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From The Germ Theory to Antimicrobials and Antibiotic Resistance

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Abstract

The recognition that microorganisms (germs) are the major cause of human disease, not surprisingly, eventually had a dramatic impact on medicine. Although there were some farsighted proponents of the germ theory during the late seventeen hundreds, until well into the mid-nineteenth century the theory that disease was caused by bad air (so-called Miasma) held sway. The recognition that microbes can cause disease led to major improvements in hygiene and eventually led to the introduction of antiseptic and then aseptic surgery. The development of penicillin and other antibiotics at last provided a means of controlling bacterial infection, allowing for the application complex medical procedures, such as open heart and transplant surgery. The appearance of antibiotic resistance is no threatening the so-called golden age of antibiotics and there is a desperate need to develop new antibiotics, or new approaches to disease control. This review aims to provide a broad account of the history of the germ theory, which highlights some common misconceptions about the work of some of the pioneers involved, and emphasises the neglected contribution of others.

Keywords: *Microorganisms; Medicine; penicillin; Antibiotic resistance; germ theory*

1. Introduction

The recognition that microorganisms exist and can cause disease in humans, animals and plants was one of the greatest developments of civilisation. It allowed for huge rises in population and improvements in human well-being. Prior to the development of the germ theory, diseases were thought to be caused by so-called "miasmas", that is literally, bad air (Last, 2001). Miasmas were thought to be generated in swamps and areas were human and animal wastes accumulated. Since pathogenic microbes also inhabit these places, the miasma theory was credible and as a result was slow to be refuted (Last, 2001).

The discovery of microorganisms is generally credited to the Dutch draper and amateur microscopist, Anton van Leeuwenhoek (Porter, 2003); the English scientist Robert Hooke however, did much to develop this initial discovery. Microbes were initially lumped together with higher microscopic organisms, like rotifers, under the term "animalcules" leading to the concept of so-called "animalcular disease". While many scientists were fascinated by animalcules, it took a long time for the view that some of these organisms might be involved in disease to become established. Many microscopists vaguely hinted at the possibility during the seventeen hundreds and the idea entered popular science and even literature without being fully accepted. A good example of this is provide by a reference to animalcules causing disease in the popular stage play, The Devil Upon Two Sticks by Samuel Foote which was a popular play in London during the late 1700s (Wainwright, 2003).

Louis Pasteur is often, erroneously, credited with the discovery of the link between microbes and human disease; a far better candidate for this honour is however, Sir John Goodsir (Wainwright, 2003). Goodsir, a Scottish anatomist, showed that the bacterium *Sarcina* caused a stomach infection in humans; remarkably he then went on to cure the infections by administering silver nitrate(check). Prior to this chemical agent, such as mercury and its salts, had been used to treat skin infections and even syphilis, with some success, but without an awareness that their effectiveness was due to their ability to kill bacteria.

It was not until the mid to late nineteenth century that the germ theory became fully developed and lead to the wide recognition that the administration of antimicrobial agents could cure microbial infections. This development was achieved by the development of methods to isolate, and culture microorganisms, and the associated division of animalcules into the main groups of microbes, namely, algae, bacteria fungi and protozoa (Wainwright and Lederberg,1992). Microbial isolation techniques were at first primitive, with bacteria, for example, initially being isolated and grown using the surface of the moist half of a cut potato (Wainwright and Lederberg,1992). However, better methods were developed, including the use of agar and the petri dish and the subsequent development of sterile technique which allowed for the isolation of specific microorganisms and their linkage with specific diseases. The introduction of Koch's postulates then enabled microbiologists to be confident that any organisms which they isolated from an infected patient was the true cause of the associated disease (Wainwright and Lederberg,1992). Having demonstrated that microbes were the cause of most human and animal disease, the next step was to find agents which could eliminate these infective agents and thereby bring about a cure.

An essential aspect of the germ theory which is often ignored or underplayed is the recognition that microorganisms (notably fungi) cause diseases in crop plants. Such recognition was initially centred on finding the cause, and prevention of potato blight, caused by the microscopic fungus. *Phytopthora infestans*. (Wainwright, 2008). This infection was prevented, and the potato crop saved, by the use of Bordeaux mixture (a copper and lime-based product) long before the cause was known. The study of potato blight and its cure, which finally prevented tragedies like the potato famine reoccurring, is forever associated with name of the Reverend Berkley (Wainwright, 2008).

The development of the science of epidemiology was also a crucial development which was dependent on the germ theory. The most famous example being the discovery, by John Snow, that cholera is a water borne disease, a link which was again made was made before the full development of the germ theory (Panaeth, 2004). Snow used epidemiology and statistics to demonstrate this association, but was unaware that the causal agent was a bacterium (*Vibrio cholera*), nor did he invoke the involvement of animalcules in general, although this possibility was raised, around the same time, by two Bristol-based scientists, Budd and Swayne (Wainwright, 2003). Even without knowing the direct cause of cholera, John Snow clearly demonstrated that a disease agent could be water borne and spread and, as a result, was able to recommend that only clean water be consumed by the people of |Soho in London; such a recognition obviously caused confusion amongst the supporters of the miasma theory, who were linking disease solely with the occurrence of bad air (Panaeth, 2004).

It soon became apparent that drinking water was not to be the only means of transmitting disease as was evidenced by studies beginning in the 1840s showing that doctors could spread childbed (i.e. puerperal fever) by hand, a fact which again markedly disturbed the miasma theory. The discovery of the bodily transfer of disease, and need for rigorous handwashing, is usually credited to the Viennese physician, Ignaz Semmelweis (Newsom,1993), who famously showed that doctors and student were transferring a disease-causing agent between pregnant women, occupying beds in lying-in wards (Newsom,1993). The standard account of this discovery sees Semmelweis being unable to convince his fellow doctors and finally dying without his ideas being fully recognised. Like Snow, Semmelweis' work depended on epidemiology and showed that at a disease could be prevented by direct intervention, in this case by making wash their hands anyone who examined women during childbirth (Newsom,1993). Like Snow however, Semmelweis was completely unaware of the nature of the infective agent involved (subsequently shown to be a bacterium) and believed that pieces of dead flesh (the so-called cadaveric principle) were being transferred between patients and directly causing the disease. Although the importance of handwashing is usually linked with Semmelweis in the standard history of medical microbiology there is clear evidence that his work was preceded by the findings of the English physician, Robert Storrs (Wainwright, 2003). Storrs was so convinced that he was carrying, and spreading, puerperal fever between expectant mothers, that he travelled to a nearby coastal town to have the causal agent "blown from off him", not surprisingly, this approach failed to work. As Semmelweis later showed, Storrs demonstrated the direct hand-transfer of a disease-causing agent, as illustrated by the case of childbed fever, but did not know, or even speculate about, was unaware of what caused the disease (Wainwright, 2003).

The history of smallpox proves yet another example of how a disease was cured before its cause had been recognised. The story of how Edward Jenner developed vaccination to treat smallpox has frequently been told (Riedell, 2005, Wainwright, 2003). As was the case of childbed fever, it is worth noting that this seminal discovery had been early achieved much earlier, in this case, by a Dorset famer called Benjamin Jesty, who developed the use of some thirty years earlier than Jenner. Jenner's

influential position however, has meant that his name has been uncritically linked with the discovery of vaccination (Wainwright, 2003). Both Jesty and Jenner were however, unaware, when they employed cow pox to prevent small pox that the causal agent was a microbe (in this case a virus).

Mention has already been made of an important contribution made by the German bacteriologist, Robert Koch, in relation to his famous "postulates" Evans (1976). Koch's other major contribution to medical microbiology was his ability to isolate bacteria from infected patients and by using first-rate microscope–technique isolate the presumed causal agent; he would then apply his postulates to show that the isolate was indeed the causal agent. Using this approach Koch isolated important bacterial infective agents such as the causal agent of tuberculosis (Broch,1999).

We have seen that the miasma theory was eventually thoroughly discredited by the work of medical scientists like John Snow and Robert Koch. However, yet another means of disease transmission, namely the spread by insect vectors had yet to demonstrated. Such insect-vector spread is exemplified by malaria and yellow fever. The cause and transmission of both of this disease was for a long tome profoundly misunderstood, and were it not for the sterling work of Ronald Ross and in relation to malaria (Schlagenhauf, 2004), and Walter Reed, in relation to yellow fever (Staples and Monath, 2008), the cause and eventual prevention and treatment of these disease would have been held back considerably.

By the late Victorian period the Germ Theory become had firmly established and the cause of many of the major infectious diseases was known, little progress had been made towards developing cures for the major diseases and sepsis developed during surgery was still a frequent killer. It was Joseph Lister who finally developed a technique that would finally dramatically reduce deaths caused during surgery.

By the simple expedient of applying the strong antibacterial agent, carbolic acid to wounds and surgical implements during operations, Lister introduced antiseptic surgery (Pitt and Aubin, 2012; Toledo-Pereyra,1976). The technique also involved spraying carbolic acid into the operating room, and following on from Semmelweis and Storrs, Lister's surgical team also washed their hands in carbolic, but did not use surgical gowns or masks. Antiseptic surgery had a major positive impact on surgery (although its effectiveness has been questioned, Toledo-Pereyra,1976).), but was eventually replaced by the aseptic approach were those present in the operating theatre (which is maintained scrupulously clean and sterile and is held at a positive pressure) where sterile gowns, masks and head gear are worn and where the surgical implements have been sterilized by autoclaving, or by some other means.

The recognition that viruses are involved in infection began in the 1930s when influenza was shown to be caused by a so-called "filterable virus" and was extended to the development of vaccines against polio and, hopefully, in the near future, AIDS (Bos, 2000).

The position at the beginning of the twentieth century, the battle against infectious disease was simple. The role of microbes, notably bacteria, as aetiological agents of the main killer diseases had been well established and individual pathogens had been shown to cause these individual infections. Yet despite this no chemical agents were available to successfully treat bacterial infections. In the early nineteen-hundreds however, things changed with the discovery of arsphenamine, an arsenic compound that when used with extreme care could cure the venereal disease, syphilis (Bosch and Rosich, 2008). Arsphenamine was sold under the trade name Salversan. It and was synthesised by Alfred Beetheim while working in the lab of Paul Erlich, although its anti-syphilis properties was discovered two years later, in 1909, by Sahachiro Hata (Lloyd, *et al*., 2005). Arsphenamine was originally called "606" because it was the sixth in the sixth group of compounds synthesized for testing (Schwartz, 2004). Eventually, members of Ehrlich's laboratory developed a safer and less problematic arsenic compound, called Neosalvarsan or neoarsphenamine. Salvarsan was by no means ideal, but it suggested the possibility that chemical compounds could be synthesised which could kill bacteria without killing the patient (Lloyd, *et al*., 2005). The next development along these lines came with discovery of Prontosil in 1932 by Gerard Domagk (Bentley (2009). Prontosil is a sulphonamide drug which has a wide antibacterial spectrum and is relatively safe to use. The sulphonamides were widely used until the advent of a new class of "wonder drugs", the antibiotics.

The Antibiotic Age

An antibiotic is defined as a product produced by microorganisms which is used in medicine to kill bacteria and thereby cure infections; the term is however often loosely used to include all antibacterial agents (Walsh, 2003). The first and most important antibiotic is penicillin which was discovered by Alexander Fleming in 1928 and developed for medicine, during the early 1940s, by a team at Oxford University lead by Howard Florey (Masters,1946).

When it first appeared, penicillin was seen as a wonder drug which revolutionised and literally saved dying patients from the jaws of death. The penicillin-group remains the most important group of antibiotics, despite the increasing appearance of penicillin resistance amongst pathogenic bacteria (Falkow, 1975).

It is difficult to appreciate the impact that penicillin had on medicine. In the late 1930s trainee doctors were often introduced to the "smell of death" by visiting so-called septic wards where dying patients were literally producing bucket loads of foul-smelling pus! Overnight these patients, who were dying of septicaemia, were saved; it appeared almost miraculous. Penicillin was used to treat a wide range of infections, including syphilis and gonorrhoea. It appeared just in time to be used in the D Day landings and did much to contribute to the Allied victory. Penicillin also allowed for the development of novel kinds of surgery, including open heart surgery.

The story of how penicillin came to be discovered has been widely told and is often used to provide an example of serendipity, that is a discovery made by accident (Bud, 2007). The simple story relates how Fleming was called back to St Mary's hospital in London doing September of 1928. He entered his laboratory and noticed a few petri dishes which he had left from earlier work. He picked one of them up and said "that's funny!" (Or in some accounts a Scottish idiom, here's a rum go!" Fleming had picked up the most famous petri dish in history Wainwright (1993,1994). On the surface of the agar, he saw a large mould colony and around it a mass of colonies of the bacterium *Staphylococcus*. The bacterial colonies close to the mould colony were killed and showing signs of being lysed. It was undoubtedly this lysis (or clearing) which so attracted Fleming's attention. The ability of fungi to kill bacteria (so-called b microbial antagonism) was commonplace and would probably have been overlooked by Fleming were it not associated with lysis. Years earlier Fleming had been interested in an enzyme called lysozyme which occurs in some human bodily fluids, including tears. Lysozyme kills bacteria by bringing about their lysis, but as Fleming showed, it has little in the way of therapeutic potential. When he saw the fungus colony causing the bacteria to lyse, Fleming thought he was observing a novel type of fungus-lysozyme, which he was obviously keen to study (Wainwright (1993,1994). He removed the fungus colony transferred it to liquid growth medium and grew the fungus. When he tested the "mould juice "against bacteria he found that the juice, which he named "penicillin" killed a wide range of pathogenic bacteria. Fleming continued to study the properties of penicillin and wrote the famous paper on his discovery which was published in 1929. Instead of an eye-catching title, Fleming chose a somewhat prosaic one, which was unlikely to catch wide-spread attention, namely *On the Antibacterial Action of a Culture of Penicillium, with Special Reference to its Use in Isolation of B. influenzae* (Fleming,1929). Fleming however, considered that this title highlighted something important about penicillin, namely that it could be used to isolated strains of *Bacillus influenza* from patients. As its name suggests, this bacterium was thought to be the cause of influenza and in the title, Fleming highlights how it could be used to isolate infective strains from individual patients, and thereby hopefully be used to create a custom-made vaccine. Since influenza is caused by a virus, this possibility never became an important reality. Fleming did however mention in his paper the potential for using penicillin as a therapeutic agent, a couple of lines which were later picked up by the Oxford team when they took an interest in penicillin some ten years later. Fleming made a number of unsuccessful attempts to purify penicillin and tried using it to cure external bacterial infections in patients, with only limited success.

The first successful use of penicillin in the form of un-purified filtrates was achieved (during the early 1930s) by a former student of Fleming, Cecil George Paine (Wainwright and Swan, 1986). Working in Sheffield, Paine used Fleming's mould to produce filtrates which he used to cure eye infections in miners and children. Unfortunately, he never gave a lecture on his successful work, nor did he write a paper on the subject, so his work had no impact at the time (Wainwright and Swan, 1987). He did however have a major effect on later events when, in 1932, he told Howard Florey about his successful use of penicillin. In 1939, armed with this knowledge, Florey and his team at Oxford began the task of purifying penicillin and making it available as a useable drug.

It is often said that Fleming gave up on penicillin soon after its discovery. His notebooks show however, that this was not the case and instead, that he attempted to find other fungal-produced antibiotics, as well as studying other potential antibacterial agents like bacteriophage (Wainwright, 1991b) clearly, Fleming understood the concept of idea of antibiosis and how important it could be in medicine Wainwright, 2004)

Fleming's paper aroused little interest until Howard Florey, Ernst Chain and Norman Heatley began attempts to purify Fleming's "mould juice", There efforts were remarkably successful and after the

American pharmaceutical companies took and interest and employed deep fermentation methods, penicillin was made available in large quantities just in time for D-Day.

The miraculous impact of penicillin led to a "gold rush" with scientists all over the world screening fungi and other microorganisms for their ability to produce novel antibiotics. Disappointingly, although thousand so antibacterial compound turned out to be toxic and could not be used in medicine. Most notably, the Rutgers-based soil microbiologist Selman Waksman began to screen the actinomycetes for antibiotics (Bush, (2010). Together with his postgraduate students discovered actinomycin, neomycin and most importantly streptomycin, the antibiotic that with combined with PAS defeated tuberculosis. Waksman was awarded the Nobel Prize for discovering the latter antibiotic, which induced one of his graduate students, Albert Schatz to claim that he was the actual discoverer of streptomycin and had been denied a share in the Prize (Wainwright,1991a). Rutgers University and the scientific establishment ostracised Schatz for his audacity, but there is no doubt that Schatz was the true and largely independent discoverer of streptomycin (Wainwright,1991a).

Surprisingly few medically useful antibiotics have been discovered since penicillin and streptomycin. However, the introduction of semi-synthetic penicillin markedly extended its range and usefulness.

Penicillin ushered in the antibiotic age a period when bacterial infections were well and truly defeated. Soon after discovering penicillin however, Fleming noticed that bacteria were quickly becoming penicillin–resistant, and in his Nobel prize oration he warned against its over and misuse. In the event, Fleming's warning was ignored and doctors around the world prescribe penicillin, and other antibiotics, for infections, like the flu, against which they have no effect whatsoever. In addition, antibiotics are available in many countries without a prescription leading to widespread self-medication (Devasahayam *et al*., 2010, Grigoryan, 2007). Prostitutes for example, often use antibiotics to self-treat syphilis and gonorrhoea (Gartin, 2010) Not surprisingly the smallest, cheapest and least effective dose is used which leads to the rapid development of pathogen resistance (Aminov, 2009, Aminov and Mackie,2007). The use of antibiotics in animal feed to increase yields only adds to the problem (Bengtsson and Wierup 2006, Jones and Rick, 2003). Doctors now predict, that since bacteria are becoming resistant to antibiotics, unless something dramatic happens to solve the problem, there will be a million more deaths in Britain and Europe by 2025 (Hegreness, 2008). Additionally, antibiotic resistant infections have major detrimental impact on nearly all surgical procedures, like open heart and brain surgery as well as hip and knee replacements. If things stay as they are then such procedures could become a distant memory.

In many parts of the world only vancomycin, remains an effective antibiotic (Walsh,2003). So desperate in fact is the current situation that we are having to resort to old fashioned remedies for the treatment of septic wounds, including, bacteriophage honey (Monk, *et al*. 2010). and maggot therapy. Honey has been used to treat infected wounds since the ancients and sterilized Manuka honey is now used, with some success, in the treatment of suppurating wounds notably in diabetics. Bacteriophage therapy was widely used on parts of the Soviet Union and successful attempts are being made to make the use the type of virus more reproducible (Levin, and Bull, 2004, Lu and Collins, 2009). Maggot therapy provides the best example of how desperate we have become in the face of the appearance of antibiotic resistance. This therapeutic approach involves use of living maggot of the green blowfly. This somewhat bizarre approach was developed by the American physician, William Stevenson Baer in the Great War, and later introduced into civilian medicine in the US, particularly in the treatment of osteomyelitis in children. Maggot, or debridement therapy, is successful because the maggots eat dead, but not living flesh, or bone and produce a range of antibacterial and wound–healing substances. The situation regarding antibiotic resistance is becoming increasingly desperate and we clearly urgently need to develop new antibiotics or new approaches to antibacterial therapy (Rahman, ,2010, Walsh 2003).

4. Conclusion

In conclusion, the journey from miasma to the present dilemma of antibiotic resistance has taken nearly 200 years, with the last hundred or so of that being taken up by advanced in the development of chemical weapons against microbial infection, antibiotics and targeted vaccines (Clatworthy *et al*., 2007). This period of history has seen incredible medical advances which have depended on our awareness of how microbes cause disease. The current situation regarding antibiotic resistance is worrying (Kumarasamy, 2010, Payne,2007), but there is no reason why new antibiotics, or novel approaches to infection control should not be developed Chopra *et al*., 2002, Devasahayam *et al*., 2010, Hancock, *et al.* 2006), so that the scourge or antibiotic resistance becomes relegated to a foot note in medical history.

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