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The Bacterial Cell Wall in the Antibiotic Era: An Ontology in Transit Between Morphology and Metabolism, 1940s–1960s

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Abstract. This essay details a historical crossroad in biochemistry and microbiology in which penicillin was a co-agent. I narrate the trajectory of the bacterial cell wall as the precise target for antibiotic action. As a strategic object of research, the bacterial cell wall remained at the core of experimental practices, scientific narratives and research funding appeals throughout the antibiotic era. The research laboratory was dedicated to the search for new antibiotics while remaining the site at which the mode of action of this new substance was investigated. This combination of circumstances made the bacterial wall an ontology in transit. As invisible as the bacterial wall was for clinical purposes, in the biological laboratory, cellular meaning in regard to the action of penicillin made the bacterial wall visible within both microbiology and biochemistry. As a border to be crossed, some components of the bacterial cell wall and the biochemical destruction produced by penicillin became known during the 1950s and 1960s. The cell wall was constructed piece by piece in a transatlantic circulation of methods, names, and images of the shape of the wall itself. From 1955 onwards, microbiologists and biochemists mobilized new names and associated conceptual meanings. The composition of this thin and rigid layer would account for its shape, growth and destruction. This paper presents a history of biochemical morphology: a chemistry of shape – the shape of bacteria, as provided by its wall – that accounted for biology, for life itself. While penicillin was being established as an industrially-manufactured object, it remained a scientific tool within the research laboratory, contributing to the circulation of further scientific objects.

Keywords: Biochemistry, Microbiology, Penicillin, Antibiotic screening, Spheroplasts, History

Introduction: In Search of New Antibiotics

In 1962, at the Merck Research Laboratories in Rahway, New Jersey, Eugene Dulaney established a new antibacterial detection assay. Known as the spheroplast method, it was based on the physical effects produced by antibiotics in the cell walls of bacteria. Although Dulaney never published on his achievements, one of his former colleagues at Merck, Lynn Silver, has recently emphasized his role in developing "the art and science of antibacterial screening". As Silver notes, Merck scientists only published this method in 1992 (Silver, 2012; Gadebusch et al., 1992). Indeed, it was considered more instrumental – and thus more in need of confidentiality – than those methods used to manufacture antibiotics in the factories, which were patented and thus became public documents.[1] Since the isolation of streptomycin, the screening program at Merck had focused on finding new antibiotics active against Gram-negative bacteria, by locating antibiotic-microorganisms in soil sample[2]. Dulaney's method of antimicrobial detection and identification of spheroplasts was instrumental in the screening program initially established in Madrid in 1956 by Merck and the Spanish penicillin manufacturing firm CEPA, enabling them to find a new antibiotic. Isolated from a soil sample and originally named phosphonomycin, it would later be marketed as fosfocina (Santesmases, 2011a, 2014).

As noted above, precise details of the screening method were not made public until quite recently: antimicrobial activity against the bacteria cell wall was revealed by a given soil sample producing a spherical shape in a bacteria culture medium (Gadebusch et al., 1992; Silver, 2012).[3] The spheroplast method designed by Dulaney had been inspired by previous research into the effects of antibiotics on the cell wall.

The vanquishing of infections through the industrial manufacture of antibiotics was one of the most eagerly anticipated possibilities of the therapeutic revolution in medicine and society that followed the devastation of the Second World War. Above the ashes of Europe, large

[1] On the history of drug patenting see Gaudillière (2008), Romero de Pablos (2011, 2014) and Hüntelmann (2012).

[2] A staining technique designed by H.C. Gram in the late 1880s, combined crystal violet dye and an iodine salt as a mordant to fix the dye. The technique divided cells into those that were tainted by this combination and those that were not. These differences acquired biological meanings that would be later researched.

[3] Silver uses a paragraph without giving reference information about the method, which she herself learnt directly from her mentor, Dulaney, while at the Merck Research Laboratories. See Silver (2012, pp. 45–46).

parts of which had been razed to the ground, penicillin, and slightly later other antibiotics, circulated as a gift – the cure of conditions ranging from syphilis to tonsillitis arising as both material therapy (Bud, 2007a, b; Hobby 1985) and a metaphor of the healing from such devastation. Beyond diplomacy, which distributed penicillin in Europe and the north of Africa at the end of WWII, and the industrial manufacture of new antimicrobial drugs in European countries, the laboratory remained a space of practices in the trajectory of penicillin.[4]

After the wonder and at times unfulfilled promise of sulfa-drugs, penicillin, in all is marketed salt forms, became the first antibiotic qualified by this new name: the term antibiotic was devised slightly later than the isolation and testing of penicillin in human infections.[5] The role of the research laboratory was increasing on two fronts: in the search for new antibiotics, and in determining the mode of action of these new substances. In the story told in this paper, exploring their mode of action became embedded in the research program searching for new antibiotics. The effects of penicillin on bacteria had been studied during the war, not only in the hunt for antimicrobial activity but also as a tool in research on the composition and structure of the bacterial cell wall, and in a combination of previous approaches to the cell envelope in the biochemistry of phage infection. Penicillin was tested in infections, but at the cellular and molecular level its action was unknown during its early clinical era. It was this combination of circumstances that made the bacterial wall an ontology in transit. Invisible as the bacterial wall was for clinical purposes, for the biological laboratory the cellular meaning in regard the action of penicillin made visible the bacterial wall both by microbiology and biochemistry. Penicillin, as later antibiotics would eventually be, acquired a wider social life besides and beyond the clinic. As a medical commodity coming from the factory, it was a combination of biological approaches that turned this new drug – medical and industrial product in itself- into agents in the era of biomedical research.[6]

[4] On antibiotic production and clinical trials, in addition to those cited, see also Marks (1997). For a review of the literature, see Santesmases and Gradmann (2011).

[5] On the history of sulfa drugs, see Lesch (2007). The coining of the term antibiotic is attributed to the Rutgers University microbiologist, Selman Waksmann, who led a research group in which streptomycin was isolated as a successful antimicrobial in the treatment of tuberculosis. On the term, see Waksman (1947). On antibiotic as a brand, see Bud (2007b).

[6] Gaudillière (2002) features postwar biomedicine as shaped by a focus on cells and molecules. In part inspired by Gaudillière, Keating and Cambrosio (2003) have suggested that biology has encircled medicine.

The clinic, and so medical practice with its own particular trajectory and agenda during the early days of penicillin, was committed to testing a new, wonder(ful) drug and establishing protocols for its use in the treatment of infections. Even the quickly apparent resistances to penicillin – and slightly later to many other antibiotics – were observed at the bedside.[7] The laboratory bench remained a parallel testing site, however, where many interactions took place, paramount among these being both the recording by Alexander Fleming of the antimicrobial activity of a microorganism named *Penicillium*, and early work on its isolation and testing in the Petri dish in Oxford. That is, penicillin – as has been the case with many drugs – was used in medical practice due to knowledge regarding its effect on bodies, while activity at the level of cells and biological molecules was unknown.[8]

The fact that the bacterial wall could be broken was well known: bacteriolysis as a technique for studying the cell wall was conceptualized as a breaking down of layers and borders. The technical practices of such a concept involved the preparation of the wall with the intention of isolating it from what was within, thereby revealing its shape – the border remained, even without its inner materiality, as a flat layer electronically microphotographed, and recomposed in a "grid" under electromagnetism – and the components were then broken down by lytic substances for further analysis.

The border was the main protagonist in defining the bacterial cell. Cell materiality – its inner materiality in addition to the wall itself – was challenged by lysis, by substances able to break the flat layer, releasing what was kept within; that is, eliminating the cell as an entity by decomposing what held it together as it was recognized – an entity in itself. As its external shape, the wall became the cell's representation.

Scientists who participated in early research on the bacterial cell wall have stated that the development of electron microscopy, together with the accumulated wealth of biochemical information and resources of classical organic chemistry and biochemistry, were the driving forces behind early achievements (Salton, 1960; Weidel and Pelzer, 1964). I suggest, however, that penicillin, and later other antimicrobial drugs, were co-agents within the field. The bacterial cell wall remained at the core of scientific narratives and research funding appeals, the target for antibiotic action. At the same time, the use of penicillin presented a double advantage: the study of its effect on the cell provided medical

[7] See Podolsky (2010, 2014). Microbiology took its part in the construction of resistance testing: see Gradmann (2013).

[8] For an inspiring conceptual approach, see Löwy (1996).

meaning and established this research as part of the biomedical agenda of the time. The search for both resistances (Creager, 2007) and antimicrobial activity (Santesmases, 2011a) would be conducted at the level of the cell.

In this essay I tell a story of the bacterial cell wall from the early 1940s until the 1960s, with the aim, on the one hand, of situating the origins of the method that led to the detection of a new antibiotic in Madrid in 1969 and, on the other, to reveal one of the paths through which the social life of antibiotics retained agency in the research laboratory, in an epistemic space that would become known as cell biology, and participating in the origins of the academic space of molecular biology.[9] I suggest that both these sets of events were embedded in the then on-going research projects on the bacterial cell wall, its components, its stereochemistry and its biosynthesis, in Europe and the US.

To achieve this I trace two almost parallel trajectories of bacterial cell wall research, back to the era of antibiotic promise during the early 1940s – when new drugs were available as cures, creating expectations of a world without infections – up until a time when drug resistance had become a significant threat. Indeed, increasing drug resistances generated not only warnings about the misuse of antibiotics but also the misuse of antimicrobial drugs themselves. As microbes resisted the known antibiotics of the time, the hunt for replacements continued.[10]

One of these trajectories originates from bacteriolytic and bacteriophage research, the other from research specifically directed at understanding the action of penicillin in a bacteria cell. The cell wall was simultaneously a target of microbiological research, of penicillin studies, and of virus studies. The antimicrobial drug appeared to join forces with older approaches that aspired to account for the shape of the bacterial cell wall, its rigidity in relation to its composition and its function as a shield against the penetration of substances, namely viruses. In turn, the cell wall remained the scientific object that circulated between laboratories and research agendas. As a research object, it accrued biological, chemical and medical meaning during the time of antibiotics, the long period during which pharmaceutical firms ran programs searching for new antibiotics to defeat the resistances built up against earlier ones. Resistances would thus be confronted by two different strategies: genetic explanations in terms of mutations would lead to medical decisions

[9] On the use of antibiotics in the same period by British biochemist Ernest Gale in Cambridge, see Rheinberger (1996).

[10] On the history of antibiotic resistances see Gradmann (2011, 2013) and Podolsky (2010, 2014).

concerning the overuse of antibiotics, while the perpetual search for new antibiotics among soil microorganisms continued. Microorganisms against microorganisms were the sought-after conflicts – animal cells could not be attacked as these lack the wall. The digestion of one microorganism by a component of another would remain the event to be witnessed in the Petri dish for decades.

The bacterial cell wall became valued ‘‘in terms of quantity of antibiotic adsorbed’’ as ‘‘the most important part of the cell’’ (Perkins and Nieto, 1974 on vancomycin). As a border to be crossed, some of the components and biochemical destruction these components produced became known throughout the 1950s and 1960s. In order to reconstruct this border's biography I firstly discuss the production of early images of the bacterial cell wall and some of the pioneering experiments carried out to reveal its components. In the second section of this article I focus on the suggestion that the bacterial wall, conceived as a bagshaped molecule, could be destroyed through the production of a new biological entity named the spheroplast. Early uses of the term spheroplast enable me to explain the biological meaning of the cell wall and spheroplasts, while negotiations surrounding this new term display the part played by the network of scientists involved in research into cell-killing as a biochemical process, produced either by phage enzymes or by penicillin.

This narrative shows the social life of a pharmaceutical, industrially-manufactured product – the new antimicrobial drug, penicillin – both as a tester and a tested substance in the study and early representation of the bacterial cell wall. This biological object, I suggest, belonged to the epistemology of the antibiotic era in which an industrial product intervened in biological and biomedical research. This interaction was a new platform both for antibiotic industrial production and for biological knowledge and practices.

Transits of the Bacterial Cell Wall

The Container and Its Borders: Biochemistry and Electron Microscopy

In this section I detail the lines of collaboration and interchanges from antimicrobial tests on penicillin up until the early findings concerning the main components of the bacterial cell wall and its shape: the evidence presented of the experiences undergone by the wall, it being emptied and dissolved into subunits. Emptying and decomposing the

wall became a process to both study the bacterial cell wall and provide evidence of its morphology.

As part of the penicillin study group led by Howard Florey and Ernest Chain at the William Dunn School of Pathology in Oxford, A. D. Gardner noticed a morphological effect, a change in the appearance of the growth of *Cl. welchii* in low concentrations of the drug. These low concentrations – less than was required for a "full inhibiting" of bacterial growth – produced an elongation of the cells, "that took the form of unsegmented filaments". He also reported that *Staphylococcus aureus* grown in the presence of penicillin produced by *Penicillium notatum* underwent spherical enlargements and was subject to "imperfect fission". Gram-negative bacteria that were resistant to penicillin displayed this shape-changing phenomenon "very well" (Gardner, 1940, p. 837).

Luis Despain Smith and Telma Hay, from the Biochemical Research Foundation of the Franklin Institute in Delaware (USA), suggested that "it seems possible that penicillin either has some action on the cellular wall of *S. aureus* or that it interferes with the assimilation of one or more growth factors necessary for the actual fission of the growing cell" (Smith and Hay, 1942, p. 602). By introducing the phenomenon of interaction between the bacterial cell wall and penicillin, they drew attention to the wall as the part of the bacteria that could be targeted by the new drug. In the presence of small amounts of penicillin, bacteria underwent lysis and released protoplasmatic substances into the culturing medium, detectable to the naked eye through the resulting turbidity. Stained by gentian violet, the culture revealed imperfect fission in the photomicrographs that darkened as the bacteria expanded: darkness that signified morphological changes.

Following postgraduate training for his PhD on "Some properties of the bacterial cell envelope" with Ernest Gale in the Biochemistry Department at Cambridge University, the Australian Milton Salton was at Manchester, also working on the bacterial cell wall (Salton and Horne, 1951; Salton, 1952). Gale had been impressed by the effects of penicillin on staphylococcal infections during WWII, and began studying the mode of action of penicillin, and later other antibiotics, on Gram-positive and Gram-negative bacteria following the war (Rheinberger, 1996, 2000). Gale would keep the biochemistry of microorganisms and antibiotics at the core of his experimental skills and research agenda. He followed Marjorie Stephenson – to whose chair at Cambridge Gale would later be appointed – in his "use of the microbe as experimental material" (Ghuysen, 1977, p. 13).

Together with A.R. Horne, soon to be a renowned electron microscopist, Milton Salton also used microbes as experimental material, and described a method for preparing the cell walls of, among other microorganisms, *E. coli* and *Salmonella pullorum*. Heating of the microorganisms was followed by successive and careful centrifugation, through which deposits would be resuspended and washed. They presented images of the different materials they obtained, including a washed suspension of bacterial walls attached to cytoplasmatic material, and in 1951 the beautiful cell walls shown here (Figure 1). According to these photomicrographs, the cytoplasmatic material expelled from the bacterial cell wall by heating retained its rod-shape. Bacterial walls had in common a thin transparent structure possessing properties of elasticity and rigidity (Salton and Horne, 1951).

Salton and Horne prepared the ''extremely thin outer cell wall enclosing the cytoplasm of the bacterial cell'', then isolated it from the cytoplasm through mechanical methods: not only heating, but with ''violent agitation, sonic and ultrasonic disintegration''. They disintegrated bacteria in what was known as a Mickle disintegrator, in which

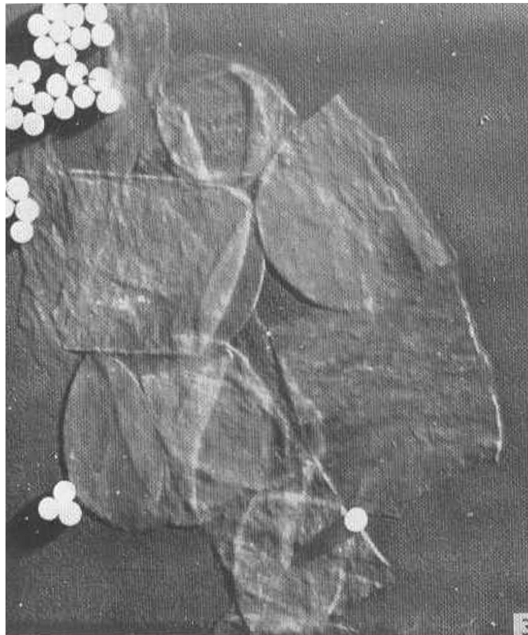

Figure 1. Electron micrograph of *B. megaterium* cell-walls preparation. The electron-dense spheres are polystyrene latex indicator particles (0.25 mm diameter). *Source* Salton, Milton R. J. 1953. Cell structure and the enzymic lysis of bacteria. *Journal of General Microbiology* 9: 512–523; plate 1. Reproduce with permission

small glass beads – *ballotini* impact grade 12 – were mixed with a sample suspended in aqueous media and agitated, the beads later being removed by filtration (Mickle, 1948; Salton and Horne, 1951). The combination of successive ultracentrifugation – to separate the wall from cytoplasmic material, and from the solvent – and electron microscopy, made the empty envelope visible, enabling images of the walls of many bacteria to be manufactured. Salton published these as cell wall representations.

Salton presented the wall as an image of a clean material. Having originated in WWII, electron microscopy was an extremely new tool, and would be instrumental in establishing cell biology as a discipline among the life sciences in many Western research institutions (Rasmussen, 1997). Cell biology proceeded as a biochemical space for experimenting with substances isolated and identified in microorganisms. Those experiments displayed the shape of cellular and subcellular entities – mitochondria and the bacterial cell wall being among the earliest – in which the metabolic transformation of such substances took place. Salton's experiments were portrayed in photomicrograph series exhibiting the successive steps toward complete isolation of the cell wall from the cytoplasmatic material it contained, the bacterial wall having been broken down by a substance. It was this representation of the cell – its wall, even if partially disintegrated – that Salton sought, and, I suggest, this was a project not only funded by antibiotic policy, but embedded in it. In 1952, Salton obtained high-quality, clear electron micrographs from Robley Williams (Salton, 1953, at a time when electron microscopes were still under-utilized at Ann Arbor; Rasmussen, 1997). The wall that protected the cell against proteolytic enzymes was shown through optical electronics to have the outward appearance of a tissue (Figure 1).

While at the department of bacteriology at the University of California, Berkeley, Salton shifted to biochemistry. Still focusing on bacterial cell wall images, he declared the effects of some enzymes to be "the most elegant method of studying the nature and location" of the bacterial cell wall and its components (Salton, 1953). Treatment of a set of Gram-negative and Gram-positive bacteria resistant to lysis by lysozyme and trypsin enabled him to characterize the set and offer "some indications of the suitability of using heat-killed bacteria for the isolation of cell-wall decomposing microorganisms" (Salton, 1952, 1953, p. 513); that is, to participate in the search for new antimicrobials. To this aim, biochemical methods were combined with electron microscopy photographs: he provided images of bacteria cell walls as evidence of his

experiments, and of the resistance to digestion by some enzymes of Gram-negative bacteria. Salton concluded that the resistance of heated Gram-positive bacteria to lysis "is largely governed by the nature of the cell walls" and suggested a method for detecting microorganism activity "capable of producing cell wall decomposing enzymes" (Salton, 1953, p. 521). Decomposed cells were illustrated by the wall's disappearance, "digested" through a combination of heat and lysozyme action. Images were attached as eye-witness evidence, the microscope amplifying turbidity and revealing the cytoplasm as rod-shaped "coagulated protoplasts", proof of the phenomena of bacterial cell wall lysis (Figure 2). Lytic substances were made visible precisely through their action – the action sought in the hunt for new antimicrobials – at a time when Salton's research in the US was funded by a Merck International Fellowship.

By the early 1950s, cellular units of Gram-positive microorganisms that had lost their rigid cell wall – that is, non-animal cells – had become

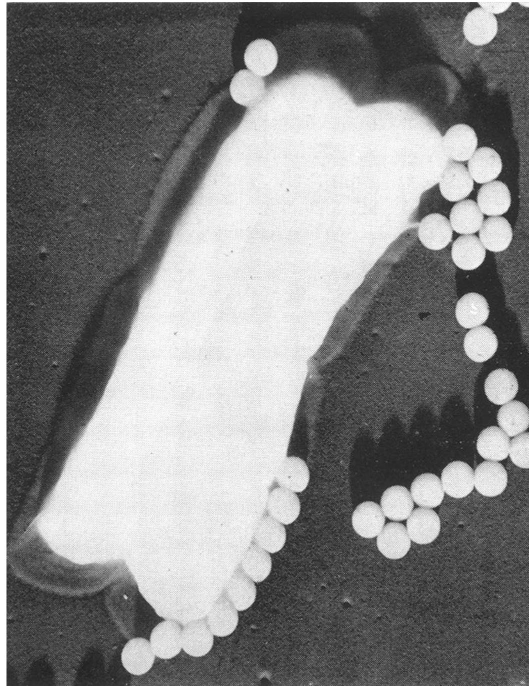

Figure 2. Electron micrograph of *B. megaterium* heat-killed cells as rod-shaped "coagulated protoplasts", proof of the phenomena of bacterial cell wall lysis. *Source* Salton (1957, plate 1, unpaged after p. 99). Reproduced with permission

known as bacterial protoplasts. The term came from botany: the living components of plant cells were known to have a membrane, but not a wall. The rigidity of that which characterized bacteria differed in permeability: the wall was a barrier while the membrane was a *passage* tissue.[11] Both the wall and the membrane contributed to an understanding of cell function and behavior, becoming mutual references, not in comparison with one another but for understanding their respective biologies – their metabolism, biosynthesis and energy transfer, as one German plant biologist phrased it (Martin, 1963, p. 1).[12]

Joshua Lederberg, then at the University of Wisconsin-Madison, identified protoplasts in bacteria as cells with their wall removed, and devised a method for obtaining them through the action of penicillin (Lederberg, 1956). Without the wall, the cell was transformed into a "spherical protoplast" and its sensitivity to a hypotonic medium produced lysis. After two hours under the action of a penicillin solution in the presence of magnesium salt, Lederberg observed the cells of *Bacilus magaterium* and *E. coli* take on a spherical shape and, once water was added, break down. These were the two features of protoplasts, a spherical shape and lysis in distilled water: in the absence of hypotonic media, protoplast suspension was able to "remain intact" for several days at 4°C.

For his 1958 Nobel lecture, Lederberg discussed his experiments with both streptomycin and penicillin, neither of which produced "direct mutation" (Lederberg, 1958, p. 10). Drug resistance had become a significant issue by that time, at least among some biomedical researchers in the US, Norway and Japan, and would continue to increase in significance, intensifying warnings, research interest and funding to combat resistance as a phenomena produced by drug use both in the microbiological laboratory and the clinic (Creager, 2007; Gradmann, 2011; Lie, 2014; Podolsky, 2010, 2014). But according to Lederberg's interpretation, presented in Stockholm in May 1959, the cell was killed by these substances before they accessed chromosomes and become mutagenic.

Together with Jacqueline St. Clair, Lederberg had by then solved the problem of bacterial shape-changing through environmental action. They had explored an environment composed of penicillin, magnesium salts and water, and the extent to which protoplasts behaved as cells or not, according to the amount of penicillin in the culture media. As Lederberg's research agenda was bacterial genetics, his main interest

[11] On the cell membrane in later years, see Grote and O'Malley (2011) and Grote (2010, 2013).

[12] See also Lederberg and St. Clair (1958).

was mutation, which did not occur in the experiments he performed with St. Clair. Their results were clearly summarized: "the mechanism of action of penicillin is to inhibit the synthesis of the bacterial cell wall" (Lederberg and St. Clair, 1958, p. 157). By inhibiting bacterial growth without affecting human cells, penicillin appeared to recover its benefits for the clinic: by inhibiting one type of cells –bacterial – it stabilized its ability to protect others – those of the human body (Figures 3, 4).

Through opposing mechanisms and questions, the cell lost its wall in Lederberg's hands, while being represented by such a wall by Salton. The images presented became evidence of the wall's very existence, its own activity, responses to substances, structure and morphology.

Border Molecules as Degradation Products

In his PhD research at the Biochemistry department of the College of Agriculture at the University of Wisconsin, James T. (Ted) Park isolated from penicillin-inhibited *Staphylococcus aureus* a very unusual nucleotide that contained uridine diphosphate (Park and Johnson, 1949). While in the armed services at Fort Detrick in Maryland, Park continued working on this unusual compound (Park, 1952a, b, c). Uridine diphosphate was linked to a sugar molecule with an unknown structure which, in turn, was linked to a peptide. Park was visited there by Jack Strominger, who had spent a year in Europe being trained by the microbial biochemists of the Carlsberg Laboratory after an unsuccessful attempt to join Gale's department in Cambridge. Park and Strominger discussed the cell wall components and began a fruitful collaboration (Strominger, 2006, 2007).

Uridine-5′ pyrophosphate derivatives – the unknown phosphate compounds – and the unusual nucleotide identified by Park in the early 1950s as components of the wall were at that time the research subject of biochemist Luis Leloir, director of the Instituto de Investigaciones Bioquímicas, Fundación Campomar (Buenos Aires, Argentina). With his early colleagues, Ranwel Caputto, Carlos E. Cardini, Raúl Trucco and Alejandro C. Paladini, he was beginning work on the metabolism of galactose which would lead to the isolation of glucose 1,6-diphosphate and uridine diphosphate glucose (UDP).[13]

A cell-wall research community was therefore being created at this time, as suggested by the letters exchanged between Park, Strominger,

[13] For an account of the experiments at the Instituto de Investigaciones Bioquímicas, see Leloir's (1971) Nobel Lecture. On Leloir in the network and lineage of physiologists and biochemists, see Santesmases (2011b).

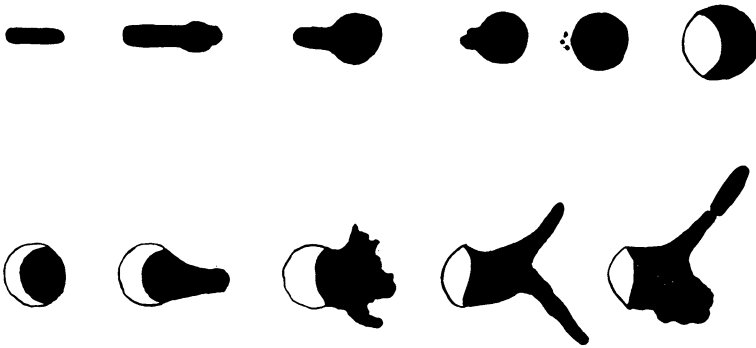

Figure 3. Transformation of bacterial rods to spherical protoplasts in the presence of penicillin (above) and reversion to rod in absence of penicillin (below). *Source* Lederberg (1956, p. 575)

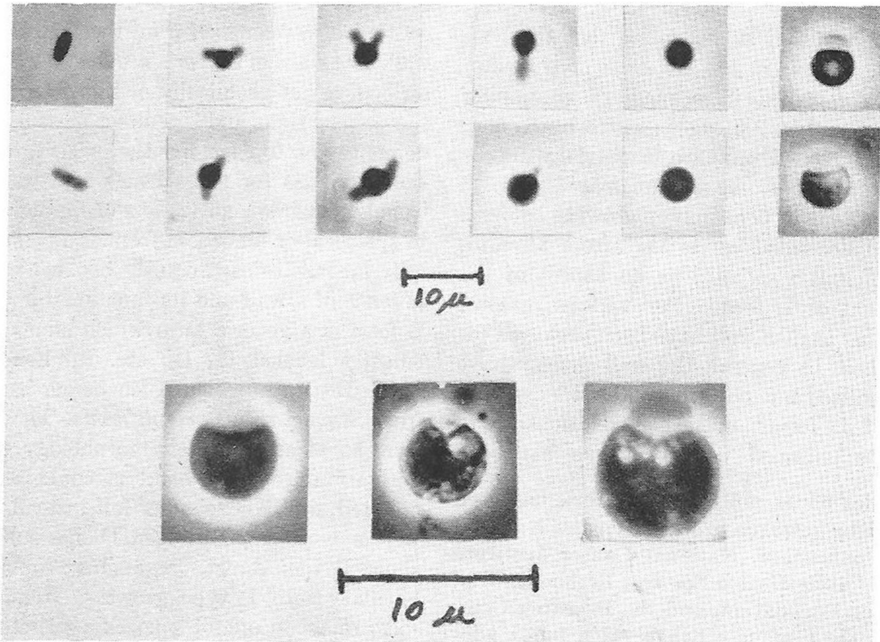

Figure 4. Phase contrasts of stages of *E. coli* cells grown in sucrose-penicillin broth are converted into protoplasts. Late stage at higher magnification. *Source* Lederberg and St. Clair (1958, p. 144). Reproduced with permission

and Lederberg,[14] who all published on the mechanisms of penicillin action in 1957, Lederberg by himself in a short note for the *Journal of Bacteriology*, Park and Strominger with a longer paper (Lederberg, 1957; Park and Strominger, 1957). Lederberg proposed a submission to the same journal but Park argued that his paper with Strominger, being longer, was better suited for *Science*.[15]

After explaining the composition findings, Park and Strominger suggested a structure for the ''principal nucleotide that accumulates'' in a mixture of *S. aureus* treated with penicillin. This included the uridine-derivative proposed by Park plus the amino-sugar found by R. E. Strange. In a biochemical style of work, they included a particular combination of the chemical units – pieces of the wall they had already identified – that represented the cell wall as a polymer. The action of penicillin, its ''selective toxicity'', was explained as an ''interference with the metabolic sequence of the biosynthesis of the cell wall, those metabolic sequences that are not found in animal cells but indeed were in bacteria''. Park and Strominger reported the materials and methods they used, a customary second detailed part of published papers in US journals of biochemistry and biological chemistry at the time: paper chromatography, paper electrophoresis and chemical tests belonged to a set of practices of earlier biochemists studying metabolism (Park and Strominger, 1957; Strominger et al., 1959). It was a style of reasoning and life representation evoking that of Otto Meyerhof, which had led him to suggest the paths of glucose breakdown in 1933. Meyerhof's research was on a physiological activity confronted as a chemical problem at the very origins of metabolic biology. As part of the trajectory of metabolic biology, the processes in which the bacterial wall was involved – its own composition, biosynthesis and degradation – included penicillin as an agent, but the wall itself also recreated the biological and medical meaning of the new drug, and of others that would be isolated as inhibitors of bacterial wall biosynthesis (Figure 5).[16]

When Park joined the Department of Microbiology at Tufts University School of Medicine in 1962, Park nucleotides already carried

[14] See Letter from Jack L. Strominger to Joshua Lederberg, Joshua Lederberg Papers, Correspondence, 1935–2002, National Library of Medicine, at http://profiles.nlm.nih.gov/ps/retrieve/Series/3. (hereafter Lederberg Papers). Accessed 15 March 2015.

[15] Park to Lederberg, October 8, 1956; Strominger to Lederberg, March 5, 1957; Lederberg Papers. Accessed 15 January 2015.

[16] On the history of metabolism, in addition to Kohler (1982), see Holmes (1992) and Landecker (2013). For a history of the term, see Bing (1971). See also Bechtel (2006) on the general history of the cell.

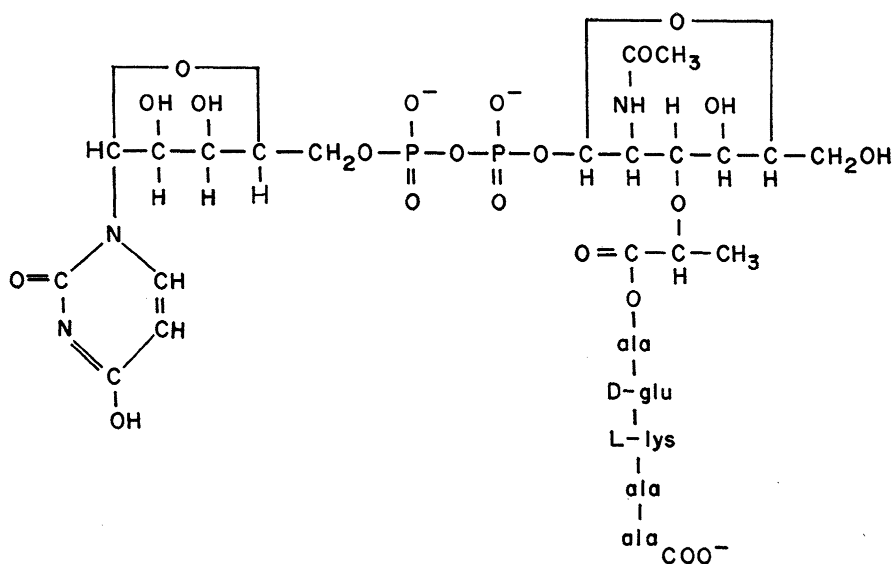

Figure 5. Proposed structure of the principal nucleotide that accumulates in penicillin-treated *Staphylococcus aureus*, that shows a transit of the bacterial wall from a cellular to a chemical ontology. *Source* Park and Strominger (1957, p. 100). Reproduced with permission

his name.[17] Through different experiments and as part of quite different research agendas – Park and Strominger in biochemistry and Lederberg in bacterial genetics – the three were studying the synthesis and composition of the bacterial cell wall by degrading it into pieces. Strominger wrote an account of the paths he had taken, from Park through Leloir, with whom he also published a paper in 1955 dealing precisely with the method for uracil derivative determination. Strominger's own paper in 1957 on the biological meaning of this derivative, uridine-5'-pyrophosphate N-acetyl-amino sugar, demonstrated that the substance accumulated when penicillin was in the test tube; to them this meant that the nucleotide was a biosynthetic precursor of the cell wall (Reissig et al., 1955; Strominger, 1957, 2006).

[17] "In 1962, Tufts University School of Medicine recruited James T. (Ted) Park to establish a Department of Microbiology and to serve as the first Chair. Park, a Big Ten collegiate tennis champion, was already well-known as a distinguished microbial biochemist for his discovery that the bacterial cell wall is synthesized from nucleotide-linked precursors (then called Park nucleotides and now known as nucleotide sugars) and for his demonstration that penicillin kills bacteria by inhibiting their ability to synthesize the cell wall". http://medicine.tufts.edu/Education/Academic-Departments/Basic-Science-Departments/Molecular-Biology-and-Microbiology/Department-History/Early-Days. Accessed 13 November 2014.

Biosynthesis and degradation of biological substances remained a broad, widely distributed research agenda in Western European and North American biochemical laboratories. From both physiology and microbiology, the biological catalyzers went beyond being biological challenges, to become tools themselves (Kohler, 1982; Santesmases, 2002; on a slightly later period, see Yi, 2009). Microorganisms retained their main role as experimental systems in their own right and as sources of substances; not only antibiotics but, as had happened previously, enzymes. The Belgian bacteriologist Jean-Marie Ghuysen, at the University of Liège, was approaching the cell wall through the isolation and testing of bacteriolytic enzymes secreted by microorganisms. Graduated in pharmacy and chemistry after WWII, he began his research career working on the isolation of RNA. Ghuysen is remembered as a researcher surrounded by a factory of enzymes that he systematically purified and characterized himself, fully focused on the chemical structure of the bacterial cell wall (Coyette et al., 2005). His expertise was strengthened by an intense development of research links he was able to establish by joining other experts in their laboratories. He collaborated with Milton Salton on the structure of the wall while in the department of Bacteriology at Berkeley under Roger Y. Stanier, the Canadian microbiologist who had also been in Cambridge following WWII with Marjorie Stephenson (Salton and Ghuysen, 1960; Ghuysen and Salton, 1960). In the early 1960s, Ghuysen visited Strominger at Washington University in St. Louis, thus becoming the enzymatic connection of the wall network (Strominger, 2006, 2007). Ghuysen's skilful practice in isolating enzymes from microbes enabled specific degradation of the bacterial envelope, and contributed to making microbiology a biochemical space of inquiry (Ghuysen, 1960, 1961, 1968).

Ghuysen remained focused on the wall in his long-standing agenda on the effects of penicillin, testing the drug at the enzymatic level at this particular space of microbial biochemistry. Microbiology methods combined with those of chemistry, such as infrared spectroscopy and gas chromatography, to isolate pieces of the wall as a puzzle that would be recomposed during the 1960s by the ever-expanding set of researchers. Ghuysen, Salton and Strominger kept penicillin at the centre of their explanatory narratives, referring to the chemical explanation of the specific toxicity of the drug and making the bacterial wall a target of their explorations. The cell was not only studied through cytology, with preparative methods developed so parts could be observed, but as morphology and biochemistry. It was becoming a biological form composed of molecules being degraded by enzymes, the substances that

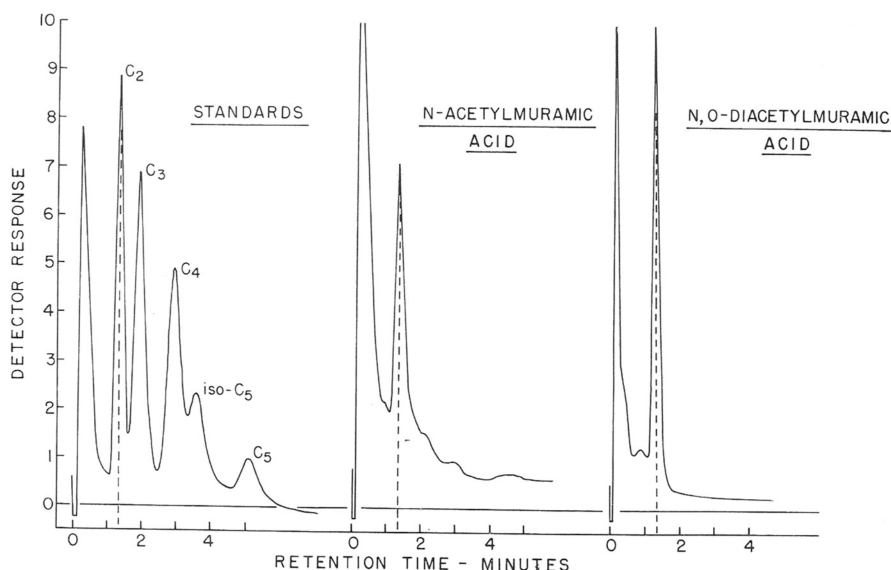

Figure 6. Gas chromatographies of bacterial wall components, a recent technique at the time, that show the style of work of biochemistry, that compared the original sample with known products so as to correctly identify them. *Source* Ghuysen and Strominger (1963, p. 1122). Reproduced with permission

were already the bases of biochemistry and metabolic studies (Ghuysen and Strominger, 1963; Strominger and Ghuysen, 1967) (Figure 6).[18]

Milton Salton, then at the University of Manchester, also contributed to this biochemical reconstruction of the bacterial cell wall in his CIBA lectures of Microbial Biochemistry, held at the Institute of Microbiology in Rutgers in 1960. He enlisted support from the electron microscope, providing images of the wall obtained in collaboration with prominent electron-microscopists of the time: among many others, Robley Williams, then in California, and Edward Kellenberg in Geneva. Thanks to electronics, morphology played a new role, providing shapes for the chemistry of the cell wall as described by Salton himself, Park, Strominger, and Ghuysen (Salton, 1960).

A Molecule that was a Bag

In 1950, while at the California Institute of Technology in Pasadena (CalTech), German virologist Wolfhard Weidel participated in the symposium *Viruses 1950*. Convened by Max Delbrück, this meeting

[18] Bechtel 2010 discusses the cell as "an integrated system". On the cytology of the time, see Santesmases and Suárez-Díaz (2015).

contributed to the framing of their virus research within the medical space, by defining viruses as "disease producing agents." The geneticists Max Delbruck and Salvador Luria, from Germany and Italy respectively, were working together at the CalTech, a lengthy research partnership that was influential in later developments of the molecular biology of viruses (Kay, 1985, 1996; Creager, 2002). It had been during Max Delbrück's visit to Tübingen in 1947 that he had agreed with the director of the Kaiser Wilhelm Institute of Biochemistry in Berlin, Georg Melchers, to grant Wolfhard Weidel a postdoctoral fellowship to spend a year at CalTech (Deichmann, 2002a, b).

After graduating in Chemistry and Medicine, and being a student of the German hormone expert and influential organic chemist Adolf Butenandt, Wolfhard Weidel began research with Erich Becker, Alfred Kühn's colleague, into Ephestia eye color pigment in 1938. This research would contribute to the identification and biochemical characterization of kynurenine, regarded by then as a *Genhormone*. By January 1940 they had published on the structure of the hormone kynurenine, responsible for determining eye color. This project, based in biochemical genetics, competed with one carried out by George Beadle and Edward Tatum at CalTech. As Brandt has pointed out, upon his arrival in Pasadena, Weidel was already known to his new colleagues (Brandt, 2004, Rheinberger, 2000).

While in California, Weidel established a method for isolating the bacterial wall, involving successive sedimentations by centrifugation. The final sediment electron micrograph showed bacterial cell walls "crumpled up and folded to give completely flat, almost circular particles of rather uniform size: the particles are nothing but empty collapsed bags with extremely thin walls". Such walls absorbed T viruses "as fast as normal living bacteria" and the electron micrographs revealed walls dissolved and disintegrated into "granular material". Weidel described the wall as "astonishingly resistant". Phage T5, once absorbed by bacteria, killed it; that is, it made it fail "to proliferate" and not a single phage was produced (Weidel, 1950, pp. 120–122). The chemical skills of Weidel contributed to representations of a phage involving its interaction with the host bacteria, explaining the event in structural terms.

Following his return to Tübingen, as he settled in at the Institute of Biology under Georg Melchers, Weidel was focused on bacteriophage research. He proposed an enzymatic perforation of the cell wall by the phage particle. His works toward identifying and characterizing the phage receptor in bacteria led him to suggest that the bacterial wall

could be considered "as one large, mainly two-dimensional molecular sheet of regular structure". Such a structure, Weidel continued, could have "all sorts of periodically repeated patterns all over the sheet" which would behave "like independent units" that would participate in the adsorption of phages through the intervention of those independent units composed of chemical groups such as carboxy-, amino- or carbohydrate. The cell's morphological epistemology was dynamic: the plasticity of the bacteria wall that Weidel proposed was based on a structure composed of "certain amino groups" as reactive points of the wall itself (Weidel, 1953, p. 157).

Weidel would become a renowned virologist, and publicized phage genetics in his homeland, writing the first book in German on molecular biology relating to viruses in 1956. Published the following year under the title *Virus: der Geschichte vom geborgten Leben*, it was translated into English in 1959 by the University of Michigan, unusual for the work of a German scientist at that time. Having trained in the US in virology, and demonstrated his chemical skills, Weidel became a member of an emerging research network of self-named molecular biologists who recognized him as an expert. He kept in touch with the small but influential group around Debrück, Luria and Gunther Stent – recently settled in Berkeley – European émigré scientists who participated in the emergence of US biological research. As part of this network, Weidel spent the academic year 1960–1961 as visiting professor at the Virus Laboratory with Stent (Brandt, 2004; Weidel, 1957, 1959, 1964).

The focus of Weidel's research on the process of bacterial infection by viruses – participating in what was then called biochemical genetics – followed, or may have been inspired by his previous experience with the insect eye pigment (Brandt, 2004, Melchers, 1964).[19] His approach to the cell wall originated from the absorption of a phage from bacteria *E. coli* as a biological and chemical problem – a central issue of Delbrück and Luria's agenda in bacterial genetics.

Weidel, provided with organic chemistry techniques for studying biological materials, regarded the infection as a chemical event, in which chemical structures were modified and chemical bonds broken and reformed. Infection remained chemical as he isolated the receptor of the phage T5 of *E. coli*. After Weidel and his colleagues in Tübingen identified the receptor as a "glycoproteid complex", he and the Swiss

[19] Weidel had a PhD and had graduated in medicine and in chemistry immediately after the war. See Lebenslaub Dr Dr W. Weidel 11.11.52. Archiv der Max-Planck-Gesellschaft, II. Abt., Rep. 1A, personal files - Weidel, Wolfhard; Berlin-Dahlem (hereafter Weidel papers, Archiv der Max-Planck-Gesellschaft). On Weidel's expertise in viruses, see Brandt (2004).

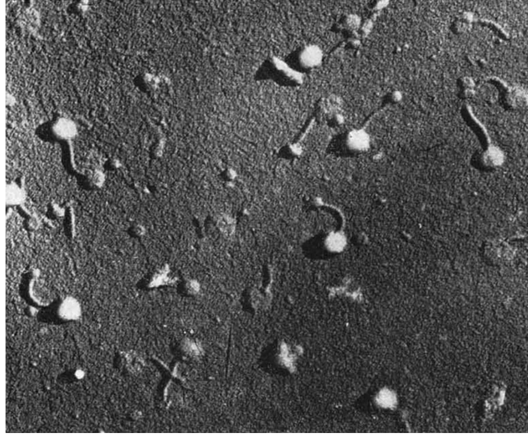

Figure 7. Suspension of phage T5 that shows phage particles with receptor spheres attached to tails. *Source* Weidel and Kellenberger (1955, p. 4, Figures 4 or 5). Reproduced with permission

electron microscopist Edward Kellenberger were able to display the kinetics of the interaction between receptor substance and phage. They published clear photomicrographs showing receptor particles attached to the tails of the bacteriophages (Figure 7) (Weidel and Kellenberger, 1955).

Alongside J. Primosigh, Weidel compared the lysis provoked in the cell by a virus with that caused by penicillin. Both bateriophages and the antimicrobial drug became "very useful and sensitive tools for helping to dissect the wall into many different, functionally intact, macro-components" (Weidel and Primosigh, 1958, p. 513). 80% of *coli* walls appeared to be composed of a layer of lypoproteins, the other 20% being "polysaccharides + lipids + a few typical aminoacids."

Chromatograms showed that *E. coli*, a typical Gram-negative organism, "hides, under a thick cover of lipoprotein, a wall which is typically 'Gram-positive in composition'". Even if smaller than the lipoprotein layer, the thinner and more complex one appeared as the wall itself. As they checked, the composition of this layer seemed to be similar to 'Park' nucleotides, "the uridine-5′-phosphate-linked complexes excreted by Park's penicillin-inhibited *Staphylococcus aureus*". "Both penicillin and the enzyme of certain virulent phages caused, under appropriate conditions, cells to lyse" (Weidel and Primosigh, 1958, p. 516). Whether caused by the action of a virus or penicillin, lysis was "linked" to the same "key component of the cell wall", namely this complex containing the three characteristic amino acids and muramic

acid, a term Weidel coined for this wall component from the Latin, *muro* (wall).

The wall was a collective scientific object of Weidel's *Abteilung* at Tübingen, where many people joined him, as demonstrated by the diversity of co-authors in Weidel's papers over the 15 years he spent at the institute. The morphology of the "rigid layer" of the wall was described as a "great number of tiny spheres linked together closely to form an extremely thin sheet", resembling a "shirt of chain mail knitted around the contents of the cell" (Weidel et al., 1960, p. 158).[20] Such a shape enabled an explanation of cell growth: "any extension of a rigid cell wall requires first its local breakdown" while the layer was extended by "cutting few links" between adjacent spheres in a "continuous process of making and remaking during growth". This would combine the action of two enzymes, "one hydrolizing and one synthesizing". Penicillin would inhibit the action of the synthesizing enzyme that appeared to "activate the hydrolysing enzyme" (Weidel et al., 1960, p. 165).

A skilful coordination of chemical knowledge enabled Weidel and his co-authors to resolve both the structure of the cell and the activity of penicillin within: objects and tools were so closely intertwined that they can barely be distinguished. The techniques of analytical organic chemistry – or biochemistry, given the size of the molecules under scrutiny and the biological environment in which the scientific problems arose – included digestion by the lysozime and T-phages, paper chromatography, chemical identification with the help of inorganic salts, fluordinitro-benzene for a coloring test, and molecular weight determination.

Biochemistry was taking form, to borrow the expression used by Lynn Nyhart (1995) in her history of German embryology. Images of the wall and its components remained the evidence presented in Weidel's publications: the powerful technicalities of electron microscopy enabled readers to witness the strength of the wall, its resistance to chemical and mechanic treatments, its characteristic shape, and the forms of its components beyond chemical identification.

Once its components were identified by Weidel's *Miterarbietern* in his *Abteilung,* and by other competing scientists – among the more mutually cited being Strominger, Park, Salton and Ghuysen – Weidel published,

[20] Weidel claimed that the term "rigid layer" was coined by his laboratory, which had "done a lot more since 1950 to clear up cell wall structure of Gram-negatives than anybody else, including Salton, ever did". Weidel to Alan Blaskett (University of Adelaida, Australia), June 1, 1964. Weidel papers, Archiv der Max-Planck-Gesellschaft. I thank Christina Brandt for calling my attention to this correspondence.

with H. Pelzer, an entire reconstruction of the characterization of the bacterial wall, its chemical shape, ''the unusual type of high polymer bacterial sacculi''. Weidel's biological epistemology was clear in the paper they published together: ''I describe my own views in great detail'', he said to Japanese bacteriologist Shozo Kotani.[21] The cell wall became, in his and Pelzer's words, a ''supporting and protective corset'', ''a tightly knitted net running back into itself'', an ''object located on that border where organic chemistry merges into morphogenesis and morphology'' which provides ''access (…) to a Biochemistry of Morphogenesis''. It was, as the paper was titled, ''a bagshaped molecule''. This particular set of synonymous expressions exhibits Weidel's morphological thinking and visual epistemology: that of a trained chemist who reoriented himself, locating an organic chemistry agenda involving bacterial and viral interactions in the antibiotics era. By that time, bacteria, both within genetics and the older disciplinary space of microbiology, had achieved high status: after Koch and Pasteur, antibiotics interacted with bacteria in the era of infection cure, an infected animal body often being cured by a product extracted from one microorganism to kill another. The mechanism of such phenomena that anti-infection became was a cellular one, which, as the network of scientists studied here showed, involved the bacterial wall.

With statements such as ''the electron microscope becomes an indispensable tool of control'', ensuring that during experiments the ''naked sacculus and its murein remain intact'', the envelope as an entity obtained its ontological status as a biologically stable object, as an explanator of phenomena – being penetrated by a phage or destroyed by penicillin – and as an experimental object. Its entire chemical identity as a large molecule should be maintained, according to Weidel and Pelzer, as the wall was ''a morphological entity'' and ''not merely a complex chemical compound'', therefore it should not be trivialized by ''chemical classification''.

A drawing of the murein sacculi, the name they gave to this ''new type of polymer from which bacterial sacculi are tailored'', was included in the paper as a graphic reconstruction (Figure 8).

[21] Weidel to Kotani, May 23, 1964. Weidel Papers, Archiv der Max-Planck-Gesellschaft.

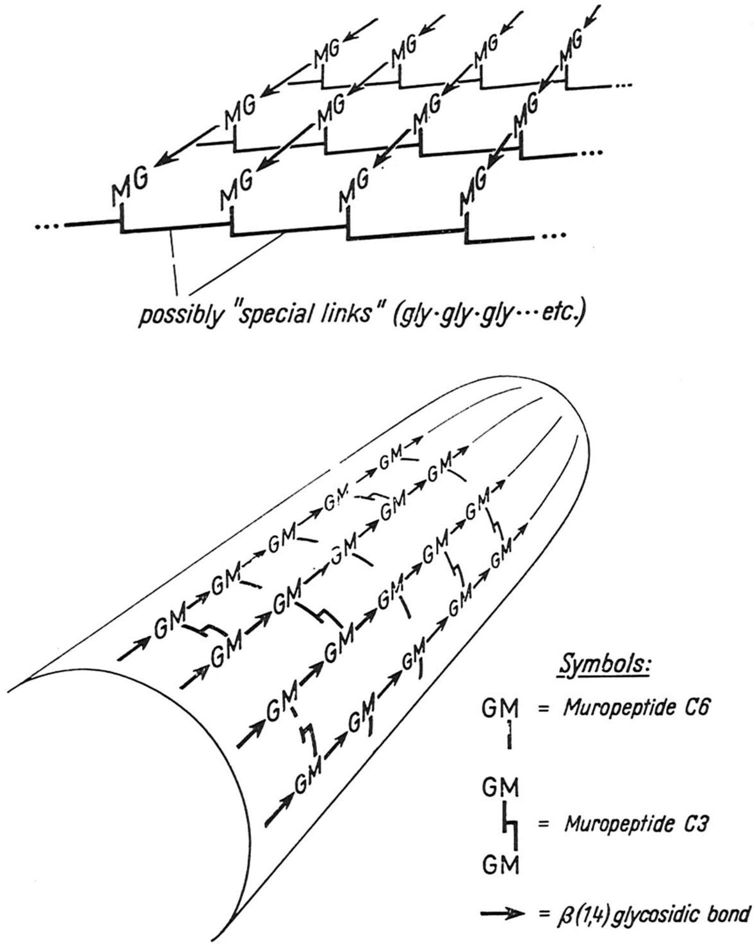

Figure 8. "Stereochemical model of *E. coli* murein sacculus" and "of a tightly knitted murein net", according to Weidel and Pelzer. *Source* Weidel and Pelzer (1964, pp. 208 and 209, Figures 7 and 9). Reproduced with permission

Negotiating Names and Meanings

The terms protoplast and spheroplast were mobilized by bacteriologists and biochemist from 1955 onwards.[22] Interchanges of letters and of references in papers suggest that negotiating the use of these terms also involved a negotiation of biological meanings and representations of the bacterial wall and its biological function: that is, reaching agreement on, among other things, what the bacterial cell wall was, its composition, chemistry, interactions with phages, morphology and osmotic sensitivity.

From the department of biochemistry at the University of Cambridge, Kenneth McQuillen, himself also a pioneer in protoplast studies, actively negotiated the meaning of the term protoplast, so as to construct an agreement for its usage. McQuillen discussed with Lederberg the convenience of defining the term protoplast as a bacterial cell that had lost its cell wall. McQuillen had described protoplasts in collaboration with Salton at Cambridge (McQuillen and Salton, 1955; Salton and McQuillen, 1955), which situated him in the earliest cell wall research network of the 1950s. He demonstrated that protoplasts from *Bacillus megaterium* were able to divide under particular conditions (McQuillen, 1955).

Protoplast, as McQuillen would argue, was a botanical name used to refer to plant cells.[23] The text McQuillen sent to Lederberg, which was also circulated by the Swiss electron microscopist Edward Kellenberger, began with a declaration of what the bacterial cell wall was composed of, and included citations of the works by those who signed, among them Weinbul, Weidel, Lederberg, Salton, Kellenberg, McQuillen himself, and many others. It is written as a scientific letter to the editor – as a short article – with 22 footnotes. After briefly presenting the knowledge and methods available on the isolation and composition of the wall, the text stated the need to avoid "a situation in which the word PROTOPLAST in one context means that part of a bacterial cell which lies within the cell wall and in another context means something dif-

[22] This discussion on terms and meanings relating to the bacterial cell wall evokes that of the protoplasmic theory of life in the late nineteenth century. See Geison (1969). On its connection with the later theory of enzymes at the origins of biochemistry, see Kohler (1973). This historiography of these previous accounts has informed biology as a historicized epistemological space.

[23] H. H. Martin, who collaborated with Weidel and later worked in the Institut für Angewandte Botanik at the Technical University of Munich, developed a reconstruction of this connection. See Martin (1963). On this collaboration, see Weidel et al. (1960).

ferent'' and proposed the term be solely used ''to describe a structure in which the cell wall is known to be absent''.[24]

On receiving a draft of the letter, Lederberg deemed McQuillen's ''question about terminology'' to be ''rhetorical''. After discussing McQuillen's interest in constructing a shared meaning of the term, he added: ''I would rather fear that if say ''spheroplast'' were adopted it would come to connote the alternative class of false protoplast rather than the inclusive class that needed to be cited''. He regarded the criticism about the characterization of protoplasts ''entirely valid'' but wondered whether it merited an approach from ''the semantic side''.[25]

Closer to biochemistry than Lederberg – a geneticist – McQuillen was unsuccessful in getting Lederberg involved. He had also been unable to include Weidel: ''the only dissentient'', as McQuillen phrased it to Lederberg, among those who received a draft of the letter. Weidel's comments appear rather derisory: ''even a megaterium protoplast is not a protoplast in the old sense…I should call this envelope the second innermost layer of the wall''.[26]

Circulating terms, even if to challenge previous meanings, made them real, and reliable. In 1958, the term spheroplast was already in use when the collective statement was finally published about the use of the wording ''bacterial protoplast'' (Brenner et al., 1958), signed by 13 researchers from 12 institutions and six countries. McQuillen further developed the meaning and normativity of the cell at the International Congress of Biochemistry held in Vienna in 1958, where he summarized the ''comparative biochemistry of bacterial cell walls, protoplasts, and spheroplasts'' and used an entire chapter of the resulting publication to insist on the meaning of protoplasts defended in the letter to *Nature*. The term ''spheroplasts is being used in this sense'', he stated, declaring the term to already have its own life (McQuillen, 1960, pp. 257–258). Later on, Weidel and Pelzer (1964) described ''the fragile structure underneath the murein skeleton of Gram-negative bacteria'' as a ''spheroplast'', ''very suitable objects for studying murein metabolism''. For Weidel, the spheroplast, obtained by removing the cell wall, rendered the cell ''mechanically fragile''.

[24] ''Proposed note to Nature. Concerning the use of the term protoplast'', undated, McQuillen to Lederberg, filed as of 1957. Capitals in the original. Joshua Lederberg Papers, Correspondence, 1935–2002, National Library of Medicine, at http://profiles.nlm.nih.gov/ps/retrieve/Series/3. Accessed 15 March 2015.

[25] McQuillen to Lederberg, 24 November 1956; Lederberg to McQuillen, December 7, 1957. Lederberg Papers.

[26] McQuillen to Lederberg, 14 December 1957. Lederberg Papers.

It was the fragility of the bacterial wall that Cepa and Merck's screening method for new antibiotics focused on. The spheroplast, a term involved in the early interchanges about the bacterial cell wall analyzed in this paper, was used by Weidel and Pelzer to name a body without the murein sacullus. The term "murein", which Weidel coined for one of the basic components of the wall (in latin, *muro*), would later be challenged. As Ghuysen's biographers noted, it was at the Symposium of the American Chemical Society in 1966 that he, together with a group of colleagues, proposed the term "peptidoglycan" for the macromolecular "skeleton" of the cell wall (Coyette et al., 2005). Both names have been in use ever since. This final compatibility of the new names embodied the two lines of research on the bacterial cell wall: one that studied the action of enzymes, phages and penicillin, while preserving the whole entity, as Weidel and his co-workers did; another that experienced the wall by eliminating it and studied its components once they had been released.

Concluding Remarks on the Geography of Bacterial Cell Walls

In the Cepa-Merck collaborative research program spheroplasts were used as markers of antibiotics against Gram-negative bacteria. The application in the Cepa-Merck screening program of the spheroplasting method designed by Eugene Dulaney led to the detection, isolation and testing of new drugs, among them phosphonomycin (Hendlin et al., 1969). This new antibiotic was detected through the knowledge and practices of bacteriological research that focused on the bacterial cell wall, its structure and composition, and its resistances and fragilities. New substances able to break the murein sacullus would be sought for during the following decade, with both the methods employed and the inspiration for this program of antibiotic screening kept confidential (Santesmases, 2014).

As an industrial, commercially available object from the late 1940s onwards, as other antibiotics would later become, penicillin became both a scientific object and a tool within the research laboratory, one which would intervene in the manufacture of knowledge about the bacterial cell wall, its shape, composition and meaning related to the identity of the cell as a whole. As such, the bacterial cell wall, or the set of bacterial cell wall types studied by the scientists mentioned here, appeared as a transatlantic object of post-WWII microbiology in many forms. From electron microscopy to biochemistry and virology, many

disciplinary, academic spaces were involved. It has been the aim of this paper to present the dynamic geography of a European and North American interchange of methods, names and knowledge relating to the wall of bacteria.

An endeavor of pharmaceutical firms – the finding of new antimicrobial drugs – intersected with research into the mechanism of the action of penicillin. The wall became the target of penicillin and, immediately after, of the biological and biomedical research that turned the wall into a scientific object. A new research agenda used the new drug and others that followed to find out about the bacterial cell wall as the shaper of bacteria, about the chemistry of its morphology. While keeping antibiotics as research objects and tools, the biological and biomedical laboratory took part in the achievements and benefits of the antibiotic era.

As a research object, the cell wall appeared to be linked to the biography of penicillin, or, perhaps, penicillin was able to become involved in the biography of the cell wall, as the life of this scientific object was older that that of the drug. The composition of this thin and rigid layer would account for its growth and its destruction. The cell was not the bag, but the wall defined its shape. It was about biochemical morphology: a chemistry of shape accounting for biology, for life itself. While penicillin was being established as an industrially manufactured object, it remained at the research laboratory as a scientific object, contributing to the circulation of other scientific objects. As a lytic substance, penicillin led to the study of what was being broken by its action: the bacterial cell wall.

The research object – a wall – appeared in the studies of infections; that is, medicine and a drug – a phage infection and penicillin – created the environment for the emergence of the cell as a biological subject provided by biochemistry, precisely by encounters with metabolic thinking and experimental practices. Research on the cell wall united with contemporary techniques for determining the structure and composition of proteins, such as through the use of bacteriolytic enzymes by Jean-Marie Ghuysen (1968). The components would be revealed, along with the shape of the cell wall, as witnessed through the electron microscope (Salton, 1960, p. 10). There were many walls of different cells, from *Bacillus subtilis* to *E. coli* and *S. aureus*, grouped into at least two sets according to their reaction to Gram staining.

The puzzle that the cell wall became began to be constructed piece by piece in this transatlantic circulation of methods and names, and images that represented the shape of the wall itself. The wall was by then a

transnational object in transit: Salton, from Australia, carried out his PhD with Ernest Gale in Cambridge; Park himself went to England, as did Strominger; Salton settled at NYU, to be joined by Jean-Marie Ghuysen, who also finally visited Strominger in 1968 at Harvard University. Salton, Strominger, Ghuysen and Park recognized each other as experts, placing themselves at the core of cell biology – even though Strominger shifted to immunology while at Harvard, and Weidel died prematurely, immediately after publishing the review with Pelzer on the bagshaped molecules.

It was in the 1950s, at the origins of the biography of the bacterial cell wall as a biological object, when the cell wall could be exhibited as an ontology in transit. It embodied a set of practices and knowledge, and chemical and physical entities, to be rendered visible by electron beams and lenses, and by the chemical action of lytic substances. From the idea of a limit, a border tissue that gives cells their rigid shape, many researchers faced the question of its composition, its synthesis and its destruction. Electron microscopy was the novel tool originating in WWII that became instrumental in establishing cell biology as a discipline among the life sciences in many Western research institutions, as a biochemical space of experimenting with substances isolated and identified in microorganisms, while displaying the shape of cellular and subcellular entities in which the metabolic transformation of such substances took place. The walls of many bacteria were seen under the electromagnetic field that would engrave them in the electron microscopy plate.

The bacterial cell wall condensed in itself many trajectories, interests, experiments and research agendas from the late 1940s until the early 1960s. Throughout my reconstruction, the bacterial cell wall remained a plastic scientific object, the plasticity of which became modeled by the successive representation of it as a tissue, as a border, as a container, and as a huge molecule manufactured in the antibiotic era while evoking the action of selective bacteriolytic enzymes, which would break the wall into fragments, at the crossroad between metabolism and morphology.

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