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Germs of Ideas

> A bacterial cause has been definitely ruled out and there is now undisputed evidence that the infectious agent belongs to the group of so-called filterable viruses.
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> Francis W. Peabody, George Draper and A.R. Dochez, *A clinical study of acute poliomyelitis*, 1912

The story so far: polio is not caused by teething, pesticides, ptomaine poisoning, electrical appliances or misaligned vertebrae in the neck. It is an infection, as was confirmed in 1908 by the grisly experiments in which Karl Landsteiner transmitted polio to live monkeys with an extract of spinal cord from a dead boy.[1]

Landsteiner also provided vital information about the nature – or at least the size – of the infectious agent. The spinal cord extract injected into the monkeys had been filtered through a 'Berkefelt candle'. The candle was a hollow cylinder of baked diatomite, a whitish silica clay made up of billions of skeletons of microscopic marine organisms. The channels through the filter were so minute and tortuous that they trapped bacteria. Whatever passed through was therefore even smaller – one of the 'so-called filterable viruses' mentioned in the quotation above, from one of the great contemporary monographs on polio.[2]

The discovery that polio was caused by a virus rather than a bacterium was not just scientific fine print. This was the essential first step towards accurate diagnostic tests and properly targeted treatments – remembering that antibiotics, our most effective weapons against bacteria, are powerless against viruses.

Landsteiner's paper came out at a time when the pace of discovery was brisk, and it seemed likely that the 'filterable virus' of polio would be pinned down within a few years. In the event, the task took nearly forty years. This was a momentous journey which generated a clutch of Nobel Prizes and pulled in some of the greatest scientific names of the twentieth century. But there were also sideshows and distractions along the way – blind alleys, red herrings and charlatans, and many instances where evidence was not allowed to stand in the way of a good story.

G. Williams, *Paralysed with Fear*

Perhaps surprisingly, many scientists did not accept that polio was due to a virus. In their well-known *Manual of infantile paralysis*, Henry Frauenthal and Jacolyn Manning gave less space to the filterable virus as a possible cause of polio than to 'Dixon's protozoon' (which fell from grace a few years later).[3] Their *Manual* was published in 1916, four years after Peabody's classic monograph, by which time Landsteiner's findings had been amply confirmed by several other groups. Years later, an eminent microbiologist was invited to talk about the cause of polio at the biggest medical conference in America. He devoted his entire lecture to the 'poliomyelitic' bacterium named after himself.[4] This was not in the dark ages of the 1920s but in 1952, long after the electron microscope had revealed the poliovirus for all to see.

To make sense of this story, we need to go back a hundred years, to an age when polio was just one of many diseases waiting to be solved by germ hunters who hoped to follow in the giant footsteps of Robert Koch and Louis Pasteur.

Causes and effects

The first decade of the twentieth century was a boom time for bacteria, genuine and otherwise. Journals devoted to infectious diseases flourished and were full of reports of weird and wonderful bacteria, previously unknown to science. One new species was *Streptothrix interproximalis*, rescued from mouths tersely described as 'habitually unclean'. It certainly looked dramatic – rods, scrolls or branches, with or without spots – but whether it did any harm was anyone's guess.[5]

The discoverers of new bacteria were also searching for a role for them, especially in diseases which had long refused to reveal their secrets. The pressure of competition, helped along by dubious experiments and wishful thinking, often stretched the imagination more than the boundaries of knowledge. Particular bacteria were identified as the causes of smallpox, rabies, measles and rubella – all of which are viral infections – and malaria, caused by a protozoon.

Other bacteria had even more to live up to, being blamed for diseases that are not even infections. Several authors claimed to have found bacteria that caused scurvy, the scourge which made teeth fall out of the bleeding gums of sailors on long-haul ocean voyages.[6] An as yet unidentified germ, reportedly spread by insect bites, was believed to cause pellagra. This was a disfiguring condition with a skin eruption so ugly that some American hospitals refused to admit sufferers, and nurses went on strike to avoid looking after them.[7] Another presumed infection was beriberi, which struck down millions in the Far East with agonising pains in the limbs and massive fluid accumulation in the legs.

In *Principles and practice of medicine* (1892), William Osler maintained, 'It is probably due to a microorganism'.[8] The more exotic candidates included Pekelharing's staphylococcus, Taylor's spirillum and Durham's looped streptococcus. The elusive bacterium of beriberi was perhaps the germ hunters' holiest grail – and one of those who joined the chase was the greatest of them all, Robert Koch himself.

Many researchers became convinced that polio was due to 'poliomyelitic bacteria', which they found in compromising situations in tissues or body fluids of polio patients. These included rod-shaped bacilli, spherical cocci and more bizarre forms such as the branched, root-shaped rhizopods. The commonest were streptococci, similar to those that cause tonsillitis and blood poisoning. Most of the purported 'poliomyelitic bacteria' soon slid back into obscurity when other researchers could not find them, or showed them to be contaminants. For example, Ivar Wickman, epidemiological detective extraordinaire, set off after the fabled 'tetracoccus' identified by Magnus Geirsvold, only to find that it was indeed a fable. No convincing case had been made by 1908, prompting the American orthopaedic surgeon B. Sachs to write, 'The infectious microorganism, whatever its character may be, has not yet been revealed'.[9] This set the stage neatly for Landsteiner to usher in his filterable virus a year later.

Landsteiner's paper did not persuade everyone to think small. The filtration candles were relatively new and were throwing up some odd results, including the heretical claim that tuberculosis was caused by a filterable virus rather than the bacillus that had won Koch his Nobel Prize in 1905.[10] Sceptics, some of them well respected and powerful, therefore clung to the belief that bacteria caused polio – and some continued to do so long after their pet microbe had been discredited.

The believers in poliomyelitic bacteria included Ludvig Hektoen, Professor of Pathology at the University of Chicago and founding editor of the *Journal of Infectious Diseases*. A prolific researcher, Hektoen had notched up his hundredth paper ('An anatomical study of a short-limbed dwarf') by his fortieth birthday.[11] In 1918, Hektoen reported in the *Journal* that he had found 'cocci' in stained sections of brain and spinal cord from polio victims. These bacteria looked 'quite like' those which others had claimed to have cultured from patients' tissues and fluids. Hektoen concluded that 'such cocci occur constantly in the central nervous system in epidemic poliomyelitis, and their presence here is not explainable as due to accident or contamination'.[12]

Hektoen's findings were a direct challenge to Landsteiner's paper, published a full decade earlier, but Hektoen had authority. His obsession with accuracy was legendary, and his *Journal* was top in the field. And he had examined a vast number of samples, including archive material

from a Norwegian epidemic in 1906, and spinal cords that had been collected with disconcerting speed, within an hour of death.

Within a few years, Hektoen's cocci were destined to follow many other poliomyelitic bacteria into oblivion. For now, though, the door was still open for those who remained unconvinced about the filterable virus. Meanwhile, those who did believe in Landsteiner were still scrabbling for ideas about what could have crept through the Berkefelt candle.

All this uncertainty provided the way in for two intriguing red herrings: Rosenow's poliomyelitic streptococcus and the globoid bodies of Noguchi and Flexner. Both should have been quickly consigned to the deep, but the power of the scientific hierarchy ensured that they continued to swim against the tide of evidence for many years.

Downsizing

The issue of the *Journal of Infectious Diseases* which contained Hektoen's article on poliomyelitic cocci was dominated by a clutch of papers about an even more promising germ. The author was Dr Edward C. Rosenow, Head of Experimental Microbiology at the Mayo Foundation in Rochester, Minnesota, one of the top research centres in the United States (Figure 4.1). Rosenow had made his reputation studying infections of the brain, but had recently set off on a crusade to prove that streptococci caused important human diseases – beginning with polio.

The massive American polio epidemic of 1916 and a smaller local outbreak the following year had provided Rosenow with fresh experimental

Figure 4.1 Edward C. Rosenow (1875–1966), tireless promoter of the 'poliomyelitic streptococcus'

material and the chance to search for streptococci. He found lots of them, in the body fluids, brain and spinal cord of polio victims.[13,14] Tonsils, removed by a friendly surgeon from patients 'who were not convalescing satisfactorily', were also a rich source. These streptococci were tricky to grow in the laboratory, but flourished in a special culture medium which Rosenow had dreamed up. The recipe began with live twenty-day-old chicks still in their shells and proceeded – badly for them – with the intervention of a mortar and pestle.

To be confident that his streptococci were responsible for polio, Rosenow had to show that they fulfilled 'Koch's Postulates'. Robert Koch had laid these down in 1884 as the essential criteria to prove that Germ X genuinely caused Disease A, and was not just an innocent fellow traveller. Germ X had to be isolated from all cases of Disease A, and never from healthy subjects. A pure culture of Germ X, grown in the laboratory, must produce Disease A when given to healthy animals. Finally, Germ X had to be isolated again from the animals with experimentally induced Disease A, and these isolates must in turn induce Disease A in fresh animals. This exercise had been straightforward in the case of cholera: easily seen, cultured and transmitted, and quick to induce the diagnostic diarrhoea. Rosenow's task was much harder, because the streptococcus was fussy, and the detection of infection inside the central nervous system was so laborious.

But he persevered, and Koch would have been satisfied by the papers which Rosenow published in 1918 – the product of four years' work and some 2,000 experimental animals. Rosenow reported that he had grown streptococci from the brain and cord of polio victims; that these caused poliomyelitis and paralysis when injected directly into the brains of monkeys or rabbits; and that the same bacteria could then be recovered and produce paralysis in healthy animals. As proof, there were photographs of unhappy rabbits trailing their useless limbs. Rosenow concluded that his 'poliomyelitic streptococcus' normally lived in the tonsils, but sometimes invaded the bloodstream, when it homed in on the spinal cord and caused paralysis.[13,14]

Being a bacterium, Rosenow's streptococcus was far too bulky to be filterable. However, it was 'pleomorphic' and could change its shape. Specifically, it underwent an extraordinary transformation when grown in the 'chick-mash' medium, forming minute 'micrococci' that were one-tenth of its normal size. The micrococci could slip easily through the Berkefelt filter – and also through the 'blood-brain barrier' which normally keeps bacteria out of the central nervous system.

Rosenow reported that the micrococci were just visible under a powerful light microscope which magnified about 1,000 times (Figure 4.2). He later described them in greater detail, thanks to the ingenuity of

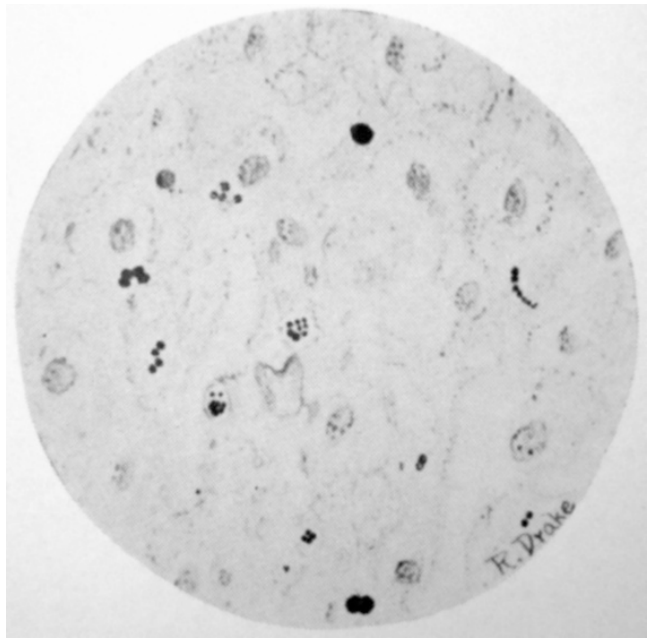

Figure 4.2 Rosenow's 'filter-passing micrococci', drawn for a paper in 1918.[13] They are marked by the arrows; the larger paired structures at the bottom of the field (circled) are normal streptococci. The 'micrococci' looked like tiny bacteria, but were artefacts. Reproduced by kind permission of the *Journal of Infectious Diseases*

Royal R. Rife, a Californian inventor known chiefly for his colourful personality and boundless energy for self-promotion. Using optical wizardry that involved polarised light and rotating quartz prisms, Rife had built a 'virus microscope' which somehow stretched the power of magnification to an incredible 8,000 times. This instrument had recently shown the causative organism of typhus (a notoriously tiny bacterium) to be perfectly round and bright blue – a claim greeted with derision by most of the medical fraternity. In July 1932, Rosenow visited Rife in San Diego, taking filtrates of his streptococcus. To his delight, the 'virus microscope' confirmed the missing link: round blobs, grey-brown in colour, small enough to be 'filter-passing'.[15]

There was more. Back in 1917, Rosenow made the stunning claim that his pleomorphic streptococcus held the key to a miracle cure for polio[16]. Animals which survived infection with the streptococcus developed antibodies against it, which then blocked subsequent attempts to infect them – mimicking the natural immunity induced by an attack of polio. Rosenow harvested large amounts of 'immune serum', rich in protective antibodies, from a horse which he had immunised with the streptococcus. This was a stoical horse, as it was injected with extracts from 40 litres of

culture broth over six months, and then bled repeatedly. The immune serum extracted from its blood was magical stuff: it could snatch back from certain death monkeys which had been given usually fatal doses of polio brain extract.

Rosenow then extended his trials to humans, treating 58 patients during a polio outbreak that hit Davenport, Iowa, during the summer of 1917.[17] He screened 'tentative' cases with lumbar puncture, even though this could not diagnose early polio with any certainty. Likely patients, such as those whose spinal fluid spurted dramatically out of the needle, were immediately given the immune serum; this was injected into an arm vein, or in babies, the jugular vein in the neck. Some subjects were already moribund when treated and, as expected, soon died. Otherwise, Rosenow reported, 'Paralysis did not develop in a single instance when treatment was begun before its onset, and all recovered'.[17] Rosenow's excitement at these astounding results was shared by the American drug company Eli Lilly, which set up cultures of the streptococcus and began manufacturing its own antiserum.

Rosenow's poliomyelitic streptococcus certainly looked the part, but other researchers were struck by his claims – and specifically that they seemed too good to be true. Even as Rosenow built up his dossier, papers appeared that comprehensively torpedoed his key findings, one by one.[18] His streptococci fell at the first hurdle of Koch's Postulates, because identical bacteria were readily found in the brain and tonsils of people and animals who had never had polio. Rosenow clearly had not checked his controls carefully enough. Worse still, the same streptoocci were found contaminating supposedly sterile glassware and the chick-mash medium. Even 'extraordinary care for sterility' – decking researchers out in caps, gowns and gloves and searing the brain surface with a red-hot scalpel before taking samples – could not exclude them. Verdict: Rosenow's streptococci were merely 'airborne contaminants or terminal invaders of sick animals'.

Next, Rosenow's germ could not be persuaded to spawn filterable offspring; put succinctly, 'the streptococci remained streptococci'. Then nobody could replicate Rosenow's finding that his germ caused poliomyelitis. Injecting a heavy culture of the streptococci directly into the brain could produce paralysis, but only because the entire skull contents became infected, just as with dozens of ordinary bacteria. The rabbit turned out to be a hopeless model for polio, as it could be paralysed by injecting virtually anything into its brain. Verdict: 'the cocci have no etiological relation to the lesions of poliomyelitis'.[18]

Finally, others could not detect Rosenow's supposedly protective antibodies. Proper trials – allowing for the variable outcome of polio – showed his immune serum to be no better than letting nature take its

course unassisted. Verdict: 'the Rosenow serum is devoid of protective power'.[19]

Rosenow's reaction to his critics was surprising for a senior professor at one of America's flagship research centres. Initially, he fought back on points of detail: their culture conditions were not quite right, the doses they injected were too small, their rabbits were too old. Then he simply turned his back on them and carried on ploughing his increasingly solitary furrow.

And a long furrow it proved to be. In 1930, 14 years after Rosenow's first paper on his poliomyelitic streptococcus, Beatrice Howitt wrote: 'Except for the work of Rosenow, it is generally accepted that poliomyelitis is due to a filterable virus'.[20] But Rosenow stuck to his guns and kept publishing. In 1933, a commentator noted:

> It must be very trying to the pundits, who assert that Rosenow's poliomyelitic streptococcus just doesn't exist, to have it continue as the subject of researches, all of which seem to indicate that the condemned microorganism is very much alive.[21]

In 1944, *Time* magazine was prompted to remark:

> For 27 years, the medical profession has looked politely down its nose at bacteriologist Edward Carl Rosenow ... a stubborn man who has persisted in his obsession that in ways no doctor understands, streptococcus plays a malignant part in infantile paralysis.[22]

And, perhaps with a note of weariness, the *Postgraduate Medical Journal* of January 1949 observed: 'Rosenow persisted in his streptococcal theory'.[23]

Rosenow continued to do so, and somehow could still pull a good crowd, long after his streptococci had been flushed away by evidence. In June 1952, Rosenow was invited to address the assembly of general practitioners at the annual meeting of the American Medical Association (AMA) in San Francisco. Now eight years into retirement, he delivered a one-man festschrift about his own 'forthright bacteriologic studies' which had led him to the bacterium that he still believed to cause polio. He acknowledged that the filterable agent was 'currently considered to be a virus', but made no concessions to his detractors. In fact, he did not even mention them. Over half of the papers that he cited were by himself, and most of the rest were the early (and by now discredited) reports by Hektoen and others of cocci spuriously associated with polio.[4]

This lecture must have been an odd event, as there was no longer any controversy over what caused polio. When the poliovirus came up on the screen of the electron microscope in 1946, it looked nothing like a

bacterium and was far tinier than Rosenow's micrococcus. Yet Rosenow spoke as though this revelation, and all the other evidence that had killed his hypothesis, had never happened.

Perhaps Rosenow was invited to the AMA so that his 'poliomyelitic streptococcus' could at last be given the decent burial which it should have had 40 years earlier. But Rosenow remained true to his mission and continued to preach the doctrine of his conviction.

Foreign bodies

Rosenow's streptococcus was not the only non-cause of polio to seize the imagination during the 1920s and 1930s. Serious competition came from 'two of the world's foremost bacteriologists' at the hugely powerful Rockefeller Institute in New York. Their contender had an alluring name: the 'globoid bodies' of Noguchi and Flexner.

The tale of the globoid bodies begins in Japan just before the turn of the twentieth century, with a bright doctor in his early twenties looking to make his name in America. Hideyo Noguchi had already shown determination to succeed against adversity from the age of two, when his left hand was badly burned. Prolonged and painful surgery left him with reasonable dexterity and a longing to be a brilliant doctor. To further this ambition, he dropped his parents' given name, Seisaku, in favour of Hideyo, which means 'excellent' (Figure 4.3).[24]

Figure 4.3 Hideyo Noguchi (1876–1928), who left Japan to work in the United States with Simon Flexner. Noguchi proved that general paralysis of the insane was a late stage of syphilis, but the organisms which he reported as the causes of rabies and yellow fever turned out to be spurious

Figure 4.4 Simon Flexner (1863–1946), founding Director of the Rockefeller Institute for Medical Research, New York, and polymath medical researcher. In addition to polio, Flexner's interests included meningitis and snake venoms. Together with Hideyo Noguchi, he reported the 'globoid bodies' as the causative organism of polio, and was convinced that the infection was spread from the nose to the brain. Reproduced by courtesy of the Rockefeller Archive Center, New York

A couple of years after graduating from medical school in Tokyo in 1897, Noguchi decided that America was ready for him. He bought a one-way ticket to Philadelphia and arrived unannounced one morning in June 1899 at the University of Pennsylvania. There, he told Dr Simon Flexner, world-famous microbiologist, that he had come to work with him (Figure 4.4). At first, Flexner did not know what to do with him, but Noguchi was clever, hard-working and eager to please. He was put to work on one of Flexner's many research interests, snake venom. Noguchi and Flexner produced several joint papers, and Noguchi went on to write a monograph on the topic, which became a classic.[25] Now firmly embedded in Flexner's team, Noguchi was regarded with affection and respect; his nickname, 'the Yellow Peril', was evidently given and received in good humour.

A few years later, Flexner was appointed chief of the Rockefeller Institute, and Noguchi followed his boss to New York to tackle the grandest challenges in microbiology. He churned out dozens of papers in top American journals. An early coup was finding the elusive corkscrew-shaped spirochaete of syphilis in the brains of victims of 'general paralysis of the insane'.[26] Noguchi's discovery settled the long-running controversy about the cause of this pitiful condition, and cemented his reputation. Noguchi went on to claim that another spirochaete was responsible for yellow fever[27] – one of the greatest killers in South America and central Africa – and that a large protozoon

'corpuscle' caused rabies. These reports impressed his peers at the time, but later came back to haunt him.

Noguchi moved into polio in 1910. Flexner had been excited by Landsteiner's findings and, together with Paul Lewis at the Rockefeller, confirmed that polio could be transmitted to monkeys by a filtrate of spinal cord extract.[28] Noguchi and Flexner now set out to hunt down the organism responsible. They used a culture medium invented by Noguchi, which had nourished various bacteria that otherwise seemed impossible to grow. This was based on human ascites fluid (which collects inside the abdomen in advanced heart failure or liver cirrhosis), sealed under paraffin oil to keep out germs and air. For the polio experiments, Noguchi popped in a piece of fresh rabbit kidney. He never explained why, but this macabre ingredient seemed to do the trick. A few days after adding a sample of poliomyelitic spinal cord, a haze appeared around the kidney fragment. Something seemed to be growing, and a high-powered microscope revealed masses of minute round forms, often in chains or clusters.

Crucially, these 'globoid bodies' were small enough to pass through the Berkefelt candle: a chain of a dozen, coiled up, could fit within the outline of a streptococcus. They did not look like any known microbe – hence their non-committal name – but Noguchi and Flexner were convinced that they transmitted polio. According to them, filtered cultures of the globoid bodies injected into the brains of monkeys produced poliomyelitis and paralysis. Globoid bodies could be cultured from the brains of these monkeys and, in turn, induced experimental polio in fresh animals. Koch would have been convinced.

In 1913, Noguchi and Flexner reported that they had cultivated 'the microorganism causing epidemic poliomyelitis'.[29] Their hefty 25-page paper in the *Journal of Experimental Medicine* seemed to have everything: ingenuity, technical brilliance, a clever solution to a thorny problem – and all coming from two of the top names in microbiology. It was also an open invitation for others to repeat the experiments – which they did, but with variable results that soon raised doubts. Harold Amoss was able to replicate the findings;[30] any technical queries would have been easy to resolve, as he worked down the corridor from Flexner, leading one sceptic to remark that the globoid bodies were a phenomenon largely confined to the Rockefeller Institute.[31] Others, however, could not persuade the globoid bodies to grow or to cause polio, and some could not find them at all. Even following Noguchi's method 'with the utmost exactness' sometimes produced nothing but peculiar tiny blobs that could only be artefacts.[31,32]

The globoid bodies wobbled along for over 20 years, generating papers and controversy along the way. They seemed to exist, at least in the depths of Noguchi's idiosyncratic medium, but whether or not they caused polio was a question that divided the polio research community into believers

and non-believers. Possibly because of Flexner's stature and intimidating personality, the non-believers were less vociferous than those who attacked Rosenow's streptococcus. Steadily, though, the balance tipped against the globoid bodies. Several researchers challenged the magic of Noguchi's ascites medium, but the killer question was the lack of proper controls. Incredibly for such high-calibre scientists, Flexner and Noguchi had not bothered to see what happened if they used samples of brain or cord from animals that did not have polio.

By the early 1930s, the globoid bodies had been inflated into 'a balloon waiting to be pricked'.[33] The decisive puncture was inflicted in 1936 by Gerald Logrippo, from Flexner's former base at the University of Pennsylvania. Logrippo laid out his stall clearly at the start of his paper:

> Although many investigators have obtained the same results [as Flexner and Noguchi], the general opinion is that the bodies have a doubtful relationship to the etiological agent of the disease.[34]

Following Noguchi's method, Logrippo recreated the tell-tale haze around fragments of spinal cord and found globoid bodies – but in the complete absence of poliovirus. He had simply performed the control experiments that Noguchi should have done two decades earlier, using spinal cord from a healthy rabbit. Logrippo photographed the bodies budding off stringy filaments that were left exposed as the spinal cord fell apart. They were nothing but tiny droplets of fat.

These minute blobs were identical to the typical Noguchi-Flexner globoid bodies that Logrippo saw when he repeated the experiment with poliomyelitic cord. To clinch his case, Logrippo rigged up a Frankensteinesque experiment with a piece of fresh cord from a healthy rabbit impaled on a silver wire, suspended between two electrodes in culture medium. Knowing that fats were attracted towards the positive end of an electrical field, he had dunked the cord in Sudan III dye, which stains fats bright red. Over a few days, a red haze emerged from the cord and spread towards the positive electrode. Under the microscope, the haze consisted of classical globoid bodies, nicely picked out in deep pink by Sudan III.

Unlike Rosenow, who never retracted his claims about his poliomyelitic streptococcus, Flexner eventually admitted that the globoid bodies – whatever they might be – were not the causative agent of polio. In fact, he had already done this in 1928, before Logrippo's experiment.[35] At that time, Noguchi was not available for comment. For reasons that will become clear, he wrote himself out of the story in that same year.

Building the picture

During the 1930s and 1940s, progress was generally slow in the hunt for the true cause of polio. The seeds of confusion sown by the likes of Rosenow, Noguchi and Flexner did not help, but a more intractable problem was the elusiveness of the virus itself. The only method for detecting it was labour-intensive, expensive, unreliable and agonisingly slow to yield results – because the detection system in question was the central nervous system of living monkeys.

As described in the next chapter, the brake on polio research was released when it was discovered in 1949 that the poliovirus could be persuaded to grow outside live animals, in cultures of various tissues. As a result, new knowledge flooded in during the 1950s.

In the meantime, several pieces of the jigsaw were slotted into place, even though the picture of the villain remained tantalisingly incomplete. One seemingly quiet advance in 1931 had extraordinarily wide consequences. This was the discovery by Macfarlane Burnet and Jean Macnamara, working in Melbourne, Australia, that the serum collected from survivors of polio neutralised some polioviruses, but not others.[36] This meant that there must be different varieties of poliovirus. This could explain some peculiarities of the disease, such as why some epidemics were more serious than others. The finding also had huge practical implications, because an effective polio vaccine would have to protect against all the different varieties of poliovirus.

Worryingly, it later turned out that there were hundreds of different 'strains' of polio, but there was also good news: they were all encompassed by just three broad 'types' of poliovirus. The three types have quite different credentials. Type 1 caused most epidemics and 80 per cent of all cases of paralysis. Type 3 was in second place for viciousness, responsible for about 13 per cent of paralytic cases. Type 2 was rather rare and benign, accounting for just 7 per cent of paralytic attacks (at least in humans; unlike the other two types, it was able to infect and paralyse mice). As the 1931 paper by Burnet and Macnamara had anticipated, an infection with one strain produced antibodies that had little or no effect against strains of the other two types. However, antibodies against a particular strain protected against all other strains of that type – meaning that a 'trivalent' polio vaccine containing just one strain from each of the three types could protect against every possible strain of the virus.

Certain poliovirus strains crop up repeatedly through the history of polio, sometimes for unhappy reasons. Type 1 strains are often represented by Mahoney, isolated from the stools of children from a family in Cleveland, Ohio in 1941. The Mahoneys' legacy included a disastrous

complication of Jonas Salk's injectable vaccine, which threatened to sabotage the entire polio vaccination programme. Another famous Type 1 strain is Brunhilde, honouring a big-breasted female chimpanzee from Johns Hopkins University in Baltimore.

The Michigan town of Lansing is immortalised in the name of a widely used Type 2 strain, which killed the source patient there in 1938. The Type 3 strain, Leon, is named after a boy who died of polio in Los Angeles in 1930 and who, like Brunhilde, provided the spinal cord which yielded the virus.

Another one to watch for is the Type 1 strain called CHAT, short for 'Charlton', the surname of the little girl from whose stools it was isolated in 1956. CHAT was used in an early oral polio vaccine that was given to hundreds of thousands of African children during the late 1950s. Later, it was catapulted straight into the realms of horror fantasy by the claim that it had been contaminated with a monkey virus which mutated into the human immunodeficiency virus (HIV) and so caused the AIDS pandemic.

All this lay many years in the future when the scientific community first considered the Burnet-Macnamara paper in 1931. To some experts, the notion that there might be different types of poliovirus was of dubious or no significance. Simon Flexner, looking down from the ivory tower of the Rockefeller Institute in New York, had little time for scientists outside America. This paper had emanated from far beyond the pale: an odd-sounding research outfit (the Walter and Eliza Hall Institute) in Melbourne, and written by authors who had not much of a track record in polio research.

Flexner never changed his belief that there was only one type of poliovirus, and even used his last public lecture on polio, in 1937, to sideline Burnet's work – proof that, right to the end, he was not always good at distinguishing winners from losers.

Small, round and beautiful

Science eventually caught up with the poliovirus.[37] It turned out to have a diameter of about 30 nanometres (3 millionths of a centimetre, roughly one-thirtieth of the size of a streptococcus), and weighed in at 10^{-17}g. Unlike bacteria and other more complicated organisms, the poliovirus is invariable in its construction. It can therefore be given a molecular weight (about 6,800,000) and a chemical formula, $C_{332662}H_{492388}N_{98245}O_{131196}P_{7500}S_{2340}$.

The structure of the poliovirus was revealed during the 1950s by X-ray diffraction and electron microscopy. X-ray diffraction had been put on the map during the 1910s by the father and son team of William Henry and William Lawrence Bragg in London, using crystals of simple

chemicals such as halite (table salt). Crystals consist of regularly stacks of molecules, which split a narrow beam of X-rays into a fan of sub-beams that show up as an array of dots on photographic film placed behind the crystal. The patterns of dots can be analysed mathematically to reveal the chemical composition and structure of the molecule in question. With heavy-duty mathematics, X-ray diffraction can yield similar information about viruses, which can also be made to pack together to form crystals.

In the early 1950s, this was cutting-edge research that attracted brilliant minds, including two men in Cambridge and a woman in London. The men in Cambridge were an Englishman and an American on a visiting fellowship from the American polio charity, the National Foundation for Infantile Paralysis. Respectively, they were Francis Crick and James D. Watson. The woman was Rosalind Franklin, who had left Cambridge for the world-class X-ray crystallography unit at Birkbeck College after falling out with Crick and Watson during the scramble to elucidate the structure of DNA.

All three helped to unmask the poliovirus. Crick and Watson were working on how small round viruses are assembled from their constituent subunits. They deduced that 60 identical subunits were packed together to form a hollow shell that appears spherical, but is actually a regular geometric solid with 20 faces, somewhat like the polygonal panels on a football.[38] This 'icosahedron' is aesthetically pleasing, and also has a nice resonance with the origins of geometry, as it was one of Plato's 'perfect solids'. It also turned out to be the structure of the poliovirus.

Franklin began work on the poliovirus in 1955. She had already cracked the structure of the tobacco mosaic virus (TMV), a hollow cylinder about 300 nm long and made up of over 2,000 spherical subunits arranged in a tight spiral. The poliovirus was a thornier problem than TMV. Coaxing it to crystallise was a major feat, yielding tiny glassy splinters half a millimetre long which tended to disintegrate under X-ray bombardment.

Even Franklin was defeated; her work had to be finished by her colleagues at Birkbeck, John Finch and Aaron Klug.[39] They used poliovirus crystals grown by Carlton Schwerdt and Frederick Schaffer in Stanford (and smuggled past English Customs by Schwerdt's wife, Patsy, in her handbag).[40] Using X-ray equipment belonging to Lawrence Bragg himself, Finch and Klug showed that the poliovirus consisted of a hollow icosahedral shell of 60 spherical subunits, enclosing a core of RNA. This structure fitted the theoretical model constructed by Crick and Watson, as well as the picture that emerged from the electron microscope: an essentially spherical particle with a finely granular surface[41] (Figure 4.5).

Rosalind Franklin missed the satisfaction of seeing all these pieces of the puzzle click together. In the summer of 1956, at the age of 36, she had been diagnosed with ovarian cancer. She did not survive to see the

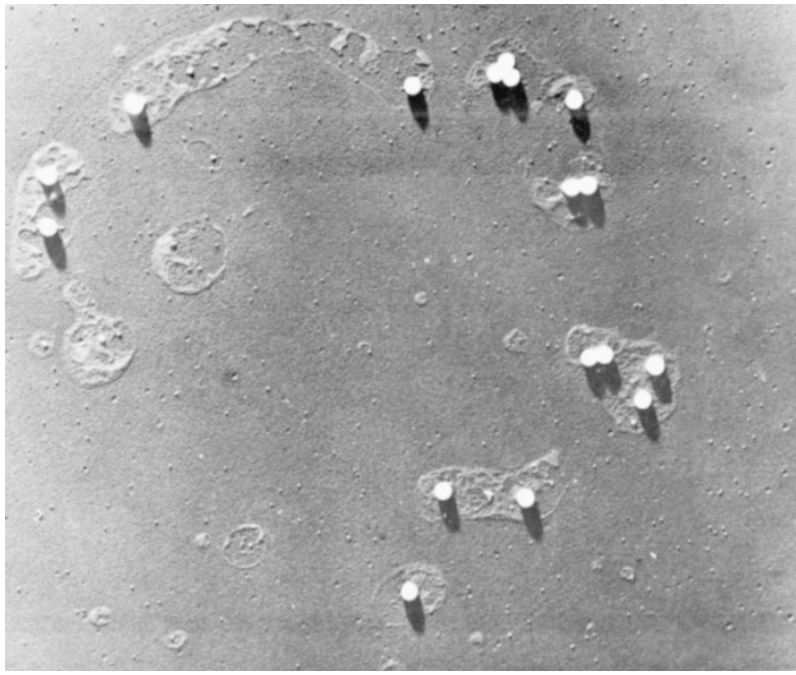

Figure 4.5 An early electron micrograph of poliovirus particles. Their spherical shape is brought out by 'shadowing' with vaporised gold fired obliquely across the sample. Reproduced by kind permission of the Centers for Disease Control, Atlanta, Georgia

sensation created by a huge model of her TMV structure when it was unveiled at the World Fair in Brussels in 1958 – or the papers published a year later by her friends at Birkbeck, on the structure of the poliovirus.

Bare necessities

Viruses are the simplest of all organisms. They are trapped in a biological limbo and only fulfil the most basic criteria for life – growth and reproduction – when they get inside their target cell, be this bacterial, plant or animal. Viruses carry their genetic information in either DNA (like bacteria, plants and animals) or in the alternative nucleic acid, RNA. The poliovirus, like those of influenza, the common cold and severe acute respiratory syndrome (SARS), is an RNA virus.

The polioviruses belong to the wider community of 'enteroviruses', so-called because they inhabit the gut. There are almost 90 different enteroviruses, of which over 60 infect humans.[42] Numerically, the 3 polioviruses are minor players; the others include 29 Coxsackie viruses (named after an Algonquin Indian settlement in New Jersey), several echoviruses and the rhinoviruses that cause the common cold.

Very rarely, other enteroviruses can cause paralysis that, under the microscope and at the bedside, is indistinguishable from classic polio. The main culprits include Coxsackie A7 and B4, and echoviruses 30 and 71. Before vaccination took hold, the poliovirus caused virtually all cases of 'acute flaccid paralysis', the clinical hallmark of polio. Now that vaccination has all but exterminated wild-type polioviruses, the rare cases of acute flaccid paralysis that occur today are caused by these other enteroviruses – or by polioviruses that have mutated from the oral polio vaccine.

Great men and great mistakes

The 'poliomyelitic bacteria' were not the only microbes which ended up in the dustbin of rejected hypotheses. Several others were killed off when 'their' diseases were shown to be vitamin deficiencies, not infections. The bacteria of scurvy were finally declared redundant with the discovery in 1930 of vitamin C (which was later touted as a miracle cure for polio). Beriberi was found to be caused by thiamine deficiency, as had already been deduced by Christiaan Eijkman, working in Djakarta in 1897. Eijkman abandoned his search for the bacterium responsible when he found that experimental beriberi in chickens could be cured simply by giving them rice husks to eat. This would have displeased Robert Koch, who had been Eijkman's mentor in Berlin and who later had microbiologists dispatched to Djakarta to resume the hunt for the beriberi bacterium.

Pellagra also turned out to be another pseudo-infection. The myth was exploded in a series of heroic experiments by the American Joseph Goldberger, who injected himself, his colleagues and family with saliva, blood, urine and faeces from pellagra victims.[43] Goldberger also proved that pellagra was due to dietary deficiency, although the missing ingredient (niacin) was not identified until 1938, nine years after his ashes had been scattered on the Potomac River in Washington, DC.

The two most famous non-causes of polio survived much longer than any of these spurious microbes. Rosenow's poliomyelitic streptococcus was particularly hardy, at least in the mind of its creator. It was the product of artefact and imagination, but still brought Rosenow fame and recognition. He was twice nominated for the Nobel Prize by a friendly Professor of Physiology in Cincinnati, where he eventually retired.[44] The first nomination, in 1938, was for 'fundamental contributions to the theory of disease'; Rosenow's case was evaluated, but went no further. In 1948, Rosenow was put forward again, for having 'established the streptococcal nature of poliomyelitis and the conversion/reversion of this streptococcus into viral form'. By then, anyone with access to the scientific

literature would have seen that this was fantasy. The nomination was not even sent out for review.

Rosenow's obsession with his poliomyelitic streptococcus had led him to commit another, graver crime against science. The evidence is buried in a wordy section of his 1918 paper which claimed wondrous effects for his immune serum.[17] Its success was boosted substantially by the deaths of several moribund patients who had not been given the serum. In fact, they had. Rosenow reallocated them posthumously to the untreated group, because they had been so ill that they were going to die anyway. This is statistical cheating at its most blatant, but he got away with it.

This brings Ludvig Hektoen, the magisterial editor-in-chief of the *Journal of Infectious Diseases*, back into the story. Hektoen was a man of legendary seriousness, with only occasional sparks of levity. Once, asked what was new in his field, he replied, 'The skirts are shorter' – but this may just have been a reference to the ongoing debate about the impact of skirt length on the infection risk of women's clothing. Hektoen was also renowned as a tough editor with an eagle eye. Rosenow's statistical sleight of hand cannot have escaped him, and we must presume that Hektoen was content to let it ride. In fact, Hektoen's own science was not perfect, even for that time. His provocative paper of 1918, showing cocci associated with polio, examined dozens of samples from polio victims – but not one from polio-free controls. It is clear from the work of many others that if he had looked, he would have found the same bacteria there too.[45] But then, of course, there would have been no discovery and no publication.

After retiring, Rosenow began a new crusade to prove that streptococci caused neurological and psychiatric diseases, ranging from multiple sclerosis to violent behaviour. This was how he came to inject streptococci from the tonsils of the criminally insane into the brains of mice.[46] It can be difficult to diagnose criminal tendencies in mice, but Rosenow did his best. He also had a simple remedy for all these illnesses: surgical removal of the tonsils, which harboured the brain-damaging streptococci.

Someone else who wobbled off the rails was Royal R. Rife, father of the 'virus microscope', which had revealed Rosenow's 'filter-passing' micrococci in 1932. Like Münchhausen's tales, Rife's inventions became ever more grandiose and fantastic. His 'Universal Microscope' (1933) was a 200-pound beast with 5,682 parts. The 60,000x magnification which he claimed can only be obtained with the electron microscope, but the Universal Microscope enabled Rife to discover the sinister 'Bacillus X' (BX), which caused polio, tuberculosis, cancer and so forth. Luckily, BX could be shaken to death by another Rife invention, his 'Ray Tube', which bombarded diseased tissues with electromagnetic radiation tuned to BW's 'mortal oscillatory rate'.[47]

Rife's career can be summarised in two newspaper headlines: 'San Diegan's cancer work may make cure possible' (*San Diego Union*, 31 July 1949) and 'Scientific genius dies; saw work discredited' (*Daily Californian*, 6 August 1971). By way of epitaph, we have the expert critique of a Rife microscope by a professor of physics at Imperial College, London. He noted that it had lots of knobs and 'had been constructed in such a way as to make the work of microscopy tedious and cumbersome'. It produced a poor image only slightly bigger than an ordinary light microscope, and was good at generating round, coloured artefacts.[48]

What of that other microbial sideshow, the globoid bodies? Long after Flexner disowned them, they continued to generate controversy. G.S. Wilson of the Public Health Laboratory Service in London was a non-believer, and in 1959 stopped short (but only just) of accusing Flexner and Noguchi of fabricating the evidence that the globoid bodies transmitted polio.[33] But 12 years later, Harold Faber from San Francisco was convinced that they had been on to something.[35] Perhaps the poliovirus managed to replicate in a few viable kidney cells, and then stuck to the surface of the fat-rich bodies? Faber even wondered whether Flexner and Noguchi should be credited with having grown the poliovirus in cell culture, 30 years before this was reported by John Enders and his colleagues – who won the Nobel Prize for their discovery.

Flexner had a tough hide, and his reputation easily survived the fall of the globoid bodies. But Hideyo Noguchi, thoughtful and reserved, was more easily bruised by criticism – especially as doubts were growing about some of his greatest discoveries. His rabies 'corpuscle' turned out to be an artefact from his ascites medium, while evidence piled up that yellow fever was caused by a virus, not the spirochaete to which he had devoted 34 papers.

In a last-ditch attempt to prove that he was right about yellow fever, Noguchi set off in late 1927 for Accra on the Gold Coast of Ghana, one of the hot spots for the disease.[24] This was to be Noguchi's last stand. Devastated because his own experiments were now showing that he had been wrong all along, he fell ill with jaundice. It was yellow fever. The virus won the final round, and Noguchi died on 21 May 1928. His last words were, 'I don't understand'.

End of an era

The demise of the bacteria supposed to cause polio and beriberi helped to bring down the curtain on the Golden Age of bacteria and the classic germ hunters such as Robert Koch and Louis Pasteur. New bacteria were waiting to be discovered, but the emphasis was already shifting towards viruses and protozoa.

The new era never quite recaptured the excitement – scientific and public – of the previous age, or the charisma of the old pioneers. The power of scientific celebrity, and its abuse, is nicely shown by Koch's two-month lecture tour to Japan in 1908, two years before his death.[49] He was accompanied by his wife, Hedwig, who had served as guinea pig for her husband's trials of his failed wonder drug, tuberculin.

By then, Koch had built up a worldwide following as the demi-god who had cracked the mystery of tuberculosis, and his trip amounted to a state visit.

He was greeted with adulation wherever he went: standing room only at his lectures, station platforms packed as if to greet the Emperor, hundreds gathering to be photographed with him. And they hung on his every word.

What was his advice about plague? Straightforward: they needed lots of cats to kill the rats that carry plague bacilli; this was so important that the police should check every house for cat ownership. Koch's suggestion of police searches was not followed up, but his other directions were. Koch single-handedly transformed the outlook for Japanese cats. Until then, their employment prospects had been limited to providing skin for the sound box of the *shamisen*, the traditional three-stringed lute. Now, thanks to Koch, they were in demand. The result was rampant feline inflation, with cats changing hands for up to $200.[49]

And what did he think about beriberi? Koch left them in no doubt. Of course beriberi was an infection, and they must send a team immediately to Djakarta to find the bacterium responsible. So they did.