The Greatest Missed Microscopical Opportunity?

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Introduction

In this article I explore the proposition that the greatest missed optical microscopical opportunity occurred in 1854, when Arthur Hill Hassall (AHH) (1817-1894) failed to recognise the cholera organism in specimens that would have been teeming with the bacteria; the same year in which the scientific community did not accept the findings of Filippo Pacini (1812-1883), who is now credited with the discovery of *Vibrio cholerae*. Had, in 1854, a majority of the 'establishment' accepted that cholera was caused by an organism, mainly transmitted in water rather than 'bad air', many lives might have been spared. (The cholera vibrio was brought to general acceptance in 1884 by Robert Koch (1843-1910)). As with many historical retrospectives, the 'facts' are contested. To make this article 'streamlined', it is presented initially as a timeline with a commentary. Seven 'rows' from the timeline, termed Exhibits, have then been selected for more detailed consideration. The timeline places perhaps undue emphasis on AHH, but he is a hero of mine who spent time on the Isle of Wight, where I live.

Background

In the seventeenth century, microscopy first revealed the presence of micro-organisms, and in the early nineteenth century the contamination of London's drinking water was made public, including through a cartoon showing what could be seen down a microscope. Progress, however, faltered as bacteria are hard to visualise in aqueous fluids, being mainly water themselves. They generally require staining or a technique like phase contrast to make them 'visible'. In addition, in the 1850s there was a strong belief in an alternative theory of disease transmission (the miasmic theory of 'bad air'), that favoured anticontagionism and the avoidance of quarantine. The first characterisation of a pathogenic bacterium (anthrax), was not complete until the 1860s, and staining of bacteria was not perfected until the late 1870s/early 1880s.

The Timeline

Date (Exhibit)	Event/publication	Commentary
c1670	Leeuwenhoek: communicated from Holland with the Royal Society	First microscopical demonstration of the existence of micro-organisms.
c1673	Leeuwenhoek: said to be first to use a stain in microscopy	Extracted dye from the saffron crocus bulb.
1828 (Exh 1)	William Heath: Cartoon 'Monster Soup' - depicting contamination in water taken from the Thames	Early attempt to make the public aware of what could be seen down the microscope.
1846	AHH: 'Microscopic Anatomy of the Body in Heath and Disease'	The first book in English on histology. Helped establish AHH as one of the leading microscopists of his day.

Date (Exhibit)	Event/publication	Commentary
1849 (Exh 2)	William Budd: Letter to The Times and other communications; claimed he had seen 'fungous cells' in association with cholera fluids	These microscopical findings were discredited by the Royal College of Physicians (correctly) and are likely to have made microscopists wary of making subsequent claims of having seen the cholera organism.
1850 (Exh 3)	AHH: 'Microscopical examination of the water supplied to the inhabitants of London etc'	Drawings of contamination seen under the microscope in samples of water supplied by various London water companies.
1850	Ignaz Semmelweiss: Advocated washing hands to prevent the spread of childbed fever	Hand washing has general application to all infectious diseases including cholera. Essential for those caring for cholera patients and handling cholera pathology samples!
1850-68	Pollender, Rayer & Davaine: 'Discovered' the anthrax bacillus	Characterised the anthrax bacillus under the microscope.
1854 (Exh 4)	John Snow: 'On the Mode of Communication of Cholera' (2nd much enlarged edition)	Snow argued that cholera was spread mainly by contaminated water, but also by touch and in food. At the time this was not generally accepted: spread by bad air was widely favoured.
1854 (Exh 5)	Filippo Pacini: 'Microscopical observations and pathological deductions on cholera'	Described the comma-shaped cholera organism. Published in Italian but noted by Farr. Recognised in 1965 – see below.
1854	Lionel Beale: 'The Microscope in Medicine'	1st ed – envisaged only very limited role in medicine. Bacteria not mentioned.
1855	AHH: 'Food its adulterations & methods for their detection'	AHH pioneer of detection, using the microscope, of what became 'criminal' adulteration of food and drugs.
1855 (Exh 6)	AHH: 'Report on the microscopical examination of Blood and Excretions, &c of Cholera Patients'	Part of a Report to both Houses of Parliament on the Cholera Epidemic of 1854. Perhaps surprisingly, AHH did not suggest that the vibriones he saw were the cause of cholera.
1863	AHH: 'Urine in health and disease'	AHH published widely on microscopy.
1866	William Farr (lead civil servant on medical statistics): Report on the 1866 outbreak of cholera	By 1866 Farr concluded that cholera was transmitted by 'cholera flux', mainly in contaminated water, and not bad air.
1867	Farr: visit to Italy where he met with Pacini	Farr praised mathematical work Pacini had undertaken on cholera. Farr did not, however, endorse Pacini as the discoverer of the cholera organism - a further missed microscopical opportunity!
1875	Karl Weigert: alcohol-methylene blue staining of bacteria	Weigert introduced into bacteriology, staining techniques in use for histology.
1876	Robert Koch: performed a series of experiments on anthrax fulfilling what came to be known as Koch's postulates	The first time a microorganism was conclusively linked with a specific disease. First 'proof' of the Germ Theory.

Date (Exhibit)	Event/publication	Commentary
1877	Robert Koch: Photograph of *Bacillus anthracis*	First photomicrograph of a bacterium.
1882	Paul Ehrlich: developed an 'acid-fast stain'.	In 1885, technique finalised by Ziehl & Neelsen.
1882	Robert Koch: announced the discovery of the main organism causing tuberculosis. 1905, awarded a Nobel Prize	Previously, Jean-Antoine Villemin had shown that TB was transmissible. Koch advanced several lines of evidence to show his identified organism is the cause of TB. Later refined into 'Koch's Postulates'.
1884	Hans Christian Gram: published his famous stain	Devised Gram stain in 1882.
1884	Robert Koch: announced the discovery of the bacterium Vibrio cholerae. 1965 organism 'reassigned'	Again, Koch advanced lines of evidence, but at that time, could not show that his cholera organism caused disease in any 'lower animal'.
1884	Correspondence about the discovery of the cholera vibrio in the Lancet, including letters from AHH	Correspondents argued that Pacini should be credited with the discovery of the cholera organism. AHH maintained he saw the organism before Pacini.
1965 (Exh 7)	International committee on nomenclature: adopts Vibrio cholerae Pacini 1854 as the correct name of the cholera causing organism.	Decision reached largely due to the urging of Prof Rudolph Hugh of USA.

Exhibit 1: Cartoon 'Monster Soup' by William Heath

Fig. 1: Cartoon Monster Soup by William Heath

In 1828, the caricaturist William Heath published a cartoon lampooning the quality of water being supplied by London water companies. This coincided with the 1827-28 Royal Commission on Metropolitan Water Supply. The growing population and increased use of drains for disposing of sewage (rather than cesspits and carting manure to fields), was polluting the River Thames, from which water companies extracted their supply.

The cartoon depicts a fashionable lady viewing a sample of drinking water through a microscope and dropping her cup of tea in response to what she saw. The caption at the top of the frame reads:

Microcosm, dedicated to the London Water Companies, Brought Forth All Monstrous, All Prodigious Thigs [sic], Hydras, and Gorgons, and Chimeras Dire. Vide Milton [Paradise Lost].

Bottom of the frame:

Monster Soup Commonly Called Thames Water, Being a Correct Representation of that Precious Stuff Doled Out To Us!!!

Exhibit 2: William Budd: described microscopic organisms he claimed to be the cause of cholera

In 1849, when cholera was affecting Bristol, the local medical society appointed a microscopical committee including Frederick Brittan, Joseph Swayne and William Budd. Subsequently, they announced widely (example below) they had found the cholera-causing organism. However, these findings were not supported by the Royal College of Physicians. The organism 'discovered' was probably a fungus and definitely not the cholera vibrio'.

London Journal of Medicine, 1849, page 987.

> *Discovery of peculiar living organisms in the "Rice-water" evacuations of Cholera'. 'Drs J. G. Swayne and Brittan, of Bristol, having been for some time engaged, under the direction of the Medico-Chirurgical Society of that city, in the microscopic investigation of subjects connected with cholera, state that they have discovered, in the rice-water discharges, and also in the atmosphere of infected places, certain living organisms, to which they are disposed to attach much importance in the etiology of cholera. Dr. William Budd, of Bristol, in a letter to the Times of September 26, confirms their statements, and mentions that he has detected similar organisms in water procured from cholera districts.*

Exhibit 3: 'A microscopical examination of the water supplied to the inhabitants of London and the suburban districts' by Dr Hassall

Arthur Hill Hassall qualified as a medical doctor, and early in his career became well known as an expert in the application of the microscope to natural history, writing books on freshwater algae, anatomy, pathology, and public health, especially on the detection of the adulteration of drugs and food. Early in his career, Hassall discovered microscopic features in the thymus gland that are still widely known as 'Hassall's Corpuscles', rather than 'Thymic Corpuscles'. (In the nineteenth century many anatomical and histological new findings were named after their discoverer; most of these 'eponymous titles' have subsequently fallen into disuse.)

Figure 2: AHH's depiction of organic matter concentrated from drinking water supplied by the New River Company

In 1850, as one of his first publications on 'adulteration' (defined in this instance as: 'making profit by supplying contaminated products, in extreme cases adding cheaper ingredients, toxic colourants etc to make a product go further or change its appearance to make it more saleable'), AHH published a microscopical review of the piped water supply sold to London inhabitants by various privately owned water companies. The review revealed extensive pollution, indicating no improvement since William Heath's cartoon 'Monster Soup'.

It is difficult to assess the impact of AHH's 1850 publication, which he previewed in the Lancet. In 1851, AHH gave evidence to a Parliamentary Committee of Inquiry on the water supplies of the Metropolis. The 1852 Metropolis Water Act resulted, but change was slow to follow and, in the end, real improvement depended on Bazalgette's sewage engineering scheme that was opened in 1865. Prior to that, in 1854, AHH undertook a further review of the microscopy of London's water supply (see Exhibit 5).

Exhibit 4: 'On the Mode of Communication of Cholera' by

John Snow (2nd enlarged edition)

4a) Involvement of Hassall by Snow in the Broad Street pump outbreak

Page 52:

> *Hassall, who was good enough to examine some of this water with the microscope, informed me that these particles had no organised structure, and that he thought they probably resulted from decomposition of other matter. He found a great number of very minute oval animalcules in the water, which are of no importance, except as an additional proof that the water contained organic matter on which they lived. The water also contained a large quantity of chlorides, indicating, no doubt, the impure sources from which the spring is supplied. Mr. Eley, the percussion-cap manufacturer of 37 Broad Street, informed me that he had long noticed that the water became offensive, both to the smell and taste, after it had been kept about two days. This, as I noticed before, is a character of water contaminated with sewage. Another person had noticed for months that a film formed on the surface of the water when it had been kept a few hours.*

4b) Snow's references to 'morbid matter'

(Snow, along with other practitioners of the time, was a follower of the 'Zymotic theory', a term that had been coined by William Farr (1807-1883).)

Page 15:

> *For the morbid matter of cholera having the property of reproducing its own kind, must necessarily have some sort of structure, most likely that of a cell. It is no objection to this view that the structure of cholera poison cannot be recognised by the microscope, for the matter of smallpox and of chancre can only be recognised by their effects and not by their physical properties.*

Page 54:

> *We must conclude from this outbreak that the quantity of morbid matter which is sufficient to produce cholera is inconceivably small, and that the shallow-wells in a town cannot be looked on with too much suspicion, what ever their local reputation may be.*

Page 109:

> *It must not be disguised, however, that medical men are not yet generally convinced that the disease is actually communicated from person to person by the morbid matter being swallowed in the drinking water, or otherwise.*

John Snow is the modern hero of cholera, and has his own Society (johnsnowsociety .org), because he got it right; cholera is mainly spread by contaminated water. He was disregarded in his day because the medical elite favoured an alternative hypothesis (cholera transmitted by 'bad air'). Snow was not himself a member of the medical elite, (although he gave chloroform to Queen Victoria in labour), and his methods and conclusions were critiqued in detail - Snow did make mistakes (Parks 1854).

Exhibit 5: 'Microscopical observations and pathological deductions on cholera' by Filippo Pacini

Like Hassall, Filippo Pacini qualified as a medical doctor and became skilled in the growing application of the microscope. By contrast, Pacini was university based, and became Professor of Anatomy at Florence in Italy. Early in his career, Pacini described a tiny nerve sensory ending that is found in skin and other tissues. This became widely known, and to this day generally retains its eponymous label, 'Pacinian Corpuscle'.

In 1854, when Florence was affected by cholera, Pacini was able to perform only a limited number of post-mortems on cholera patients, but conducted histological examinations of their intestinal mucosa. Pacini published a paper that was subsequently key, in 1965, to the decision of the International Committee on Nomenclature. However, at the time an anonymous reviewer, published in The British and Foreign Medico-Chirurgical Review 16: 144-145, stated:

> *The Florentine micrographer prefaces his memoir with the statement, that the results recorded in it are few and incomplete, owing to the smallness of his opportunities for the practical study of pathological anatomy.*

Four cases of Asiatic cholera were the bases of these observations. In the only two cases in which he examined the vomited matter it contained sarcinae ventriculi; in one he additionally found some vibrios of the genus Banksium. Only once did he examine the faeces, and then without particular result. In three of the cases he examined the intestinal fluid, and found floating in it a large quantity of epithelium, and some detached villi. In one of the cases he additionally found many of the choleraic fungi, and an immense number of vibrios. On examining the corresponding mucous membrane, it appeared anaemic, and completely stripped of its epithelial covering; this condition, by permitting the extraordinary serous extravasation, he regards as the first and principal pathological condition of cholera. To develop this proposition, our author engages in lengthened arguments, which however ingenious, have failed to remove from our mind an impression of regret that before their publication a much larger number of observations was not instituted, and the micrographer's power of judging their value increased by his studying the disease en clinicier. We cannot but think that, in studying cholera, the renowned discoverer "dei nuovi organi" if he has not actually mistaken an effect for a cause, has raised an incident to the unmerited position of an essential and fundamental feature.

Pacini was unable at the time to convince his peers or the authorities that he had discovered an organism that was associated with, and the cause of, cholera. In 1867, he was unable to convince William Farr when he visited Florence, in spite of Farr's recent recognition that cholera was transmitted by contaminated water.

Exhibit 6: 'Report on the microscopical examination of Blood and Excretions, &c of Cholera Patients' by Dr Hassall

Arthur Hassall, who had become an established, medically qualified microscopist, was commissioned by the government (Board of Health) during the course of the 1854 cholera epidemic to produce two reports, the first on microscopy of the public water supply (to an extent replicating work he had undertaken in 1850 (exhibit 3)) and the second on the microscopy of rice water diarrhoea, blood, urine etc., from cholera patients. Only the second of Hassall's reports is featured here, and then only the microscopy of rice water evacuations.

Hassall related that he had examined samples from 25 patients including material that was fresh (passed within 2 hours of examination), and a sample obtained at post-mortem from the small intestine. Hassall found 'myriads of vibriones' in all of these samples. Hassall noted:

- *Of these vibriones many formed threads more or less twisted, while others were aggregated into masses, which under the microscope, presented a dotted appearance.*
- *It thus appears that vibriones are constantly present in the rice-water discharge of cholera, and that they are developed in it during life, and while still retained in the small intestines.*
- *It is possible that they may obtain entrance into the stomach and bowels by means of the atmosphere, and it is perfectly certain that they do frequently gain admission through some*

Figure 3: AHH's 1855 depiction of 'Rice water evacuation of cholera'. (d) Acicular crystals (e) Fragments of muscular fiber (f) Vibriones (in a chain) (g) Masses of vibriones

of the impure waters consumed, in which I have not unfrequently detected the presence of vibriones, sometimes in considerable numbers.

- *Once introduced into the alimentary canal, they are brought into relation with conditions highly favourable to their development and propagation, both of which take place with almost inconceivable rapidity.*
- *I have made two or three examinations of healthy and natural faecal evacuations at the time of their being passed, and in these I have detected the presence of comparatively a very small number of vibriones.*

In 1855, AHH concluded, in retrospect surprisingly:

> *Without, however, at all supposing that there is any essential or primary connexion between these vibriones and cholera, their occurrence in such vast numbers in the rice-water discharges of that disease is not without interest, and possibly is of importance.*

In 1884, following the announcement of Koch's discovery, Hassall wrote three times to the Lancet and was at pains to underline his belief that he saw vibriones before Pacini. He then included the sentence:

> *I would remark, however, that while I recognise the importance of this discovery I did not go to the length of describing it as the cause of cholera.*

But that is the point, Pacini consistently claimed that what he had seen was the cause of cholera – even though he was not generally believed.

In his autobiography published in 1893, AHH noted

> *… from the characters exhibited and which are fairly represented in the figure (reproduced above), and from their general agreement with Koch's description, there is not the smallest doubt that the cholera bacillus was present in the discharges in nearly every case and was first seen by me during the cholera epidemic of 1854, now nearly 40 years since.*

Although AHH reflected on many things in his autobiography, he did not reflect on the elephant in the room, why had he not suggested there was a primary connection between the vibrions and cholera?

Exhibit 7: International committee on nomenclature: Adopts Vibrio cholerae Pacini 1854 as the correct name of the cholera causing organism.

In his 'Request for an opinion' to the International Committee on Nomenclature, Prof Rudolph Hugh referred to the 1854 paper by Filippo Pacini and concluded:

> *Pacini saw the curved etiologic agent of Asiatic cholera in tremendous numbers in denuded intestinal epithelium and intestinal contents of victims. The pathological changes in the intestine and the organism itself were described in such a manner that they may be recognised.*

The International Committee, with a hundred years' experience of the germ theory, sided with Pacini and the name of the vibrio was changed from *Vibrio cholerae* Koch 1884 to *Vibrio cholerae* Pacini 1854.

Conclusion

Was 1854 a credible opportunity for the visualisation of the cholera vibrio and its demonstration to the medical establishment?

Factors mitigating against:

- William Budd's 1849 announcement of the discovery of the cholera organism had been discredited.
- By 1854, no bacterial organism had been discovered and no staining of bacteria had taken place.
- Snow's contention that cholera was mainly spread by contaminated drinking water and caused by 'morbid matter', that he believed was an organism, had not been generally accepted by the medical establishment

Factors mitigating for

- AHH (along with others) was an expert of microscopy whose opinion was likely to have been respected.
- AHH described and illustrated 'vibriones', that in retrospect were the cholera vibrio.
- AHH knew Snow personally, and is likely to have been well acquainted with his ideas, but for some reason he

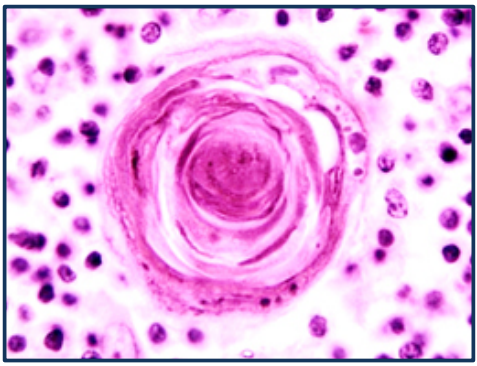

Fig. 4: Hassall's corpuscle

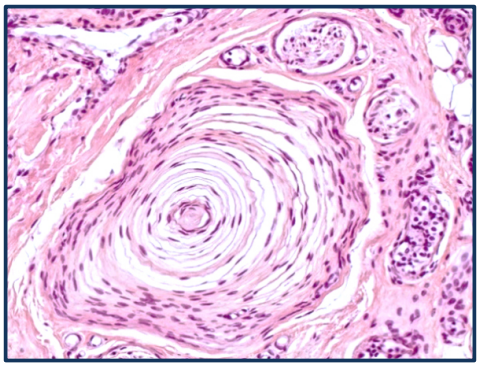

Fig. 5: Pacinian corpuscle

did not integrate Snow's ideas with his vibriones.

Arguably Pacini had less opportunity (few cases of cholera to study and he was not government sponsored) than AHH. In the view of a small number of contemporary supporters, and in retrospect, the International Committee on Nomenclature, he not only described the cholera vibrio but hypothesised it was the cause of cholera. At the time, however, he was not generally believed.

Should Cholera vibrio have been renamed?

Pacini may have been the first to 'discover' the cholera vibrio, but that made no apparent difference. Robert Koch appears initially to have believed that he was first to have discovered the vibrio. Although change was slow, his 'discovery' did make a difference.

Are there other contenders for the greatest missed optical microscopical opportunity?

For this question, it is over to readers for as many nominations as possible. If there were to be enough interest, PMS could take a vote!

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