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STUDYING MICROBIOLOGY WITH GLENN F. WEBB

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Figure 1. Glenn F. Webb and Martin Blaser on a trail in the Colorado Rockies, 2004. Uphill, there are many pauses in the conversation, but downhill the words flow much faster. The natural beauty all around us facilitated our discussions about the mathematics of biology.

I began working with Glenn Webb in 1997. At that time, I was on the faculty of Vanderbilt University, in the School of Medicine, in the Department of Medicine, in its Division of Infectious Diseases. As with mathematics, modern medicine has its different disciplines (e.g. Surgery and Internal Medicine), and then further subdivisions (e.g. Cardiology and Infectious Diseases). Within Internal Medicine, most of the divisions are based on the treatment of conditions that relate to a single organ or group of organs – the heart, the lungs, the kidneys, the digestive system. But the discipline of Infectious Diseases was based on a different concept: the war between humans and microbes.

With the advent of antibiotics, important new weapons in the perennial battles, and the growth of hospitals as a place for the treatment of the very ill, the intrinsic

complexity required specialists who could provide state-of-the-art care. Initially, the specialty was mostly hospital-based, helping in the care of very ill persons. Some patients came from their homes with community-acquired infections like pneumonia, or infections of a heart valve, or malaria, depending on where they had been traveling. But others acquired their infections in the hospital. Interference with normal anatomy and physiology such as surgical intrusions, placement of tubes across tissue planes, medications that altered important functions all predisposed patients to infections. And most hospital patients were already ill – that's why they were there. This combination of sick people with aggressive therapies has lead to more infections, necessitating more complex approaches to treatment. In a sense, it has been an arms race, compounded because the very treatments we use select for the resistance of the microbes. It has been sufficient to fuel the growth of new profession, the specialists in Infectious Diseases, over the past sixty years.

As such a specialist, I was focusing on a microbe that lives in the human stomach, bacteria called *Helicobacter pylori*. These organisms were discovered in 1982, and found to be pathogens, causing ulcers of the stomach and duodenum, a condition known as peptic ulcer disease [1]. The use of antibiotics to clear the stomach of these organisms cured people of ulcers, and revolutionized their treatment [2]. Later, we and others showed that these organisms also were responsible for most cases of stomach cancer.

But their biology was puzzling. They lived only in humans, and once acquired, almost always by a child, they persisted for decades, often for the entire life of their host. Only a small fraction of people became ill; most carried the organism with no apparent consequence (later we found that there actually was benefit). The longer a person had the organism, the greater their risk of developing gastric cancer. This was straightforward, but for ulcers, the peak was in the third to fifth decades of life, and even though males and females were equally infected, the disease rates differed markedly.

To me, one of the keys to understanding how best to deal with *H. pylori* was to understand how it persisted. In 1990, at a meeting in England, I postulated that there must be an equilibrium between *H. pylori* and its host, but my views on how it operated were very incomplete [3]. I had a sense that mathematicians could help me better understand the relationships. At a party in Nashville, I was introduced to a graduate student in mathematics, Marty Fryd, and when I told him about my work and ideas about *H. pylori*, he immediately suggested that I contact Denise Kirschner, who was interested in such subjects.

Denise Kirschner was a Postdoctoral Fellow working with Glenn F. Webb. It took a while for us to actually meet, but when we did, it became clear to us both that this was a very fertile area for exploration. Denise and I began working together in 1994, and made a great deal of progress developing models for the persistence of *H. pylori*, in the human stomach. It was Denise who introduced me to the world of mathematical biology. Although I had the intuition that the problem I was pursuing – how *H. pylori* is able to persist in the stomach – could be approached by math, Denise showed me how it could be done. I formulated the essentials of the model and she could reduce these into a series of differential equations. Four variables, four equations; she could solve for each [4]. Later, we added a fifth equation to deal with another important variable [5]. Over the years, we refined and expanded the model [6, 7], but it was ultimately based on the simultaneous equations.

When Denise was planning to leave Vanderbilt to take her first faculty job at Texas A & M, she strongly recommended that I meet Glenn F. Webb and suggested that we would have much in common. Her prediction was quite accurate. From a strong professional relationship has come a beautiful friendship. This is one of the dividends of an academic career. Some of our best work has come on the trail, while hiking in the high mountains of Colorado (**Figure 1**). There, amidst the glories of nature, we sought to understand her rules about the interactions of microbes and humans. It has been most rewarding, and a great deal of fun.

The first problem we studied again related to *H. pylori* persistence in the stomach. It was based on the observation that the bacteria expresses surface molecules that also are present on the surface of the cells lining the stomach, where it lives. These molecules are called the Lewis (Le) antigens; they are small sugars called oligosaccharides. The human stomach expresses Le^a, Le^b, Le^x, and Le^y among other Le antigens, and *H. pylori* has the genetic machinery to express each of these as well. These antigens are only found on a very few microbes, and not with that degree of complexity, so the fact that they may be expressed on the surface of *H. pylori* cells that colonize the stomach did not seem to be just random. Further, each strain appears to have the full genetic machinery to allow it to express one or another of the antigens. In a clinical study, we had shown that people whose stomach surface is dominated by Le^a and Le^x are preferentially colonized by strains that express Le^x, whereas a parallel relationship was found for those with Le^b/Le^y stomachs colonized by Le^y strains [8].

Now we wanted to study the problem in a model system. We could colonize rodents – mice and Mongolian gerbils – with *H. pylori* for long periods of time. This permitted characterizing the strains isolated from their stomachs to examine how the Le phenotypes varied over time. Glenn built a mathematical model to examine the evolution of the phenotypes in the animals [9]. The model was based on the concepts that phenotype expression varies in a continuum with boundaries, that the phenotypes vary in steps, with each generation of bacterial daughter cells, that each host's stomach has a maximal carrying capacity which constrains the system, and that variation is governed by mutation and selection. Glenn used a partial differential equation to capture the variables, balancing the rate in change in time with the rate in change in phenotype distribution. Mutation created the population of variant alleles, diffusing away from the founder population over time, but the work provided a model in which selection determined who would win. The work showed that within the boundary conditions examined, even a small selective difference – equivalent to being a red organism in a red stomach – could over time, over generations, lead to dominance of a population of cells expressing red (e.g. particular Le antigens). This work, submitted for publication in 2001 after several years of study, provided a model of phenotype evolution in a natural setting for relatively large population ($10^4 - 10^5$ individuals) over a long evolutionary time scale ($10^3 - 10^4$ generations).

Anthrax

On September 11, 2001, terrorist attacks on the United States were launched, and shortly afterwards, letters containing anthrax spores were mailed to prominent people. Between October and December 2001, 22 people became ill, and five people died. Of these 22 cases, 11 occurred in postal workers, and 7 were recipients of the letters or were in their work environment. Their sources of exposure were obvious,

but what about the other 4 persons with anthrax, who had no such exposure? There were essentially no background cases of anthrax in the United States, so it could be assumed that all must in some ways be related to the terrorism, but no solution was obvious. Living in New York City then, I was part of the Mayor's Taskforce on Bioterrorism, and charged with understanding what was happening, along with the FBI and law enforcement officials everywhere. In late November, a case of anthrax was detected in an elderly woman in Connecticut who mostly stayed at home. Her sources of exposure were very limited. From that case, a hypothesis presented itself to me – as with the others, she was exposed by spores in the mail. But as far as anyone knew, no letter had been mailed to her; she was not a prominent person in government or the media. My hypothesis is that ordinary letters that she had received had been cross-contaminated by spores from one of the envelopes that were laden by spores. I immediately called up Glenn and proposed this idea to him. He thought that it was plausible, and when I asked him whether he could come up with a model for this, he immediately said "yes." His model consisted of vectors describing the number of contaminated letters reaching a series of nodes in the postal system (the letterbox, the postal station, the sorting facility, etc.). One vector described the number of cases from receiving the letters, and then there were a series of matrices that transition the vectors. As in our prior studies of mice, it was based on the concept of discrete generations, in this case the postal nodes. A spore-laden letter would leak some spores that would contaminate adjacent letters, and at the next node, they each would contaminate other letters. Eventually many letters could become contaminated, but with a distribution of spore counts – a few with many and many more with fewer and fewer. When we took into account what was known about attack rates, and about age differences in susceptibility to infection in the matrix model, the predicted number of cases approximated the number of cases of inhalational anthrax that were observed. We now could explain all of the cases. We submitted this work for publication on Dec 21, 2001, and it was published a few months later [10]. We know that the FBI and the Centers for Disease Control were interested in our findings, and although the workings of the Postal Service are appropriately confidential, we believe that our studies improved safety.

SARS

In 2003, a new disease was recognized in Asia that was called SARS (severe acute respiratory syndrome). This was first recognized as causing an outbreak in Hong Kong, but cases spread all over the world. Many of the people who became ill were patients already in hospitals, but others were in the community, and had no obvious hospital contact. Within a short time, it became clear that the cause was an infection with a novel type of coronavirus. A critical question that concerned scientists all over the world was how was the virus being transmitted? From Hong Kong, SARS spread to Beijing, but not to Shanghai. It spread to Toronto but not to Vancouver. The United States was almost completely spared. Such a transmission pattern was unusual. Several initial studies suggested an R_0 that was around 2 or 3. But with such values, the outbreak would just spread from city to city, and there would not be the kinds of differences just described. In Toronto, there was extensive spread in hospitals. We teamed up with three mathematicians there – Huaiping Zhu, Sten Ardal, and Jianhong Wu to further analyse the data [11]. We had the hypothesis that transmission was not uniform, with different (and higher)

rates in the hospital than in the community. We used a typical four compartment model with susceptibles (S), exposed (E), infectives (I), and removed (R; individuals who were infected and now no longer are susceptible). The SEIR model is common in modeling the transmission of infectious agents. Our novel feature was to split it into 2 SEIR models that interlocked – one for the general public in the community, and the other within hospitals. With our sectored model, we found an R_0 for hospitalized patients to be closer to 4.5, whereas in the community the R_0 was about 1.6. This large differential emphasized the importance of infection control policies in the hospital, and the need for strong quarantine measures in the community. With quarantine, the outbreak dies down because R_0 becomes less than 1. In fact, in Toronto, when the quarantine was relaxed prematurely, there were new cases. This modeling, in the face of an epidemic, provided a new view of the outbreak, with increased precision, and able to pinpoint control measures that could be important in future outbreaks.

Influenza

In 2009, there was another outbreak of respiratory illness that caught the world's attention. But it was not a novel pathogen; it was influenza, however, it was a new strain. Influenza A visits the population every winter, but each year it is a somewhat different strain. In some years, the changes from the prior year's strains are minor; we call that kind of viral evolution "drift". But in other years, often every 8–12 years, there is a major strain difference; this is called "shift", and in 2009, this is exactly what happened. Starting in Mexico, a new influenza A strain (H1N1) began infecting the population. From the very first, it became clear that younger individuals were the major targets. This was not surprising, since the new strain shared important characteristics with the major H1N1 strain that had circulated throughout the world between 1949 and 1957, before it was replaced by a "shift". According to the theory, anyone who had already been born before 1957, which meant persons 52 years old or older in 2009, had at least some partial immunity, and possibly complete immunity. The epidemiological evidence supported the theory; most of the illness was in young adults and children.

When a shift occurs, with the absence of substantial immunity, there often is higher mortality than in 'drift' years. In 2009, it was uncertain whether this would be the case or not. In the past, public health authorities had used maneuvers such as quarantine, and shutting of schools to slow the transmission of infection, and to dissipate the force of infection (the R_0), to attempt to lower the overall attack rate. The important scientific and public health question is whether such actions would be effective, and if so, what are the exact characteristics of the infection that would permit effectiveness. Understanding these phenomena could save lives in the event of a highly lethal outbreak.

It is well-known that influenza may be infectious before it is symptomatic. That property allows spread before public health or common sense measures prevent transmission. Thus, the duration of the pre-symptomatic phase of the infection is directly relevant to transmission risk. We employed an SEIR model, similar to that in the studies of SARS, but now introduced elements in the model that distinguished between infectious and symptomatic carriage. This permitted us to examine the effects of quarantine and school closures on model communities. These studies permitted us to define the parameters of the infection and its effects on

susceptible individuals for which such public health measures as school closures and quarantine.

The future

I am happy to say that after working together for 20 years, we are still at it. For the past eight years we have been developing a study of the effects of late-in-life infections on overall host population size and community fitness. This work was just published [13] in *mBio*, the journal of the American Society for Microbiology. But beyond that, we have other plans for using the tools of mathematics to help us understand the natural phenomena in hosts that infectious diseases, and the normal organisms that inhabit us represent. It has been a great journey working with Glenn, and we are still near the start of the trail.

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