visceral side of the ribs have also been linked to tuberculosis, but are not viewed as pathognomonic of the disease because they have been linked to other conditions. Low rates of bony changes, coupled with the fragmentary nature of many skeletal remains, makes large scale examination of tuberculosis in skeletal populations difficult, and as such the field has largely been dominated by case studies. The advent of aDNA studies using sectioned bone has allowed researchers to identify the presence of tuberculosis in skeletal remains with no associated osteological changes. The invasive and destructive nature of this technique, however, has limited its usefulness. This is the first time tuberculosis DNA has been isolated from historic, pre-historic, or ancient dental calculus. This method will greatly expand the study of tuberculosis in the past, allowing researchers to tap into a new reservoir of aDNA for study.

Dental calculus, calcified dental plaque, preserves DNA from degradation, much like bone. Until now, tuberculosis has never been identified in dental calculus. The

successful amplification and sequencing of the *IS6110* sequence has demonstrated that calculus is a reservoir for tuberculosis aDNA, and provides a new method for identifying tuberculosis in skeletal populations. Sampling dental calculus is not invasive, and less destructive, and so may be more useful for widespread study of tuberculosis in the past.

Social Life of a Disease

My study demonstrates that *M. tuberculosis* can be isolated from dental calculus, and provides a new method to investigate tuberculosis in skeletal collections. Two of the individuals that tested positive, 318420 and 317896, had phthisis, an early name for tuberculosis, listed as their cause of death. These samples serve as positive controls, and although the sample size is very small, it is promising that both of the samples with tuberculosis listed as a cause of death tested positive. This shows that, under at least some circumstances, *M. tuberculosis* bacteria, and thus DNA, is incorporated into dental calculus, where the DNA is protected from degradation. Tuberculosis aDNA from dental calculus can thus be isolated, amplified, and sequenced, allowing identification of the disease in past skeletal populations.

Even more promising are the positive results from two of the unaccessioned mandibles (110 and 173**)** and individuals 319625 and 318066. The latter two did not die of tuberculosis but from apoplexy and Bright's disease, respectively. Apoplexy is an archaic term for internal bleeding associated with having a stroke, and Bright's disease is a chronic inflammation of the kidney (Leak et al. 2014; Bricker et al. 1960). The two unaccessioned mandibles from the pilot study had no demographic information associated with them, making it impossible to tell if they died of tuberculosis. The positive identification of tuberculosis in skeletal remains with no other markers, be they

historical or osteological, of the disease is the ultimate application of this method. We now know that 319625, 318066, 173, and 110 were exposed to tuberculosis at some point in their lives. It seems likely that all four of these samples had an active infection at some point, given that *M. tuberculosis* would be incorporated into plaque following coughing fits which brought cells to the mouth.

Within the Huntington collection, tuberculosis is the most common cause of death. 52% of German immigrants (n=130), 59% of Italian immigrants (n=96), 49% of Irish immigrants (n=179), and 63% of U.S.-born individuals (n=418) are listed as having died of tuberculosis. Tuberculosis was the recorded cause of death for over half of the total skeletal population. The second most common cause of death among Irish immigrants is kidney disease, which accounts for 13% of listed causes of death among the Irish bodies in the Huntington Collection (Pearlstein 2015). The overall high rates of tuberculosis listed as a cause of death in the Huntington Collection is likely due to the epidemic proportions of the disease in NYC at the end of the 19th century and beginning of the 20th century, although overall rates and mortality had declined from the beginning of the 19th century (Smith 2003). It is estimated that 70% to almost 100% of the population of major cities was infected with tuberculosis at the end of the 19th century, although they may not have been symptomatic ("Tuberculosis in Europe and North America," 2017). In 1900, tuberculosis was the leading cause of death for New York City residents, causing 237 deaths per 100,000 individuals, although this number declined to 126 deaths per 100,000 individuals by 1920 (Lerner 1993).

It is likely the low socio-economic status of the individuals represented in the Huntington collection also affected the high death rate. Irish immigrants in NYC at the

beginning of the 20th largely lived in closely packed, poorly ventilated, unsanitary tenements, and sometimes in shantytowns (Ernst 1949; Burrows and Wallace 1999; diZerega Wall et al. 2008). These conditions facilitate the spread of tuberculosis, making it unlikely that only 49% of those listed as dying from tuberculosis had the disease. It is likely that most of the Irish immigrants in the collection, especially the older ones or ones who died in the first decade of the 20th century, were exposed to tuberculosis at some point in their life. They may or may not have contracted the disease, but a weak immune system resulting from poor access to nutrition, clean water, and heavy overall disease loads likely compounded the problem (Roberts and Buikstra 2004).

The lives and deaths of 319625, a 68-year-old male who died in 1901, and 318066, a 37-year-old male who died between 1894 and 1895, have become more nuanced now that we know they had tuberculosis. They are likely to have experienced the pain of frequent, violent coughing fits, the night fevers, and the fatigue. Did it make it difficult to work? How did they treat their symptoms? Did they feel alone? Did they have support? Were they ever diagnosed? These questions cannot be answered without additional documentary information about both individuals, which I do not have at this point in time. We also know from death records, and confirmed in this study, that 318420, a 54-year-old female who died between 1895 and 1896, and 317896, a 78-year-old male who died in 1902, had tuberculosis.

All these individuals would have had tuberculosis during the second half of the 19th century, a time when public health efforts were being pushed in Europe and America, partially as a response to the "polluting" effects of immigrants (see chapter one; Douglas 2002 [1966]; Barnes 2006; Craddock 2001; Arnold 2012). Tuberculosis became

inexplicably tied up with middle class fears over degeneration of the population and the "white race", and was transformed into a disease of poor hygiene and overcrowding rather than constitution. The middle-class demanded state-level public health programs to deal with the dirt and filth of the lower class, particularly for the "mass treatment and *isolation* of the tubercular" (Arnold 2012, 7). Large scale public health measures were not implemented until the end of the 19th and beginning of 20th century, but there were growing concerns about "germs" during the second half of the 19th century. Perceived as foreign invaders, germs came to be understood as the cause of most illnesses.

Thus, the tubercular body of the second half of the 19th and 20th century is the literal embodiment of a threatened, invaded, impure, and dangerous body. Sociocultural understandings of tuberculosis and body cannot be disentangled from the biological "reality" of tuberculosis. Instead, "social" and "biological" discourses and realities of tuberculosis must be understood as informing each other to make the tubercular body. Bodily experiences of tuberculosis, or how tuberculosis is *enacted* and *understood* through the body are not static, but exist in specific geographic, historical, and social contexts. Any examination of tuberculosis in the past should take into account the broader socioecological context in which individuals with the disease lived, and died.

As I asserted in my first chapter, tuberculosis cannot be understood as a single entity, but multiple diseases. This is because the biological experience and reality of tuberculosis is affected by both the life history of the sufferer as well as sociocultural understandings of the disease. The six individuals in this study who had tuberculosis likely understood this affliction, and its manifestations in this context. Perhaps this caused emotional distress, or shame as their bodies came to embody dirt and

degeneracy—at least according to others. Certainly the inadequate conditions in which these individuals lived, their limited access to the fresh food, and the general stress they were under exacerbated the course of the illness, perhaps leading to more severe symptoms. Further study, with more historical data, is needed to fully unpack how tuberculosis was experienced and understood by these individuals. Yet the identification of 319625 and 318066 as individuals who were infected has already contributed to our understanding of their lives. These findings not only demonstrate the benefits of this method for examining tuberculosis in past skeletal populations, but they also lead us to ask new questions about the ways in which social, political economic, and historical environments make and are made by the bodies inhabiting them.

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