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History of Microbiology

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Glossary

Antibiotics Antimicrobial agents produced by living organisms
Bacterial genetics Study of genetic elements and hereditary in bacteria
Chemotherapy Systemic use of chemical agents to treat microbial infections
Molecular biology Science concerned with DNA and protein synthesis of living organisms
Monoclonal antibodies Specific antibodies produced by *in vitro* clones of B cells hybridized with cancerous cells

ALTHOUGH MICROORGANISMS were first observed using primitive microscopes as early as the late 1600s, the science of microbiology is barely 150 years old. In this time, major developments have been made in our understanding of microbial physiology, ecology, and systematics. This knowledge has been successfuly applied to broaden our awareness of the nature and etiology of disease, with the result that the majority of the traditional killer diseases have now been conquered. Similar strides have been made in the use of microorganisms in industry, and more recently attempts are being made to apply our knowledge of microbial ecology and physiology to help solve environmental problems. A dramatic development and broadening of the subject of microbiology has taken place since World War II. Microbial genetics, molecular biology, and biotechnology in particular have blossomed. It is to be hoped that these developments are sufficiently opportune to enable us to conquer the latest specter of disease facing us, namely AIDS. Any account of the history of a discipline is, by its very nature, a personal view; hopefully, what follows includes all the major highlights in the development of our science.

The period approximating 1930–1950 was a 'vicennium" of extraordinary transformation of microbiology, just prior to the landmark publication on the structure of DNA by Watson and Crick in 1953. We have important milestones for the vicennium: Jordan and Falk (1928) and "System of Bacteriology" (1930) at its start are magisterial reviews of prior knowledge and thought. Dubos (1945) and Burnet (1945) anticipate the modern era, and Werkman and Wilson (1951) and Gunsalus and Stanier (1962) document its early and continued progress in monographic detail. The *Annual Review of Microbiology*, starting in 1947 (and several other *Annual Reviews*), and *Bacteriological Reviews*, starting in 1937, offer invaluable snapshots of the contemporary state of the art. These works can be consulted for many of the pertinent bibliographic citations, and they will be explicitly repeated here only when important for the argument.

This account will center on the fundamental biology of microbes and give scant attention to continuing advances in the isolation of etiological agents of disease and of vaccines and immunodiagnostic procedures. Most of the agents of common bacterial infections had been characterized by "1930," but

[1]For the period from 1930.

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the vicennium was distinguished by important work on the classification of enteric (diarrheal) bacteria and, above all, by the isolation and new study of viruses and rickettsia with methods such as cultivation virus in the chick embryo (Kilbourne, 1987).

I. Observations without Application

Macroscopic manifestations of microbial growth such as bacterial and algal slimes have been recognized since antiquity. However, it was the Dutch microscopist van Leeuwenhoek (Fig. 1) who provided the first observations of bacteria at the microscopic level. van Leeuwenhoek, a draper in Delft, Holland, ground his own lenses to make microscopes with short-focal length lenses giving magnifications of between ×30 and ×266. Descartes had earlier described a similar crude form of microscope, but the quality of his lenses did not allow for magnifications sufficient to see bacteria. van Leeuwenhoek, in contrast, used his homemade microscopes (Fig. 2) to examine microorganisms in rainwater, well water, and seawater as well as water infused with peppercorns. His observations were forwarded to the Royal Society in London on October 1676 and were later published in the Society's *Philosophical Transactions*. In 1683, van Leeuwenhoek contributed a second letter to the Society describing his various microscopical investigations, including novel observations on bacteria present in the scurf of teeth. Published in 1684, these observations include the first drawings of bacteria ever to appear. These drawings are stil extant and clearly show that van Leeuwenhoek observed bacilli, streptococci, and many other characteristic forms of bacteria. van Leeuwenhoek's meticulous drawings also show protozoa such as *Vorticella*, *Volvox*, and *Euglena*. At about the same time, Huygens also reported observations on a number of free-living protozoa, including species of *Paramecium*. Van Leeuwenhoek also gains credit for describing the first parasitic protozoan, when in 1681 he observed his own fecal stools during a bout of diarrhea and described large populations of what later became known as *Giardia lamblia*.

Figure 1 Anton van Leeuwenhoek (1632–1723).

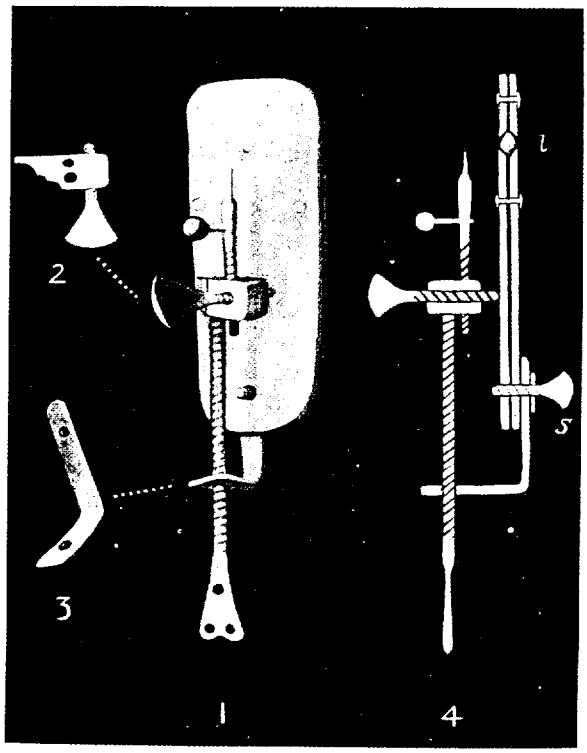

Figure 2 Dobell's reconstruction of van Leeuwenhoek's microscope.

Although van Leeuwenhoek also observed yeast cells in beer, the first illustrations of filamentous microscopic fungi were provided by Robert Hooke, again in a letter to the Royal Society, this time in 1667. However, the most important early work on molds appeared in the following century when the Tuscan botanist, Pietro Antonio Micheli described some 900 species, including important genera such as *Aspergillus* and *Mucor*. It is also worth noting that molds have been used from ancient times to treat infections, an approach termed mold therapy, which was based on folk medicine rather than on any scientific rationale.

Because the connection between microorganisms and fermentation or disease was never made during this period, observations made by the first microscopists had surprisingly little impact on human affairs. Despite this, Cicero and the Renaissance scholar Fracastorius had previously suggested that fevers might be caused by minute animals, collectively described as *contagium vivum*, but it was to be many centuries before the role of microorganisms in disease became recognized, eventually to replace the view that disease resulted from odors or other invisible "miasmas."

II. The Spontaneous Generation Controversy

The view that life arises *de novo* from inanimate objects was widely held from the Middle Ages until remarkably recent times; Van Helmont even provides us with a recipe for the production of mice. The tenacity with which spontaneous generation lingered on is highlighted by the fact that H. Charlton Bastian, one of the concept's chief proponents, died in 1915, still totally convinced of its merit. Although a scientific rationale was apparently provided to account for spontaneous generation by Needham and Buffon as early as 1745, these ideas were quickly dismissed by Spallanzani in the following year. Further developments then had to await the work of Schwann, who in 1837 showed that "air which had been heated then cooled left unchanged a meat broth which had been boiled." Yet by the middle of the seventeenth century, the concept of spontaneous generation held on tenaciously. Then, in 1858, Pouchet published a paper entitled "Proto-organisms . . . Borne Spontaneously in Artificial Air and Oxygen Gas." The French Academy of Sciences was moved by Pouchet's work to offer a prize to anyone who could settle the controversy once and for all. Despite discouragement from his friends who cautioned against becoming embroiled in the controversy, Louis Pasteur (Fig. 3) realized that if microbiology was to advance as a rational science the idea that microorganisms arose spontaneously would need to be experimentally defeated.

Pasteur's studies were published in memoir in 1861 and effortlessly took the prize offered by the Academy. He first of all showed that when air is filtered through cotton wool, large numbers of microorganisms are held back. Pasteur then successfully repeated Schwann's work, but his most famous and successful experiments involved the use of swan-necked flasks, with which he showed that heat-sterilized infusions could be kept sterile in an open flask as long as the open part was tortuous enough to allow any microorganism to settle on the sides of the tubes before reaching the liquid.

It is often assumed that Pasteur's experiments

Figure 3 Louis Pasteur (1822–1895).

immediately brought about the defeat of the theory of spontaneous generation, but this is far from true. Pouchet, for one, remained convinced that Pasteur's experiments did not defeat the concept. The controversy continued over the next quarter of a decade or so. Proponents of Pasteur's views included British scientists such as Huxley, William Roberts, John Tyndall, and the American Jeffries Wyman. The main counter-arguments were provided by the last and most dedicated of the important heterogenesists, H. Charlton Bastian. How this rearguard action by Bastian and others nearly carried the day is remarkable. However, experiments by the mathematician and physicist John Tyndall on the existence of heat-stable forms of certain bacteria (the removal of which involved the process of repeated heating and rest, referred to as tyndalization) finally convinced the scientific establishment of the error of Bastian's arguments. Bastian summed up his views on spontaneous generation in his book *The Evolution of Life* (published as late as 1905), and then died in 1915, still a confirmed believer.

III. Tools of the Trade

The science of microbiology needed two major developments to assure its progress. The first involved improvements in microscopes and associated means by which microorganisms could be better visualized, and the second involved developing methods for culturing microorganisms, thereby ironically liberating the science from total dependence on microscope-based observation.

Compound microscopes first began to appear in Germany at the end of the sixteenth century, and during the following century Robert Hooke developed instruments with magnifications of 3–500×. Although Hooke made major advances in observing microorganisms, he also recognized cellular structure in a variety of life forms. His microscopes, like those of his contemporaries, suffered from chromatic aberration (whereby a ring of colored light prevents accurate focusing on small objects such as bacteria). It was not until the early nineteenth century, when achromatic lenses were introduced by Professor Amici of the University of Medina, that this problem was solved, thereby enabling the light microscope to be developed to its full potential.

The next major development was the introduction of staining procedures, which allowed the fine visualization of microorganisms to occur. The staining of histological specimens was first carried out by the German botanist Ferdinand Cohn in 1849, his work being based on vegetable dyes such as carmine and hematoxylin. By 1877, Robert Koch (Fig. 4) was using methylene blue to stain bacteria, a process in which he developed the standard techniques of preparing dried films, and with the aid of coverslips was preparing permanent preparations. By 1882, Koch had succeeded in staining the tubercle bacillus with methylene blue, employing heat to encourage the stain to penetrate the waxy envelope. Two years later, the Danish pathologist Hans Christian Gram introduced his famous stain, which allowed bacteria to be characterized as gram-positive if they retained the violet dye or gram-negative if they did not. This distinction was later to be correlated with differences in biochemical and morphological characteristics, allowing bacteria to be classified into the two broad groupings still in use today.

Differential staining techniques soon followed, allowing Frederick Loeffler in 1890 to demonstrate the presence of bacterial flagella. During this period, rapid developments occurred in methods for identi-

Figure 4 Robert Koch (1843–1910).

fying bacteria and demonstrating their involvement as causal agents of specific diseases.

The light microscope was eventually developed to its theoretical limits and further progress in microscopy had to await the appearance of the ultraviolet microscope in 1919 (which for the first time allowed certain elementary viruses to be seen). Then, in 1934, the Belgian physicist Marton built the first electron microscope, which achieved magnifications of 2–300,000×, compared to 1200× and 2500× achieved by the light and ultraviolet microscopes, respectively. A further major development in microscope technology came in 1965 with the introduction of the scanning electron microscope.

The first semisynthetic medium designed for cultivating bacteria was introduced in 1860 by Pasteur and consisted of ammonium salts, yeast ash, and candy sugar. Prior to this, meat broths had been used for bacterial growth medium, an approach that persisted well into this century in the laboratories devoted to medical bacteriology. Mycologists, too, tended to rely on undefined media such as potato dextrose agar, although the introduction of Czapek Dox medium eventually provided an ideal defined substrate on which molds could be grown.

In 1872, Ferdinand Cohn developed the idea of the basal medium, to which various additions could be made as required. These early media were always liquid-based and it was not until the introduction first of gelatine and then agar in 1882 that the use of solid media became commonplace. The latter introduction of silica gel media then allowed for rapid advances to be made in the study of chemolithotrophic bacteria such as *Thiobacillus thiooxidans*.

By 1887, a simple and prosaic development revolutionized microbiology when Petri, one of Koch's assistants, introduced the Petri dish. This simple invention provided a far more versatile means of culturing microorganisms than did use of the bulky bell jars employed previously.

From 1898 onward, the Dutch school of microbiologists led by Beijerinck developed the art of enrichment culture, which led to the isolation of both nitrifying and cellulolytic bacteria. Studies on gas gangrene during the first war encouraged McIntosh and Fildes to develop the anaerobic jar. A vast array of selective media were then developed that involved amendments such as tetrathionate broth, tellurite, and crude penicillin. Finally, the introduction of central media supplies after the war liberated the microbiologist and their technicians from the tedium of preparing media in-house. No longer did mycologists, for example, have to spend hours peeling and boiling potatoes when potato dextrose agar was available ready to rehydrate, sterilize, and use.

None of the preceding developments in media preparation would have been useful without the introduction of an efficient means of sterilization. Pasteur's colleague, Chamberland, developed autoclaves—essentially large pressure cookers—in 1884. More recently, gamma rays and ethylene oxide sterilization have allowed for the introduction of factory-sterilized plastics including Petri dishes, another relatively simple development that has, nevertheless, had a marked stimulatory effect on the recent progress of microbiology. [*See* STERILIZATION.]

IV. Microorganisms as Causal Agents of Disease

In 1788, an epidemic of smallpox broke out in the English county of Gloucestershire. Edward Jenner, a country doctor and pupil of the famous anatomist John Hunter, decided to try and prevent his patients from contracting the disease by employing the standard method of inoculation using a mild dose of the infection. Jenner, who had suffered under the blood purgers and inoculists in his youth, was himself immune to smallpox. He aimed to make the traditional inoculation method as rational and reliable as he could. While on his regular rounds, he was surprised to find that patients who had already suffered from cowpox did not react in the normal way to inoculation with smallpox. Although Jenner was aware of the old wives' tale suggesting that cowpox gave protection against the disease, it was not until 1796 nearly a quarter of a century after he had first heard these suggestions, that he decided to act. His first experimental inoculation involved a local boy named James Phipps, who, after receiving cowpox, became immune to smallpox. In June 1798, Jenner presented a paper on his work to the Royal Society, and the effect was remarkable— within a few years, vaccination was commonplace.

Despite Jenner's breakthrough, there was still no convincing explanation to account for the appearance and spread of infections, and by the mid-1800s there was still little that could be done to counter infectious disease. Childbed or puerperal fever was a particularly terrible blight that affected every one of the lying-in hospitals in Europe. During a single

month in 1856 in a Paris hospital, 31 recent mothers died of the infection. Vienna of the 1840s had a particularly bad reputation for this disease, despite having one of the most enlightened hospitals in Europe. It was here that the Hungarian doctor, Ignaz Semmelweiss joined the staff of the lying-in clinic of the Vienna General Hospital in 1844. In his first few months of practice, he heard yet another wives' tale, this one associating the high death rate from childbed fever found in the teaching division of the hospital with the high frequency of examination by doctors and their students. Semmelweiss began to collect statistics and soon became aware that the highest rates of infection and mortality occurred in the teaching clinic. This information led him to surmise that the contagion was being transmitted by the doctors and medical students, many of whom examined the wombs of patients without washing their hands, even after coming directly from mortuary duty. Semmelweiss suggested that anyone examining patients should first wash their hands in chlorine water. The results of this simple remedy were phenomenally successful, with mortality rates being reduced from around 11 to 3% within 1 yr. Semmelweiss was slow to write an account of his work, but eventually in 1857 he provided a rambling and highly egotistical survey of his work, which completely failed to make any impression.

Eventually, however, the view that infection was spread by some organic particle did at last become widely accepted, although the exact nature of such particles was unknown. The effect of this ignorance was devastating; during the Crimean War of 1853–1856, for example, a single regiment of the British Army lost 2162 men, with 1713 dying not from wounds or the effects of trauma but from disease. The infamous hospital diseases of erysipelas, pyemia, septicemia, and gangrene made surgical wards nightmares of suffering and death. The causes and mechanisms of disease transmission remained essentially unknown. By 1865, however, Pasteur had concluded that disease must be airborne, a view that galvanized the English surgeon Joseph Lister into action. Lister reasoned that he could reduce mortality due to sepsis by covering wounds with dressings containing chemicals that killed these airborne germs without preventing the entry of air. He knew that carbolic acid had recently been used to sterilize sewage, and with the help of the chemist Anderson he obtained a supply of the sweet-smelling dark liquid that was commonly called German creosote. Lister published his findings in *The Lancet* in 1867. In contrast to Semmelweiss's efforts, Lister's work attracted immediate attention—The age of antiseptic surgery was soon underway.

Once it became realized that microscopic organisms present in the air were responsible for transmitting disease, the next important development was to isolate these organisms and then conclusively demonstrate their role as causal agents of any given disease. Yet, some authorities continued to argue that microorganisms were not the cause of disease, but merely grew on the weakened infection site. In May 1882, Robert Koch dismissed this view when he announced the discovery of the tubercle bacillus; the search for other disease-causing microorganisms then gathered momentum. The introduction by Koch of his famous postulates finally established a means of conclusively demonstrating the involvement of a microorganism as a causal agent of a given disease, and the way lay open to disease prevention and cure.

Major developments were next made in our understanding of immunity. The first rational attempts to produce artificial active immunity was made by Pasteur in 1880 during his work on fowl cholera. By 1882, the Russian biologist Metchnikoff had made the first observations of cellular immunity and coined the term phagocyte. By 1891, Ehrlich had distinguished between active and passive immunity, and 6 years later Kraus published the first account of precipitation reactions when immune sera were added to cell-free filtrates of homologous bacterial cultures.

Nearly 250 million people have been vaccinated against tuberculosis with the bacille Calmette–Guérin (BCG) vaccine, yet its originator, Charles Calmette, remains a largely unknown figure. Calmette, a disciple of Pasteur, was the first Director of the Pasteur Institute in Lille, France, and later became Assistant Director of the Pasteur Institute in Paris. With Guérin, he set about to prepare a protective vaccine against tuberculosis. He spent 13 years developing an attenuated virus, which by not recovering its lost virulence remained both stable and safe. This vaccine, BCG, was first used in 1921, but because of considerable resistance to its use was not widely accepted until after Calmette's death in 1933.

Modern developments in immunology include the work of F. Macfarlane Burnet, who in 1957 published his clonal selection hypothesis.

V. Chemotherapy and Antibiosis

The origin and early development of the concept of chemotherapy is somewhat unusual in that it can be credited to the work of one man, the German chemist Paul Ehrlich. Ehrlich had the vision to apply his knowledge of specific staining of bacteria to the search for chemical compounds that would inhibit the growth of pathogenic bacteria *in vivo*. In 1891, he showed that methylene blue was useful for the treatment of malaria, but because this dye showed no advantage over quinine it was not widely used. By 1902, Ehrlich was concentrating his attention on the organic arsenic compounds, which he hoped would defeat experimental trypanosomiasis in mice. At this point, he and his Japanese bacteriologist assistant Shiga found that atoxyl (sodium arsanilate) was ineffective against mouse trypanosomiasis. This turned out to be a somewhat inexplicable error when the British bacteriologist Thomas was soon to show that atoxyl was in fact extremely effective against trypanosomiasis in mice.

A second equally inexplicable error followed when Schaudinn and Hoffman concluded that *Treponema pallidum* was a protozoan. Ironically, this error proved productive because it pointed to the likelihood that the antiprotozoal agent atoxyl, or a similar compound, might cure syphilis. In 1906, Robert Koch used atoxyl to treat trypanosomiasis in humans. This was the year in which Ehrlich became director of the newly opened George Speyer Institute, which was devoted to chemotherapy research. It was here that the first major chemotherapeutic agent salvarsan was developed. Salvarsan was first discovered in 1907 and was initially found to be inactive against the experimental mouse trypanosomiasis system. Then in 1909, a young Japanese scientist, Hata, joined Ehrlich's laboratory, bringing with him a system that he had developed for the artificial transmission of *T. pallidium* in rabbits. To his evident surprise, Hata found that salvarsan was in fact effective against syphilis in mice; by 1909, the drug was proving spectacularly successful in treating the disease in humans.

Following Ehrlich's death in 1915, research continued into chemotherapy, but little progress was made, with the exception that in 1932 Atebrin became available as the first synthetic drug for prophylactic use against malaria. The next major advance in chemotherapy came in 1935, when Domagk discovered the antibacterial effect of the red dye prontosil. This compound had a dramatic effect on lobar pneumonia in humans, reducing death rates by by two-thirds. In the same year as its discovery, the French scientist Trefouel showed that the active ingredient of prontosil was not the chromophore, but the sulphonamide moeity (sulfanilamide). Sulphonamides were widely used with success to treat bacterial infections from the mid-1930s until the middle of the following decade.

The concept of chemotherapy reached its zenith with the sulphonamides, but such compounds were soon eclipsed by the arrival of first penicillin and then a range of other antibiotics.

Antibiotics (cf. Waksman, MacFarlane, Wilson) had a spectacular beginning with the famous discovery of penicillin by Fleming in 1928, a mold spore having accidentally lodged on agar plates seeded with staphylococci. The story of the discovery of penicillin by Alexander Fleming is probably the best known in the history of medicine, although much that has been written on the subject borders on fairy tale. The important point about Fleming's initial observation, made during the late summer of 1928, was that it represented an extremely rare phenomenon, not merely an example of microbial antagonism, but one of bacterial lysis brought about by mold contaminant. Fleming probably initially thought that he had discovered a fungal variant of lysozyme, a lytic substance that he had previously found in various body fluids. It was this lytic phenomenon that distinguished Fleming's observations from the numerous observations of microbial antagonism that had been reported since Pastuer's time. It is likely that had he observed microbial antagonism, rather than lysis, Fleming would have ignored his observations, regarding them as an example of common phenomenon that was of little interest.

Fleming, however, understood the significance of what he observed. He soon showed that the contaminant produced the antibacterial substance in culture broth, which he called penicillin. Then, with help from various surgeon colleagues, Fleming used crude penicillin-rich filtrates to treat superficial bacterial infections, unfortunately without much success. The first documented cures with penicillin were in fact achieved (using the crude broths) by a former student of Fleming's, Cecil George Paine, who worked at Sheffield University.

In his first famous paper on penicillin, Fleming detailed its properties and antibacterial spectrum and suggested that it, or a similar substance, might

find a use in medicine. Unfortunately, neither he nor his colleagues could purify penicillin, an obvious necessary first step for its successful introduction into medicine. It is worth pointing out, however, that Fleming was not alone in being unable to achieve this essential purification step; other attempts such as those made by famous fungal product biochemist Harold Raistrick also proved unsuccessful.

Fleming's notebooks show that despite being unable to purify penicillin, he continued working on crude penicillin throughout the 1930s, during which time he also attempted to isolate other microorganisms capable of producing antibacterial products. Unfortunately, this work was not published and the medical potential of antibioisis remained undeveloped until the discovery of gramicidin in 1939. This substance, which was discovered by Rene Dubos, was unfortunately too toxic for intravenous use; therefore, it was limited to use on a number of superficial infections.

At about the time when gramicidin was being first developed, Florey, Chain, and Heatley managed to purify penicillin and demonstrate its remarkable antibacterial effects when used systematically. The isolation of the antibiotic from the crude culture filtrates was a formidable chemical task, but it was undertaken successfully in the late 1930s by Florey and Chain in England. Industrial production of penicillin soon followed as a joint U.S.–British war project. For this to be feasible required a substantial effort in strain improvement, which was conducted, however, along empirical rather than rational genetic lines (Wilson 1976). This was nevertheless the forerunner of the modern fermentation industry and biotechnology; its antecedents had been the production of butanol and acetone as munitions solvents during World War I and the peacetime production of citric acid by a mold fermentation. Penicillin's introduction into medicine as the first successful antibiotic stimulated the search for similar compounds. A particularly successful antibiotic screening program, devoted to soil actinomycetes, was carried out by Selman Waksman and his students at Rutgers. The first major product of this research, actinomycin, was, like gramicidin, too toxic to be of medical use as an antibiotic, although it was later used as an anticancer agent.

S. Waksman and R. J. Dubos had been studying the biochemical and ecological interrelations of soil microbes. The role of secreted antibiotics in ecological competition provided a rational for seeking these substances. *Tyrothricin* (Dubos, 1939; cf. Crease, 1989) was the first antibiotic to be clinically applicable, but its systemic toxicity limited its application to topical treatment. In Waksman's hands, the same paradigm led to the discovery of streptomycin (1944), which when used in conjunction with periodic acid–Schiff and isoniazid helped to defeat tuberculosis. Thereafter, a continued stream of new antibiotics with untold human benefit. It would be some time before the mode of action of antibiotics would even begin to be understood (cf. Gottlieb and Shaw, 1967) and to allow rational principles to assist in their improvement.

Although Waksman received the Nobel Prize for streptomycin, his triumph was marred by his tardy treatment of the codiscoverer of the antibiotic, Albert Schatz. Schatz, one of Waksman's graduate students, successfully sued Waksman and Rutgers for a share of the royalties for streptomycin. His later attempts to gain a share of the Nobel Prize for his work (he was senior author on the first streptomycin papers and coassignee with Waksman of the streptomycin patents) were, however, unsuccessful.

Streptomycin was soon followed by antibiotics such as chloramphenicol, neomycin, tetracycline, and the first effective antifungal antibiotic, nystatin (discovered by Elizabeth Hazen and Rachel Brown). Penicillinase-resistant penicillins such as methicillin then appeared, followed by semisynthetic penicillins, and finally broad-spectrum compounds like ampicillin.

VI. Microbial Metabolism and Applied Microbiology

Developments in the study of microbial metabolism were, from the outset, closely associated with attempts to use microorganisms for industrial purposes, a trend that continues in modern biotechnology. It is not surprising then to find that the first scientific paper devoted to microbial metabolism (appearing in 1857) can also be regarded as the first citation in applied microbiology or biotechnology. Again Pasteur was responsible for this development, the paper being devoted to an explanation of the causes of the repeated failures of industrial alcohol fermentations. This was an important paper for two reasons: first, because it laid the foundation of the view, later to be amply validated, that microbial

activity was responsible for many industrially important fermentations, and, second, because it introduced quantitative treatment of data on microbial growth and metabolism.

Pasteur also addressed problems associated with the microbiology of wine-making. Among the suggestions that he made was a method for improving the keeping qualities of wine by heating it to 68°C for 10 min, followed by rapid cooling, a process subsequently referred to as pasteurization. By 1872, Pasteur's work had been developed to the point where Ferdinand Cohn could suggest that microorganisms play a major role in the biological cycling of the elements responsible for soil fertility and the proper functioning of natural ecosystems. The first fruits of Cohn's theory came in 1888 when the Dutch microbiologist Beijerinck isolated the symbiotic N-fixing bacterium *Rhizobium* from the root nodules of legumes. During these studies, Beijerinck also developed the enrichment technique to the isolation of microorganisms, an approach later to be refined and developed by the Dutch school of microbiologists.

Many of the following breakthroughs in microbial metabolism were associated with studies on soil microbiology and its association with soil fertility. In 1889, Winogradsky described the autotrophic iron and sulfur bacteria, and in the following year the free-living N-fixing *Azotobacter* and the nitrite-oxidizing bacterium *Nitrobacter*. Although many of Winogradsky's so-called pure cultures appear to have been contaminated, his work was nevertheless important because he was the first to appreciate the concept of chemoautotrophy and to relate this growth strategy to the major natural cycles. It was a lack of appreciation of this concept that had hindered the work of others interested in processes such as nitrification. Despite this, the soil chemist Warrington nevertheless did important work on the factors that influence this process in agricultural soils.

Waksman and Joffe isolated and described *T. thiooxidans* in 1922, and over the next quarter of a century, major contributions to the science of microbial physiology came from, among others, Wieland, who in 1900 demonstrated the importance of biological oxidations using microorganisms. Other work of note came from Marjorie Stephenson and J. H. Quastel on enzymes. In 1924, A. J. Kluyver published an important article entitled "Unity and Diversity in the Metabolism of Micro-organisms," a paper that demonstrated the fundamental unity underlaying the apparent diversity of microbial metabolism. By 1930, Karstrom had established the concept of constitutive and adaptive enzymes. By now, microbiology had begun to be a cornerstone of biochemistry and the boundaries between the subjects were soon blurred. In 1941, Lipmann advanced the concept of the high-energy bond, and major developments in theories on the working of enzymes came from Monod's lab.

While most of the early developments in microbial metabolism were centered on bacteria, fungal metabolism, because of its importance to many industrial fermentation (e.g., citric acid production), was by no means neglected. The seminal work in this area came in 1940, when Jackson Foster published his "Chemical Activities of the Fungi." Studies on fungal metabolism obviously gained impetus following the introduction, while the isolation of antibiotics such as streptomycin also gave a boost to the study of a neglected group of organisms—the actinomycetes. It was Selman Waksman who initiated work in these organisms during the early part of this century, a period when the actinomycetes were regarded as fungi rather than bacteria.

VII. Nutrition, Comparative Biochemistry, and Other Aspects of Metabolism

Microbes, first yeast and then bacteria, played an important part in the discovery of vitamins and other growth factors. Growth could be measured in test tubes far more expeditiously and economically than in mice, rats, or humans. Conversely, the realization that microbes shared virtually all of the complex growth factor requirements of animals was an important impetus to "comparative biochemistry," the view that they had a common evolution and a similar underlying architecture. One of the essential amino acids, methionine, was first discovered by Mueller (1922) as a growth factor required by diphtheria bacilli. Mueller joined a school founded by Twort (1911), including Lwoff, Fildes, Knight, and Tatum, that made nutrition a branch of general biochemistry. They perceived that the requirement for a growth factor belied a loss or deficiency of synthetic power; lacking internal synthesis, the organism had to look to the nutrient environment for supply of substance. This also implied that organisms with simple nutrition had to be empowered with complex biosynthetic capability—leaving us humili-

ated by our species' inferiority to *Escherichia coli*, but that in turn is less capable than the green plant! Besides the practical utility of these findings, they led to a well-founded respect for the complexity of microbial cells.

By "1930," a number of growth factors had been shown to be important in bacterial nutrition, including factors V and X, later shown to be diphosphopyridine-nucleotide and heme, respectively, for hemophilic bacteria; *Mycobacterium phlei* factor, later shown to be vitamin K, for *M. pseudotuberculosis;* and tryptophane for *Salmonella typhi* (Fildes, 1936). Starting with the work of W. H. Peterson, H. Wood, E. Snell, and E. L. Tatum at the University of Wisconsin and B.C.J.G. Knight and P. Fildes in England, a number of bacterial growth factors were identified with B vitamins (extensively cataloged by Johnson and Johnson, 1945). By "1950," most of the known trace growth factors had been identified and associated with nutritional requirements of particular bacteria, as also had been most of the amino acids and a host of other metabolites (Snell, 1951). During the vicennium, most of the vitamins were also identified as co-enzymes, playing a role in the function of specific metabolic enzymes [e.g., thiamin for keto-acid decarboxylases, niacin for dehydrogenases, pyridoxal for transaminases, pantothenate in the citric acid cycle (Schlenk, 1951)]. The 20 canonical amino acids were listed and could be shown to be incorporated into bacterial protein.

A host of other biochemical pathways were also detailed with the help of new methodologies of radioisotopic tracers and chromatography. Of special significance in bacterial metabolism was the demonstration of heterotrophic assimilation of CO_2. This view of CO_2 as an anabolite was contrary to its usual image as a waste product. The specific requirements for CO_2 as a nutrient helped to clear up difficulties in the cultivation of fastidious bacteria and eventually of tissue cells.

By 1941, microbiology and genetics overlapped when G. W. Beadle, Tatum, and coworkers at Stanford University began to use the red bread mold *Neurospora crassa*, an approach in which mutants were employed to help elucidate genetic mechanisms, thereby allowing a number of microbial pathways to be worked out for the first time.

In due course, especially after Beadle and Tatum (1941), the power of synthesis came to be understood as the capability of individual specific genes. This in turn led to concepts and experiments on the genetic underpinnings of metabolism.

The birth of molecular biology followed the work of Watson and Crick in 1853, when microbiology entered into a new phase, allowing it to overlap with many other sciences, leading to the appearance of numerous exciting developments.

The major conceptual theme of change in microbiology during the vicennium was the convergence of the discipline with general biology. As noted by Dubos (1945),

> To the biologist of the nineteenth century, bacteria appeared as the most primitive expression of cellular organization, the very limit of life. Speaking of what he considered "the smallest, and at the same time the simplest and lowest of all living forms," Ferdinand Cohn asserted: "They form the boundary line of life; beyond them, life does not exist, so far at least as our microscopic expedients reach; and these are not small." The minute dimensions of bacteria were considered by many to be incompatible with any significant morphological differentiation; it encouraged the physical chemist to treat the bacterial cell as a simple colloidal system and the biochemist to regard it as a "bag of enzymes."

Still dominated by the medical importance of microbes, the views of microbiologists in "1930" had not evolved much further, although "System" (1930) does have a brief chapter on bacterial cytology and allusion to ongoing controversy over the existence of nuclear structures. Far more attention is given to the Gram stain!

While a few differences in the detail of intermediary metabolism and biosynthetic options have been discovered (e.g., for lysine), it remains true that pathways conveniently noted in bacteria have usually been reliable predictors of the same steps in higher plants and animals. It is possible today to relate this functional conservatism to evolutionary affinity with currently available tools of DNA sequencing.

A. Induced Enzyme Formation, or "Enzymatic Adaptation"

One of the most intriguing phenomena of bacterial physiology is the plasticity of enzyme expression

dependent on the chemical environment. For example, *E. coli* grown on a glucose medium exhibits very low levels of β-galactosidase (lactase). When glucose is replaced by lactose, there is a growth delay followed by the abundant production of lactase. Thousands of comparable examples are now known, and the pursuit of the mechanism of this phenomenon has been of outstanding importance in the development of molecular genetics. Anecdotal reports of enzyme adaptation can be traced back to Wortmann (1882, cited *in* Karstrom, 1930); they were collected, together with new experimental observations, by Henning Karstrom for his doctoral dissertation in Virtanen's laboratory in Helsinki. In this turning-point review [Karstrom, 1930, followed by the more accessible Karstrom, 1937; Dubos 1940 (1945)], bacterial enzymes are classified as constitutive or adaptive according to their independence, or otherwise, of the cultural environment. Except for glucose metabolism, most sugar-splitting enzymes are adaptive—resulting in substantial biosynthetic economy for a bacterium or yeast that may only rarely encounter, say, maltose now, or lactose next week. During the vicennium, the work of Stephenson and Yudkin (1963) and Gale (1943) furnished additional clearcut examples of the adaptive response, and Dubos (1945) offers a critical appraisal of the fundamental biological issues. Several theories allowed for the stabilization of preformed enzyme by a substrate, or a Le Chatelier-like principle of mass action, to encourage enzyme synthesis. They shared the presumption that the enzyme molecule itself was the receptor of the inducing substrate. Other hypotheses lent the substrate an instructive role in shaping the specificity of the enzyme. Further progress would depend on the postulation of an enzyme-forming system distinct from the enzyme—and this would emerge under the impetus of genetic studies to be described later. At the very end of the vicennium, Lederberg *et al.* (1951) described a noninducing substrate of lactose, the analog altrose-β-D-galactoside, which pointed to a separation of those specificities. This substrate also allowed the selection of constitutive-lactase formers, showing that lactose was not required for the conformation of the enzyme, but that the latter could be derived directly from the genetic constitution. The debate continued until the mid-1950s (see Lederberg, 1956, p. 51; Monod, 1956); it was mooted by the spectacular progress of the Pasteur Institute group in showing that enzyme induction was the neutralization of an endogenous repressor that inhibited the expression of the lactase gene in the absence of an inducer (Jacob, 1965).

The simultaneous induction of several steps in a metabolic pathway, usually by an early substrate, was exploited to delineate the later steps, notably in the oxidation of aromatic compounds by pseudomonads.

Among technical innovations, one of the most ingenious was the chemostat (Novick and Szilard, 1950). This allowed microbial populations to be maintained for the first time in a well-defined steady state, albeit under limitation for one specific nutrient.

VIII. Microbial Genetics

During the last two decades of the nineteenth century, it was realized that bacterial species were not as stable as had first been thought. Pure line cultures that had been maintained for many generations suddenly underwent dramatic changes in morphology, metabolic properties, and pathogenicity. As more pure cultures were obtained, this variability, or dissociation as it was called, became even more apparent. Then in 1925, R. M. Mellon published a paper describing a primitive from of sexuality in colityphoid bacteria. This work had little contemporary impact on the contemporary view that bacteria were anucleate organisms that reproduced without sexuality by binary fission.

Bacterial genetics was substantially nonexistent in 1930. As late as 1942, the eminent British biologist Julian Huxley would suggest of bacteria that "the entire organism appears to function both as soma and germ plasm and evolution must be a matter of alteration in the reaction system as a whole" (Huxley, 1942. Such ideas gave little encouragement to efforts to dissect out individual genes along the Mendelian lines that had been so successful with *Drosophila* and other animals and plants. Some work with fungi had gotten off to a promising start early in the century (Blakeselee, 1902). Authentic but sporadic observations of bacterial mutation (Beijerinck, 1901) were outnumbered by wooly-minded speculations that embraced variations of colony form as manifestations of cellular life cycles among the bacteria (see Dubos, 1945; Lederberg, 1992). These

clouds of speculation probably discouraged more serious-minded experimentation.

Mention has already been made of the impact of the work of Beadle and Tatum on mutants in *Neurospora* on our understanding of microbial physiology. However, by initiating the field of biochemical genetics, these studies had even greater impact on the science of genetics. Prior to 1941, genetic research was dominated by work on the fruit fly, *Drosophila melanogaster*. Much was learned from studying morphological mutations in this organism, but efforts to disentangle the biochemical basis of these characteristics resulted only in frustration. Beadle and his microbial biochemist colleague Tatum turned their attention to studying the red bread mold *N. crassa* and soon obtained mutants with nutritional defects such as blocks in the biosynthesis of vitamins like pyridoxine and thiamine. This allows for rapid improvements to be made in genetic analysis, an approach that was subsequently extended by other workers using bacteria. The first fruits of such application came in 1943 when Luria and Delbrück showed by means of their "fluctuation test" that spontaneous mutations occurred in bacteria, to both phage resistance and streptomycin resistance at similar frequencies, as had been observed in other organisms.

The study of bacterial genetics was dramatically advanced during the 1940s following the recognition of antibiotic resistance in pathogenic bacteria. Here was a practical problem, the solution to which provided an obvious impetus to studies aimed at determining its cause. [*See* ANTIBIOTIC RESISTANCE.]

Bacteria did of course suffer from the serious methodological constraint of the apparent lack of any recombinational (sexual or crossing) mechanism by which to analyze and reconstitute gene combinations. They would prove, however, to be marvelous material for mutation studies (cf., e.g., Ames, 1975) once the concepts were clarified, for which a major turning point was the work of Luria and Delbrück (1943). In a fashion that reminds one of Gregor Mendel, they studied bacterial mutation by quantitative counts. They used resistance to (bacterio)phage as the marker. Like resistance to antibiotics, or growth on a nutritionally deprived medium, the phage is an environmental agent that makes it easy to count exceptional cells against a preponderant background that can be selectively wiped out. Most importantly, they distinguished between mutational events, which engender resistant clones, and mutant cells, which are counted when you plate a population with the selecting phage.

Luria (1984) in his charming book "A Slot Machine, A Broken Test Tube," recounts how his observation of a jackpot in a gambling den inspired his premonition of the skewed statistics that would govern the numbers of mutants. The fit of experimental numbers to those statistics is subject to great theoretical uncertainty, but they were a corroboration of the clonal model. One of the first articles on bacteria to be published in *Genetics*, the paper promptly attracted broad attention and was widely regarded as having proved "that bacteria have genes." The gist of the demonstration was that mutations to phage resistance agree with a clonal distribution and, thus, render more likely their "preadaptive" occurrence, that is, within the growth of the population rather than at the time of the challenge with the selective agent. It therefore harkens more to Darwin than to Mendel; nevertheless, it was a turning point in geneticists' appreciation of bacteria. The statistical methods, which are helpful in the quantitative estimation of mutation rate, have been improved (Sarkan, 1991).

The themes of nutrition and mutation among microbes had occasional false starts, with observations of strain variability and the "training" of exacting bacteria to dispense with growth factors (Knight, 1936). However, lacking a conceptual framework of "genes in bacteria," these had little fruit prior to the work of Beadle and Tatum (1941) on *Neurospora*. Beadle had begun his research program with Ephrussi on the genes for eye color in *Drosophila* (Burian, 1989). Tatum was engaged to do the biochemical work but found the material almost intractable—When he approached success, he was scooped by Butenandt on the identification of kynurenine as a pigment precursor. Nor was it clear how much closer to the primary gene product this chemistry would bring them. The following account is taken from J. Lederberg's memoir on E. L. Tatum, who was his teacher from 1946 to 1947 (Lederberg, 1990).

> This jarring experience, to have such painstaking work overtaken in so facile a fashion, impelled Beadle and Tatum to seek another organism more tractable than *Drosophila* for biochemical studies of gene action.
>
> In Winter Quarter 1941, Tatum offered a new graduate course in comparative biochem-

istry. In it, he called upon his postdoctorate experience with Kogl in Utrecht, in 1937, and recounting the nutrition of yeasts and fungi, some of which exhibited well-defined blocks in vitamin biosynthesis. Beadle, attending some of these lectures, recalled the elegant work on the segregation of morphological mutant factors in *Neurospora* that he had heard from B. O. Dodge in 1932. The conjunction was that *Neurospora* had an ideal life-cycle for genetic analysis with the immediate manifestation of segregating genes in the string of ascospores. *Neurospora* also proved to be readily cultured on a well defined medium, requiring only biotin as a supplement. By February 1941, the team was X-raying *Neurospora* and seeking mutants with specific biosynthetic defects, namely nutritional requirements for exogenous growth factors.

Harvesting nutritional mutants in microorganisms in those days was painstaking hand labor; it meant examining single-spore cultures isolated from irradiated parents, one by one, for their nutritional properties. No one could have predicted how many thousands of cultures would have to be tested to discover the first mutant: isolate #299 in fact required pyridoxine. Furthermore, the trait segregated in crosses according to simple Mendelian principles, which foretold that it could in due course be mapped onto a specific chromosome of the fungus. Therewith *Neurospora* moved to center stage as an object of genetic experimentation.

In their first paper, they remarked "that there must exist orders of directness of gene control ranging from one-to-one relations to relations of great complexity." The characteristics of mutations affecting metabolic steps spoke to a direct and simple role of genes in the control of enzymes. These were therefore hypothesized to be the primary products of genes. Indeed, insome cases, genes might themselves be enzymes. This was an assertion of what came to be labeled the one-gene : one-enzyme theory, which has become the canonical foundation of modern molecular genetics, albeit with substantial correction and elaboration of detail, especially with regard to the intermediating role of messenger RNA, which could hardly be thought of in 1941. It would be a mistake to focus too sharply on the numerical 1 : 1 assertion; more important was the general assumption of simplicity, and that the details of gene expression could be learned as an outcome of such studies—as indeed they were (see also Horowitz, 1990).

The recruitment of *Neurospora* for what have become classical genetic studies offered further encouragement that bacteria, albeit somewhat more primitive, might be handled in similar fashion. By 1944, Gray and Tatum had produced nutritional mutants in bacteria, including some in a strain that has dominated bacterial genetics ever since, namely *E. coli* strain K-12. These mutants were soon to be put to a most striking use.

In 1944, O. T. Avery and his colleagues concluded that the transforming principle involved in transformation in pneumococci was DNA. This was a major breakthrough, because until then it was thought that the significant part of the nucleoprotein of the chromosome molecule was the protein, the nucleic acid merely acting as a sort of binding agent. The role of DNA was initially puzzling, because it was difficult to see how a polymer that contained only four bases could possibly code for the complex phenotype of even the simplest of organisms. Meanwhile, classic genetic approaches were yielding a wealth of new discoveries. In 1945, Tatum showed that the mutant rate of bacteria could be increased using X-rays, whereas 2 yr later, Tatum and Lederberg demonstrated genetic recombination between two nutritionally defective strains of *E. coli K12*.

The first gene map of *E. coli* K12 appeared, and over the next few years progress was made in explaining the phenomena of conjugation, transduction, and transformation. William Hayes, working at the postgraduate medical school in Hammersmith announced in 1952 his discovery that in conjugation recombination occurred due to the one-way transfer of genetic material, and during the same year Lederberg and Cavalli coined the terms fertility plus (F^+) for donor cells and fertility minus (F^-) for recipient cells. The recognition of these mating types made it clear that conjugation was a primitive form of sexuality, with the recipient F^- cell being the zygote. More advances came when Lederberg, Cavalli, and Lederberg discovered high-frequency recombinant mutants from the F^+ type of *E. coli* K12, a finding that was subsequently confirmed by Hayes. These mutant strains (Hfr) differed from the wild-type F^+ strains, first in transferring various genetic

markers at a rate hundreds of times greater than the original strains and, second, in not producing an alteration in the mating type of the recipient cell. However, although the frequency of transfer of the various markers differed, it was the same for any given strain of Hfr.

Between 1955 and 1958, Jacob and Wollman used their famous "interrupted mating experiment" to determine the mechanism of gene transfer in *E. coli* K12. Jacob and Wollman coined the term episome, and in 1963 Cairns confirmed the circular nature of the bacterial chromosome using autoradiography. Bacterial genetics further progressed following the report published in 1961 by Watanabe explaining infectious drug resistance.

A. The Pneumococcus Transformation

What might be regarded as the first major breakthrough in microbial genetics came in 1928 when Griffith published on detailing "transformation" in pneumococci, a study that laid the foundation for later work by Avery and his colleagues. A further development in our understanding of transformation came in 1933, when Alloway showed that rough type 1 cells could be changed into genetically stable smooth type II cells, by growing them in the presence of a cell-free extract of a heat-killed broth culture of smooth type II cells. This work demonstrated the existence of a soluble "transforming agent." [*See* GENETIC TRANSFORMATION, MECHANISMS.]

Apart from cataclysmic happenings in global war, 1944 will also be remembered for the publication of "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types," by Avery, MacLeod, and McCarty.[1] The pneumococcus transformation was stumbled upon by Fred Griffith in London, in 1928, in the course of his studies on the serosystematics of pneumonia. Extracts of one serotype evidently could transform cells of another into the type of the first. In retrospect, it is hard to imagine any interpretation other than the transmission of a gene from one bacterial cell to another, but this interpretation was inevitably dimmed by the poor general understanding of bacterial genetics at that time.

This vagueness was compounded by two outstanding misinterpretations: (1) that the transmissible agent was the polysaccharide itself and (2) that the agent was a "specific mutagen." Concerning the first, it is sometimes overlooked that Griffith understood the distinction well enough. Better than many of his followers, he had at least the germ of a genetic theory: "By S substance I mean that specific protein structure of the virulent pneumococcus which enables it to manufacture a specific soluble carbohydrate." In regard to the second misinterpretation, Dobzhansky wrote that ". . . we are dealing with authentic cases of induction of specific mutations by specific treatments—a feat which geneticists have vainly tried to accomplish in higher organisms." This formally correct attribution, from a most influential source, obfuscates the idea that the agent is the genetic information. Muller had much greater clarity: In his 1946 Pilgrim Trust Lecture to the Royal Society, he remarked,

> . . . in the *Pneumococcus* case the extracted "transforming agent" may really have had its genetic proteins still tightly bound to the polymerized nucleic acid; that is, there were, in effect, still viable bacterial "chromosomes" or parts of chromosomes floating free in the medium used. These might, in my opinion, have penetrated the capsuleless bacteria and in part at least taken root there, perhaps after having undergone a kind of crossing over with the chromosomes of the host. In view of the transfer of only a part of the genetic material at a time, at least in the viruses, a method appears to be provided whereby the gene constitution of these forms can be analyzed, much as in the cross-breeding test on higher organisms. However, unlike what has so far been possible in higher organisms, viable chromosome threads could also be obtained from these lower forms for in vitro observation, chemical analysis, and determination of the genetic effects of treatment.

Other "classical" geneticists had virtually nothing to say about Griffith's work and would have judged themselves incompetent to assess its experimental validity. They began to pay closer attention

[1] It is awkward to have such a nondescript term as "transformation" applied to such an important, specific phenomenon. But when it was first discovered and named, there was no warrant to give it any narrower connotation. Avery had the power of new coinage but was hardly the likely personality.

after 1944, but again had little training in bacterial chemistry to enable them to form critical judgments about the claims presented them.

In Avery's world, however, Griffith was a central figure and his observations could not be ignored. His basic observations were confirmed in Avery's laboratory (see Dubos, 1986), and in due course Avery felt compelled to pursue the chemical extraction and identification of the substance responsible for the transformation. Sixteen years after Griffith, this was achieved, and DNA was thrust into the scientific consciousness as the substance of the gene.

In retrospect, it is difficult to give proper credit to the logical validity of a large range of alternative interpretations and to reconstruct the confusions about what was meant by "gene" and "genetic." Recall that until 1951 the only marker observed in transformation was the capsular polysaccharide, the biosynthesis of which was itself subject to many conjectures [e.g., about the role of starter fragments in self-assembly (discussed by Lederberg, 1956)]. Avery undoubtedly somewhat intimidated by Dobzhansky's authority, was reluctant to put his speculations about the genetic significance of transformation in print; his famous letter to his brother Roy surfaced only years later. There, but not in the paper, he remarks that the . . . [transforming substance is] thereafter reduplicated in the daughter cells and after innumerable transfers [it] can be recovered far in excess of the amount originally used. . . . Sounds like a virus—may be a gene. But with mechanisms I am not now concerned—One step at a time—and the first is, what is the chemical nature of the transforming principle? Someone else can work out the rest (quoted *in* Dubos, 1976). As late as 1948, so distinguished a geneticist as G. W. Beadle still referred to the phenomenon as a "first success in transmuting genes in predetermined ways" (note transmuting, not transmitting!). This obscuration of the pneumococcus transformation became less troublesome with the overall development of bacterial genetics.

Indeed, the controversy raged on the chemical claim that the substance was DNA (and nothing else!). [This story is detailed by Judson (1979) and in McCarty's personal memoir (1987).] Alfred Mirsky, Avery's colleague at the Rockefeller Institute, was a vocal critic of the chemical identification of the transforming agent. Some believe he was quite persuaded that this was an instance of gene transfer, but the more reluctant to concede that the evidence to date settled so important a question as the chemical identity of the gene as pure DNA (versus a complex nucleoprotein). Avery himself had cause to worry—There had been much resistance to his earlier proofs that pneumococcal polysaccharides, free of protein, were immunogenic. Wendell Stanley's first claims that crystalline tobacco mosaic virus was pure protein had to be subject to humiliating correction when ribonucleic acid was also found therein. We should recall that when most biologists of that era used terms such as protein, nucleic acid, or nucleoprotein, it can hardly be assumed that they had today's crisp connotations of defined chemical structure. These issues could only be settled by the few experts who had worked with these materials experimentally—and it was a daunting task to prove that there were too few molecules of any contaminating protein in the "DNA" to account for its genetic specificity. Maclyn McCarty's meticulous work continued to provide ever more persuasive evidence that it was DNA, and the contemporaneous studies of Chargaff showed that DNA was far more complex than Levene had figured it to be and, therefore, capable of the subtlety demanded of a "gene." Rigorous proof about "DNA alone" was really not furnished prior to the production of genetically active synthetic DNA three decades later. By 1952, Hershey and Chase gave evidence from an independent quarter that DNA alone penetrated the phage-infected cell. In the following year, the structural models of DNA as a double helix (Watson and Crick, 1953) lent final plausibility to "DNA alone."

This episode is sometimes painted as unreasonable resistance to a new idea (Stent, 1972). This is hardly a fair assessment of a controversy that was settled within 9 years and that required the emergence of a new class of workers, and conversion of some of the old ones, to deal with new techniques and experimental materials. That controversy continued is appropriate to the spirit of scientific skepticism—more to worry about when challenging new ideas are merely ignored.

All these discoveries, taken together, gave substance to Luria's vision of the virus as a genetic element that is coordinated with the genome of the host, but with pathogenetic consequence that has evolved to suit the needs of the parasite. The host may also co-evolve to reach an equilibrium compatible with the survival of both partners—a general principle in the evolution of pathogenicity (Th. Smith, 1934).

Prospects of cytoplasmic heredity fascinated many workers, even during the working out of the nuclear (Mendelian) basis of microbial biology, perhaps as a carryover of Huxley's idea of the persistent soma. In the course of the discussion, there were angry ripostes as to whether a given entity was really a plasmagene, or perhaps a virus, or perhaps a symbiont. The term and concept "plasmid" was introduced (in 1952) to stress the operational vacuity of those distinctions. A particle could be at the same time a virus (if one focuses on pathology), or symboint, or plasmagene (if one focuses on the genetic role). As a prophage, it may even be integrated into the chromosome, with a potential reappearance later. And it would be impossible to say whether a virus had evolved its pathogenicity, having once been a benign organelle, or vice versa, or both at different evolutionary epochs. One might even revive Altmann's old picture of the mitochondria as originally symbiotic bacteria, an allusion founded merely on the limitations of cytological analysis.

The vicennium worked a transformation—the "biologization" of the microbe. It was an extraordinarily exciting and fertile time, with new phenomena to be found in every culture dish. One could even learn to treasure one's contaminations.

IX. Viruses and Lysogeny: The Plasmid Concept

A. Biology of the Virus

The cardinal discovery for virology was the isolation and crystallization of the tobacco mosaic virus (Stanley, 1935), which sharpened many questions about this boundary of living existence (Pirie, 1937). A more convenient system for virus biology proved, however, to be the viruses attacking bacterial hosts, the (bacterio)phages, especially in the hands of the Delbrück school (Adams, 1959). Their life cycle was worked out in some detail, eventually culminating in two cardinal experiments:

Hershey and Chase (1952): The DNA of the attacking phage particle is sufficient to initiate infection. The DNA (not the entire phage) replicates in the host bacterium and then generates the capsid and assembles itself into mature, infectious phage particles.

Hershey (1946): Different phage genomes can undergo genetic recombination, enabling the construction of linkage maps. These would eventually be constructed in ultimate detail, matching the DNA sequence of the nucleotides.

Viruses were defined by Luria (1953, p.) as "submicroscopic entities, capable of being introduced into specific living cells and of reproducing inside such cells only." He pointed out that this is a methodological rather than taxonomic criterion; such a definition might well embrace a wide range of diverse entities. By 1950, he insisted that the phages exhibited "parasitism at the genetic level," taking over the metabolic direction of the host cell and exploiting a wide repertoire of its genetic capabilities. Whether or not other viruses, in plant and animal cells, would share these attributes remained to be seen (Luria, 1953; Adams, 1959; Hayes, 1964; Galpern, 1988; Burnet, 1945).

B. Lysogeny

Not long after the Twort-d'Herelle discovery of the bacteriophages (1915–1917), bacterial cultures were found that appeared to have established a durable symbiosis with a resident phage. The Delbruck school tended to dismiss these as contaminants, despite persuasive arguments of Burnet and Lush (1936). Lwoff and Gutmann (1950) reentered the controversy and showed that lysogenic *Bacilli* carried a "prophage," a genetic capability of producing the phage. At the same time, Lederberg and Lederberg (1951, 1953) had discovered that *E. coli* K-12 was lysogenic, for a phage they named "lambda," as a parallel (or so they thought) for the kappa particles in *Paramecium*. Crosses of lysogenic with sensitive strains, however, showed that the capacity to produce lambda segregated in close linkage with a chromosomal marker (gal); therefore, they invoked Lwoff's concept and terminology of prophage. However, the working out of that story, and of the phenomena of phage-mediate transduction, belongs to the next era.

X. Virology

The term virus was originally an unspecific term coined by Pasteur to mean any living organism that caused disease. This terminology was used well into

the 1930s; thus, the word antivirus was used by Besredka to refer to bacterial filtrates that could apparently cure infections.

The realization that disease could be transmitted by inoculation of cell-free lesions from plant and animal infections led to the introduction of the concept of "filterable virus." Iwanowski's discovery in 1892 of tobacco mosaic disease in plants is usually credited as the first demonstration that a filterable virus could cause disease. Then in 1898, Loeffler and Frosch showed that a filterable virus was apparently the cause of foot and mouth disease. In the same year, S. M. Chapman introduced the use of fertile hens eggs as a means of cultivating viruses. This approach was later to be used by Pyton Rous in his work on the fowl sarcoma that bears his name. By 1915, a new class of virus affecting bacteria but neither plants nor animals, was discovered by F. W. Twort. His observations were extended in 1917 by D. Herrelle, who over the next 13 years published a series of papers on what was initially called the Twort–Herelle phenomenon, but which later became known as bacteriophage.

The development first of the ultraviolet microscope and then of tissue culture techniques in the 1920s added impetus to research on virus structure and cultivation. Maitland's work in 1928 was a major advance in tissue culture techniques, but because of the tedious nature and lack of antibiotics to control bacterial contaminants they were not widely adopted.

By 1931, the potential of the fertile egg for culturing viruses was finally appreciated in the work first of Goodpasture and then of the Australian Macfarlane Burnet. Burnet used this approach to culture the influenza virus, which previously had to be grown in ferrets.

John Enders did much to develop the art of culturing viruses, which finally enabled the development of a range of vaccines. Enders' outstanding contribution to the study of viruses began with his work on mumps when he showed that a virus could be grown in chick embryos and, after successive generations, would loose its ability to cause the disease while retaining the capacity to immunize against it. In this way, the modified virus could be used to prepare vaccines to control the disease. Prior to 1949, for example, the poliomyelitis virus could only be propagated in monkeys. Enders showed that the virus could be grown in culture of nonnervous tissues, and by using this technique Salk developed his famous vaccine, which essentially defeated infantile paralysis. The application of Enders' tissue culture techniques led to the isolation of many other viruses: in 1954, the year when he received the Nobel Prize, Enders himself, for example, succeeded in isolating the measles virus.

The introduction of the electron microscope in 1934 proved a great asset to research on viruses. In 1956, Watson and Crick proposed on theoretical grounds that virus particles must be made up of a nucleic acid core and a surrounding shell comprised of protein subunits, a structure later seen in 1959 under the electron microscope by Horne and Nagington.

Antibiotics aided virus research, allowing for contamination-free studies, so that by 1949, poliomyelitis virus could be grown on nonneural tissues such as minced monkey kidney.

In 1952, the name of the patient Helen Lane became cryptically immortalized when Gay and his colleagues established the famous continuous cell line of HeLa cells, derived from a carcinoma of the patient's cervix uterus. Then, in the following year, Scherer succeeded in growing poliomyelitis virus in these cells.

In 1954, Younger published his technique for growing trypsinized cells in monolayers on glass. This allowed viral infection of cells to be recognized by detecting the cytopathic effect, which allowed for the routine screening for the presence of viruses.

XI. Mycology and Protozoology, Microbiology's Cinderellas

Filamentous fungi and protozoa (i.e., molds and animacules) were observed soon after the earliest microscopes were developed. Studies of these organisms continued largely unnoticed as bacteriology developed. The fact that neither of these groups of microorganisms cause major diseases in the developed world tended to hinder the rapid development of both mycology and protozoology. The principle motivation for studying fungi came from their ability to infect important crop plants. This resulted in a close association between mycology and botany, with the unfortunate result that many microbiologists in the past, as today, regard fungi as lying outside the orbit of their subject.

As early as 1767, Torgioni-Tozetti advanced the view that rust diseases of cereals are caused by microscopic fungi, but experimental proof of the role

of fungi as phytopathogens had to await the monograph by Prevost, who in 1807 described experimental smut infections. Prevost also showed that fungal infections could be prevented by soaking seeds in a solution of copper sulfate and thus, he inadvertently became the originator of the pesticide industry. It was Anton de Bary, however, who did the most to develop the science of plant pathology.

During the early part of this century, attention was also focused on the role that fungi play in soil fertility. It soon became evident that while fungi are not as metabolically diverse as soil bacteria, they nevertheless play an important role, principally as agents of decay of organic forms of carbon and nitrogen, in the degradation of leaf litter and humus. Waksman and his colleagues were particularly active in demonstrating the role played by fungi in soils.

Waksman was also one of the first microbiologists to appreciate the industrial importance of molds, he and his group investigating the production of butyric acid and butyl alcohol from starch-rich materials, and then, in 1930, examined lactic acid production by species of *Rhizopus*. The foundation for the development of studies on mold metabolism was laid by Raistrick and his numerous collaborators working at the London School of Hygiene and Tropical Medicine during the 1920s.

Ringworm was the first human disease to be shown to be caused by fungi: described in 1839 by Schoenlein, it was soon followed by the recognition by the Swede F. T. Berg that *Candida albicans* was the causal agent of thrush. Medical mycology was slow to develop, however, and it was not until 1910 tha Sabouraud introduced a medium suitable for the isolation and growth of pathogenic fungi. Systemic mycoses were discovered at the turn of this century, while it was as late as 1934 before Monbreun conclusively demonstrated that histoplasmosis is caused by *Histoplasma capsulatum*. Medical mycology has tended to lag behind other aspects of medical microbiology, although the importance of fungal infections such as pneumocystis pneumonia and candidiasis in the AIDS syndrome is likely to accelerate developments in this area of the subject.

The development of protozoology as a science is almost exclusively devoted to the role of protozoa as agents of disease. Although initially referred to as animacules, by 1764 Wrisberg had introduced the term infusoria, while the first generic name for a protozoan, *Paramecium*, was introduced by Hill in 1752. The term protozoa was first used by the German Goldfuss in 1817. By 1836, Alfred Donne working in Paris had shown that a flagellate was responsible for vaginal discharge in women. It was, however, the colonial expansion of the European powers that provided the stimulus to studies in medical protozoology. The first observations of parasites in the blood of malaria sufferers was made in 1880 by Alphonse Laveran. A long list of diseases were then shown to be caused by protozoa including Texas cattle fever in 1893, Malta fever in 1895, malaria in 1898, sleeping sickness in 1902.

Protozoa have yet to be widely used in industrial microbiology and biotechnology, and their role in the environment has been subject to only limited study; therefore, the history of the development of protozoology in these areas will have to await future developments.

XII. The Modern Period

What then of the landmarks of the recent history of science? Without a doubt, the most obvious development in our science that has taken place since the last war has been the rise in the status of a single organism, the colon bacterium *E. coli*. Using this single organism, scientists such as Niremberg, Holley, Jacob, and Monod have revolutionized our thinking on biology. One practical outcome of this work was the development of an *E. coli* strain by W. Gilbert and others in 1978 that produces human insulin.

A perusal of the list of awards for the Nobel Prize for research in microbiology in the widest sense shows that since 1958 particular recognition has been given to work on genetics, virology, and immunology. Knowledge derived from such studies have had a profound effect on our understanding of the life process, and recent developments in biotechnology have provided real benefits in our lives. [*See* BIOTECHNOLOGY INDUSTRY: A PERSPECTIVE.]

The key technique that has made genetic engineering possible was devised by Herbert Boyer and Stanley Cohen. Boyer working at the University of California collaborated with Cohen of Stanford University to develop a method of splicing genes from a donor into a recipient bacterium. In 1973, they took a gene from the plasmid of one organism and spliced it into a plasmid from another to produce recombinant DNA. When inserted into a recipient bacterium, the foreign genes not only survived but also

affected the host in the way it had affected the donor, and was also copied as the cell divides. Boyer later used this approach to insert genes from human proteins into bacteria, and, thus, heralded a biotechnological revolution. A similar revolution was initiated by the production of monoclonal antibodies by Kohler and Milstein (Interestingly, what might be termed "natural monoclonal antibodies" had been observed a few years earlier by Joseph Sinkovicks.)

A microbiologist who left science even as late as the mid-1970s to follow other pursuits would now hardly recognize his or her former subject. Studies on the genetics and molecular biology of microorganisms have made particularly rapid progress in the intervening years. We have also seen major improvements in the way we apply microorganisms in biotechnology and, more recently, to address environmental problems. The appearance of AIDS has once and for all shattered our cozy belief that we had all but conquered infectious disease. HIV will undoubtedly not be the last new infectious agent to confront us in the future; if for no other reason than to combat such infections, our science will need to continue to develop at the rapid rate seen in the past few decades.

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