DISCOVERY and EXPLORATION

of the MICROBIAL UNIVERSE:

1665 to "MODERN TIMES"

by HOWARD GEST

Distinguished Professor Emeritus
of Microbiology
Adjunct Professor, History and
Philosophy of Science

Indiana University, Bloomington, IN 47405

SEQUENCE OF TOPICS

Introduction

Why study the history of scientific discovery?

An orienting timeline

Fresh views of discoveries by Robert Hooke and Antoni van Leeuwenhoek

Metchnikoff, Elie (1845-1916)

Yersin, Alexandre (1863-1943)

Avery, Oswald (1887-1955), MacLeod, Colin (1909-1972), McCarty, Maclyn (1911-2005)

Fleming, Alexander (1881-1955), Florey, Howard (1898-1968), Chain, Ernst (1906-1979), Heatley, Norman (1911-2004)

Robert Hooke, as viewed by J. Desmond Bernal

Bassi, Agostino (1733-1856); the doctrine of pathogenic microbes

Pasteur, Louis (1822-1895), Koch, Robert (1843-1910), Jenner, Edward (1749-1823)

Molish, Hans (1856-1937) Photosynthetic bacteria

Cohn, Ferdinand (1828-1898) Driving force of "modern" microbiology

Winogradsky, Sergei (1856-1953) Pioneer microbial ecologist

INTRODUCTION

These "Historical Adventures" focus on microbiologists, biochemists and others who have contributed significantly to advancing understanding of microbial life and its basic principles (biochemical, biophysical etc.), many during my active years in research. A major aim is to inform and educate students and young scientists who are so overwhelmed by the pressures of modern scientific life that they have little time, if any, to read the extensive history of how microbiology and biochemistry developed to its present state of sophistication. Current textbooks have become encyclopedias of facts, with little space devoted to history; 1,000 page texts typically have only about 15 pages devoted to the major discoveries of the past century. I hope that readers will become convinced that these fields have a long way to go before we will really understand microbial life in comprehensive detail. . Most of our predecessors certainly felt that *they* were at the "cutting edge" even though we now can clearly see that they were discovering only glimpses of complex phenomena.

A view of science in 1923

The dedication of the classic book on thermodynamics by Lewis and Randall (1923) notes: "The fascination of a growing science lies in the work of the pioneers at the very borderland of the unknown, but to reach this frontier one must pass over well traveled roads; of these one of the safest and surest is the broad highway of themodynamics." There are, of course, a number of highways. For biology, biological chemistry is certainly a

particularly major thoroughfare. In turn, history has amply demonstrated that the advance of biochemistry was especially facilitated by the study of microbial cells.

I have always been fascinated by the inspired Preface of Lewis and Randall's book, which begins:

"There are ancient cathedrals which, apart from their consecrated purpose, inspire solemnity and awe. Even the curious visitor speaks of serious things, with hushed voice, and as each whisper reverberates through the vaulted nave, the returning echo seems to bear a message of mystery. The labor of generations of architects and artisans has been forgotten, the scaffolding erected for their toil has long since been removed, their mistakes have been erased, or have become hidden by the dust of centuries. Seeing only the perfection of the completed whole, we are impressed as by some superhuman agency. But sometimes we enter such an edifice that is still under construction; then the sound of hammers, the reek of tobacco, the trivial jests bandied from workman to workman, enable us to realize that these great structures are but the result of giving to ordinary human effort a direction and a purpose. Science has its cathedrals, built by the efforts of a few architects and many workers."

The foregoing is a truly remarkable perspective of how science advances, but I would take issue with implications of the last sentence, at least in respect to biology.

"Workers and architects"

During the eighty four years after the publication of Lewis and Randall's book, the distinction between "architects" and "workers" in microbiology and biochemistry became a "gray" zone in which certain "workers" who had deeply ingrained curiosity coupled with intense drive opened doors to explanation of previously obscure complex phenomena. Frequently, they were not "architects" in the usual sense, but rather keen observers who could capitalize on a serendipic observation or had a sudden insight into a puzzling set of facts. As science marches on, several kinds of scientists make significant contributions. Some tend to explore a variety of problems within a particular area. More rarely, some pursue one basic problem with great persistence for many years or decades. When the latter types are successful, they may achieve "architect-like" status and are celebrated....at least for a while.

Recognition of "architects"

It may seem curious that even "architects" are soon forgotten. Clearly, this has several causes. Of particular importance: the explosion in the number of scientists during the past fifty years, and the attendant escalation of media-driven publicity. In the Introduction to his Historical Essays, Fruton (1972) notes: "In these essays, the work of some famous men will be given special attention because of their influence on the interplay of chemistry and biology, but there are also many men of lesser renown whose findings were important in their time; can we say that the latter deserve lesser attention as individuals? To accord each of these people a brief "potted" biography would hardly do justice to any of them, and would

increase inordinately the size of this book. We must therefore leave to others the task of remedying the biographical deficiencies of these essays." Fruton's "Index of Names" lists about 2,000! As far as I can tell, no one has taken up his challenge.

Some comments on "molecular biology

Many major advances in biochemistry and "molecular biology" were based on studies with microorganisms. Thus, the distinctions between these fields have become blurry. A "working group" headed by John Kendrew (Nobel Laureate 1962) came to the following conclusion in 1968: "The fact is that the fashionable term 'molecular biology' is unfortunate, on several grounds. Much of the research commonly held to be within this field (e.g., into the mechanism of protein synthesis and of DNA replication) is actually quite inseparable from biochemistry; in consequence, the term is resented by many biochemists who feel that in the eyes of the world they have no part in currently fashionable fields which in reality are their own territory, and which in a sense they were the first to explore." [quoted in Fruton 1972].

Some relevant remarks of Sydney Brenner on molecular biology in 1988 follow. They were made in a "relaxed" lengthy interview published in 1988 (Wolpert and Richards):

"Question: "Was molecular biology a particularly competitive subject in the early days, and is it now?"

Answer: "Well, I think molecular biology now is viciously competitive and that's because it's enormous. You can judge a field by looking to see how many people simultaneously discover the same thing. I can show you cases

where the hit rate is three or four. Three or four groups discover the same things, and they produce papers, which are almost identical. They haven't copied from each other but you know they're all looking at the same piece of nature with the same techniques. And that's a sign of an overpopulated science. Of course a sign of an underpopulated science is one where nothing is ever confirmed by anybody else and you don't know whether it's true or not....Also, the literature is now enormous and you can only follow it by living in a sort of cooperative, a reading cooperative. Or, as they are now, xeroxing cooperatives. Because hardly anybody reads anymore—people only xerox things. I once asked a student who had a big xerox bill whether he'd neuroxing some papers. So he asked me what that was. I said 'It's a very easy and cheap process. You hold the page in front of your eyes and you let it go through there into the brain. It's much better than xeroxing.'....The amount of information is enormous. People have reached a specialization that is unbelievable. I have people in this laboratory who can't understand each other." The unique book by Wolpert and Richards (1988)was based on interviews for BBC Radio 3, and there is much to be learned from it.

Creative and hum-drum research

Important advances in science are usually not made in the course of "routine" kinds of research. At present, there is a great abundance of what I perceive as hum-drum research. The creative advances typically result from dedicated work of researchers who have a passion, very strong curiosity, and the willingness to make sustained efforts to solve problems. For the most part, their names are unknown to the general public. In recent years, with the

explosive accumulation of scientific facts, historical accounts made available to students of various kinds are usually minimal. Scientists who achieved great fame 50-100 years ago were, important explorers at the periphery of a vast unknown territory of biological sciences. Their work set the stage for expeditions into the jungles of microbiology, biochemistry, genetics etc. that gradually revealed details of the complex mechanisms that control cell growth and reproduction. As the details emerged, scientists were, and still are, faced with ever-increasing complexity of cellular mechanisms. As biological sciences progressed after the end of World War Two, it became apparent that microorganisms of various kinds are particularly good experimental systems for analyzing basic details of cellular growth processes. The passions of many biochemists focused on microbial cells, and this inevitably led to "molecular biology." The borders of the earlier jungles became indistinct and new analytical tools were profitably used by scientists who were once labeled "botanists," "zoologists" etc.

Max Perutz examines how important discoveries are made

Max Perutz was awarded the Nobel Prize in Chemistry in 1962 for pioneering work on the structure of hemoglobin and other macromolecules. He published two noteworthy books on "Science, Scientists, and Humanity" (see References). He was particularly interested in what quality the best scientists, shared with great writers, musicians and artists. To Perutz, it was *creativity.* Where does that come from? He comments on creative scientists and artists (Perutz 1989):

"They both tend to be single-mindedly devoted to their work. Renoir painted every day of his life, and when old age had made his fingers too arthritic to hold a brush, he got someone to tie the brush to his hand. Haydn rose early each morning to compose; if ideas failed him, he clasped his rosary and prayed until Heaven sent him fresh inspiration. Tolstoy rewrote *War and Peace* seven times. When Newton was asked how he had arrived at his insights, he answered 'By keeping the problem constantly before my mind.'

There is little benefit in following scientists' daily grind but much in tracing the unique combinations of theoretical knowledge and manual skills, the web of personal encounters and accidental observations, the experience, temperament, moods and clashes that go into the making of discoveries, even though the crucial leap of the mind is often impenetrable, There is also something to be said for finding out why others, seemingly just as able, were too blind to grasp what Nature tried to tell them."

The 21th Century; pioneering scientists become "one-liners" or disappear from the literature

In the 21st century many investigators whose research led to significant unraveling of cellular mechanisms and/or important changes in research directions became "one-liners" in text book tables….a total of one line for a name plus 5 or 6 words for major accomplishment. Time-lines are useful and informative, but without additional information they tell us nothing about the human element---the dynamics and excitement of the research, and associated circumstances in world history.

For those who believe that the recent successes of molecular biology (genomics, proteomics, and other "omics) present an immediate gateway to understanding the comparatively few remaining mysteries of life, a note of caution from A. Cornish-Bowden [Putting the systems back into systems biology. *Persp. Biol. Med.* 49, 475-489, 2006]: "Returning to genome sequences, the problem is not so much that they contain no phenotypic information, but that we do not have reliable methods for undertaking all of the steps involved in deducing a phenotype from them.... Present methods of sequence analysis allow a genome sequence to be converted into a list of genes without much difficulty, but transforming the list of genes into a list of enzymes has a high failure rate, perhaps 50%. In any case, however, this is only the beginning of the process. A list of putative gene products, or even a list of putative enzymes, is not a phenotype, and converting it into a phenotype requires construction of a plausible metabolic map, which then needs further work to convert it into a possible phenotype. Finally, the possible phenotype can only become a real phenotype when all relevant kinetic and regulatory properties are taken into account, together with information about how all the components are organized into a three-dimensional whole—even a four dimensional whole, given that the times when different components are made may be just as important as where they are placed."

A similar caution is expressed in a recent book review by M. Pigliucci ("Postgenomic musings"; Science 317: 1172-1173, 2007), who says: "As much as genes are fundamental to the evolutionary process, there is much more to biology than genes and their dynamics. The very

fact that molecular biologists are now talking (albeit often naively) about higher-level "-omics," all the way to phenomics means that they appreciate that genomes are only a part of the story, arguably the simplest part to figure out."

During the past decade, some molecular biologists (and others) claim that a myriad of short nucleic acid "signatures" found in natural samples indicate that there must be hundreds of thousands (or millions!) of still unknown species of bacteria. Since there are only about 6000-7000 "type strains" in collections and the definition of a bacterial species is still debatable, I believe such claims are myths. The burden of proof rests with "molecular taxonomists", who will have to isolate living organisms to support their notions. Those interested in the origin of microbial diversity should keep in mind Ernst Mayr's view that evolution is a matter of phenotype, not genotype [E. Mayr: *What Evolution Is.* Basic Books, 2001].

Why study the history of scientific discovery?

The answer should be obvious, but is hardly evident from current major textbooks in microbiology/biochemistry. This state of affairs was addressed in 1990, by the eminent microbiologist Joshua Lederberg (who was then President of Rockefeller University) in an introduction to *The Excitement and Fascination of Science: Reflections by Eminent Scientists*, Vol. 3, Part 1 (Ann. Reviews, Palo Alto, CA; 1990). Following are quotations, selected from his eloquent discussion. First, is a footnote about autobiographies of scientists.

> "Literary geniuses have often expressed themselves in autobiography, but we do not often find such practiced expository skill among scientists; and the problem of rapport with a broad readership on arcane subject matter is an additional grave hindrance. The knack of simplification is a gift. This truth and the fact that simplifications must distort complex knowledge have deterred most scientists of genius from autobiography.

> "....Accounts of the lives of scientists have enjoyed only a limited vogue in recent decades, both within the profession and in popular culture. Thus "what one does," adduced to justify one's findings, comprises the primary scientific literature, while "who one is" is omitted as a potential contaminant of objective scientific judgment. In science the personal life has been considered far less relevant to the

search for truth than in more self-expressive fields such as literature and the arts. Hence tradition in scientific writing has discouraged use of personal pronouns and other manifestations of self.

"....While the scientist's restraint from self-description may have helped to preserve the purity of the logic of justification, the indispensable critical function in science, it has also deprived us of insight into the personal and social processes that motivate discovery and pervade the scientific effort. We are left with narratives of chase, competition, and interpersonal stress rather than accounts of imagination gratified and cooperation achieved. Today's youngsters contemplating scientific careers indeed deserve more life-sized and sophisticated portraits of their role models than my generation had in de Kruif's *Microbe Hunters* (1926)—but also truer portraits than the melodrama that now makes the bestseller lists and electronic media.

"....*The Sociology of Science*

Missing from most primary literature in science are all but the faintest clues about the social context of discovery—how the scientific community is shaped by its operating norms and institutions, as well as by its fraternal and intergenerational networks. The proliferation of multiple authorship does suggest imperatives of collaboration, especially as the technology of experimentation becomes more specialized; and appended acknowledgments of the funding of ever more costly instruments give some hint of the dependence of science on

the larger community. Likewise, the application of science to the search for solutions to many of humankind's gravest problems manifests the institution's social aspect.

Biography depicts directly the personal relationships among scientists, their mutual debts, their etiquettes, sometimes their jealousies and transgressions. Rarely among our pages, however, do we find signs of a competition as intense as that attributed by Watson to the race for 'The Double Helix.' Perhaps the stakes of that race are matched only a few times in a century, so that such a chase engenders a ferocity foreign to even the highest accomplishments of less notoriety. For the most part our authors have not attained, nor did they seek, the degree of public attention that warrants full-length biography. Their personalities, though less flamboyant that those celebrated in the daily headlines, are far more typical of practicing scientists.

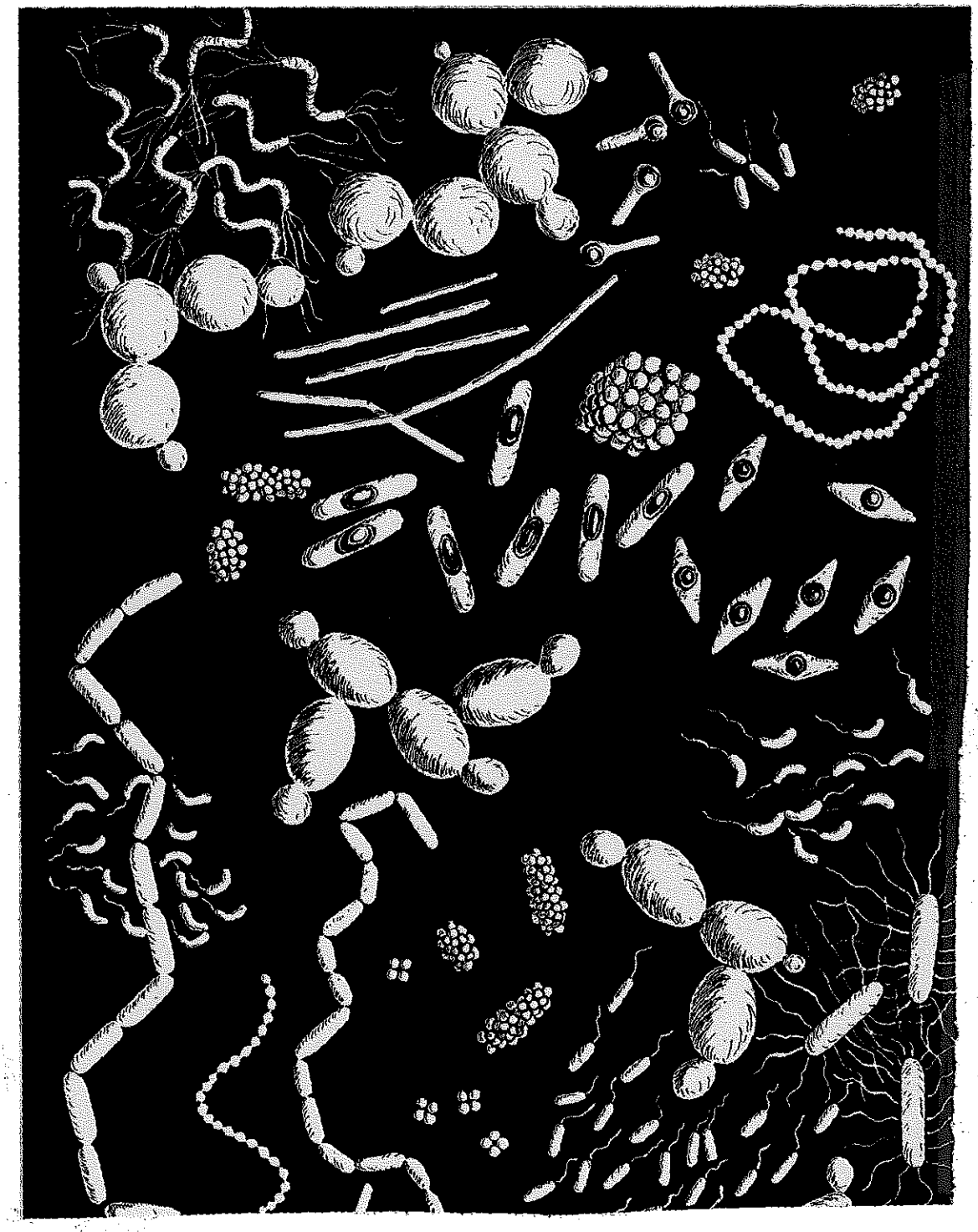
Enmeshed in society, scientists may also find themselves with extra-scientific responsibilities and roles, though each of these is grounded in the fundamental one of discovering and telling the truth.

History

"No contemporary scientist has worked and thought in a vacuum; the presentation and solution of problems are part of a history of ideas. The greatest discontinuities pose the greatest challenge to understanding. Why are some ideas so "premature" as to meet fatal resistance when first published? One thinks of Gregor Mendel, whose

far-reaching experiments were ignored during his lifetime, as an uncontroversial example.

Because the scientific method in practical use is so complex, the course of science is subject to numerous noncognitive, social influences. We know little, for example, about what informs the creative imagination."



An Orienting Timeline

Raymond Beck's *"A Chronology of Microbiology/In Historical Context"* (ASM Press, 2000) is a valuable source of information for the history of microbiology/biochemistry. A good example is given by the entries for 1956. I selected 7 of 19 entries to illustrate the flavor of the timeline (some are shortened):

D. Bacterial Physiology: *β-Galactoside Permease*

Howard V. Rickenberg, Georges N. Cohen, Gerard Buttin, and Jacque Monod, after investigating the uptake of thio-β-D-galactoside, discover a stereospecific permeation system for □-galactosides in *Escherichia coli.* They apply the genera name "permease" to such systems.

F. Bacterial Genetics: *Circular Chromosome*

After analyzing data from interrupted mating experiments, Elie Wollman, Francois Jacob, and William Hayes conclude that the chromosome of *Escherichia coli* is circular.

H. Bacterial Genetics: *Specialized Transduction*

M. L. Morse, Joshua Lederberg, and Esther Lederberg discover that bacteriophage lambda in *Escherichia coli* K12 transfers only the genes associated with galactose metabolism.

M. Molecular Biology: *Messenger RNA*

Elliot Volkin and Lazarus Astrachan, in studies of T-phage infection of *Escherichia coli*, find an unusual type of RNA that follows the base composition of the infecting phage. Although they determine that it is not a

precursor of the phage DNA, they are unable to assign a function to it. These observations contribute to the development of the concept of messenger RNA (mRNA).

O. Molecular Biology: *DNA Polymerase*

Arthur Kornberg and his coworkers partially purify an enzyme from *Escherichia coli* that polymerizes triphosphate to form a DNA polymer. The enzyme is subsequently named "DNA polymerase" (later called DNA polymerase I). Kornberg and Severo Ochoa share the 1959 Nobel Prize in physiology or medicine.

R. Society and Politics

Egypt announces that it is assuming control over the Suez Canal, instigating an international crisis involving Israel, France, England, and the United Nations, Israel invades the Gaza Strip and the Sinai Peninsula, while Britain and France seize control of the Mediterranean end of the canal. A United Nations Emergency Force is assembled to assist in ending the conflict, and the invading troops withdraw.

S. The Arts: *Music*

The musical version of George Bernard Shaw's play *Pygmalion* appears as *My Fair Lady*, with music by Frederick Loewe and lyrics by Alan Jay Lerner.

A small sampling of entries from Beck's Chronology (many are in short form)

1862: **Microbiology:** *Thermophiles*

Ferdinand Cohn reports that different species of algae and blue-green algae (cyanobacteria) grow at different temperatures in hot springs.

1882: **Bacterial Disease:** *Germ Theory of Disease/Koch's Postulates*

Robert Koch isolates *Mycobacterium tuberculosis*, the bacillus that causes tuberculosis, and publishes perhaps his best paper, establishing that a specific bacterium causes a specific disease. In this paper, and in 1884, he states concepts that become known as Koch's postulates, but which are in fact best described by Friedrich Löffler in 1883. In 1905, Koch receives the Nobel Prize in physiology or medicine for his work on tuberculosis.

1885: **Bacterial Species:** ***Escherichia coli* and *Enterobacter aerogenes***

Theodore Escherich describes two bacteria isolated from the feces of infants and names them *Bacterium coli commune* and *Bacterium lactis aerogenes*, the latter organism causing the clotting of milk more actively than the former. In 1919, Aldo Castellani and Albert Chambers change the name of *Bacterium coli* to *Escherichia coli.*

1887: **Bacterial Physiology:** *Chemoautotrophs*

Sergei Winogradsky publishes a study of the sulfur bacteria that includes a key to their classification. He includes such genera as *Beggiatoa* (created by V. Trevisan in 1842) and *Chromatium* (named by Maximilian Perry in 1852), later recognized to be photosynthetic. Winogradsky formulates the concept of chemoautotrophic life (chemolithotrophy) to describe organisms that obtain metabolic energy through the oxidation of inorganic substances such as ammonia and hydrogen sulfide.

1891: **Bacterial physiology:** *Nitrifying Bacteria*

Sergei Winogradsky isolates nitrifying bacteria by growing them in media free of organic matter and solidified with silica gel instead of agar. His discovery that carbon dioxide is the source of carbon and that energy is derived from the oxidation of ammonia by *Nitrosomonas* and the oxidation of nitrite by *Nitrobacter* proves that they are autotrophs.

1894: **Bacterial Physiology:** *Sulfate-reducing Bacteria*

While investigating the accumulation of calcium sulfate in the steam boilers in a yeast factory, Martinus Beijerinck isolates a sulfate-reducing bacterium that he names *Spirillum desulfuricans*. The name is changed to *Desulfovibrio desulfuricans* in 1936 by Albert Jan Kluyver and Cornelis B. van Niel.

1895: **Bacterial Physiology:** *Nitrogen Fixation*

Sergei Winogradsky is the first to isolate a bacterium capable of fixing nitrogen in culture. By repeated subculturing from soil cultures, he isolates an anaerobic sporeformer that he names *Clostridium pastorianum*, later changed to *Clostridium pasteurianum*.

1901: **Bacterial Physiology:** *Nonsymbiotic Nitrogen Fixation*

Martinus Beijerinck isolates aerobic nitrogen-fixing bacteria, free-living in the soil and not associated with plant roots. He names the genus *Azotobacter* and describes two species, *Azotobacter chroococcum* and *Azotobacter agilis*.

1909: **Biochemistry:** *Ribonucleic Acid (RNA) and Deoxyribonucleic Acid (DNA)*

Phoebus Aaron Theodore Levene and Walter Abraham Jacobs identify the sugar *d*-ribose (now D-ribose) as being a component of yeast nucleic

acid. In 1929, Levene finds deoxyribose in thymus nucleic acid, thus establishing the terminology ribonucleic acid (RNA) and deoxyribonucleic acid (DNA).

1915: **Bacteriophage:** *Discovery*

Frederick William Twort reports that a lytic phenomenon in cultures of micrococcus may be an ultramicroscopic bacterial virus. Nevertheless, he believes the responsible agent to be a lytic enzyme that induces other bacteria in the culture to produce the same enzyme. The phenomenon is later independently discovered by Felix d'Herelle, who coins the name "bacteriophage."

1917: **Bacteriophage:** *Discovery*

Felix d'Herelle, independent of Frederick Twort's observation in 1915, discovers a bacterial virus that lyses the dysentery bacillus. Giving it the name "bacteriophage," he also notes that it causes the appearance of clear zones called "plaques" in a film of bacterial growth on an agar plate.

1929: **Antibiotics:** *Penicillin*

Alexander Fleming discovers an antibacterial substance in a culture of the mold *Penicillium notatum*. He names the substance penicillin and develops an assay to follow its production in the mold culture. Upon testing its killing effect of a few species of bacteria, he notes that it is most effective against gram-positive bacteria and least effective against gram-negative bacteria.

1929: **Biochemistry:** *Adenosine Triphosphate*

Karl Lohmann and, independently, Cyrus Fiske and Yellapragada Subba-Row, discover a compound called adenylpyrophosphate in muscle. They show that the compound can be split into adenylic acid and pyrophosphate. In 1935 Lohmann determines its structure to be adenosine 5′-triphosphate (ATP).

1935: **Bacterial Physiology:** *Heterotrophic Carbon Dioxide Utilization*

Harland G. Wood and Chester Hamlin Werkman report the first of a number of experiments on how heterotrophic bacteria utilize carbon dioxide. In carbon balance calculations from studies of the fermentation of glycerol by propionibacteria, they conclude that CO_2 is consumed.

1940: **Antibiotics:** *Penicillin*

Howard Florey begins clinical trials with penicillin that is purified by Enrst Chain, assisted by Edward P. Abraham and Norman G. Heatley. Florey and Heatley later attempt to obtain larger quantities of penicillin for further trials. Researchers at the U.S. Department of Agriculture Northern Regional Research Laboratory find that *Penicillium chrysogenum* is a better source of penicillin than the original strain of *Penicillium notatum* isolated by Alexander Fleming in 1929.

1941: **Microbial Genetics:** *One Gene-One Enzyme Concept*

George Beadle and Edward L. Tatum use X rays to create nutritional mutants of the fungus *Neurospora crassa.* After studying a series of such mutants, they conclude that a single gene codes for a single enzyme, thus formulating the "one gene-one enzyme" concept. At this time, when the role of DNA is not known, genes are thought to be either protein or

nucleo-protein. Beadle, Tatum, and Joshua Lederberg share the 1958 Nobel Prize in physiology or medicine.

1944: **Bacterial Genetics:** *Transforming Principle*

Oswald T. Avery, Colin MacLeod, and Maclyn McCarty, in one of the important steps toward an understanding of the biochemistry of genes, prove that the "transforming principle" of *Diplococcus pneumoniae* (*Streptococcus pneumoniae*) is DNA.

1953: **Bacterial Physiology:** *Feedback Inhibition*

Studying the biosynthesis of tryptophan in *Escherichia coli,* Aaron Novick and Leo Szilard find that high concentrations of the amino acid block the formation of one of the intermediates in the pathway, indole 3-glycerol phosphate. They suggest that a high intracellular concentration of tryptophan inhibits the pathway of biosynthesis, whereas a low concentration permits it functioning. This type of process is later called "feedback inhibition" of enzyme activity.

1964: **Molecular Biology:** *Colinearity of Genes and Proteins*

Charles Yanofsky, Donald R. Helinski, and colleagues demonstrate a linear correspondence between one segment of gene A and the A protein of tryptophan synthetase. Their studies, begun in 1961 with *Escherichia coli,* show that distances between amino acid residues in the protein are representative of the values obtained by genetic recombination of the genome.

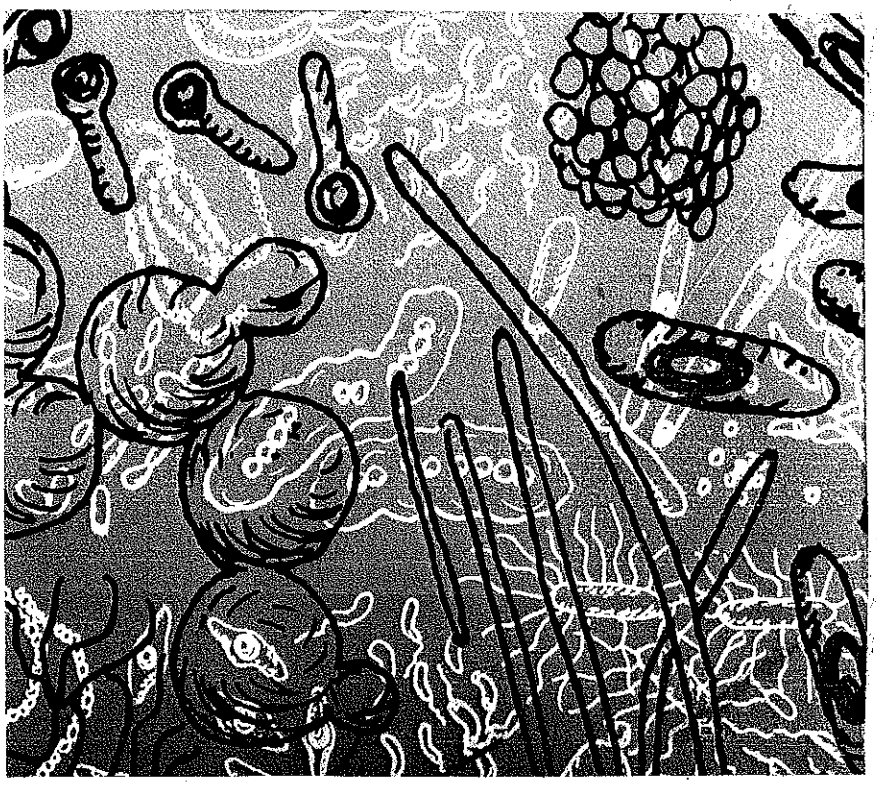
1979: **Microbial Ecology:** *Deep-Sea Bacteria*

Two groups of researchers, one led by Holger W. Jannasch and Carl O. Wirsen and a second led by John B. Corliss, reinvestigate the

hydrothermal vent communities of invertebrates found in 1977. They discover concentrations of sulfur-oxidizing bacteria in the waters of hydrothermal vents at temperatures up to 300°C and at depths of up to 3,000 meters.

1987: **Molecular Biology:** *Polymerase Chain Reaction*

Kary Mullis and Fred A. Faloona describe a polymerase chain reaction (PCR) that exponentially amplifies a nucleic acid sequence in vitro. In 1988, the technique is greatly improved by use of a heat-stable DNA polymerase derived from a thermophilic bacterium, *Thermus aquaticus*. Mullis shares the 1993 Nobel Prize in chemistry with Michael Smith, who develops site-specific mutagenesis.



Biographical Notes, Sketches and Commentaries

Some sources cited:

ASM= Microbiology's fifty most significant events during the past 125 years…a poster supplement to ASM News, vol. 65, No. 5, 1999

Bulloch= W. Bulloch: *The History of Bacteriology*, Dover Publications, New York, 1979 (republication of the classic published by Oxford University Press in 1938)

Gest= H. Gest: *Microbes/An Invisible Universe,* ASM Press, 2003

MICROBE (Oct. 2007)

Fresh Views of 17th Century Discoveries by Hooke and van Leeuwenhoek

Recently found writings by Hooke fill historical gaps and correct some myths about the early days of microbiology

Howard Gest

Robert Hooke and Antoni van Leeuwenhoek are credited with discovering the microbial world during the 17th century. However, some of the details about their contributions are garbled, leading to an unintended mythology. According to microbiologist Milton Wainwright of Sheffield University in Sheffield, England: "Unfortunately, much of what is taught about the history of microbiology has been oversimplified to the point where plain untruths are being told; at best a fascinating and convoluted story has been reduced to the minimum for easy, uncritical consumption."

Thus, a critical study of 17th-century documents reveals that the primary role of Robert Hooke was diminished, starting in the 1920s. Greatly oversimplified accounts, such as the popular book published in 1926 by Paul de Kruif, dramatized the unusual life story of Antoni van Leeuwenhoek, identifying him as the "First of the Microbe Hunters." More than one million copies of de Kruif's book were sold, and his romanticized essays described the lives of a dozen famous scientists. However, some of those tales strayed from the truth. One of the essays led Nobel Laureate Sir Ronald Ross to castigate de Kruif for statements that Ross considered libelous. Meanwhile, the early discoveries of Hooke were either ignored or given short shrift.

Summary

- Critical readings of 17th-century documents reveal that the role of Robert Hooke in discovering microbiology has been understated compared to that of Antoni van Leeuwenhoek.
- Hooke's interests ranged over physics, mechanics, astronomy, chemistry, geology, and biology, and he also was a prolific inventor, especially in connection with microscopes and telescopes.
- Hooke's *Micrographia* includes an exact description of how to make a single-lens microscope, whereas van Leeuwenhoek never disclosed his approaches for grinding lenses or illuminating samples.
- Recently uncovered records of Hooke's writings from 1677 and 1678 are particularly important regarding the first observations of microorganisms and help to pinpoint his experiments to confirm Leeuwenhoek's claims of seeing "little animalcules," i.e., bacteria.

Early in His Career, Hooke Showed Broad Curiosity and Talent

In 1653, Hooke enrolled as a "poor scholar" at Christ Church, University of Oxford. Although there is no record of his receiving a bachelor's degree, he was awarded an M.A. in 1663. Awarding him that M.A. "may have been brought about by the influence of people in high places who wanted to ensure that the Royal Society's newly appointed Curator of Experiments was a fully incorporated member of the learned establishment," according to historian Allan Chapman of Oxford University in Oxford, England. The degree was likely awarded in absentia and was not recorded in the University Register.

Hooke's outstanding abilities in "mechanics" were recognized at a very young

Howard Gest is Distinguished Professor Emeritus of Microbiology and Adjunct Professor of History and Philosophy of Science at Indiana University, Bloomington.

pp. 483-488.

Orig. Op.	OPERATOR:	Session	PROOF:	PE's:	AA's:	COMMENTS	ARTNO:
1st XX, 2nd YY	lambertr	5					

zjs= zjss=

age and led to his appointment as Curator in 1662 and subsequent election as a Fellow in 1663. As curator, his duties were to conduct "considerable" experiments at the Society's weekly meetings, and to do research officially recommended to him. Hooke became a commanding intellectual presence in the Society, and as curator provided the main substance of many meetings. His interests ranged over physics, mechanics, astronomy, chemistry, geology, and biology. Moreover, he was a prolific inventor, especially in connection with microscopes and telescopes.

Hooke's *Micrographia* is the First Systematic Account of the Microscopic World

After *Micrographia* appeared in bookshops in January 1665, it quickly became a best seller. Its profound impact is aptly described by Chapman in his critical biography of Hooke:

> [*Micrographia*] possessed a dazzling, immediate quality, being written in an easy style. . . . It was, moreover, the first proper *picture book* of science to come off the press, for its 60 Observations were accompanied by 38 beautiful engravings of the objects seen with the new instruments. . . . Modern science is replete with visual images, and in our own time the televisual image is the most powerful medium through which its ideas are now communicated to the lay public. So we must not forget that this tradition of visual communication in science largely begins with Hooke's *Micrographia.*

In his studies, Hooke used a microscope that was about six inches long and had two convex lenses. He examined mainly biological specimens, including sponges, wood, seaweed, leaf surfaces, hair, peacock feathers, wings of flies, eggs of silkworms, mites, a flea, and a louse, even though his primary interests were in mechanics and the physical sciences. The printed illustrations of microscopic views in *Micrographia* were prepared from engravings based on Hooke's excellent drawings, which attest to his acute powers of observation and skill as a draftsman.

The 1663 Journal Book of the Society has an entry for April 22 which says: "Mr. Hooke brought in two microscopical observations—one was of Leeches in Vinegar; the other a

Portrait of Antoni van Leeuwenhoek taken from his book *Arcana Naturae detecta* (1695) (SPL/Photo Researchers, Inc.).

blueish mould upon a mouldy piece of leather." Hooke's studies on the "mould" resulted in Observation XX of *Micrographia.* This chapter is devoted to the microfungus *Mucor,* which includes a detailed drawing of its reproductive structures (sporangia). Thus, *Mucor* was the first microbe described and depicted in the scientific literature (Figure 1).

Hooke described white spots of "hairy mould" on the red sheepskin covers of a small book:

> These spots appear'd, through a good Microscope, to be a very pretty shap'd vegetative body, which, from almost the same part of the Leather, shot out multitudes of small long cylindrical and transparent stalks, not exactly straight, but a little bended with the weight of a round and white knob that grew on the top of each of them; many of these knobs I obsrv'd to be very round and of a smooth surface, such as A A, etc. others smooth likewise, but a little oblong, as B.; several of them a little broken, or cloven with chops at the top, as C; others flitter'd as 'twere, or flown all to pieces, as DD. The whole substance of these pretty bodies was of a very tender constitution, much like the substance of the softer kind of common white Mushroms, for by touching them with a Pin, I found them to be brused and torn; they seem'd each of them to have a distinct root of their own; for they grew neer together in a cluster,

Orig. Op.	OPERATOR:	Session	PROOF:	PE's:	AA's:	COMMENTS	ARTNO:
1st XX, 2nd YY	lambertr	5					

zjs= zjss=

Much effort has been spent in trying to find an authentic portrait of Hooke, with no success. The drawing above appears in historian Allan Chapman's 2005 book (A. Chapman, England's Leonardo: Robert Hooke and the Seventeenth-Century Scientific Revolution, Institute of Physics Publishing, Bristol and Philadelphia, 2005) and is by Rachel Chapman. Chapman's book has an appendix which discusses the mystery of why an original portrait has never been found.

> yet I could perceive each stem to rise out of a distinct part or pore of the Leather; some of these were small and short, as seeming to have been but newly sprung up, of these the balls were for the most part round, others were bigger and taller, as being perhaps of a longer growth, and of these, for the most part, the heads were broken, and some much wasted, as E.

Hooke significantly advanced the techniques of microscopy, several of which are discussed in *Micrographia* and an important compilation that was published 13 years later in 1678, *Lectures and Collections*. This collection is in two parts. The first, "Comet A," consists of an extended discourse on the Comet of 1677. The second part, *Microscopium,* includes a detailed description of microscopic techniques as well as a discussion of "Mr. Leeuwenhoek's two Letters concerning some Late Microscopical Discoveries" and "The Author's Discourse and Description of Microscopes, improved for discerning the nature and texture of Bodies."

Contrasting Hooke and Leeuwenhoek

The 28-page preface to *Micrographia* is a remarkable document. For instance, it includes Hooke's precise description of how to make a single-lens, hand-held miniature microscope of the kind Leeuwenhoek later improved and used extensively.

Many microbiologists mistakenly believe that Leeuwenhoek invented this kind of hand-held microscope. However, in contrast to Hooke's lengthy published descriptions of such devices, Leeuwenhoek was notoriously secretive about his own methods and microscopes. He never disclosed the techniques that he used for grinding lenses or his conditions for illuminating samples.

As late as 1685, Leeuwenhoek's approach to microscopy remained obscure. In 1685, the Royal Society sent Thomas Molyneux to visit Leeuwenhoek in the hope of obtaining more information about van Leeuwenhoek's experimental techniques. Molyneux reported to the society that Leeuwenhoek refused even to let him see the "best" microscopes. Expressing skepticism about Leeuwenhoek's grasp of what others were thinking and observing, Molyneux wrote:

> As for the microscopes I looked through, they do not magnify much, if any thing, more than several glasses I have seen, both in England and Ireland: but in one particular, I must needs say, they far surpass them all, that is in their extreme clearness, and their representing all objects so extraordinarily distinctly. For I remember we were in a dark room with only one window, and the sun too was then off of that, yet the objects appeared more fair and clear, than any I have seen through microscopes, though the sun shone full upon them, or though they received more than ordinary light by help of reflective specula or otherwise: So that I imagine 'tis chiefly, if not alone in this particular, that his glasses exceeds all others, which generally the more they magnify, the more obscure they represent the object; and his only secret, I believe is making clearer glasses, and giving them a better polish than others can do. I found him to be a very civil complaisant man, and doubtless of great natural abilities; but contrary to my expectations, quite a stranger to letters, master of neither Latin, French or English, or any other of the modern tongues besides his own, which is a great hindrance to him in his reasonings upon his observations; for being ignorant of all other mens thoughts, he is wholly trusting to his own, which, I observe, now and then lead him to extravagancies, and

Hooke Exhibit at the National Library of Medicine

The History of Medicine Division (HMD) of the National Library of Medicine is currently exhibiting "Hooke's Books: Books that Influenced or Were Influenced by Robert Hooke's Micrographia." It is located in display cases in the HMD Reading Room, on the first floor of Building 38, National Institutes of Health, Bethesda, Md. The exhibit is open Monday through Friday, 8:30 AM to 5:00 PM, through November 1, 2007. The exhibit features a selection of books from the NLM collection, plus a facsimile of Hooke's own microscope. It is a companion to NLM's latest "Turning the Pages" production, a digital selection from Micrographia, which can be viewed at http://ttp.nlm.nih.gov.

> suggest very odd accounts of things, nay, sometimes such, as are wholly irreconcilable with all truth.

Leeuwenhoek, a Cloth Merchant and Lens Maker, Was Curious about Biology

In 1654, at the age of 22, Leeuwenhoek set up shop as a draper in Delft, Holland. He remained in that city during his long life, at times serving as a functionary in municipal offices. Some historians suggest that Leeuwenhoek's use of a magnifying glass to inspect cloth helped to trigger his scientific career. He eventually developed the skills to make very small lenses of excellent quality for use in miniature microscopes that Hooke designed.

Despite limitations such as those that Molyneux described, Leeuwenhoek was a keen observer and had extraordinary curiosity about the living world. With simple single-lens microscopes, he made many important discoveries. These were described in numerous, frequently lengthy, letters sent to the Royal Society. This remarkable shopkeeper, who had little formal education, was the first to describe protozoa and yeast cells, as well as the sperm cells of animals and red blood cells.

In regard to Leeuwenhoek's relationship with Hooke, there is clear-cut evidence of translators providing Leeuwenhoek with information from *Micrographia* before he began communicating his own findings to the Royal Society. For example,, Leeuwenhoek's first letter to the Royal Society, dated 28 April 1673, was submitted by a Dutch corresponding member of the Society.

The profound differences between Hooke and Leeuwenhoek in their practices in regard to making new technical knowledge available to other scientists needs no further comment.

Hooke's Old Records, now called the Hooke Folio, Were Discovered in 2006

In 1677, Hooke became Secretary of the Royal Society. Thus, in addition to conducting experimental demonstrations, he was obliged to record all activities of the Society for subsequent publications in its journals, including the *Journal Book of Ye Royal Society*. The records during Hooke's tenure are incomplete, and many temporary binders were left empty.

Unexpectedly in 2006, more than 650 pages of Hooke's missing notes were discovered in a cupboard of a private country house in England. Hours before those notes were to be auctioned, the Royal Society secured funds from more than 150 donors to purchase what is now called the "Hooke Folio."

I recently visited the Royal Society in London to pore over that Folio, especially with the aim of examining entries for 1677–1678 that might address Hooke's confirmation of Leeuwenhoek's discovery of bacteria ("little animalcules"). In addition, the visit gave me the opportunity to reexamine Hooke's other major publications, including journals of the Royal Society and relevant letters.

The records of 1677 and 1678 are particularly important in regard to the first observations of bacteria. Folio entries help to pinpoint the timeline of Hooke's experiments aimed at checking Leeuwenhoek's claims of seeing very large numbers of tiny "animalcules" in a single drop of pepper-water infusion. It is easy to understand why in the 1670s Leeuwenhoek's claims of seeing more than 1 million per drop were considered dubious.

Nevertheless, Hooke was determined to see for himself. Here are several excerpts from his Folio (edited slightly, mainly to use modern English spellings):

> "The Society met at 4 and ye President being Absent Mr Henshaw ye Vice president took the chair. The first thing exhibited was the Expt. charged on Mr. Hooke the Last Day of Exam-

Orig. Op.	OPERATOR:	Session	PROOF:	PE's:	AA's:	COMMENTS	ARTNO:
1st XX, 2nd YY	lambertr	5					

zjs= zjss=

ining pepper water with better microscopes and thinner & smaller pipes" [i.e., *glass capillary tubes*]. *At this point, several improvements in the microscope are described.* "But not withstanding the pepper mixture was very strong being made of Rainwater & whole black pepper Steepd for 3 days and not withstanding the microscope was much better than was shewed ye Last Day/yet we could see nothing of Mr Lewenhooks animals. Mr Henshaw conjectured with a great Deal of Reason that twas very likely that it might not now be a proper season for their generation. . . . It was further added that a person who had seen those creatures in holland this Last summer with a microscope of his own could not within this fortnight find any such in pepper water made here. Dr Whistler conjectured that these small imagined creatures might indeed be nothing else but the small particles of the pepper Swimming in ye water. But Dr Mapletost answered that Mr Lewenhooke affirmed to shew them both alive & dead. Dead when he put vinegar to the said tincture. [from Folio page 189 on 8 November 1677]

That section is followed by a description of a method that Hooke then was developing to measure the size of objects using a capillary tube "not bigger than a pigs bristle" that would appear as a cylinder of about 3 inches diameter once magnified.

The Society met at the usual place and the President being Absent mr Henshaw the vice president took the chair. The first Experiment exhibited was the pepper water which had been made with Rain water & a small quantity of common black pepper put whole into it about 9 or 10 days before. In this RH had all this week Discovered great numbers of exceeding small animals swimming to & fro in the water and by all that saw them they were verily believed to be animals and that there could be no fallacy in the appearance. They were seen by mr Henshaw, Sir Chris. Wren, Sir John Hoskins and diverse others so that there was no longer any doubt of mr. Leeuwenhoek's Discovery. Notice was ordered to be taken of this Discovery and further trial was Desired to be made upon Raine water alone & upon Rainewater in which had been steeped wheat barley and other seeds & graines. The shape of the microscope and the manner of Examining the liquor was as follows. [Folio pages 111–114; 15 November 1677]

Many details are given including how the samples were illuminated. Subsequent discussion, in which Sir Christopher Wren was prominent, concerned the desirability for further control experiments.

During November 1677, Hooke was also very busy (as usual) with many other matters, including experiments on respiration and blood circulation in higher animals. The Catalogue of Manuscript Letters of the Royal Society lists a letter written by Hooke to Leeuwenhoek, identified as H.3.54 from December 1677. It is signed: Your very great admirer and honorer, RH.

Microscopium (1678, pages 81–104) contains the text of two of Leeuwenhoek's important letters and an extensive discourse on microscopy, describing further improvements in detail. For example, he [Hooke] notes: "By this means I examined the water in which I had steeped the pepper I formerly mentioned; and as if I had been looking upon a Sea, I saw infinite of small living Creatures swimming up and down in it, a thing indeed very wonderful to behold."

In a letter dated 8 February 1680, Hooke informed Leeuwenhoek of his being elected as a fellow of the Royal Society by a unanimous vote. Leeuwenhoek became a celebrity and was visited in Delft by many notables, including Tsar Peter the Great of Russia.

Coda

There is little doubt that when the Hooke Folio is fully transcribed, other aspects of Hooke's genius will emerge. What we know about him gives us reason to anticipate fresh surprises about this distinguished early experimental scientist. Historian Chapman points out:

> . . . Robert Hooke was one of the greatest experimental scientists of all time. While modern historical scholarship can now place that genius within a wider intellectual and social context, and enable us to develop a balanced understanding as a man, what cannot be denied is that he, more than anyone else, showed that the experimental method actually worked, and could transform mankind's understanding of nature. And this he achieved through the communication of his findings by writing, by demonstration, and by the spoken word—to the wider world, wherein they could inspire scientists, inventors and poets."

ACKNOWLEDGMENTS

I am indebted to librarians and archivists of the Royal Society. Also to Anna Winterbottom and Jennifer Thomas, graduate students at Queen Mary (University of London) who are transcribing the Hooke Folio as part of a project of the Centre for Editing Lives and Letters; they are working at the Royal Society under the supervision of Dr. Robyn Adams and Prof. Lisa Jardine (Queen Mary), Director of the Centre.

SUGGESTED READING

Adams R., and L. Jardine. 2006. The return of the Hooke folio. Notes and Records of the Royal Society London 60:235–239.
Bennett, J., M. Cooper, M. Hunter, and L. Jardine. 2003. London's Leonardo—the life and work of Robert Hooke. Oxford University Press, Oxford.
Chapman, A. 2005. England's Leonardo: Robert Hooke and the Seventeenth-Century Scientific Revolution. Institute of Physics Publishing, Bristol and Philadelphia.
De Kruif, P. 1926. Microbe Hunters. Harcourt, Brace, New York.
Ford, B. J. 1985. Single lens. Harper and Row, New York.
Gest, H. 2004. The discovery of microorganisms revisited. ASM News 70:269–274.
Gest H. 2004.The discovery of microorganisms by Robert Hooke and Antoni van Leeuwenhoek, Fellows of the Royal Society. Notes and Records of the Royal Society London 58:187–201.
Gest, H. 2005. The remarkable vision of Robert Hooke/first observer of the microbial world. Persp. Biol. Med. 48:266–272.
Hooke, R. 1665. Micrographia, or some physiological descriptions of minute bodies made by magnifying glasses with observations and inquiries thereupon. J. Martyn and J. Allestry, Printers to the Royal Society, London.
Hooke, R. 1678. Lectures and Collections; Microscopium. J. Martyn, Printer to the Royal Society, London.
Jardine, L. 2004. The curious life of Robert Hooke: the man who measured London. Harper Collins, New York.
Leeuwenhoek, A. v. Alle de Briefen van Antoni van Leeuwenhoek/The Collected Letters of Antoni van Leeuwenhoek. Eleven volumes published intermittently between 1939 and 1983; edited and annotated by committees of Dutch scientists. Swets and Zeitlinger, Amsterdam.
Wainwright, M. 2003. An alternative view of the early history of microbiology. Adv. Appl. Microbiol. 52:333–355.

ining pepper water with better microscopes and thinner & smaller pipes" [i.e., *glass capillary tubes*]. *At this point, several improvements in the microscope are described.* "But not withstanding the pepper mixture was very strong being made of Rainwater & whole black pepper Steepd for 3 days and not withstanding the microscope was much better than was shewed ye Last Day/yet we could see nothing of Mr Lewenhooks animals. Mr Henshaw conjectured with a great Deal of Reason that twas very likely that it might not now be a proper season for their generation. . . . It was further added that a person who had seen those creatures in holland this Last summer with a microscope of his own could not within this fortnight find any such in pepper water made here. Dr Whistler conjectured that these small imagined creatures might indeed be nothing else but the small particles of the pepper Swimming in ye water. But Dr Mapletost answered that Mr Lewenhooke affirmed to shew them both alive & dead. Dead when he put vinegar to the said tincture. [from Folio page 189 on 8 November 1677]

That section is followed by a description of a method that Hooke then was developing to measure the size of objects using a capillary tube "not bigger than a pigs bristle" that would appear as a cylinder of about 3 inches diameter once magnified.

> The Society met at the usual place and the President being Absent mr Henshaw the vice president took the chair. The first Experiment exhibited was the pepper water which had been made with Rain water & a small quantity of common black pepper put whole into it about 9 or 10 days before. In this RH had all this week Discovered great numbers of exceeding small animals swimming to & fro in the water and by all that saw them they were verily believed to be animals and that there could be no fallacy in the appearance. They were seen by mr Henshaw, Sir Chris. Wren, Sir John Hoskins and diverse others so that there was no longer any doubt of mr. Leeuwenhoek's Discovery. Notice was ordered to be taken of this Discovery and further trial was Desired to be made upon Raine water alone & upon Rainewater in which had been steeped wheat barley and other seeds & graines. The shape of the microscope and the manner of Examining the liquor was as follows. [Folio pages 111–114; 15 November 1677]

Many details are given including how the samples were illuminated. Subsequent discussion, in which Sir Christopher Wren was prominent, concerned the desirability for further control experiments.

During November 1677, Hooke was also very busy (as usual) with many other matters, including experiments on respiration and blood circulation in higher animals. The Catalogue of Manuscript Letters of the Royal Society lists a letter written by Hooke to Leeuwenhoek, identified as H.3.54 from December 1677. It is signed: Your very great admirer and honorer, RH.

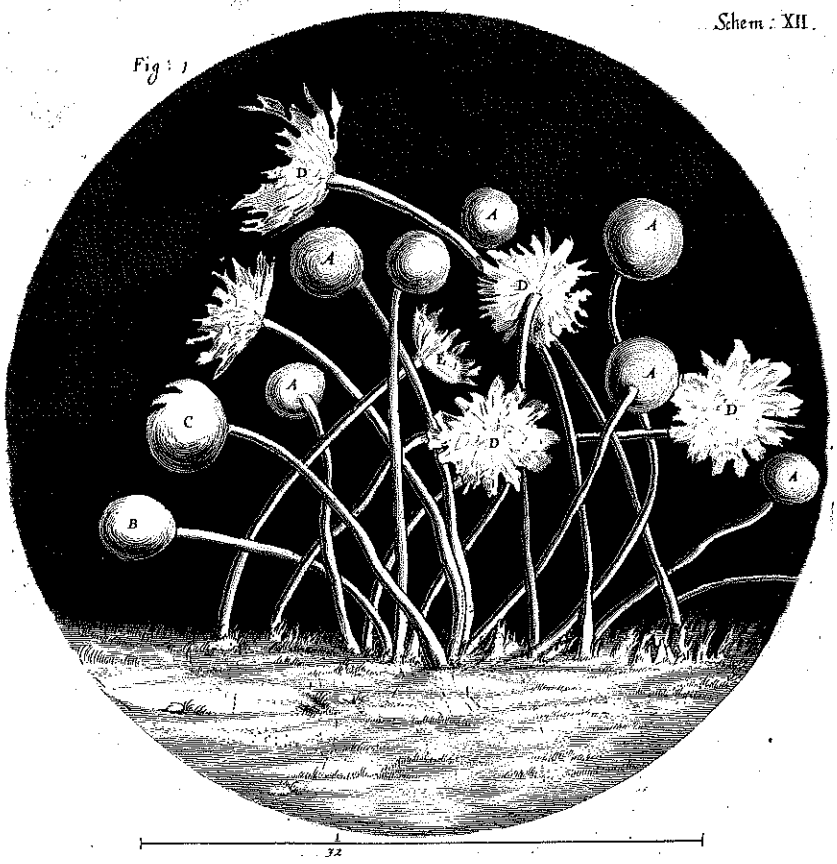
Microscopium (1678, pages 81–104) contains the text of two of Leeuwenhoek's important letters and an extensive discourse on microscopy, describing further improvements in detail. For example, he [Hooke] notes: "By this means I examined the water in which I had steeped the pepper I formerly mentioned; and as if I had been looking upon a Sea, I saw infinite of small living Creatures swimming up and down in it, a thing indeed very wonderful to behold."

In a letter dated 8 February 1680, Hooke informed Leeuwenhoek of his being elected as a fellow of the Royal Society by a unanimous vote. Leeuwenhoek became a celebrity and was visited in Delft by many notables, including Tsar Peter the Great of Russia.

Coda

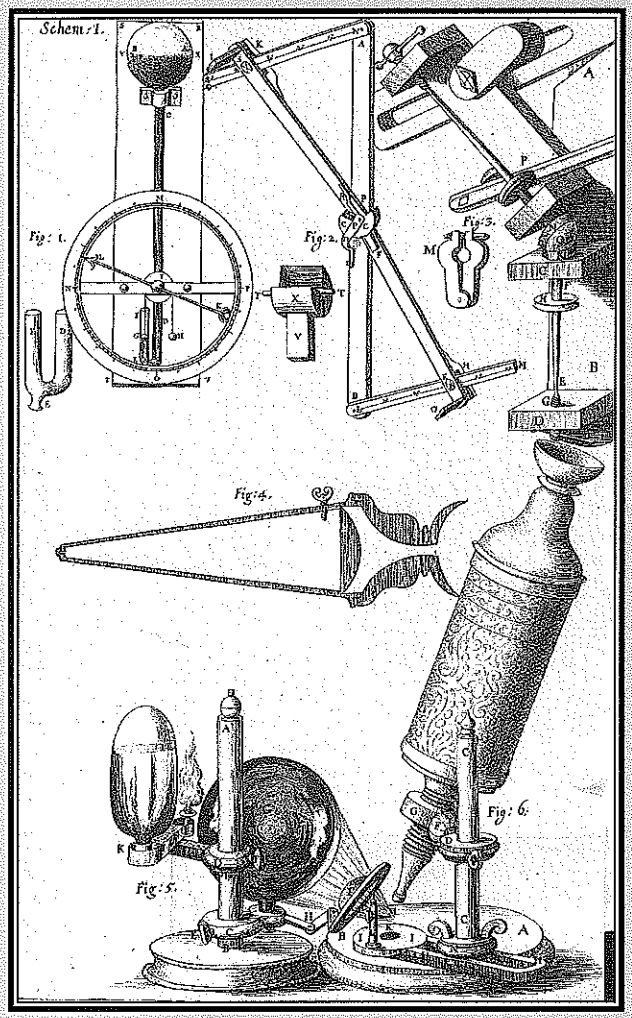
There is little doubt that when the Hooke Folio is fully transcribed, other aspects of Hooke's genius will emerge. What we know about him gives us reason to anticipate fresh surprises about this distinguished early experimental scientist. Historian Chapman points out:

> . . . Robert Hooke was one of the greatest experimental scientists of all time. While modern historical scholarship can now place that genius within a wider intellectual and social context, and enable us to develop a balanced understanding as a man, what cannot be denied is that he, more than anyone else, showed that the experimental method actually worked, and could transform mankind's understanding of nature. And this he achieved through the communication of his findings by writing, by demonstration, and by the spoken word—to the wider world, wherein they could inspire scientists, inventors and poets."



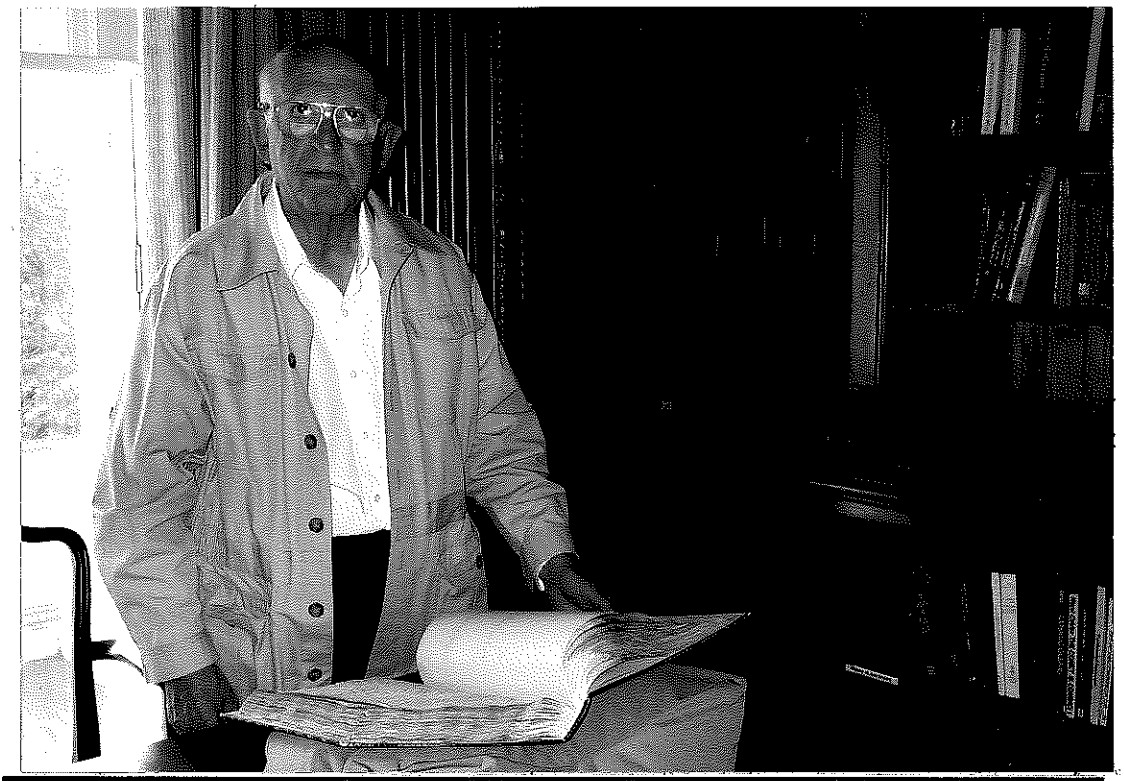
Hooke's drawing of *Mucor* growing on leather (1665)
"The whole substance of these pretty bodies was of a very tender constitution, much like the substance of the softer kind of common white Mushroms, for by touching them with a Pin, I found them to be brused and torn; they seem'd each of them to have a distinct root of their own ; for they grew neer together in a cluster, yet I could perceive each stem to rise out of a distinct part or pore of the Leather; some of these were small and short, as seeming to have been but newly sprung up...."

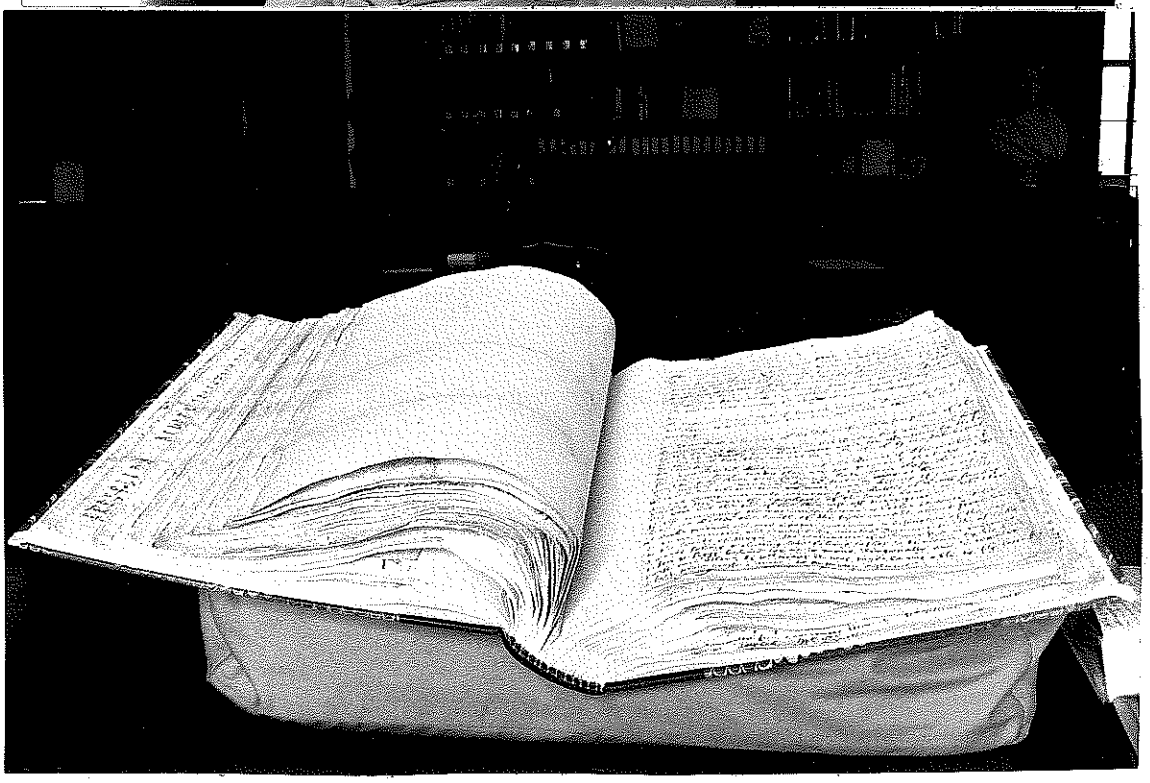
PERSPECTIVES
IN BIOLOGY AND MEDICINE



Summer 2009

Cover Illustration: "Schematic 1" of Robert Hooke's *Micrographia* (1665). See "Homage to Robert Hooke (1635–1703): New Insights from the Recently Discovered Hooke Folio" by Howard Gest, which begins on page 392.





Howard Gest with the "Hooke Folio" (650 pages of Hooke's notes on 17th century meetings of the Royal Society). At the Royal Society Library, London, October 2008.

METCHNIKOFF, Elie (1845-1916)

ASM

1882: Ilya Ilich (Élie) Metchnikoff demonstrates that certain body cells move to damaged areas of the body, where they consume bacterial and other foreign particles. He calls the process phagocytosis. He proposes a theory of cellular immunity. With Ehrlich, Metchnikoff is awarded the Nobel Prize in Medicine or Physiology in 1908.

Bulloch: Metchnikoff, Elie, Russian zoologist, embryologist, and pathologist. Founder of the phagocytic theory of immunity. Born in province of Charkow (Russia). Educated at Univ. of Charkow. Later, studies at Giessen and Naples. Became Prof. in Odessa. Worked in Messina. Became Director of Bacteriological Institute in Odessa 1886, but left in 1887 and went to Paris, where he resided till the end of his life. Sub-director of the Pasteur Institute. He received the Copley medal of the Royal Society in 1906. Metchnikoff was a prolific worker in many different branches and exercised a great influence on the development of the doctrines of immunity. He received the Nobel Prize 1908.

From Roger Reid: *Microbes and Men*, 1975 (Saturday Review Press, Dutton)

"Metchnikoff's early years could well have provided the material for a Chekhov play. The youngest son in a land-owning Russian family, he saw his mother's inheritance frittered away by his father. The mother considered that her weakly child had sensibilities which were too feminine to allow him to

study medicine; for this reason he had to reject it in favour of zoology. He was a highly temperamental youth and frequently threw himself into a frenzy when his studies were disturbed by anything as apparently ordinary as a dog's bark or a cat's mew. As an adult he became a manic depressive: a condition he observed with the same fascination as he did any other of his own physiological states. Even when he suffered a serious heart attack he took detailed notes of its effects on himself. And when he attempted to commit suicide for the second time, by injecting himself with relapsing fever, he was interested to discover whether the disease could be inoculated through the blood. The answer, though in his case not fatally so, was in the affirmative....

....At the peak of his manic moods he was at his most attractive and creative: Olga [Metchnikoff's wife] was fond of comparing his demeanour, his pale bearded face and his saintly manners, to those of Jesus Christ. And the ideas he plucked as though from nowhere, whether they were concerned with marriage, ageing, natural selection, or directly with his own subject, though many of them were plainly naïve, had the most appealing simplicity and originality."

Gest, 2003: Phagocytes were discovered by Metchnikoff, who visualized the animal body as a battlefield fought over by warring microbes and protective phagocytes. It's a quite remarkable story:

"In 1882, thanks to an inheritance acquired from his wife's parents, Metchnikoff was carrying out some research on zoological specimens in the Mediterranean. He had rented a small apartment overlooking the straits of Messina, in which he had settled his wife and the rest of her

inheritance – her five younger brothers and sisters. It was an ideally beautiful place in which he could pull himself out of a great period of depression which had followed his forced resignation from the University of Odessa because of his radical political views. His wife had an inheritance from her parents, so they decided to move to a town on the shore of the Mediterranean where he could pursue his research on zoological marine specimens. It is rare for scientists to remember the exact moment of dramatically new insights, and Metchnikoff's recollections are of interest in this connection (Metchnikoff, 1921).*

I was resting from the shock of the events which provoked my resignation from the University and indulging enthusiastically in researches in the splendid setting of the Straits of Messina. One day when the whole family had gone to the circus to see some extraordinary performing apes, I remained alone with my microscope, observing the life in the mobile cells of a transparent star-fish larva, when a new thought suddenly flashed across my brain. It struck me that similar cells might serve in the defense of the organism against intruders. Feeling that there was in this something of surpassing interest, I felt so excited that I began striding up and down the room and even went to the seashore to collect my thoughts.

I said to myself that, if my supposition was true, a splinter introduced into the body of a star-fish larva, devoid of blood vessels or of a nervous system, should soon be surrounded by mobile cells as is to

* Olga Metchnikoff: *Life of Elie Metchnikoff*, 1845-1916; Houghton Mifflin, 1921. Boston, New York.

be observed in a man who runs a splinter into his finger. This was no sooner said than done. There was a small garden to our dwelling, in which we had a few days previously organized a "Christmas tree" for the children on a little tangerine tree; I fetched from it a few rose thorns and introduced them at once under the skin of some beautiful star-fish larvae as transparent as water.

I was too excited to sleep that night in the expectation of the result of my experiment, and very early the next morning I ascertained that it had fully succeeded.

That experiment formed the basis of the phagocyte theory, to the development of which I devoted the next twenty-five years of my life."

From Bulloch (1938):

METCHNIKOFF, ÉLIE [Russian ILYA ILYICH] (born 1845, died 1916). Russian zoologist, embryologist, and pathologist. Founder of the phagocytic theory of immunity. Born in province of Charkow (Russia). Educated at Univ. of Charkow. Later, studied at Giessen and Naples. Became Prof. in Odessa. Worked in Messina. Became Director of Bacteriological Institute in Odessa 1886, but left in 1887 and went to Paris, where he resided till the end of his life. Sub-director of the Pasteur Institute. He received the Copley medal of the Royal Society in 1906. Metchnikoff was a prolific worker in many different branches and exercised a great influence on the development of the doctrines of immunity. He received the Nobel Prize 1908. (Metchnikoff, Olga, *Vie d'Élie Metchnikoff*, Paris, 1920, 272 pp.; *Ann. de l'Inst. Pasteur*, 1915, xxix. 357–63 [Roux]; *J. Path. and Bacteriol.*, 1917, xxi. 215 [portrait]; *Proc. Roy. Soc. Lond.*, 1917, B. lxxxix, pp. li–lix [Ray Lankester].)

YERSIN, Alexandre (1863-1943)

From Zinsser's Textbook of Bacteriology, by D. T. Smith et al. 1952; Appleton-Century-Crofts, New York:

> "The history of epidemic diseases has no more terrifying chapter than that of plague. Sweeping time and again over large areas of the civilized world, its scope and mortality were often so great that all forms of human activity were temporarily paralyzed. In the reign of Justinian almost 50 per cent of the entire population of the Roman Empire perished from the disease. The "black death" which swept over Europe during the fourteenth century killed about 25 million people. Smaller epidemics, appearing in numerous parts of the world during the sixteenth, seventeenth and eighteenth centuries, have claimed innumerable victims. In 1893 plague appeared in Hong Kong. During the epidemic which followed, *Pasteurella pestis*, now recognized as the etiologic agent of the disease, was seen in smears by Kitasato but isolated and identified by the Swiss bacteriologist, Yersin. The organism was found in the pus of afflicted individuals and could be demonstrated in enormous numbers in the cadavers of victims. This evidence was strengthened by accidental infections with laboratory cultures which occurred in Vienna in 1898."

The foregoing is from the text used for teaching medical students when I was on the faculty of the Dept. of Microbiology, Western Reserve University School of Medicine. Isolation and identification of the bacterium that caused

plague by Alexandre Yersin was a very important accomplishment, made under primitive and difficult conditions. We now know many of the details from a reminiscence published in 1995 by Ludwik Gross [How the plague bacillus and its transmission through fleas were discovered: Reminiscences from my years at the Pasteur Institute in Paris. Proc. Nat. Acad. Sci. 92:7609-7611, 1995]. When first isolated, the etiologic agent of plague was called *Pasteurella pestis*. It is now designated *Yersinia pestis*. In the following, sections in quotes are from the paper by Gross.

Yersin was working at a hospital in Paris at the time Pasteur introduced a vaccine treatment for rabies. "Yersin was performing an autopsy on the spinal cord of a patient who died following a bite by a rabid wild dog; during the dissection of the spinal cord, Yersin cut his finger; he immediately proceeded to Pasteur's laboratory. Pasteur called his assistant, Emile Roux, and asked him to start vaccinating Yersin against rabies. That was the beginning of a long friendship that developed between Yersin, Pasteur, and Roux."

In time, Yersin became a member of the French Medical Colonial Corps. When a plague epidemic developed in Hong Kong, Pasteur suggested to French authorities that they should send Yersin there to study the outbreak and try to isolate the causative agent.

"The problem was not as simple as it might have appeared. Yes, the epidemic was in full swing. People died by the hundreds. The city hospital was full of sick and dying patients. But Yersin had no access to the morgue. The hospital director, Dr. Lawson, did not give him permission. After many interventions and appeals, even to the governor, Yersin finally, as a gesture of

good will, received permission to have a small table in a corner of a dark corridor, next to the patients' room, where he could leave his microscope, a notebook, and a few cages with guinea pigs, mice and rats. That was the limit of his allowance. He had no access to the morgue, where he was anticipating piercing the enlarged lymph nodes (bubos) of a patient who died from plague in order to look for the causative bacillus. Frustrated, Yersin in the meantime, developed a friendship with an English priest, Father Vigano, who helped him build a small shack outside, but adjoining, the hospital, where Yersin could have a small folding bed and a very small makeshift laboratory. At the advice of Father Vigano, Yersin gave a few dollars to two English sailors who were helping to take care of the morgue at the hospital. Yersin was now able to go with the two sailors into the morgue for a few minutes and have access to the corpse of a patient who just died with plague. Yersin punctured the patient's swollen inguinal lymph node – i.e., bubo – with his sterile pipette and ran to his small laboratory, where one part of the fluid was placed under the microscope, another part was injected into a few guinea pigs, and the rest was prepared for immediate shipment to Roux at the Pasteur Institute in Paris. Yersin was excited after he looked into the microscope; he wrote in his notebook: "June 20, 1894. The specimen is full of microbes, all looking alike, with rounded ends, staining very poorly (Gram-negative); this is without question the microbe of plague.

....A new, very important observation followed shortly. Yersin, intrigued by the large number of dead rats lying on the streets, around the morgue, and in hospital corridors, decided to examine, under the microscope, the blood, nymph nodes, and other organs of these rats and found that they

were full of the same bacilli that he found in patients dying with the plague. He now realized that plague affects not only humans but also, and perhaps predominantly, rats. As a matter of fact, Yersin recorded that rats have long been known to be affected not only at the time of plague epidemics but also often preceding such epidemics in humans; ancient people knew about it, and mountain inhabitants in Chinese villages as well as villagers in parts of the mountains in India and also on the island of Formosa knew that when hundreds and thousands of rats lie around on the roads and in houses, they precede the outbreak of the fatal epidemic in humans.

....The mystery of transmission of plague from rat to rat, or from rats to humans, was solved a few years later by [Paul-Louis] Simond, a young French colonial Army physician, who was delegated to Indo-China by Pasteur to take over the research on this devastating disease and to follow and expand the initial observations of Yersin. He worked in the former Metchnikoff's laboratory at the Pasteur Institute in Paris (and where, in fact, I had the privilege of working some 40 years later).

Roux suggested to Simond to go to Indo-China to try to follow up Yersin's work and particularly to try to treat patients suffering from plague with a serum prepared from horses, immunized with the bacillus isolated by Yersin. Simond accepted with enthusiasm his new mission with the orders from the French government to proceed to Long-Tcheon in Indo-China where plague was ravaging."

Simond became convinced that the rat flea (*Xenopsylla cheopis*) transmits the disease from rats to man, and developed simple, but clever

experiments to prove his thesis. His results were published in Ann. Inst. Pasteur 12: 625-687, 1898.

Coda:

Finally, Gross added the following "interesting story." Edouard Dujardin-Beaumetz "showed me a tube filled with live bacilli of plague. He told me that not only humans and rats but also monkeys, guinea pigs, mice, and many other species are susceptible to the plague bacillus. But not the chicken. Among the species resistant to plague, is the chicken. 'Look at this tube full of live bacilli of plague': said Dujardin-Beaumetz to me, taking out of a cabinet a small tube marked with a red pencil B.P. 'This small tube contains sufficient quantity of live plague bacilli to infect and kill the population of an entire district of Paris,' he continued. 'We injected a similar quantity of live plague bacilli into the peritoneal cavity of a young chicken in our laboratory,' Dujardin-Beaumetz told me, 'and the chicken remained in good health; in fact, the next day she laid an egg. Surprisingly, the chicken got lost, presumably flew out of a small open window in the adjoining laboratory. We were frantic and looked for this animal all over, afraid that it may spread the deadly disease but we could not find the chicken. Only several days later did we learn that the chicken was caught by a house superintendent, residing on a street adjoining the Institut, on rue Falguiere; not realizing the chicken came from our laboratory, he roasted the chicken and consumed it, sharing the unexpected meal with his family. The plague bacilli were presumably destroyed by roasted the chicken. Nothing happened to them. They all remained alive and well."

AVERY, Oswald T. (1887 – 1955)

MacLEOD, Colin (1909 – 1972)

McCARTY, Maclyn (1911 – 2005)

ASM, 1944 - Oswald Avery, Colin MacLeod, and Maclyn McCarty show that transformation of *Streptococcus pneumoniae* from an avirulent phenotype to a virulent phenotype is the result of the transfer of DNA from dead smooth organisms to live rough ones. They also show that the transforming principle is destroyed by pancreatic deoxyribonuclease, which hydrolyzes DNA but is not affected by pancreatic ribonuclease or proteolytic enzymes.

One of the truly great discoveries in the 20th century was made by Oswald Avery and two younger coworkers, Colin MacLeod and Maclyn McCarty. Their research with *Pneumococcus* (*Streptococcus pneumoniae*) published in 1944 in the *Journal of Experimental Medicine* concluded, "that a nucleic acid of the deoxyribose type is the fundamental unit of the transforming principle of *Pneumococcus* Type III." In other words, that genes are composed of DNA. This was not accepted by the pundits of the day who generally believed that the "genetic material must be protein." Why? It seemed that proteins, with ca. 20 amino acids, were more likely to provide the many combinations required for coding than nucleic acid, with only 4 bases. In 1988, Crick remarked: "It is astonishing how one simple incorrect idea can envelop the subject in a dense fog."

MacLeod left Avery's laboratory in 1941, and much of the subsequent work was done by McCarty. This epoch-making research was detailed in a 1985 book by McCarty, entitled "The Transforming Principle: Discovering that Genes are Made of DNA" (W. W. Norton). McCarty presented the team's research in a June, 1941 Cold Spring Harbor Symposium on "Heredity and Variation in Microorganisms," and reports in his book: "At the end of the session at which I had presented the paper, one of the geneticists actually came up to me and said, 'Now that you fellows have shown that nucleic acid is not responsible for transformation, why don't you get to work and find out what really is?' I recovered quickly enough from this sally to reply that I was under the impression that this was exactly what we had done – shown that it was DNA, thus abruptly ending the conversation."

Sydney Brenner published an excellent review of McCarty's book (*Nature*, 317: 209-210, 1985) which begins: "For most young molecular biologists, the history of their subject is divided into two epochs: the last two years and everything else before that. The present and very recent past are perceived in sharp detail but the rest is swathed in a legendary mist where Crick, Watson, Mendel, Darwin – perhaps even Aristotle – coexist as uneasy contemporaries. It would not surprise me to find that most graduate student have not heard of Avery, MacLeod and McCarty or of their discovery that the transforming principle of the pneumonococcus was DNA. The general ignorance of our times might easily consign this book, with the wonderful title *The Transforming Principle*, to the "Religion and Occult" section of a bookshop, as once I found Levi-Strauss's *The Raw and the Cooked* under "Recipes"."

Ending his review, Brenner asks: "Did this work deserve a Nobel Prize? Of course it did, and probably more so than many of the ones given since. But as McCarty suggests, the Committee was more careful than wise and let it go by. Thus the story ends on a somewhat sad note, and leads the reader to contemplate the ruthlessness of the process of scientific research, and why, as one grows older, one comes to value books such as this, not so much for their scholarship, but for the memories of men and the humanity they bring with them."

For additional enlightenment on this remarkable episode in the history of microbiology/biochemistry see Perutz (2003; pages 197-205).

I Wish I'd Made You Angry Earlier

Essays on **SCIENCE, SCIENTISTS,** *and* **HUMANITY**

EXPANDED EDITION

with nine new essays by the author
and an Appreciation by John Meurig Thomas

Max F. Perutz

Formerly Chairman and Member of the MRC
Laboratory of Molecular Biology
Cambridge, England

Evolution of the discovery of penicillin

A saga of serendipity, intuition, ingenuity and hard work

FLEMING, Alexander (1881-1955)

FLOREY, Howard W. (1898-1968)

CHAIN, Ernst B. (1906-1979)

HEATLEY, Norman G. (1911-2004)

ASM

1929: Alexander Fleming publishes the first paper describing penicillin and its effect on gram-positive microorganisms. This finding is unique since it is a rare example of bacterial lysis and not just microbial antagonism brought on by the mold *Penicillium*. When penicillin is finally produced in major quantities in the 1940s, its power and availability effectively launch the "Antibiotics Era." With Florey and Chain, Fleming is awarded the Nobel Prize in Physiology or Medicine in 1945.

Gest, 2003:

Fleming, Alexander. *1881-1955.* British bacteriologist. Born in Lochfield, Scotland. Educated at St. Mary's Medical School at the University of London and returned to teaching there after serving in the army medical corps during World War I. Professor at the Royal College of Surgeons. Discovered penicillin in 1928. Admitted to the Royal Society in 1943, knighted in 1944, and awarded the Nobel Prize in 1945.

Florey, Howard W. *1898-1968.* Australian scientist who became head of the University of Oxford group that purified penicillin, determined its chemical structure, and demonstrated its antibacterial properties in laboratory animals. Florey's group also performed the first clinical trials with the antibiotic. Florey, his associate Ernst B. Chain (1906-1979) and Alexander Fleming (1881-1955) were awarded the 1945 Nobel Prize in Physiology or Medicine.

The existence of antibiotics, the most useful and potent agents for fighting disease, was discovered by accident in 1928 by Alexander Fleming, a microbiologist working at St. Mary's Hospital and Medical School in London. He was hired by Almroth Wright, a famous physician, who devoted much of his time to research on how immunity to typhoid fever could be achieved. Wright was a close friend of George Bernard Shaw, who frequently visited Wright's laboratory in the evening, after the theater, for a cup of tea. Wright often did his research from evening until 3 or 4 A.M., and Shaw made him the hero of his play *The Doctor's Dilemma.* (The play contains quite a lot of microbiology, including talk about white blood cells "eating" microbes.)

Fleming's research centered on ways of killing pathogenic bacteria with antiseptics, and he frequently used staphylococci as the test organism. Fleming was not a particularly tidy researcher; in fact, he was often teased for being disorderly. Typically, his laboratory bench was piled high with old Petri dish cultures that should have been discarded. One day in 1928, while talking to a young assistant, he lifted the lids of a few old dishes and glanced at the agar cultures. These had become contaminated with moulds; this

frequently occurs when cultures are allowed to sit around for months. He muttered to his assistant: "As soon as you uncover a culture dish something tiresome is sure to happen. Things fall out of the air." Suddenly, he stopped talking, and then said, "That's funny...." He was struck by an unusual sight. On the particular dish he was examining, there was a large fungus colony on the agar next to where he had been growing some yellow colonies of *Staphylococcus* bacteria. However, the bacterial colonies near the fungus growth on this dish seemed to have dissolved and looked like small drops of dew. Fleming eventually identified the fungus as *Penicillium notatum*, which naturally secretes an organic chemical substance of relatively simple structure that kills a number of bacterial species very effectively. The substance was appropriately named *penicillin*, and it became the first antibiotic to be discovered.

It is clear that Fleming did not realize the potential value of penicillin for treatment of infectious diseases, and it was not until 1938 that this idea began to take root. Early in that year, Ernst Chain of the University of Oxford came across Fleming's 1929 report and convinced his department chairman, Howard Florey, that further research on penicillin would be of interest and scientific value.

By 1940, Chain and Florey and their colleagues were in the midst of a rapidly expanding pioneering effort to isolate penicillin in pure form and test its chemotherapeutic effects on bacterial infections of humans. The first "miraculous" cures were effected in 1941 and led inevitably to a burst of research activity aimed at finding other antibiotics. In 1954, Florey, Chain, and Fleming were awarded a Nobel Prize for their pioneering work; since then

over 1,000 antibiotics from various fungi and bacteria have been isolated and characterized.

Many books and articles have been written about Fleming and Florey. The foregoing summarizes the essence of the observations leading to the discovery of penicillin and early tests of its use in treating human infections. But it does not do historical justice to several scientists whose efforts were crucial to determining the chemical nature of penicillin and how it could be produced in large quantities. Among these, Ernst Chain and Norman Heatley deserve particular attention.

Ernst Chain

In many ways, Chain was the major driving force of the penicillin "story". In 1971, he spoke at a symposium on the history of penicillin and related antibiotics. The title of his talk was "Thirty years of penicillin therapy." The published account (see references below) is of great interest and gives many insights into how this outstanding research developed. Following are several quotations in Chain's own words.

"In 1935, a few months after his appointment to the Chair of Pathology at Oxford, I was invited by Professor H. W. Florey, as he then was, to join his staff at the Sir William Dunn School of Pathology. Though Florey had no specific biochemical training, he was very conscious of the importance of biochemistry for progress in all the biological sciences, and particularly his own subject, experimental pathology, and he felt that a Department such as

the one he intended to build up, could not be fully successful without some internal biochemical support....

I was then a refugee from Hitler's Germany. I had left my native town, Berlin, on that fateful day, 30 January 1933, when Hitler acceded to power and Europe was temporarily plunged into a darkness in comparison with which the darkest Middle Ages now appear as a blaze of light. After a short interlude in London at University College, I had the great good fortune to be accepted by Hopkins in his Department as a research worker, largely through the good offices of the late Professor J. B. S. Haldane, and spent there two very happy years. One of my main scientific interests at that time was the study of the biochemical mode of action of neurotoxic snake venoms....

When Hopkins asked me whether I would like to go to Oxford to join Florey's staff, I was at the same time both extremely surprised and delighted, for I never expected such exceptionally good fortune to come my way in my unsettled condition with a very uncertain future in front of me. He introduced me to Florey in his office immediately after our talk and I naturally accepted the offer without any hesitation....

I collected about 200 references on growth inhibitions caused by the action of bacteria, streptomycetes, fungi and yeasts on one another. It was evident that in many cases the growth inhibition was caused by specific metabolites produced by the various micro-organisms. However, next to nothing was known about the chemical or biological nature of the inhibitory substances, and it seemed an interesting and rewarding field of exploration....

When I saw Fleming's paper for the first time I thought Fleming had discovered a sort of mould lysozyme, which in contrast to mould lysozyme, acted on a wide range of gram positive pathogenic bacteria....

"In 1943, we proposed the thiazolidine-β-lactam structure of penicillin which is now universally accepted. It was one of the last structural investigations which was carried out mainly with chemical methods though we received considerable help from X-ray crystallographic analysis carried out by Dorothy Hodgkin. She succeeded in 1945 in obtaining the complete structure of the penicillin molecule by X-ray analysis and in proving unequivocally the presence of the four membered β-lactam ring which was doubted by many organic chemists. It took Dorothy Hodgkin about two years to calculate the structure of the penicillin molecule, using mechanical calculators then at her disposal; today, with the modern computers, the job would be completed in two weeks, or maximally a month."

In 1948, Chain left Oxford to organize the International Centre for Chemical Microbiology at the Istituto Superiore di Sanita in Rome. There, he and his colleagues pursued research in a number of fields. A new strong interest was development of industrial-scale fermentation pilot plants as research tools. This continued when Chain moved back to England in 1964 to become head of the Department of Biochemistry at the Imperial College of Science and Technology (London); Chair's activities in Rome and Imperial College are reviewed in a 1991 article in Nature (see ref. to B. Chain). The keynote caption of the latter is "The discovery of penicillin remains one of the greatest advances in medical science. From the success of the discovery the biotechnology industry became established."

From B. Chain, 1991: "Influenced by his experiences during the first frustrating attempts at scaling-up penicillin production in the Oxford laboratories using antiquated and inappropriate technologies, Chain was convinced that progress in isolation and characterization of biologically active substances (not only antibiotics, but vitamins, hormones, growth factors and other biological molecules active at very low concentrations) absolutely required large scale production of biological material . . . . Chain's own career also predisposed him to an interdisciplinary approach to scientific problems. He trained as an organic chemist, turned later to biochemistry, and ultimately became interested in bioengineering . . . . Both in Rome and later in London, Chain's ambitions to work on a scale unprecedented within an academic biochemistry department were fulfilled."

References:

Chain, Benj. Penicillin and beyond. Nature 353: 492-494, 1991.

Chain, E. Thirty years of penicillin therapy. Proc. Roy. Soc. Lond. B179: 293-319, 1971.

Gest, H. 2003

MacFarlane, G. Howard Florey/The Making of a Great Scientist. The Scientific Book Club (London), 1979.

Maurois, A. The Life of Sir Alexander Fleming. Penguin Books, 1979.

HEATLEY, Norman: "Penicillin's Forgotten Man"

Without Heatley's efforts, the success of the Oxford team would no doubt have been very much delayed. He received a PhD degree in biochemistry from Cambridge University in 1936. In 1937, Chain asked Florey to engage Heatley as a junior member of his (Chain's) biochemical team, to manage growing *Penicillium* and develop methods for assaying penicillin concentration.

Heatley had great ingenuity and technical skills. He designed a simple penicillin assay procedure that became known as the "cylinder plate test" which greatly facilitated the research. Another major problem facing the Oxford team was how to grow sufficient *Penicillium* (on agar) with the limited supplies available during wartime. For this purpose he assembled an astonishing assortment of sterilized bottles, trays, pie dishes, gasoline cans and biscuit tins. He came to the conclusion that the most practical containers for the surface growth of *Penicillium* were porcelain bedpans in use at the Radcliffe Infirmary. This led him to design a modified version that could be manufactured in potteries. These became invaluable in establishing the world's first penicillin factory in the laboratories of the Sir William Dunn School of Pathology.

One of Heatley's obituaries noted an entry in his diary concerning trials on 8 mice during May 1940:

"After supper with some friends, I returned to the lab and met the professor to give a final dose of penicillin to two of the mice. The 'controls' were looking very sick, but the two treated mice seemed very well. I stayed at

the lab until 3:45 a.m., by which time all four control animals were dead. It really looks as if penicillin may be of practical importance."

Heatley was eventually honored with an OBE (Order of the British Empire) and an honorary Doctorate of Medicine from Oxford University; he was the only person to receive that award in the university's 800-year history. For more details, see C. L. Moberg: Penicillin's Forgotten Man – Norman Heatley; Science 253: 734-735, 1991.

Proc. R. Soc. Lond. B. **179**, 293–319 (1971
Printed in Great Britain

With warmest best wishes
Ernst Chain
19. March 1973

Thirty years of penicillin therapy

By Sir Ernst Chain, F.R.S.
Department of Biochemistry, Imperial College, Imperial Institute Road, London, S.W.7

Three decades have passed since the publication in the *Lancet* (Abraham *et al.* 1941) of the paper by our group at Oxford in which it was, for the first time, reported that the mould metabolite penicillin exhibited remarkable chemotherapeutic effects in clinical bacterial infections, including those caused by *Staphylococcus aureus*, against which no member of the *only* then known group of antibacterials possessing *in vivo* chemotherapeutic activity, the sulphonamides, was fully effective. A year earlier, in 1940, we had published, also in the *Lancet* (Chain *et al.* 1940), our first paper on the chemotherapeutic power of penicillin in experimental bacterial infections in mice which was dramatic and of unprecedented magnitude.

The introduction into clinical medicine of penicillin therapy and of the antibiotics therapy stemming from it has, by general consensus of opinion, completely revolutionized the treatment of bacterial infections in both man and animals, and rendered the large majority of them, including the most severe ones, amenable to successful therapeutic control.

The passage of thirty years—conventionally accepted to represent the life span of a generation—which have lapsed since the introduction of penicillin into clinical medicine, would seem an appropriate occasion to mark this event—and it is, I believe, an event well worth marking. This is the purpose of this symposium, gratifyingly organized under the joint auspices of the two leading professional scientific and medical organizations of this country, the Royal Society and the Royal College of Physicians, and, in itself, representing a historic event—for, as far as I am aware, no joint function of this kind has ever been held in the long history of these distinguished bodies.

HOOKE, Robert, as seen by J. D. Bernal (1901-1971)

At the age of 26, J. D. Bernal was appointed Assistant Director of Research at the Cavendish Laboratory, University of Cambridge. He later became Professor of Physics at Birkbeck College, University of London. Bernal was a founder of molecular biology through his pioneering work on X-ray structure of biologically important molecules. He had no doubt that some of the explanations of the origin of life would come from the geometry and structure of proteins and nucleic acids. In Cambridge, Bernal became the PhD supervisor of Max Perutz (Nobel Laureate 1962), who recalled his first meeting with Bernal as follows [Perutz, 2003]:

> "In 1936, after four years of chemistry at Vienna University, I took the train to Cambridge to seek out the Great Sage, and asked him: 'How can I solve the riddle of life?' 'The riddle of life is in the structure of proteins,' he replied, 'and it can be solved only by X-ray crystallography.' The Great Sage was John Desmond Bernal, a flamboyant Irishman with a mane of fair hair, crumpled flannel trousers and a tweed jacket. We called him Sage, because he knew everything, from physics to the history of art. Knowledge poured from him as from a fountain, unselfconsciously, vividly, without showing off, on any subject under the sun. His enthusiasm for science was unbounded."

For more details of the life of Bernal, see the recent biography by Andrew Brown: J. D. Bernal, The Sage of Science; Oxford University Press,

2005. Aside from his scientific research, Bernal was an impressive historian of science. In the Preface of his monumental book, *Science in History* (3rd ed., 1965) he noted: "In the second half of the twentieth century the great revolution has been in biology; not in one branch or another, but in the common fusing together of all branches of biology, from genetics to molecular structure.... "Discoveries are beginning to show how the nucleic acids, the carriers of heredity, transfer the information built into them, according to a code locked in the chromosomes, to the formation of the specific enzyme proteins which carry out the current living processes. The discovery and elaboration of these mechanisms, which has only just begun, should completely transform our understanding of life, making precise what was previously vague or incomprehensible..."

From Bernal (1965):

In many ways Robert Boyle contrasts with his first assistant and lifelong friend, Robert Hooke. If one was a nobleman, condescending to science, the other was a poor man who had to make his living out of science while he pursued it. The son of a clergyman in the Isle of Wight, Hooke managed to secure a servitorship at Oriel College at the time when Boyle had come to Oxford. He early attached himself to him and, in fact, probably made all his apparatus and carried out most of his experiments on the vacuum pump and gases. Boyle certainly did not shine as an experimenter after Hooke left him. Hooke was made curator of experiments of the Royal Society when it was founded, and as well as carrying out his heavy duties managed to supplement

his meager and irregular salary by being largely responsible for the plans of the new City of London after the Great Fire of 1666.

If he had been in a more secure social position and had not suffered from his ugliness and chronic ill health, he would not have been the difficult, suspicious, and cantankerous person he was, and his quite decisive role in the history of science would have been fully recognized. If Boyle was the spirit behind the Royal Society, Hooke provided it with eyes and hands. He was the greatest experimental physicist before Faraday, and, like him, lacked the mathematical ability of Newton and Maxwell. His interests ranged over the whole of mechanics, physics, chemistry, and biology. He studied elasticity and discovered what is known as Hooke's law, the shortest in physics; *ut tensio sic vis* (extension is proportional to force); he invented the balance wheel, the use of which made possible accurate watches and chronometers; he wrote *Micrographia*, the first systematic account of the microscopic world, including the discovery of cells; he introduced the telescope into astronomic measurement and invented the micrometer; and he shares with Papin the credit of preparing the way to the steam-engine.

Probably his greatest contribution to science in only now beginning to be recognized: his claim to have originated the idea of the inverse square law and universal gravity. Here, as we shall see, he was outclassed by the superb mathematical achievement of Newton, but it now seems that the basic physical ideas were Hooke's and that he was quite unjustly robbed of the credit for them. Hooke's life illustrates both the opportunities and the difficulties that the gifted experimenter could find in the seventeenth century."

BASSI, Agostino (1773-1856)

Bulloch: By his demonstration (1835) of the parasitic nature of the muscardine disease of silkworms Bassi may be regarded as the founder of the doctrine of pathogenic microbes. He was born in Mairago, near Lodi, and was educated for the law but studied natural science in Pavia. He held various civil posts under the French and Austrian Governments but had to give them up on account of persistent ill health and failure of vision. Reduced to great financial straits he tried farming but was unsuccessful. By means of a legacy inherited from a relative he was able to discharge his debts and to help the needy and infirm. He wrote on the cultivation of potatoes (1812, 1817), on cheese (1820), vinification (1823), contagion (1844), pellagra (1846), cholera (1849). His great work, *Del mal del segno calcinaccio o moscardino*, was published in Lodi in 1835 and 1836.

Gest, 2003: Students of the history of biological science are taught that the first perceptive insights into the nature of infectious disease were advanced by Girolamo Fracastoro (ca. 1478-1553). He was an acute observer and published an important early work on syphilis. Other books dealt with the essence of contagion. He spoke of the "seminaria" of disease; the word is translated as "seeds" or "germs," and some scholars believe that he considered them to be living entities. By the end

of the 16th century, however, his work had been forgotten, mainly because of the lack of scientific communication during the 17th century.

In 1835, Agostino Bassi (1773-1856) published the first definitive evidence for microbial causation of an infectious disease in animals, in the form of a monograph. Bassi was an Italian lawyer and naturalist who abandoned public posts in 1816 to devote full time to agriculture. At the time, muscardine, a disease of silkworms, was ravaging the silkworm industries in Italy and France. The prevailing notion was that death of the worms was due to some vague environmental cause (state of the atmosphere?). Bassi had the idea that the disease was caused by an "extraneous germ," and he soon discovered that a white material which always developed on dead worms was the infectious matter. He concluded that every outbreak of the disease could be traced to infected silkworms or use of contaminated cages or utensils. Moreover, he demonstrated that suitable precautions could prevent outbreak of the disease, for example, disinfection of silkworm eggs with alcohol and disinfection of all instruments and implements used in the nursery.

Despite his failing eyesight, Bassi identified the culprit of muscardine as a microscopic fungus. It was an organism known as *Botrytis paradoxa*. An Italian botanist confirmed the identification and renamed the fungus *Botrytis bassiana*. Because of the onset of blindness, Bassi could no longer continue microscopic work, but pursued development of his "parasite theory of disease" in connection with plague, syphilis, cholera, and other infectious processes. This pioneer received a number awards from both Italian and foreign

academies, but his momentous research was not properly appreciated by a number of subsequent investigators, including those of the "Pasteur school."

MICROBES

AN INVISIBLE UNIVERSE

HOWARD GEST (2003)

DEL MAL DEL SEGNO

CALCINACCIO o MOSCARDINO

Malattia che affligge

I BACHI DA SETA

E SUL MODO

DI LIBERARNE LE BIGATTAJE

ANCHE LE PIÙ INFESTATE

Opera

DEL DOTTORE AGOSTINO BASSI

DI LODI

la quale oltre a contenere molti utili precetti intorno al miglior governo dei Filugelli, tratta altresì delle Malattie

DEL NEGRONE E DEL GIALLUME

LODI

DALLA TIPOGRAFIA ORCESI

1835

PASTEUR, Louis (1822-1895)

KOCH, Robert (1843-1910)

ASM

1876: Robert Koch publishes a paper on his work with anthrax, pointing explicitly to a bacterium as the cause of this disease. This validates the germ theory of disease. His work on anthrax was presented and his papers on the subject were published under the auspices of Ferdinand Cohn.

1880: Louis Pasteur develops a method of attenuating a virulent pathogen, the agent of chicken cholera, so it would immunize and not cause disease. This is the conceptual breakthrough for establishing protection against disease by the inoculation of a weakened strain of the causative agent. Pasteur uses the work "attenuated" to mean weakened. As Pasteur acknowledged, the concept came from Jenner's success at smallpox vaccination.

1881: Robert Koch struggles with the disadvantages of using liquid media for certain experiments. He seeks out alternatives, and first uses an aseptically cut slice of a potato as a solid culture medium. He also turns to gelatin, which is added to culture media; the resulting mixture is poured onto flat glass plates and is allowed to gel. The plate technique is used to isolate pure cultures of bacteria from colonies growing on the surface of the plate.

1884: Robert Koch publishes *The Etiology of Tuberculosis*, in which he followed three steps: 1) the presence of the tubercule bacillus (as proved

by staining) in tubercular lesions of various organs of humans and animals, 2) the cultivation of the organisms in pure culture on blood serum, and 3) the production of tuberculosis at will by its inoculation into guinea pigs. Koch was awarded the Nobel Prize in Physiology or Medicine in 1905.

1885: Louis Pasteur oversees injections of the child Joseph Meister with "aged" spinal cord infected with rabies virus. Pasteur uses the term "virus," meaning poison, but has no idea of the nature of the causative organism. Although the treatment is successful, the experiment itself is an ethical violation of research standards. Pasteur knew he was giving the child successively more dangerous portions.

Bulloch: Pasteur, Louis – Great French chemist and bacteriologist. Born, the son of a tanner, at Dôle (Jura) 27 Dec. 1822. Spent early life at Arbois, and was educated there and at Besancon and the École normale, Paris. Was Prof. of physics at the Lycée of Dijon 1848, and of chemistry in Strassburg 1852. Dean of the faculty of science at Lille 1854. Director of Studies in the École normale, Paris (Rue d'Ulm). In 1848 he discovered the true nature of tartaric acid and revealed the connexion between right and left handedness of crystalline form (enantiomorphism) and optical activity. Received Rumford medal of the Royal Society (1848) for his discovery of the nature of racemic acid, and its relation to polarized light. Pasteur carried out epoch-making researches demonstrating the connexion between various fermentations and the activity of living micro-organisms. Lactic fermentation 1857, alcoholic

fermentation 1858-60, butyric fermentation 1861, acetic fermentation 1861-64. *Études sur le vin*, 1866; *Études sur la bière*, 1876. His *Les maladies des vers à soie* dates from 1865 to 1870. By his researches on spontaneous generation (1860-1) he destroyed this ancient belief. In 1877 he turned his attention to the study of the causes and prevention of infective diseases in man and animals. He discovered the protective properties of attenuated virus in fowl cholera 1880, anthrax 1881, swine erysipelas 1882, rabies 1884. He was elected a foreign member of the Royal Society 1869 and received its Copley medal 1874. In his honour the Pasteur Institute in Paris was founded 1888. He died at Villeneuve l'Etang, near Garches, on 28 Sept. 1895, and received a State funeral with military honours. He was buried in a magnificent crypt in the Institut Pasteur, Paris. Statues of him have been erected in Dôle, Arbois, Besancon, Lille, Alais, Melun, Chartres, Marnes, and three in Paris. To his memory a great monument was inaugurated in Strassburg in 1923.

Koch, Robert – By common consent the greatest pure bacteriologist. Born in Clausthal, Hanover, the son of a mining engineer. Studied in Univ. of Göttingen under Wöhler, G. Meissner, and J. Henle. Graduated as doctor of medicine 1866. Practised in Niemegk and Rakwitz. Served as surgeon in Franco-Prussian war, and in 1872 became Kreisphysikus in Wollstein. Published his classical research on anthrax in 1876, on the technical methods of bacterial examination in 1877, and on the etiology of traumatic infective diseases 1878. Became associated with the Gesundheitsamt in Berlin and founded famous school of bacteriology

there. In 1881 he solved the problem of pure bacterial cultures and his methods were universally employed. Discovered tubercle bacillus 1882, and cholera Vibrio in 1883. In 1890 made known his discovery of tuberculin, and in 1900 his views on the non-identity of human and bovine tuberculosis. From 1891 to 1904 Koch was director of the Institut f. Infektionskrankheiten in Berlin. In 1896 he investigated rinderpest in S. Africa and sleeping-sickness in Uganda. Travelled extensively studying protozoal diseases in the East. Received Nobel Prize 1905 and was enobled with the title of Excellenz. Foreign member of Royal Society 1897. Died of cardiac failure 27 May 1910, aged 67. His ashes deposited in the Institut für Infektionskrankheiten, Berlin.

Without doubt, there has been more written about Pasteur and Koch than any other microbiologists. Following his important work on microbial fermentations and his disproof of spontaneous generation, Pasteur embarked on study of infectious diseases. From 1875 to 1890, Pasteur and Koch were in the limelight of research advances, and they became fierce competitors in seeking recognition for their discoveries. Unfortunately, their interactions became acrimonious and had nationalistic overtones. Pasteur was a formidable "warrior" and Koch was also combative. They both attended an International Congress of Hygiene in 1882 where they displayed heated antagonism and traded professional insults. A 1995 book by Gerald Geison, *The Private Science of Louis Pasteur* (Princeton Univ. Press) claims that Pasteur was guilty of deception, of stealing other people's ideas, and of unsavory and unethical conduct, In a vigorous defense of Pasteur, Perutz

(2003; "Deconstructing Pasteur", pages 135-145) concludes: "Pasteur may have been domineering, intolerant, pugnacious, and, in his later years, a hypochondriac who searched every slice of bread for bacteria before eating it; but he was courageous, compassionate, and honest, and his scientific achievements, which have much reduced human suffering, make him one of the greatest benefactors of mankind."

More about the accomplishments and lives of Pasteur and Koch can be found in Gest (2003) and in books by T. Brock: *Milestones in Microbiology*, and Robert Koch: *A Life in Medicine and Bacteriology* (1999; ASM Press). Brock's "*Milestones*" includes excerpts from important papers by Pasteur and Koch, translated by Brock, who added interpretive comments.

Three Giants of Infectious Disease Research: Pasteur, Koch and Jenner

From Gest (2003):

Pasteur's later researches focused on infectious diseases, at first on diseases of silkworms. Emile Duclaux described how this came about. Duclaux (1840-1904) was a French chemist and bacteriologist who served as a professor at several French universities and succeeded Pasteur as Director of the Pasteur Institute in Paris. Duclaux's biography of Pasteur, *Pasteur—History of a Mind*, was published in 1896, one year after Pasteur's death. According to Duclaux, during a protracted epidemic that was affecting silkworms and ruining the French silk industry, Senator J. B. Dumas convinced Pasteur to work on the problem. Curiously, Duclaux discusses the background of information that was available on silkworm diseases without mentioning Bassi. It is ironic that Pasteur's misinterpretation of certain observations led him temporarily astray as to whether or not one of the apparently infectious diseases of silkworms was in fact caused by a microbe. In any event, this research was Pasteur's introduction to later study of infectious disease in more highly evolved domestic animals and humans. Pasteur had remarkable insight and imagination, excellent technical skills, good organizational ability, and political acumen and, in addition, was a formidable warrior. A biography published three years after his death (Frankland and Frankland, 1898) gives the essence of his disposition:

"It is in this connection that we realize that Pasteur was not only a savant content to seek the truth and find it, but that when he had in any matter suc-

ceeded in the difficult task of convincing himself, he was impelled with almost a fanatic's zeal to force his conviction on the world, nor did he put up his sword until every redoubt of unbelief had been taken, every opponent converted or slain."

Koch, 21 years younger than Pasteur, was also combative, and in some ways excelled Pasteur as an experimenter, at least in bacteriology. Koch's initial research (while practicing as a country doctor) was concerned with anthrax, primarily a disease of cattle, sheep, and horses, but which can also affect other domestic animals and humans. At the time, anthrax epidemics were commonplace in Europe and had ruinous effects on small farms. Koch isolated the bacterium *Bacillus anthracis* from diseased animals in pure culture and showed by the most rigorous criteria that this organism was the causative agent of anthrax. This was the first instance in which a specific microbe was demonstrated to be the cause of an infectious disease in a higher animal. Koch later isolated the bacteria that cause tuberculosis (1882) and cholera (1883). In his research he refines that strategy required to unambiguously identify the cause of microbial disease, the so-called Koch's Postulates. One version of these is as follows:

1. The microbe must be present in every case of the disease.
2. It must be isolated from the diseased host and grown in pure culture.
3. The same specific disease must result when a pure culture of the microbe is inoculated into a healthy susceptible host.
4. The microbe must be recoverable once again from the experimentally infected host.

These criteria proved to be important in much later research, but in some instances they could not all be met easily. An outstanding example in this connection is leprosy. The bacterium responsible, *Mycobacterium leprae*, was identified in 1872, but a susceptible laboratory animal was not discovered until 1971 (surprisingly, the armadillo).

Vaccination and Immunity

The last phase of Pasteur's meteoric career was concerned primarily with prophylaxis against infectious disease, in particular by *vaccination* procedures. This was not a new concept; inoculation to induce immunity to smallpox had been practiced for centuries. According to F. F. Cartwright (*Disease and History*, T. Y. Crowell, New York, 1972), physicians in ancient China "removed scales from the dying pustules of a person suffering from mild smallpox, ground the scales to a fine powder, and blew a few grains of this into the nostril of the person to be protected." Another procedure was publicized in 1717 by a remarkable 29-year-old woman, Lady Mary Montagu, wife of the British ambassador to Turkey. She observed that every September a group of old women made rounds of houses in Constantinople, where families would gather for 'ingrafting" ("inoculation parties"). Each practitioner carried, in a nutshell, a small sample of pus collected from a victim of a mild attack of smallpox. She would quickly scratch open a vein on a limb of the "customer" with a needle, dip the needle into the pus, smear it on the open vein, and then bind the wound. Lady Mary wrote to a friend about the response of children treated in this way:

"... they play together all the rest of the day, and are in perfect health to the eighth. Then the fever begins to seize them, and they keep their beds two days, very seldom three ... and in eight days' time they are as well as before their illness ... Every year thousands undergo this operation; and the French ambassador says pleasantly, that they take the smallpox here by way of diversion, as they take the waters in other countries. There is no example of anyone that has died in it; and you may believe I am very well satisfied of the safety of this experiment, since I intend to try it on my dear little son."

Which she did. Her son became the first known Englishman to be vaccinated against smallpox. By 1722, King George I was persuaded to have two of his grandchildren similarly inoculated (beforehand, six prisoners under sentence of death volunteered to be guinea pigs on promise of reprieve). Lady Montagu became a celebrity.

The inoculation procedure worked well most of the time, but there were occasional failures. The method was totally empirical, and sometimes the child would actually become ill with smallpox. This happened to the young Edward Jenner (1749-1823) during a severe epidemic in England. He recovered and was thereafter immune to the disease, which became a definite advantage in his later work. Jenner became a country doctor and used the procedure himself on children of his patients. Jenner was aware of the old wives' tales that people who suffered from the mild disease "cowpox" became resistant to smallpox. Cowpox would first appear on the teats of infected cows as inflamed pustules and would quickly spread throughout the herd. Dairymaids and milkmen would then develop sores on the ends of their

fingers and at the finger joints. The sores would spread to other parts of the body and a fever would set in, which usually subsides after a few days.

Jenner hypothesized that cowpox was a form of smallpox, and he closely observed numerous cases. In May 1796, he performed one of the classic experiments in the history of medicine. In his words: "I selected a healthy boy, about eight years old, for the purpose of inoculation for the Cow Pox. The matter was taken from a sore on the hand of a dairymaid who was infected by her master's cows." Jenner smeared pus into several deep scratches on the arm of James Phipps. Seven days later, the boy had an eruption on his arm at the site of the scratches and discomfort in his armpits, but he recovered within a few days. On July 1, Jenner inoculated James with "matter" from the pustules of a person ill with smallpox. The smallpox matter had no effect, and Phipps was subsequently inoculated many times in the same fashion with no ill effect. Jenner had reinvented vaccination *as a scientific procedure.*

MOLISCH, Hans (1856-1937)

Molisch was an exceptionally gifted and productive researcher who had broad interests in plant biology, physiology and biochemistry. In addition, he pioneered in isolating a number of species of purple photosynthetic bacteria in pure culture which led to his discovery of basic aspects bacterial photosynthesis. Molisch demonstrated conclusively that molecular oxygen is not produced by photosynthetic bacteria, and discovered the photoheterotrophic growth mode. The range of Molisch's research accomplishments was impressive, and he emerged as a major figure in the history of photosynthesis research. Aside from his work on photosynthesis, he did notable research on iron metabolism in plants and microorganisms, and on luminescent bacteria. At the time of his formal retirement in 1922, he was Professor of Botany and Director of the Plant Physiology Institute, University of Vienna.

Molisch was born December 6, 1856 in Brünn, Moravia, now called Brno, Czechoslovakia. When he was nine years old, he had a scientific encounter that was to remain a vivid memory for the rest of his life. During Molisch's youth, Brünn was a city with a strong commitment to the natural sciences, and Molisch describes the memorable incident in his autobiography (Molisch 1934):

"As a nine-year-old boy I had an interesting experience at a grape harvest festival. One of the (Molisch's) vineyards, called the "King", lay just behind the walls of the monastery of the same name and the prelate Gregor

Mendel was the head of the Augustinian chapter there. Earlier, as a secular priest, he was professor of natural sciences at the high school (Brünn Technical Secondary School) in the Johannesgasse where my two brothers, Ferdinand and Edmund, had the good fortune of being his pupils. Mendel was very favorably disposed to our family. When Ferdinand was in Dresden for a year at the well-known Wagner nursery in order to learn especially about azaleas, rhododendron and heather, the abbot visited him while passing through, which one can fully appreciate today, after Mendel has become famous as a biologist. On one of the days of the grape harvest, the prelate came over to us from the neighboring monastery and greeted my mother, who was pleasantly surprised by this important visit. I still remember that charming man, of medium height, wearing the priest's black cassock, highly polished boots, gold-rimmed glasses, and engaging features. As my mother presented him the sweetest of grapes on a tray, he spoke to me and my sister, at times seriously, at times joking in an affable manner. At the time, none of us had any inkling that he would achieve world fame in science equal to that of Darwin." This encounter occurred in 1865, the same year in which Mendel published his findings on heredity.

Research on iron bacteria

A continuing interest in the biological roles of iron led to Molisch's classic monograph *Die Eisenbakterien* (Molisch 1910). His experimental results contradicted some interpretations of Winogradsky on the physiology of iron bacteria, and were summarized in Marjory Stephenson's influential book *Bacterial Metabolism* (Stephenson 1949) as follows: "One of the

principal opponents of Winogradsky's views was Molisch, who himself made important contributions to the study of the group. He was first to obtain *Leptothrix ochracea* in the pure culture, and showed that it is not a strict autotroph, but can grow in peptone, with or without iron. Furthermore, he showed that if in such media manganese replaces iron, the oxide of the former metal is deposited in the sheath of the organism. From these observations he concluded that iron plays no essential part in the metabolism of this organism, and that the deposit of oxides of iron (or manganese) in the sheath is due to adsorptive processes and has no connection with any metabolic function, being, in fact, paralleled by iron accretions in certain *algae*, moulds, *infusoria*, and flagellates, where no physiological role is assigned to it."

Die Purpurbakterien (1907)

Molisch's important contributions to our knowledge of photosynthetic bacteria were described in his classic monograph of 1907. On the basis of numerous experiments, he established that the nonsulfur purple bacteria are widely distributed in nature, and also demonstrated that many types could be readily enriched by providing sources of organic substances, light, and restricted oxygen supply. He isolated and characterized pure cultures of type species, including *Rhodobacter capsulatus*, *Rhodospirillum photometricum* and *Rhodopsewdomonas palustris.* The type species *Rhodospirillum rubrum* is designated as 'Esmarch/Molisch' because Molisch demonstrated that the organism *Spirillum rubrum*, isolated as an ordinary heterotroph by Esmarch (1887) from the dried residue of a dead mouse, was in fact a photosynthetic purple bacterium.

One major conclusion in Molisch's monograph: "For the present I am satisfied that the nutritional experiments with the purple bacteria have familiarized us with a *new type of photosynthesis* in which organic substance is assimilated in the light, and whereby the two pigments, the bacteriochlorin and bacteriopurpurin, probably play an analogous role to that of chlorophyll and carotenoid in the carbonic assimilation of green cells."

A second major aspect was concerned the absence of O_2 production by purple bacteria. Using very sensitive O_2-detection methods, he was able to make critical test for photosynthetic oxygen production. The results were invariably negative. It is astonishing that the notion of possible production of O_2 by purple bacteria lingered on until 1954 when an investigation using ^{18}O as a tracer confirmed once again that O_2 is not produced by *Rhodospirillum rubrum*.

Molisch's firm conclusion that the purple bacteria are incapable of producing molecular oxygen should have settled the question in 1907. It is interesting that although Molisch's main interests focused on green plants, he did not feel compelled to "explain" anoxygenic photosynthesis as a variation of the oxygenic process. Misguided efforts to develop a 'unitary' theory of photosynthetic processes based on "rationalizing" the absence of oxygen production by the bacteria probably delayed conceptual advances for some time.

A serendipic adventure based on misinterpreting Molisch's remarks:

The notion of testing earthworm contents as possible sources of purple photosynthetic bacteria was suggested to H. Gest and J. Favinger by a hasty

reading, and misinterpretation, of remarks made by Hans Molisch in his autobiography. He was a visiting professor at the Bose Institute in Calcutta during 1928/1929, and noted the following experience: "From time to time during the preparation of the lectures, something unexpected would take place. One day I explained to my assistant, a very devout Hindu who belonged to a particular sect whose followers were not allowed to kill animals, how one could obtain purple bacteria in the laboratory with relative certainty. For this purpose it was only necessary to fill a long glass tube with *tap water*, put in pieces of a chopped up earthworm, cover the water with a layer of oil and then let the whole thing stand in the sun, whereupon the purple bacteria appear in 1-2 weeks and color the water red. When I checked up after a few days on whether the assistant had initiated the experiments correctly, I saw that everything was in order, except that the earthworm was not cut up, but moving around in a lively fashion at the base of the tube. When I drew the assistant's attention to this, he looked at me almost in fear, with wide eyes, and asked quite remorsefully for forgiveness for not having been able to bring it upon himself to kill the earthworm. Attention may not be drawn to such events in India when one considers that here widespread sects of the Jains kill no animals at all, not even fleas, lice and mosquitoes; they even have the evening meal before sunset, because if they would eat after sunset, many insects would then fly into their lamps and be killed."

Assuming that Molisch knew that earthworms harbored purple bacteria, and believing that this was a reasonable proposition, we set up enrichments using earthworms puree as inoculum for media containing organic acid carbon sources. Then, we reread Molisch's 1907 paper to see if earthworm

enrichments were described in his classic monograph. It became clear that minced earthworms were used by Molisch in 1907 not as sources of photosynthetic bacteria, but rather to provide organic carbon substrates for growth of the purple bacteria present in Prague tap water! Molisch states that the tap water in Prague at that time was undrinkable (he and his family drank only boiled water imported from elsewhere), but was a veritable [El] "Dorado" of interesting microorganisms of many kinds. The tap water in Calcutta in 1928/1929 must also have been a "microbiological zoo".

Although we had set up our enrichment cultures on a mistaken premise, we allowed them to incubate, and were pleasantly surprised to observe that a number of them developed luxuriant growth of purple bacteria. From these we isolated most commonly *Rhodomicrobium vannelii* and *Rhodopseudomonas palustris*. There are at least 1800 species of earthworms, and the worm batches used in our trials were not necessarily of a single species. It is likely that other species of purple bacteria could be obtained this way by modifying the composition of the enrichment medium. So, we proved once again that serendipity is alive and well! (See Gest and Favinger 1992).

References:

Molisch, H. (1907) Die Purpurbakterien nach neuen Untersuchungen. Gustav Fischer, Jena.

Molisch, H. (1934) ErinMerungen und Welteindrücke eines Naturforschers. Emil Haim. Vienna and Leipzig.

Gest, H. (1991) The legacy of Hans Molisch, photosynthesis savant, Photosyn. Res. 30: 49-59.

Gest H. and Favinger, J.L. (1992) Enrichment of photosynthetic bacteria from earthworms. FEMS Microbial. Lett. 91: 265-270.

Gest, H. (1997) Serendipity in scientific discovery: a closer look. Persp. Biol. Med. 41: 21-28.

Serendipity in Gest's experiments with photosynthetic bacteria, 1948

For the summer of 1948, I was a "research student" in C.B. van Niel's laboratory at the Hopkins Marine Station in Pacific Grove, CA. My project involved testing the vigor of photosynthetic growth of *Rhodospirillum rubrum* in various kinds of media. I was astonished to observe that in one particular recipe, the bacterium produced copious amounts of molecular hydrogen. This phenomenon had never been observed before.

Utilization ("fixation") of nitrogen gas (N_2) by photosynthetic bacteria

Back in St. Louis during the fall of 1948, my attempts to demonstrate the time course of (light-dependent) H_2 production in a sensitive way that could be measured with accuracy were frustrated. For these experiments, I used *R. rubrum* cells obtained from H_2-producing cultures, resuspended in a dilute solution of mineral salts under an atmosphere of N_2. In experiment after experiment, not a trace of H_2 was produced. After many trials, in January 1949, I performed a deliberate experiment based on rather "loose" reasoning, in which I replaced the supposedly *inert* gas phase consisting of 100 percent N_2 with 100 percent H_2. For the first time, I observed rapid production of H_2 as I watched the detection device. This and related experiments quickly showed that N_2 was *not* inert for *Rhodospirillum rubrum*, and that the bacterium could use atmospheric N_2 gas as a source of nitrogen for growth.[8,9,10]

Before 1949, only three kinds of bacteria were known to use ("fix") N_2, and these were identified before 1900. Thus, *R. rubrum* was the first new nitrogen fixer found in the 20th century, and this stimulated other investigators to renew the search for other kinds. Of the 60 known species of photosynthetic bacteria, 58 can use N_2. There are reasons to believe that this capacity as well as the related process of H_2 production are ancient kinds of metabolic reactions that probably were used by bacteria on the early Earth, that is, before the atmosphere contained oxygen gas. Experiments with *R. rubrum*[11,12] gave the first indications that both the utilization of N_2 and the production of H_2 were catalyzed by the same enzyme system.

Homage to Ferdinand J. Cohn, Driving Force in the Emergence of Modern Microbiology

[Postscript to "Historical Adventures in Scientific Discovery/Microbiology/Biochemistry"

https:scholarworks.iu.edu/dspace/handle/2022/3358 (2009)]

1875

Untersuchungen über Bacterien.

Von

Dr. Ferdinand Cohn.

Mit Tafel III.

Als ich vor nahezu 20 Jahren meine ersten Untersuchungen über Bacterien veröffentlichte (Ueber die Entwicklungsgeschichte mikroskopischer Algen und Pilze Nova Acta Ac. Car. Leop. nat. cur. XXIV. I. 1853), waren es überwiegend morphologische nnd entwicklungsgeschichtliche Fragen, an die sich das Interesse für diese kleinsten aller Organismen, durch welche nach Ehrenbergs sinnigem Ausspruch die Milchstrasse der lebenden Wesen hindurchgeht, knüpfte, und noch wenig entwickelt waren die Gesichtspunkte, welche in den letzten Jahren die Geschichte der Bacterien mit den wichtigsten Problemen der allgemeinen Naturwissenschaft in Zusammenhang gebracht haben. Zwei Männer sind es vor allem, deren Arbeiten, wenn auch von ungleichem Werth, doch fast in gleicher Weise dazu beigetragen haben, das Interesse für die Bacterien in den weitesten Kreisen anzuregen. Pasteur hat zwar die Bacterien nur beiläufig berührt und die Schwäche seiner mikroskopischen Bestimmungen beeinträchtigt seine Arbeiten, so weit sie diese Organismen berühren, in viel höherem Grade als bei seinen epochemachenden Forschungen über die Hefepilze; dennoch müssen alle neuern Untersuchungen zunächst an die Pasteur'sche anknüpfen. Auf der andern Seite gebührt Hallier unzweifelhaft das nicht gering anzuschlagende Verdienst, dass derselbe zuerst die Frage von den Beziehungen der Fermente und Contagien zu den Bacterien, welche früher meist nur auf theoretischem Wege erörtert worden waren, zum Object directer und Jahrelang fortgesetzter mikroskopischer Untersuchungen gemacht, und es ist nicht genug zu bedauern, dass alle spätern Beobachter, deren nicht wenige in Deutschland und England zunächst durch Hallier angeregt worden sind,

The history of microbiology spans almost 350 years, starting with the discoveries of Robert Hooke and Antoni van Leeuwenhoek in the 17th century (Gest 2004, 2009a). From my studies, I conclude that "modern" microbiology emerged in the late 19th century from the singular efforts of a relatively small number of gifted investigators. Prominent among them were: Ferdinand Cohn (1828-1898), Louis Pasteur (1822-1895), Robert Koch (1843-1910), Martinus Beijerinck (1851-1931), and Sergei Winogradsky (1856-1953). This essay focuses on Cohn, who is not well known to most contemporary microbiologists still active in research. Pasteur and Koch are much more familiar; they are lauded, even in the skimpy historical sections of current textbooks. The important roles of Beijerinck and Winogradsky in developing understanding of microbial ecology, diversity, and chemical activities of microbes in the biosphere are discussed in Gest 2009b and 2009c. Here, I focus on Cohn, who deserves to be remembered and celebrated as a "prime mover" into the modern era.

How important scientific discoveries are made

Cohn was a truly creative scientist in pioneering the development of "modern" microbiology at a particularly important time. "Spontaneous generation" of microbial life was a major topic of current discussion and Cohn's discoveries were crucial in ending the debate. The sources of creativity in science and art was of special interest to Max Perutz (Nobel Laureate 1962), who pinpointed their major features (Perutz 1989): "Great scientists and artists have one [other] trait in common--they both tend to be single-mindedly devoted to their work. Renoir painted every day of his life, and when old age made his fingers too arthritic to hold a brush, he got someone to tie the brush to his hand. Haydn rose early each morning to compose; if ideas failed him, he clasped his rosary and prayed until Heaven sent him fresh inspiration. Tolstoy rewrote *War and Peace* seven times. When Newton was asked how he had arrived at his insights, he answered 'By keeping the problem constantly before my mind.' There is little benefit in following scientists' daily grind but much in

tracing the unique combinations of theoretical knowledge and manual skills, the web of personal encounters and accidental observations , the experience, temperament, moods and clashes that go into the making of discoveries, even though the crucial leap of the mind is often impenetrable." There is no doubt that Cohn had the creative gift, as well as energy, drive, and foresight.

<u>From botany to micobiology</u>

The present article presents an account of Cohn's life and important accomplishments, and also provides references to pertinent literature. I begin with a condensed "biographical notice" from William Bulloch's great classic of 1938...The History of Bacteriology:

"COHN, Ferdinand" (born 1828, died 1898). Great German botanist and one of the founders of bacteriology. Born in Breslau, where he was for many years Prof. of Botany. He early took to the study of microscopic algae and fungi and made many important discoveries. From 1860 onwards devoted himself

particularly to the study of bacteria and became the leading authority on the subject. He was one of the first to hold that bacteria can be arranged in genera and species which exhibit a high degree of constancy. Much of our knowledge is based on his work. He supported Pasteur's ideas on spontaneous generation in opposition to Pouchet and Bastien, and first clearly described bacterial spores. He wrote a great deal and most of it was accurate. He discovered Robert Koch and befriended him. In Cohn's *Beiträge zur Biologie der Pflanzen* appeared many of the classical papers on bacteriology by Cohn, Schroeter, Koch, and others. Cohn was a man of great diligence and talent and personally a fine character."

In discussion of Cohn's research, Bulloch also comments: "His researches were the result of many years laborious work and he was successful in disentangling almost everything that was correct and important out of a mass of confused statements on what at that time was a most difficult subject to study. *His work was entirely modern in its character and expression,*

and its perusal makes one feel like passing from ancient history to modern times [my italics]. He was clear, explicit, and fair in his judgment to other workers, and on every page it is apparent that he wrote from first-hand knowledge. In his paper of 1872 he at once raised the fundamental question whether, like other plants or animals , bacteria can be arranged in genera and species."

It is relevant that a number of fundamental aspects of bacterial evolution, classification, and nomenclature are still unresolved (e.g., there is still no generally accepted definition of a bacterial species). In 1946, C.B. van Niel wrote an important essay on these subjects in which he reviewed Cohn's ideas. Van Niel noted "....Cohn appreciated the great significance which attaches to a stable and generally accepted nomenclature," and described Cohn's contributions "for the time he worked, masterly: he furnished sufficiently complete descriptions of several species so that the organisms could be recognized by others, using the same general approach, and he supplied a sort of

key for the allocation of a bacterium to one of the six genera which he proposed and consolidated into four tribes."

The first journal that can be understood as a microbiological journal in the modern sense was established and published by Cohn himself...Beiträge zur Biologie der Pflanzen [i.e., Contributions to the biology of plants]. Volumes 1 and 2, dated 1875-1877, were bound together and contained 235 pages devoted to basic research on bacteria. Cohn was the author of 188 pages, and Robert Koch authored a 31 page paper describing his epoch-making discoveries on the etiology of anthrax. One of Cohn's papers included a section on the behavior of bacteria to "extreme temperatures," foreshadowing current preoccupation with "extremophiles" by about a century.

<u>Cohn's academic career</u>

The following is a composite of accounts based largely on Bulloch (1938), a profile in the Dictionary of Scientific Biography by Geison (1971), and papers by Gerhart Drews (see below).

Cohn began studies of natural sciences (major subject, botany) in Breslau in 1844. His application for the doctoral program at the university was refused because of his Jewish faith. Undaunted, he proceeded to the University of Berlin in 1846 and received his doctorate in botany in 1847, at the age of 19. He returned to Breslau where he completed a second dissertation (Habilitation) and became a lecturer in 1857. Eventually, in 1872, he was promoted to full professor rank. Meanwhile, he had agitated for establishment of an institute of plant physiology. "In 1866, the Breslau authorities finally acceded to Cohn's long-standing request and acquired a nearby building that had once been a prison. In these inauspicious surroundings Cohn founded the first institute for plant physiology in the world, and soon launched the second great creative period of his career....About 1870, Cohn turned his attention primarily to bacteria, and it is for his researches in this area that he is best known. In 1870 he founded a journal, *Beiträge zur Biologie der Pflanzen*, designed primarily to publish the work that came out of

his institute. In this journal appeared the founding papers of modern bacteriology" (Geison 1971).

Cohn and Robert Koch

As indicated earlier, Cohn was instrumental in launching Koch's transformation from country doctor to great fame (Nobel Prize 1905). Bulloch: " We are introduced to Koch by Ferdinand Cohn (1876), who tells us [*in Beiträge*] that it was with great pleasure that he received a letter, dated 22 April 1876, from Dr. Koch to the effect that after prolonged investigations he had discovered the complete life-history of the anthrax bacillus, and that he was prepared to come to Breslau to demonstrate his work to Cohn. The meeting took place in Cohn's institute on 30 April 1876, and lasted three days, in which time Koch completely convinced his audience of his discovery. The occasion is historic....Koch's discovery, published (1876) under the aegis of Ferdinand Cohn, immediately became widely known, and it was at once recognized that a great investigator had arisen in the field of bacteriological research. The early hopes raised by Koch's first

publication were not frustrated, for, along with Pasteur, he remains to-day the greatest exponent of bacteriological science. In connection with his rise to fame I cannot refrain from adding a tribute to the memory of Ferdinand Cohn, who behaved towards Koch in a most generous way. Along with [Julius] Cohnheim he was largely responsible for giving Koch a proper start in his scientific career, and they did everything in their power to further his worldly interests and set him free from the hum-drum of medical practice so that he could get scope for his great talents."

The historic 1876 letter from Koch to Cohn is included in the biography of Cohn by his wife Pauline (Cohn, P. 1901). Brock's biography of Koch (1998) gives an English translation of the letter, which follows: "Honored Professor!

I have found your work on bacteria, published in the *Beiträge zur Biologie der Pflanzen*, very exciting. I have been working for some time on the contagion of anthrax. After many futile attempts I have finally

succeeded in discovering the complete life cycle of Bacillus anthracis. I am certain, now, as a result of a large number of experiments, that my conclusions are correct, However, before I publish my work I would like to request, honored professor, that you, as the best expert on bacteria, examine my results and give me your judgement on their validity. Unfortunately, I am not able to send you preparations which would show the various developmental stages [*including spores*] as I have not succeeded in conserving the bacteria in appropriate fluids. Therefore, I earnestly request that you permit me to visit you in your Institute of Plant Physiology for several days, so that I might show you the essential experiments. If this request is agreeable to you, perhaps you might inform me of a suitable time that I could come to Breslau.

Very sincerely yours,

Dr. Koch, Kreisphysikus"

Koch's title indicates that he was in practice as a District Medical Officer for the province of Wollstein, Prussia.

Cohn's career and contributions reviewed by G. Drews

Two lengthy articles by microbiologist Gerhart Drews (1999, 2000) review Cohn's personal life, scientific career, and impacts on the development of microbiology. The 1999 article covers Cohn's botanical research on plants and microalgae thoroughly; 153 references including a comprehensive list of Cohn's major publications. His 2000 paper has a somewhat different perspective, which is evident from the title; the paper discusses scientific progress in biology and chemistry in the 17th and 18th centuries and then focuses on the 19th century in respect to early classification of microorganisms, concepts of taxonomy, and the "spontaneous generation of living organisms" controversy. He gives a detailed history of the latter because Cohn played a major role (together with Pasteur and Tyndall) in its demise. One of Cohn's major discoveries was the fact that certain bacteria produce heat resistant spores, especially *Bacillus* species. "The results of Cohn and Tyndall explained many of the controversial results of the advocates and

opponents of the doctrine of spontaneous generation, especially the observation that hay infusion, which very often contains heat-resistant spores, resists boiling" (Drews 2000). Bulloch (1938) devotes 58 pages to this topic!

Discovery of spore formation in bacteria

An English translation, by Thomas Brock, of one of Cohn's classic research papers became available in 1961 (see Suggested Reading). Part of one section of the paper describing the formation and generation of spores of *Bacillus subtilis* is an excellent example of Cohn's astute observations and clear writing style: "The process of spore formation can only be observed by careful observations with very strong immersion systems. Although the *Bacillus* filaments seem to be without cross walls even under the strongest magnification, this is in reality not the case. The single members which make up he filament are four times as long as wide. In each member a spore develops, which does not fill the cavity completely, but is separated from the empty cell membrane on each side. The spores are

1.5-2.2 microns long and 0.8 microns wide....In their development they seem to resemble those of Nostocaceae (*Cylindrospermum, Nostoc. Spermosira,* etc.) the most. Depending on whether the *Bacillus* filaments are shorter or longer, out of two or more members, we find the spores in a filament arranged in short chains of two or more. By decomposition of the *Bacillus* filaments, single members become isolated which contain only single spores. When these have completely separated from their mother cell, they show a delicate, jelly-like enclosure (spore membrane) and a strongly refracting interior.... With the maturation, release and settling out of the spores, the development of the *Bacillus* is ended and no further changes take place in the hay infusion...."

The same paper gives Cohn's account of the extraordinary visit of Koch to Breslau: "To my great pleasure, I received a letter from Dr, Koch in Wollstein on 22 April. He has been occupied with studies on the anthrax contagium for a long time and has finally been able to discover the complete life cycle of *Bacillus*

anthracis*. He was willing to demonstrate this to me at my plant physiology institute and obtain my opinion of his discoveries. Dr. Koch came to Breslau from 30 April to 3 May and with anthrax material he had brought along performed in our institute inoculations into living frogs, mice and rabbits. Through this series of experiments I was given the opportunity to convince myself of the complete correctness of his discoveries on development of the anthrax bacillus.... Herr Dr. Koch reports the results of his experiments at the end of this paper and indicates the highly important conclusions which these studies yield for the nature and spread of the anthrax contagium. I will only remark here that the life history of the anthrax bacillus agrees completely with that of the bacillus of hay infusions. Indeed, the anthrax bacillus does not have a motile stage, but otherwise the similarity with the hay bacillus is so perfect that the drawings of Koch can serve without change for the clarification of my observations, and some of my drawings could serve as illustrations of the of the anthrax rods." *In fact, the figures for the paper by

Cohn are on the very same published plate with those of Koch's succeeding paper on B. anthracis.

Cohn and Charles Darwin

Cohn had an active correspondence with Darwin from 1874 to 1882, largely on botanical subjects. Darwin obviously was impressed by Cohn's wide knowledge and research acumen. Cohn clearly understood the great importance of Darwin's observations and theories, but did not hesitate to criticize certain conclusions of Darwin on plant physiology. Their correspondence has been documented by T. Junker and M. Richmond in the form of telegraphic summaries (in English) of the subject matter of each letter [Charles Darwins Briefwechsel mit Deutschen Naturforschern; Basilisken-Presse, Marburg an der Lahn, 1996]. Some relevant examples follow.

From Cohn, 21 Aug 1875: Acknowledges presentation copy of *Insectivorous plants*. Studying *Drosera* on vacation in Bohemia. Thinks CD has erred in considering 'aggregation' to have occurred in the protoplasm. Suggests it is result of exosmosis of vacuole.

To Cohn, 24 Aug 1875. Thanks for good opinion of *Insectivorous plants.* Responds to FJC's criticism regarding 'aggregation' as it occurs in protoplasm.

To Cohn, 26 Sept 1876. Invites him to visit Down.

From Cohn, 31 Dec 1876. Acknowledges presentation copy of *Cross and self fertilization.* Thanks for visit to Down. Praise for CD's theories. *The visit by Cohn and his wife is described by Mrs. Cohn in her biography of FJC.*

From Cohn, 31 Dec 1877. Sends details of Robert Koch's work on bacteria, including the first photographs. Sanderson's and Koch's collaboration on systemic fever. Thinks movement of Francis Darwin's *Dipsacus* filaments is an artifact.

To Cohn, 3 Jan 1878. Comments on discovery of micro-organisms in disease. Describes experiments carried out by Francis Darwin on the filaments of *Dipsacus.*

From Cohn, 26 Dec 1880. Response to *Movement in plants.* Setting out to confirm CD's experiments.

Believes plant cell motion, like that of animals , depends on protoplasm more than water.

Cohn and bacterial species

Drews (1999) summarizes Cohn's conceptions of bacterial species at length. Thus: "The first of his comprehensive articles on bacteria (1875) was a critical evaluation of the available data on shape and properties of the four groups of bacteria he proposed: I. Sphaerobacteria (sphere-shaped) *Micrococcus,* II. Microbacteria (rod-like) *Bacterium,* III. Desmobacteria (filamentous bacteria) *Bacillus, Vibrio,* and IV. Spirobacteria (screw-like bacteria) *Spirillum, Spirochaete*....Cohn designated the new genus *Bacillus* and the formation of endospores (light-scattering bodies) as a possible stage of propagation."

What is a bacterial species?

As a student in van Niel's renowned microbiology course (in 1947), I quickly learned that van Niel (like Cohn) had a clear-cut *practical* understanding of what a species is. He was fond of quoting the famous remark of mycologist Oscar Brefeld: "If one does not work with

pure cultures, you end up only with nonsense and *Penicillium glaucum* (i.e., *blue mold*)." What is a pure culture? It is commonly understood to be a culture of morphologically homogeneous cells, derived from successive single colony transfers, that show a consistent profile of physiological and biochemical characteristics. Such pure cultures gave us the ca. 7000 organisms, regarded as species, which are in bacterial culture collections. Arduous experimental studies of the properties of these organisms provided the basis of a "Mt. Everest" of contemporary molecular biological speculations.

<u>100 years later; *Bacillus* spores, a model system for research in developmental microbiology</u>

The Royal Society of London Leeuwenhoek Lecture for 1975 was delivered by Prof. Joel Mandelstam (1919-2008) on "Bacterial sporulation: a problem in the biochemistry and genetics of a primitive developmental system." [see Mandelstam 1976]. The lecture summarized an impressive series of investigations by Mandelstam and his colleagues at the

University of Oxford [Microbiology Unit of the Biochemistry Dept.] They analyzed the complex series of morphological, biochemical and genetic events that occur in the formation of spores by *Bacillus subtilis.* Further progress during the following decade in defining the sequence of gene expression in spore formation (regulated by at least 50 operons!) was described by Mandelstam and Errington in 1987. In the same year, Gest and Mandelstam (1987) reported observations on the longevity of bacterial spores in natural environments. We also conducted experiments to test the possibility that the survival of *Bacillus* spores over very long periods of time might be limited by the lethal effects of natural radiations. We concluded that the calculated half-life of the stored *B. subtilis* spore population that we tested would be about 7000 years. "Using this value, and assuming an exponential rate for death resulting from radiation damage, it can be estimated that a population containing 10^{10} spores initially would have a measurable number still viable after 200,000 years."

CODA:

I am indebted to Prof. Donald A. Klein (Colorado State University) for bringing my attention to Cohn's unusual efforts to communicate the latest scientific advances to the public: "In 1872, he wrote a delightful essay for non-specialists entitled 'Bacteria, the smallest living organisms.' An English translation by C.S. Dolley was published by the Johns Hopkins Press in 1939. According to Dolley, this was one of the 'earliest (such) works to be translated into English,' and had a wide-spread influence on making information on the new field of bacteriology available to Americans."

Recognition of Cohn's eminence

Geison (1971) notes a number of honors awarded to Cohn: "Cohn held an honorary doctorate from the faculty of medicine at the University of Tübingen and was named a corresponding member of the Accademia dei Lincei in Rome, the Institut de France in Paris, and the Royal Society of London [*Note: he was named a Foreign Member of the Royal Society in 1897*] . In 1885

he was awarded the Leeuwenhoek Gold Medal and in 1895 the Gold Medal of the Linnean Society."

SUGGESTED READING

Brock, T. D. (Transl. and Ed.) 1961. Milestones in Microbiology. Prentice-Hall, Englewood Cliffs. See especially "Studies on the biology of the bacilli," pages 49-56, translation of part of Cohn's 1876 paper in Untersuchungen über Bacterien. IV. Beiträge zur Biologie der Pflanzen , vol. 2, pp. 249-276.

Brock, T. D. 1998. Robert Koch/A Life in Medicine and Bacteriology. ASM Press, Washington.

Bulloch, W. 1938. The History of Bacteriology. Oxford University Press, Oxford.

Cohn, P. 1901. Ferdinand Cohn/Blätter der Erinnerung. [Leaves of Remembrance]. Kern's Verlag, Breslau.

Drews, G. 1999. Ferdinand Cohn: A Promoter of Modern Microbiology. Nova Acta Leopoldina NF 80, Nr. 312, 13-43.

Drews, G. 2000. The roots of microbiology and the influence of Ferdinand Cohn on microbiology of the 19th century. FEMS Microbiol. Revs. 24: 225-249.

Geison, G.L. 1971. Cohn, Ferdinand Julius. *In:*

Dictionary of Scientific Biography. C.C. Gillispie, Ed. in Chief, vol. III, pp. 336-341. Charles Scribner's Sons, New York.

Gest, H. 1994. A microbiologist's odyssey: Bacterial viruses to photosynthetic bacteria. Photosyn. Res. 40: 129-146.

Gest, H. 2004. The discovery of microorganisms by Robert Hooke and Antoni van Leeuwenhoek, Fellows of the Royal Society, London. Notes and Records of the Royal Society 58:187-201.

Gest, H. 2006. Associations with outstanding scientists during a research career in microbiology and biochemistry. https://scholarworks.iu.edu/dspace/bitstream/handle/2022/1083/1/Gestfinal.pdfsequence=1

Gest, H. 2009a. Homage to Robert Hooke (1635-1703): New insights from the recently discovered Hooke Folio. Persp. Biol Med. 52: 392-399.

Gest, H. 2009b. Rediscovering Pioneering Research in Microbial Ecology. Microbe 4 (No. 10): 440-441.

Gest, H. 2009c. Historical Adventures in Scientific Discovery: Microbiology/Biochemistry. https://scholarworks.iu.edu/dspace/handle/2022/3358

Gest, H. and J. Mandelstam. 1987. Longevity of

microorganisms in natural environments. Microbiol. Sciences 4: 69-71.

Mandelstam, J. 1976. The Leeuwenhoek Lecture, 1975. Bacterial sporulation: a problem in the biochemistry and genetics of a primitive developmental system. Proc. Roy. Soc. Lond. B. 193: 89-106.

Mandelstam, J. and J. Errington. 1987. Dependent sequences of gene expression controlling spore formation in *Bacillus subtilis*. Microbiol. Sciences 4: 238-244.

Perutz, M. 1989. Is Science Necessary? Essays on Science and Scientists. E. P. Dutton, New York.

van Niel, C.B. 1946. The classification and natural relationships of bacteria. Cold Spring Harbor Symposia on Quantitative Biology. Vol. XI. Heredity and Variation in Microorganisms, pp. 285-301. The Biological Laboratory, Cold Spring Harbor.

WINOGRADSKY, Sergei N. (1856-1953)

From Gest, 2003

Winogradsky, Sergei N. Winogradsky was forced out of Russia by the 1917 revolution and resumed his career at the Pasteur Institute in Paris. He developed new methods of studying soil microbes, especially those involved in the nitrogen cycle (N_2 fixation, oxidation of ammonia to nitrite and nitrate). In 1893-1895, he isolated *Clostridium pasteurianum*, an anaerobe capable of using N_2 as a sole nitrogen source. Winogradsky also made early fundamental studies on autotrophic bacteria, demonstrating that colorless sulfur bacteria can obtain growth energy by aerobic oxidation of H_2S to elemental sulfur and sulfate.

ASM

1890: Sergei Winogradsky succeeds in isolating nitrifying bacteria from soil. During the period 1890-91, Winogradsky performs the major definitive work on organisms responsible for the process of nitrification.

Winogradsky had a long peripatetic life during which he made important contributions to our knowledge of microbial physiology and ecology. His father became a wealthy banker and acquired vast estates in the Ukraine. Many of those were lost during the 1917 Revolution. Selman Waksman's biography of Winogradsky divides his life into six periods: 1881-1884, St. Petersburg, 1885-1888, Strassburg; 1891-1905, St. Petersburg

again; "Rest", on his estates in the Ukraine, 1905-1922; Pasteur Institute at Brie-Comte-Robert (near Paris), 1922-1940. Waksman[1] (originally from the Ukraine) visited Winogradsky many times and his book gives rich detail on Winogradsky's personal life and research accomplishments. At Waksman's suggestion, Winogradsky's complete works were assembled and finally published in 1949 (see refs.).

Winogradsky is particularly well known as the discoverer of chemo-synthetic autotrophy, especially of bacteria important in the Earth's S and N cycles ("sulfur bacteria", *Nitrosomonas*, *Nitrosococcus*, *Nitrobacter*).[2] He referred to them as "anorgoxydants". This research required meticulous microbiological techniques as well as development of innovative procedures. Brook's "Milestones in Microbiology" (1961) includes a translation of one of Winogradsky's important papers on nitrifying bacteria. This is followed by a "Comment", which highlights the significance of the nitrifiers.

Winogradsky, S. 1890. Sur les organisms de la nitrification. Comptes rendus de l'Académie des Sciences, Vol. 110, pages 1013-1016.

Before summarizing the work on nitrification which has occupied me for the past year, I would like to recall several of my previous works which were the point of departure for the present report.

Besides the organisms which are the subject of the present note, two groups of organisms have been studied which have the ability to oxidize

inorganic substances. I have designated them by the names sulfur bacteria and iron bacteria.

The first group live in natural waters which contain hydrogen sulfide and do not grow in media lacking this substance. This gas is absorbed extensively and oxidized by their cells and is converted into sulfur granules. These latter are in turn degraded and sulfuric acid is excreted. The second group are able to oxidize iron salts, and their life is also closely connected with the presence of these compounds in their nutrient medium.

My efforts to elucidate the physiological significance of these phenomena have led me to the concept that these inorganic compounds are the fermentable materials (in the largest sense of the word) in the life of these beings, instead of the organic materials which are fermentable substances for the large majority of the microbes. This concept leads to the logical conclusion, confirmed by experience, that these beings comprise a group with certain physiological properties which can be summarized as follows. All of the energy necessary for their vital activity would be furnished by the oxidation of mineral substances, and their dependence on organic compounds for growth would be quite slight. In addition, inorganic compounds of carbon which are not utilizable by other organisms that lack chlorophyll would be used by them as a source of carbon.

The remarkable work of MM. Schloesing and Müntz has thrown light on the role of lower organisms in the process of nitrification. However, although their work makes it highly likely that a special agent exists for nitrification, they have not succeeded in demonstrating the process away from the soil, which is a natural medium with a wide variety of microorganisms.

The principal requirement for all microbiological experiments today is the isolation in pure culture of the agent responsible for the process. Because of the difficulties involved, a number of workers have failed to isolate the nitrification ferment, so that the conclusion of MM. Schloesing and Müntz concerning the existence of this ferment has not been confirmed by bacteriologists and botanists.

This question must be clarified first. I have found that the failures of my predecessors are due to the fact that they used media which had been solidified with gelatin, such as are used to so often today for the isolation and culture of microbes. The nitrifying organisms will not grow on such media, so that if a mixture of microbes taken from a soil that is in the process of nitrifying are placed in such a medium, all of the organisms that are active die, and one only isolates those which are ineffective. It is possible, with some difficulty, to eliminate one by one all of the foreign species and to obtain pure and in large numbers the nitrifying species, by using a medium that is favorable to it but unfavorable to the other organisms. These cultures are able under the usual microbiological experimental conditions to carry out the nitrification process just as intensely as M. Schloesing has recently shown it to occur in the soil.

This organism has been more difficult to experiment with than any of the other very delicate organisms which I had previously worked with. However, its physiological properties not only confirm my conclusions, but have revealed a new fact which I would like to report to the Academy.

I applied to this study the ideas which I had already acquired concerning the nutrition of organisms which oxidize mineral substances. I cultivated the

nitrifying microbe from the beginning in a liquid which did not contain organic matter, but only a natural water that was very pure. Since the addition or organic compounds did not seem to promote its growth, I have used for its culture a mineral solution that is completely devoid of organic carbon. Although this medium does not have any other carbon compounds in it but carbonic acid and carbonates, the action of the nitrifying organism has not diminished in its intensity over several months.

We must conclude that this organism is able to assimilate carbon from carbonic acid, and this conclusion is confirmed by the amounts of organic carbon in the cultures. This demonstrates that there has been an accumulation or organic carbon by the action of this organism.

The nitrifying organism, which is colorless, is able to synthesize completely its cell substance from carbonic acid and ammonia. It carries out these syntheses independently of the light, and without other sources of energy than the oxidation of ammonia. This new fact is contradictory to that fundamental doctrine of physiology which states that a complete synthesis of organic matter cannot take place in nature except through chlorophyll-containing plants by the action of light.

It is hardly likely that the nitrifying organism exhibits a chlorophyllous action, since a release of oxygen has never been observed. Another hypothesis, that it is an amide, perhaps urea, that is the first stage in the synthesis occurring in this organism, seems to me to be the only plausible one.

Further studies on the physiology and morphology of the nitrifying organism are in progress.

Comment

Winogradsky was able to show in a clear way for the nitrifying organisms that they obtained their energy from the oxidation of ammonia and use this energy for the assimilation of carbon dioxide. His earlier studies on the sulfur and iron bacteria had pointed this way, but these organisms had proven harder to work with. This discovery was really one of the most important in physiology, since it shows, as Winogradsky realized, that carbon dioxide is convertible into organic carbon without the intervention of light energy through chlorophyll. With the addition here of a third group of bacteria that could obtain energy from the oxidation of inorganic compounds, the chemosynthetic bacteria appeared to be fairly common.

The process of nitrification turned out to be more complicated than it appears here, and Winogradsky was instrumental in clarifying this picture. He described two genera of bacteria, one which oxidized ammonia to nitrite, and the other which oxidized nitrite to nitrate. This process is important agriculturally, since ammonia is easily lost from the soil, while nitrate is more stable and serves as a good nitrogen source for plants. As he mentioned, the isolation of these organisms in pure culture was quite difficult, mainly because the soil is so rich in bacteria that other forms, which grow much faster than the nitrifying bacteria, will take over on agar plates containing organic media. Further, the nitrifying bacteria seem to be inhibited by organic matter, so that it is necessary to find a substitute for agar or gelatin. Winogradsky later did this, using silica gel, and succeeded in this way in isolating pure cultures of each of the nitrifying bacteria. He was then able to

demonstrate this process in pure culture and show that a different organism was responsible for each stage.

The biochemical aspects of chemosynthetic organisms are just beginning to be worked out. We know that the process of carbon dioxide fixation in the sulfur bacteria is quite similar to the process in green plants, using the same enzyme systems. The difference is in the source of energy. The sulfur and nitrifying bacteria derive their energy from the oxidation of these inorganic compounds, and these oxidations are coupled to phosphorylation, giving ATP. The energy from ATP is used in the process of carbon dioxide fixation. Only a small amount of modern work has been done on these interesting organisms, and many new things remain to be dis-covered.[3]

In addition to his pioneering research on autotrophs, Winogradsky productively studied the bacteriology of cellulose decomposition and microbiology of the soil. He clearly understood the great complexity of soil microbiology, which is now being rediscovered by the current generation of microbiologists and "nucleic acid sequence hunters."[2]

References:

Winogradsky, S. (1949) *Microbiologie du sol.* Problems et methodes. Cinquante ans de recherches. 853 pages, 35 plates. Masson et Cie, Paris.

Waksman, S. A. (1953). *Sergei N. Winogradsky, His Life and Work*/The Story of a Great Bacteriologist, Rutgers University Press, New Brunswick.

Footnotes:

1. Waksman received a Nobel Prize in 1952 for the discovery of streptomycin. The real discoverer was his graduate student Albert Schatz. Eventually, Schatz sued Waksman in order to gain his rightful recognition.
2. Winogradsky worked with living microorganisms, not "computer bacteroids", i.e., partial nucleic acid sequences. The fashion of renaming actual bacteria on the basis of such sequences led H. Gest to publish a satire called "Gest's Postulates." (ASM News 65: p. 123, 1999). The latter specifies penalties for scientists who publish short nucleic acid sequences of "computer bacteroids", but fail to provide evidence for corresponding living cells. See also: H. Gest (2000) Report from the year 2025 meeting of the American Microbiological Society: Discovery of the bacterial "taxonomy gene." Microbiology Today 27: 28-30.
3. Marjory Stephenson's *Bacterial Metabolism* (1969) has an excellent chapter on the early history of research on chemosynthetic autotrophs.

110

Microbe

October 2009 • The News Magazine of the American Society for Microbiology • Vol. 4 • No. 10

Rediscovering Pioneering Research in Microbial Ecology

Science risks retracing its own tracks if early research is forgotten

Howard Gest

"Those who are ignorant of history are condemned to repeat it." This is one of the many variations of George Santayana's famous quotation of 1905. It is particularly apt in describing some current and recent research efforts in microbial ecology. Thus, NASA's publicity machine has seized on "extremophiles," giving the impression that such microbes have been discovered recently, just in time to bolster faltering hopes that evidence for past or present microbial life on Mars will be forthcoming. The ubiquity of bacteria, well known for over a century, was rediscovered by NASA in 2007 in disconcerting circumstances . . . reported under a New York Times headline (October 9, 2007): "In NASA's Sterile Areas, Plenty of Robust Bacteria. . . . Researchers have found a surprising diversity of hardy bacteria in a seemingly unlikely place—the so-called sterile clean rooms where NASA assembles its spacecraft and prepares them for launching." The leader of the study (C. Moissi et al., FEMS Microbiol. Ecol. 61:509–521, 2007) is quoted as saying that their findings will advance the search for life on Mars and other worlds "by sparking improved cleaning and sterilization methods." Thanks, but we already have excellent methods!

Presently, we see articles rediscovering the complexity of microbial ecology in milieus such as soils, waters, and the animal intestine (e.g., see "Gut Reactions," Science 324:1136–1137, 2009). Since current texts have become heavy, encyclopedic tomes with scant coverage of the history of microbiological research, it is evidently time for guiding "molecular ecologists" and multidisciplinary research teams to important basic literature. A wag once said that sometimes six months in the lab can save an hour in the library. Research during the early decades of the 20th century established the fundamental facts that microbes of great physiological diversity occur in huge numbers in natural circumstances. I recommend H. G. Schlegel's *General Microbiology* 7th ed., 1986 (Cambridge Univ. Press) for a succinct description of basic ecological facts. Schlegel points out that "the number of symbiotic associations between animals and microorganisms is immense." The latter and other major aspects of microbial ecology are comprehensively described by Klein (2005), who has also advanced the field with a "postgenomic perspective" (2007). He emphasizes that "DNA-based analyses of natural microbial assemblages using bulk extraction-based approaches do not provide specific information on the active microbes functioning in particular locations and conditions."

Winogradsky and Beijerinck. Roger Stanier (1951) noted that Sergei Winogradsky and Martinus Beijerinck share the honors for revealing two great concepts of microbiology: "the physiological specificity of different microbial types, and the essential role of microorganisms in maintaining the cycle of matter." This required the development of special methodologies in discovery of the chemosyntheic autotrophs and major bacterial catalysts of the earth's nitrogen cycle (Winogradsky), and the technique of "selective enrichment culture." Analysis of the microbial ecology of soil by such methods led to a cornucopia of knowledge in general microbiology and identification of important species whose properties are grist for the mill of "molecular ecological" studies.

Winogradsky, in particular, had deep insights into the complexities of microbial ecology, and introduced novel methods of study which are probably largely unknown to present day researchers. For example, the technique of "separative enrichment," in which a solid medium of silica gel is impregnated with suitable nutrients and inoculated at spaced intervals with small particles of soil. Such enrichments closely simulated the physical conditions in soil and permitted isolation of the more slowly growing types in a given physiological group. In 1925, Winogradsky proposed that microorganisms found in an ecosystem can be classified in either of two categories—*autochthonous*: those indigenous and always present in a given ecosystem (soil, intestine, etc.), and *allochthonous*: organisms dependent on an occasional increase in concentration of certain nutrients or in the presence of specific nutrients.

p. 440

Howard Gest is Distinguished Professor Emeritus of Microbiology and Adjunct Professor of History & Philosophy of Science I at Indiana University, Bloomington.

gest@indiana.edu

Beijerinck had insatiable curiosity and was a prodigious worker. Among the numerous bacteria he discovered and characterized between 1888 and 1904 were: *Rhizobium leguminosarum, Azotobacter chroococcum, Thiobacillus thioparus, and Desulfovibrio desulfuricans.* Beijerinck published a large number of important researches dealing with fundamental problems in the physiology of bacteria, the bacteria of soil, and infectious diseases of plants. From his intermittent research on "tobacco mosaic disease," he concluded that the pathogen was a *"contagium vivum fluidum."* The latter was later understood as the concept of a virus. For the complete lengthy reference to Beijerinck's collected works see Gest 2009.

Winogradsky had a very long career, and his collected works were published as *Microbiologie du Sol* in 1949 (861 pp). This remarkable epic, covering 50 years of research, consists of 10 parts, the last one entitled *The Principles of Ecological Microbiology.* Stanier's 1951 perceptive analysis of Winogradsky's great contributions ended with the statement "His elaboration of the concept of an ecologically defined microbial species may well prove one of the cornerstones of future bacterial taxonomy."

Tidbits on Extreme Ecology. The great diversity of physiological/metabolic patterns among microorganisms accounts for the fact that a number of species can thrive in "extreme" environments. The absence of visible life forms gave the highly saline Dead Sea its name. Aristotle (384–322 B.C.) wrote that the sea was so "salty bitter" that fish could not live in it. An expedition in 1863 reported that despite all efforts, "no living creatures were found in the waters of the Dead Sea." But in 1936, Benjamin Volcani demonstrated the presence of viable osmophilic bacteria as well as many species of algae in "Dead" sea water. His studies also focused on organisms present on the sea bottom sediment, overlain by water containing salt concentrations of 25 to 32%. Two of Volcani's reports in *Nature* (145:975, 1940; 152:274–275, 1943) on Dead Sea organisms described *Scenedesmus quadricauda*, diatoms, and positive enrichment cultures for various kinds of aerobic and anaerobic bacteria.

Volcani received the first doctoral degree in microbiology awarded by the Hebrew University. His dissertation on Dead Sea halophiles was written in Hebrew, and *Haloferax volcanii* was named in his honor. For many years, Volcani was a professor of microbiology at the Scripps Institution of Oceanography (University of California, San Diego), where he did notable research on diatoms. Although silicon was believed to be biologically inert, Volcani discovered that in diatoms it was required for many biochemical pathways.

As early as 1881, bacteria that could grow at 60–70°C were isolated from the Seine River. During the past 40 years, considerable attention has been given to thermophilic extremophiles. The microbiology of hot environments, however, was not investigated systematically until Thomas Brock undertook a major study. From 1965 through 1971, the laboratory work of Brock and his students, associated with extensive field work, was conducted at Indiana University in Bloomington, and subsequently at the University of Wisconsin-Madison. Their research on thermophiles in hot pools of Yellowstone National Park and other thermal areas yielded a comprehensive body of basic ecological information and pure type cultures of novel thermophiles of importance. The latter included *Thermus aquaticus*, which contains thermostable Taq polymerase, a major tool in molecular biology research; *Thermoplasma*, which can grow at 55°C and pH 2; and the hyperthermophile *Sulfolobus*, capable of growing at temperatures as high as 90°C and pH of 1–2. The work of the Brock group is described in detail in Brock's 1978 classic book on thermophiles (see also Brock's chapter "The origins of research on thermophiles" in Reysenbach et al. 2001).

The Outlook. There is little doubt that the further development of powerful Web-based research engines will eventually make the detailed history of microbiological research more readily available than it is at present (for an update, see "The Unseen Scholars," in Los Alamos Science and Technology Magazine, December 2008, p. 20–23). However, scientists now doing research on microbial ecology are well advised to make special efforts to learn of past accomplishments to avoid falling into the trap that Santayana warned against. At present, there is no substitute for browsing in a well-developed physical library of books.

SUGGESTED READING

Brock, T. D. 1978. Thermophilic microorganisms and life at high temperatures. Springer, New York, Heidelberg, Berlin.

Gest, H. 2009. Historical adventures in scientific discovery: Microbiology/biochemistry. https:scholarworks.iu.edu/dspace/handle/2022/3358

Klein, D. A. 2005. Microorganism interactions and microbial ecology, p. 577–613. *In* L. Prescott, J. P. Harley, and D. A. Klein, Microbiology, 6th ed. W. C. Brown-McGraw Hill, Dubuque.

Klein, D. A. 2007. Seeking microbial communities in nature: a postgenomic perspective. Microbe 2:591–595.

Reysenbach, A-L., M. Voytek, and R. Mancinelli (ed.). 2001. Thermophiles/Biodiversity, ecology and evolution. Kluwer Academic/Plenum Publishing, New York.

Stanier, R. Y. 1951. The life-work of a founder of bacteriology. A review of Microbiologie du Sol (Winogradsky). Quart. Rev. Biol. 26:35–37.

Final notes on Cohn, Koch, Beijerinck and Winogradsky

COHN: Volumes 1-2 (1875-1877) of Cohn's *Beiträge zur Biologie der Pflanzen* are now accessible on-line at http://www.biodiversitylibrary.org/title/5917#9 These volumes contain Cohn's classic papers on bacteria and Koch's famous contribution on anthrax.

KOCH: As noted earlier, details of the accomplishments and life of Koch can be found in Brock's book "*Robert Koch: A Life in Medicine and Bacteriology,*" ASM Press 1999.

BEIJERINCK: In 1983, Brock's Science Tech, Inc. republished "*BEIJERINCK HIS LIFE AND HIS WORK*" by G. van Iterson, Jr., L.E. den Dooren de Jong and A.J. Kluyver. The latter was originally included in *Verzamelde Geschriften van Martinus Willem Beijerinck*, vol. 6, 1940. The Forward to Brock's book, by C.B. van Niel is entitled " The 'Delft School' and the Rise of General Microbiology." Van Niel's article is a reprint from *Bacteriological Reviews* 13: 161-174, 1949.

WINOGRADSKY: H.G. Thornton's 1953 obituary of Winogradsky in *Obituary Notices of Fellows of the Royal Society* 8(22): 635-644 gives an extensive overview of his life and work.

Discovery of the thermophilic bacterium *Thermus aquaticus*, great boon to molecular biological research

T. aquaticus was discovered as part of the research program of Prof. Thomas Brock and his students in the Dept. of Microbiology at Indiana University (Bloomington). I happened to be chairman of the department at the time. An undergraduate student, Hudson Freeze, played a key role in discovering *T. aquaticus,* which lives in a thermal pool in Yellowstone National Park. From samples brought back to Bloomington, Freeze isolated the bacterium, which thrives at 160°F. Brock and Freeze published a paper on the bacterium in 1969, which also described isolation of the bacterium from sources on the Indiana University campus. The bacterium was deposited in the American Type Culture Collection, making it available to all researchers. *T. aquaticus* is, of course, the source of

Taq polymerase. From Google: "In 1989, Science magazine named Taq polymerase as its first "Molecule of the Year.....The *T. aquaticus* enzyme makes millions of dollars annually for Hoffman-LaRoche, the Swiss pharmaceutical company that paid the Cetus Corporation $300,000,000 for the Taq patent. As the commercial potential of Taq Polymerase became apparent in the 1990's the National Park Service labeled its use as the "Great Taq Ripoff." Researchers now working in National Parks are now required to sign 'benefits sharing' agreements that would send a portion of later profits back to the Park Service."

Freeze made a recent visit to our campus, and I asked him to write a personal summary account of the isolation of *T. aquaticu,* which follows.

THE FIRST TAQ MAN

Hudson Freeze PhD

Burnham Institute for Medical Research, La Jolla, California

I met Dr. Tom Brock in the spring of 1966 near the end of my sophomore year at Indiana University. I was thrilled when he invited me to join his research effort in Yellowstone National Park that summer. I arrived in early August and after a bit of training, I did routine analysis of the bacteria and algae samples we collected from the hot springs. Returning to IU in September gave me the opportunity to work in Tom's lab for research course credit. He soon gave me a series of samples they had isolated from various hot springs in Yellowstone, and after quickly jotting down a series of different media, temperatures, and sample identification tags, I was off to assemble the equipment and supplies. It was not easy finding water baths that would maintain a range of very high temperatures. I used all of them in Brock's lab and in Dr. Arthur Koch's lab next door. Each of the tubes of media received just two drops of each of the various Yellowstone spring water samples. I didn't have a regular bound notebook, but I did have one that I had used the year before in Dr. Dean Fraser's lab. It had just a few observations, and was clearly distinct from the work I was doing with the bacteria and algae. So, I skipped a few pages to define the new project and then drew up an orderly matrix listing media, temperature and samples. Every day—sometimes twice a day—I would come to the lab, pick up the tubes, give them a swirl and look for evidence of bacterial growth. Nothing hazy appeared for several days. Once I thought I saw something, but when I examined the materials under a microscope it was simply a salt precipitate. No organisms at all. Then it all changed.

I picked up one tube from the 72°C water bath containing water from Mushroom Spring and the yellow colored 0.1% peptone/0.1% yeast extract broth medium along with a few vitamins. I gave the tube a flick and there it was: A swirl of life. Just a beautiful mass of.... maybe it was only some precipitated inanimate salt crystals again, and not bacteria at all. A quick trip to the phase contrast microscope dispelled all doubts. I saw all of these long, long filaments of rod shaped bacteria more than 200uM long looking like large bundles of straw or smaller rosettes. Some of the short chains appeared to be wrapped around a large bubble. No doubt that these were bacteria that were growing at 72 degrees. The organisms from that tube were later designated as Yellowstone Thermophile I or YT-1. Eventually the organism was given the proper name *Thermus aquaticus*, and that isolate retained the YT-1 designation.

A popular song of the time from the Broadway musical "Hair" heralded the "Dawning of the Age of Aquarius" and, of course, in the lab, we transformed that into the "Dawning of the Age of Aquaticus". Every undergraduate hopes his or her own little project will be the breakthrough discovery of a new age. Surprisingly, in this case it was. Not because of any particular talent, but because of simple curiosity about how life survives in the most inhospitable environments.

Potential commercialization of *Thermus aquaticus* certainly had humble beginnings. Procter & Gamble representatives from Cincinnati visited the lab one day, and I suspect Tom looking for an escape route, pushed them in my direction, saying, "Here is the guy who did the work". Their interest was modest but practical: Could an extract of *Thermus* with all of those high temperature enzymes be added to Tide or Cheer to make clothes even cleaner? It was a short conversation about the bright yellow bacteria that might not easily generate "whiter and

brighter" clothes come laundry day. As far as I was aware, there was no more commercial interest in *Thermus* at that time, but times change.

The biggest and most significant change was when Kerry Mullis envisioned the polymerase chain reaction, PCR, as a clever way of amplifying the tiniest trace amounts of the genetic material, DNA. *Thermus aquaticus*, which generally became known in the business as *Taq*, provided the critical component, a thermostable DNA polymerase. PCR can amplify DNA but it requires the presence of the thermostable enzyme to work at temperatures where the double helix DNA melts. Repeated cycles of amplification are possible using the thermostable polymerase because it does not denature when the DNA melts, and hence does not need to be added at each cycle. Taq polymerase is the critical component for amplifying DNA millions of times. As luck would have it, *Taq* polymerase was isolated from the *Thermus aquaticus* YT-1that Tom Brock and I sent to the American Type Culture Collection (ATCC). Not only was this technique an ingenious use of a little known thermophile, but it revolutionized, perhaps even created, the modern science of molecular biology. So important was this technique that Mullis was awarded the 1993 Nobel Prize in Chemistry.

The idea that thermophile could provide heat stable enzymes gave rise to an entire industry of companies trying to find even more extremeophiles including organisms that live in thermal events in the ocean where the temperature far exceeds 100°C.

What started out as a project of curiosity by an undergraduate student looking for "a far-out project," specifically, one as unusual as my high school science fair project "testing for life on Mars," eventually turned into a basic element that established molecular biology in the 20th century.

As you look through the notebook, you will notice a few experiments testing the amino acid and vitamin nutritional requirements and antibiotic sensitivities of *Thermus aquaticus*. We did these to try to understand if *Thermus* would be similar to other prokaryotes that were already quite familiar. Those results were important, too, because they provided an evolutionary connection, but not exactly in the way we thought because *Thermus* and other similar thermophiles discovered later defined an entirely new kingdom of life called Archea that is distinct from prokaryotes and eukaryotes.

Many stories in the popular press describe the discovery of *Taq* and its rise to prominence in molecular biology and biotechnology. The articles sometimes focus on the practical benefits and unanticipated fruits of taking good care of our National Parks. Others stress that Yellowstone National Park has not received enough financial benefit from the discoveries it enabled. A trip to nearly any of the popular Yellowstone hot springs or park museums now carries a description (sometimes with pictures) of the organisms and how they have been used to promote a better, healthier life for all. In the end, I hope that these articles, pictures and rest stop posters will catch the eye of young boys or girls who will say, "Yes, I could do that, too!". It's an adventurous life being a scientist, and they could find the same excitement in making discoveries that we have all known through our work in basic science. We have been fortunate to witness marvelous applications of discoveries from the most humble of beginnings.

THE DELFT "CONNECTIONS"

BEIJERINCK, Martinus W. (1851-1931)

KLUYVER, Albert J. (1888-1956)

Van NIEL, Cornelis B. (1897-1985)

ASM

1889: Martinus Beijerinck uses enrichment culture, minus nitrogenous compounds, to obtain a pure culture of the root nodule bacterium *Rhizobium*, demonstrating that enrichment culture creates the conditions for optimal growth of a desired bacterium.

1899: Martinus Beijerinck recognizes soluble living microbes, a term he applies to the discovery of tobacco mosaic virus. A filtrate free of bacteria retains ability to cause disease in plants even after repeated dilutions. He calls the agent "contagium vivum fluidium"—contagious living fluid.

1926: Albert Jan Kluyver and **Hendrick Jean Louis Donker** propose a universal model for metabolic events in cells based on a transfer of hydrogen atoms. The model applies to aerobic and anaerobic organisms.

1931: C. B. van Niel shows that photosynthetic bacteria use reduced compounds as electron donors without producing oxygen. Sulfur bacteria use H_2S as a source of electrons for the fixation of carbon dioxide. He posits that plants use water as a source and release oxygen.

Bulloch: BEIJERINCK, Martinus Willem. Great Dutch microbiologist. Born in Amsterdam. Educated in Haarlem. Lecturer on botany, physiology, and physics at Agricultural School at Warffum (Groningen). Lecturer in Utrecht and in Wageningen for ten years. In 1897 bacteriologist to Yeast works in Delft, and from 1898 to 1921 as Prof. of General Bacteriology in the High School at Delft. B. published a large number of highly important researches dealing with fundamental problems in the physiology of bacteria, the bacteria of soil and plants, and plant infective diseases. Foreign member of the Royal Society 1926. His *Verzamelde Geschriften van M. W. Meijerinck ter Gelengenheid van zijn 70sten Verjaardag*, publ. at Delft, 1921-2, 5 parts. Biog.: *Proc. Roy. Soc.* B, cix, pp.i-iii.

Beijerinck's early education and research were in the field of botany. With no previous microbiological training, he accepted a position, in 1884, as a bacteriologist at the Dutch Yeast and Spirit Factory in Delft. Eleven years later, he became professor of microbiology at the Polytechnical School in Delft and remained there for the rest of his career. Beijerinck published more than 140 papers on botany, microbiology, chemistry and genetics. When re retired in 1921, colleagues presented him with five volumes of his published scientific papers: "Verzamelde geschriften van M. W. Beijerinck", a major microbiological classic. [see ref. at end]

Personally, Beijerinck was acerbic and taciturn. According to Ronald Bentley (ASM News 60: 3-4, 1994), during his 1960 course, van Niel noted that Beijerinck did not like to teach and in his lectures he emphasized his own

current interests. He published mostly under his own name or with himself as first author. The latter is consistent with the gist of van Niel's remarks reported by Bentley: "With his assistants, he [Beijerinck] was possessive of information; he would pick up a culture plate and say, 'Did you see that? Remember, I saw it first? Some assistants kept their cultures in the toilet, since this was the only room that he never visited – his house was next door with his own private toilet." Nevertheless, Beijerinck left a great scientific legacy.

During 1885 to 1900, Beijerinck did intermittent research on the "tobacco mosaic disease." His experiments showed that the agent was unique in that it was "fluid" or "non-particulate." Also, he noted that the pathogen was unable to reproduce outside of the host and seemed to multiply only in parts of the plant undergoing rapid cell division. Beijerinck concluded that the disease was caused by a "*contagium vivum fluidum*." This was a revolutionary idea which, of course, was later understood as the concept of a *virus*. This research was virtually forgotten until Wendell Stanley crystallized the tobacco mosaic virus in 1935.

Beijerinck developed the enrichment culture technique (simultaneous with Winogradsky) and exploited it in isolation of a number of important physiological types of bacteria, which are listed below:

1888 *Rhizobium leguminosarum*

1889 *Vibrio fischeri*

1895 *Desulfuvibrio desulfuricans*

1898 *Acetobacter aceti*

1901 *Azotobacter chroococcum*

1901 *Lactobacillus fermentum*

1901 *Sporosarcina ureae*

1904 *Thiobacillua thioparus*

Verzamelde geschriften van M. W. Beijerinck; G. van Inerson, Jr., L.E. den Dooren de Jong, and A. J. Kluyver, eds., 5 vols. (The Hague, 1921). Vol. VI (The Hague, 1940) contains papers by Beijerinck that appeared after 1921, indexes to all 6 vols., and biographical material.

The Microbe's Contribution To Biology

A.J. Kluyver
and
C.B. Van Niel

Harvard University Press
Cambridge, Massachusetts
1956
Printed version of the "J.M. Prather lectures" (1954)

KLUYVER

Kluyver was trained in chemical engineering and spent six years in the Dutch East Indies as an advisor to the Netherlands Indies Government on promotions of native industries. In 1922, he returned to the Netherlands to succeed Beijerinck in the chair of general and applied microbiology at The Technical University of Delft. Throughout his career, Kluyver maintained a strong interest in commercial uses of microorganisms, and many of his students went on to work in industry. He became head of a group of advisors to the Netherlands Yeast Factory when it expanded its manufacture of pharmaceuticals, and also was an advisor to the Royal Dutch Shell Laboratory. In 1924, Kluyver began study of *Acetobacter suboxydans* and recognized its importance in the production of sorbose, an intermediate in the commercial manufacture of ascorbic acid.

Kluyver's study of *A. suboxydans* stimulated his interest in oxidation/reduction reactions, and this led him to develop the basis for the publication by A. J. Kluyver and H. J. L. Donkers: Die Einheit in der Biochemie, in *Chemie der Zelle und Gewebe* 13:134-90 (1926). In essence, this paper stated the principle of hydrogen transfer as the fundamental feature of all metabolic processes. Early in his career, C. B. van Niel became an assistant to Kluyver, and he revered Kluyver as "the Master." van Niel enthusiastically adopted Kluyver's thesis of "hydrogen transfer" as the fundamental basis of "comparative biochemistry," and this was later reflected in van Niel's famous course on microbiology (see later). My remarks on Kluyver's "comparative biochemistry" follow the summary of van Niel's career.

VAN NIEL, Cornelis B. (1897-1985)

After receiving a chemical engineering degree in Delft, van Niel became an assistant to A. J. Kluyver while pursuing graduate studies. The latter involved purple photosynthetic bacteria, but the major subject of his PhD dissertation was the biochemistry and taxonomy of propionic acid bacteria. In 1928, van Niel joined the staff of the Hopkins Marine Station of Stanford University (Pacific Grove, CA). In addition to research on sulfur purple and green bacteria, he developed a major study of the nonsulfur purple and brown bacteria. This encompassed their general physiology, photosynthetic pigments, and classification of some 150 strains isolated from natural sources. These studies were summarized in a large monograph published as a special issue of Bacteriological Reviews (8: 1-118, 1944).

Early studies by Hans Molisch (1904) showed that purple bacteria do not produce O_2. This apparent conflict with the accepted definition of photosynthesis led to doubts that the purple bacteria were, in fact, photosynthetic. Eventually, however, it was realized that Molisch's work had revealed the existence of an *anaerobic* type of photosynthesis. During the 1930's, van Niel proposed a "comparative biochemical" hypothesis to rationalize green plant and bacterial photosyntheses. His basic assumption was that water is the "direct" H donor for CO_2 reduction in all photosyntheses ($HOH \rightarrow H+OH$). It was presumed that in green plants OH is disposed of by conversion to O_2 and H_2O. The bacteria evidently lack the latter mechanism, and consequently, require an "accessory" H donor (e.g., H_2 or H_2S), which reduces OH without formation of O_2. This conception enjoyed great popularity for sometime, but

it proved to be untestable. van Niel's idea was abandoned after one of the main common denominators of photosynthetic processes was discovered in the 1950's, namely photophosphorylation of ADP, yielding ATP. For details of the lengthy and tortuous history of this subject, see H. Gest, The comparative biochemistry of photosynthesis: milestones in a conceptual zigzag. In: *From Cyclotrons to Cytochromes*, ed. By N. O. Kaplan and A. Robinson, Academic Press, New York, 1982.*

van Niel's microbiology course

van Niel was a superlative teacher and one obituary noted that "his greatest contribution to science may well have been his teaching of general microbiology and comparative biochemistry." He developed an intensive ten-week summer "laboratory" course, in which he accepted a maximum of 14 students. Lectures and laboratory experiments were in the same room. van Niel would suddenly interrupt lab work at particular times and give relevant spell-binding lectures, sometimes for several hours! Otherwise, he was always in the room interacting with each student. A major feature of the lectures was intermittent Socratic dialogue with the students.

A major feature of the course was preparation of enrichment cultures for various physiological types of microorganisms, followed by isolation in *pure culture*. When I took the course in 1947, by midsummer we had isolated and done simple experiments with the following: *Pseudomonas*, aerobic spore-formers, mycobacteria, actinomycetes, coli-group organisms, acetic acid

* This essay also contains my critique of "overly comparative biochemistry."

bacteria, *Azotobacter*, *Rhizobium*, H_2-bacteria, spirilla, luminous bacteria, as well as cellulose and agar-decomposing bacteria. When the course ended, I had a list of 82 stock-cultures in my collection.

van Niel had great histrionic skill, and with his extensive knowledge of the history of science, he emphasized the special talents and idiosyncrasies of outstanding investigators. As his reputation as a teacher spread, noted mature scientists in various fields asked van Niel if they would be allowed to attend the course as silent observers on the sidelines. In time, the number of observers equaled the number of students. The list of students and auditors between the 1940's and 1960's reads "like a Who's Who" of biological science. There is no doubt that directly and indirectly through students and auditors, van Niel exerted a great influence on teaching and research in general microbiology for a generation.

A number of "van Niel-type" courses proliferated in the U.S., notably at the Marine Biological Laboratory in Woods Hole. van Niel's contributions were recognized by numerous awards and honors, including the U.S. National Medal of Science in 1964.

To convey other personal impressions of van Niel's great course, I include parts of accounts by Arthur Kornberg (Nobel Laureate in Medicine, 1959) and Konrad Bloch (Nobel Laureate in Medicine, 1964).

From A. Kornberg: *For the Love of Enzymes/The Odyssey of a Biochemist.* Harvard University Press. Cambridge, 1989.

"My metamorphosis from physician to biochemist and my growing awareness of genetics had convinced me that instruction of medical students

in the basic biochemistry and genetics of bacteria, viruses, and parasites would be more valuable than exclusive attention to the latest techniques in culturing and staining each of the many pathogenic microbes. As chairman of a department that would try to teach these aspects of general microbiology, it seemed to me that I should take some formal instruction in the subject. I also sought refuge for myself and my family from the steamy heat of a St. Louis summer.

Van Niel's course provided a superb historical review of microbiology and a powerful antidote to medically oriented bacteriology. He dwelled on the good microbes in the environment and forbade the mention of pathogens, except those few that figured prominently in the history of microbiology. Progress was described in the exploits of his heroes: Anton van Leeuwenhoek, Louis Pasteur, Sergei N. Winogradsky (1856-1953), the Russian soil microbiologist, and not least, the yeast cell. Van Niel traced his own Dutch lineage with reverence to Albert J. Kluyver (1888-1956), his teacher, and farther back to Martinus W. Beijerinck (1851-1931), who taught Kluyver.

....One during the course I gave a seminar on my previous work on the isolation of enzymes from the cellular juices of microbes. Afterwards, van Niel told me: 'This is beautiful work, I know it needs to be done. I myself would not have the heart to grind up the little beasties.'"

From K. Bloch: *Blondes in Venetian Paintings, the Nine-Banded Armadillo, and other Essays in Biochemistry.* Yale University Press, New Haven, 1994.

"Except for the occasional excursions mentioned, my early research had been limited to rats, whole animals, and isolated liver preparations. During the 1950s biochemists, many among them middle-aged, began to seize the opportunities provided by the remarkable developments in molecular biology. The use of microbial mutants, introduced by Beadle and Tatum with the mold *Neurospora*, had been extended to bacteria with the aid of the elegant penicillin technique. Joshua Lederberg and Bernard Davis discovered it independently in 1952. *Escherichia coli* became the organism of choice for biochemists whose primary interests were biosynthetic processes. I was particularly impressed by the elucidation of the pathways to the aromatic amino acids tryptophan, tyrosine, and phenylalanine, totally unknown previously and an outstanding example of the power of the mutant technique.

In order the ease the transition from familiar to unfamiliar biological systems, I decided in 1957 to go back to school. I had been told of a popular microbiology summer course taught by C. B. van Niel at the Hopkins Marine Station in Pacific Grove, California – located on the Monterey Peninsula, an added attraction. My application to enroll in the course was accepted, along with those of some fifteen biochemists, all anxious to be introduced to microbiological techniques (the average age of those in the class was forty-two). We heard a rumor that applicants who had already taken a microbiology course elsewhere were not admitted.

The exceedingly demanding course taught me important lessons that were to influence and redirect my research from then on. Van Niel, a classic bacterial physiologist in the tradition of the Dutch school of Beyerinck and Kluyver, first made the class aware of the rich variety of microorganisms

("beasties," he called them) and their diverse lifestyles. Second, we learned from van Niel that Nature provides the investigator with numerous organisms to choose from, some uniquely suited for studying a specific biological phenomenon....

Another piece of helpful information van Niel mentioned – almost casually – in his lectures benefited me perhaps more than any other for planning future research. The class was told that common brewer's yeast, *Saccharomyces cerevisae* – the organism that led to Pasteur's fundamental discovery, which he termed "la vie sans air" – is in fact microaerophilic, not strictly anaerobic. This information came from a 1954 paper by Andeassen and Stier in the *Journal of Cellular and Comparative Physiology*, a periodical then unfamiliar to me. These authors noted that in the *strict* absence of oxygen, yeast fails to grow unless supplies with cholesterol and an unsaturated fatty acid. Obviously oxygen, albeit at very low atmospheric pressures, is essential for the biosynthesis of these lipid molecules. This realization was essential to our later research and also stimulated my interest in the role of oxygen in the evolution of biochemical pathways and organisms."

SUMMER 1947

C.B. van Niel, "Het Mikroben Koenig" (The Microbe King) takes a break with some of the students during his intensive microbiology course. We are standing outside of the Hopkins Marine Station of Stanford University (Pacific Grove, CA). Elliot Juni, Maurice Sussman and I were graduate students at Washington University (St. Louis). Juni is third from left; fourth is van Niel, I am fifth, eighth is Bob Weatherwax. kneeling, Barbara Kalckar Wright, Maurice Sussman.

The following summer, I was back in van Niel's lab as a "research student." While investigating growth of the purple photosynthetic bacterium *Rhodospirillum rubrum*, I discovered massive H_2 production when the usual N source, an ammonium salt, was replaced by glutamate. van Niel, an authority on photosynthetic bacteria, was skeptical and suggested that I must have contaminated my cultures with clostridia. Controls, however, showed that my cultures were pure. Back in St. Louis in the fall, I pursued these observations which resulted in the discovery that *R. rubrum* had the capacity use N_2 as the sole N source for growth. Subsequent research showed that all anoxygenic photosynthetic bacteria are N_2 fixers.

Howard Gest & Cornelis van Niel, 1947

129

BIOCHEMISTRY AND MOLECULAR BIOLOGY EDUCATION
Vol. 30, No. 1, pp. 9–13, 2002

Mini-Series: Significant Contributions to Biological Chemistry Over the Past 125 Years

Landmark Discoveries in the Trail from Chemistry to Cellular Biochemistry, with Particular Reference to Mileposts in Research on Bioenergetics*

Received for publication, December 14, 2001

Howard Gest‡

From the Department of Biology and Department of History and Philosophy of Science, Indiana University, Bloomington, Indiana 47405

"The fascination of a growing science lies in the work of the pioneers at the very borderland of the unknown, but to reach this frontier one must pass over well traveled roads" [1].

Molecular oxygen was discovered in 1775 by Joseph Priestley. He also made the important observations that oxygen is produced by plants and that gases (O_2 and CO_2) mediate the interdependence of plant and animal life. The biological experiments of Priestley dealt with aerobes, the most advanced forms of life. It was the research of Louis Pasteur, however, on fermentation of sugar by anaerobic microbes that provided a system that was the first to reveal significant clues to the biochemistry of bioenergetics. Analysis of the detailed mechanism of anaerobic sugar fermentation by yeast during the first third of the 20th century and the later study of energy-yielding aerobic respiration and energy conversion in photosynthesis required development of many new techniques. These became the tools that were exploited to yield the basic outlines of cell biochemistry. Included were chromatography, metabolic gas manometry, spectrophotometry, the use of stable and radioactive isotopes as tracers of intermediary metabolism, and procedures for purification of proteins and other macromolecules. Noteworthy advances included discovery and characterization of the (Krebs) tricarboxylic acid cycle, "activated" intermediates such as acetyl coenzyme A, electron carriers and coenzymes necessary for energy conversion and reductive biosynthesis, ATP (the universal energy "currency") and a multitude of enzymes involved in catabolic and biosynthetic metabolism.

The founding of the American Chemical Society was closely related to discoveries that had great importance in the history of research in biological chemistry. I refer, in particular, to discoveries made by the English minister Joseph Priestley (1733–1804). Priestley was a self-taught polymath who discovered molecular oxygen in 1775 and demonstrated that the gas is produced by green plants. Using the mouse as an experimental system, Priestley also did clever experiments showing that the oxygen produced by plants was essential for the life of animals [2].

In 1794, Priestley left the United Kingdom and emigrated to Northumberland, PA, where he continued research in a combination home/laboratory. In 1874, 77 chemists met at Priestley's house to commemorate the centenary of his discovery of O_2 and discussed the possibility of forming a national association. The idea was rejected, because many at the meeting felt there would never be enough chemists in the United States to support such an organization. Two years later, in 1876, some of the same group met again and voted to establish the American Chemical Society. There are now more than 163,000 members! In the Chemical & Engineering News of August 8, 1994 (p. 9), there is a photograph showing Priestley House on the occasion of its dedication in 1994 as the third National Historic Chemical Landmark.

After Priestley's discoveries, about 100 years elapsed before it was recognized that there are microorganisms that do not require molecular oxygen and, in fact, are inhibited by oxygen. The so-called "anaerobic" life style became the focus of the research of Louis Pasteur in the 1870s, and this had far-reaching consequences. Pasteur concluded that anaerobic fermentation of sugars by organisms such as yeast was the result of life without air and was always intimately associated with cell growth. In other words, he believed that fermentation occurred only in living cells.

Pasteur died in 1895. If he had lived two more years, he would have been stupefied by an accidental discovery made in 1897 by Hans and Eduard Buchner. The Buchners were trying to make cell-free protein preparations from yeast for use in immunological experiments and decided to add sugar to a batch of yeast "juice" to act as a

* This work was supported by National Institutes of Health Grant GM 58050. This article is based on a talk delivered at the 125th meeting of the American Chemical Society in Chicago, IL, July 29, 2001.

‡ To whom correspondence should be addressed. Tel.: 812-855-9612; Fax: 812-855-6705; E-mail: hgest@bio.indiana.edu.

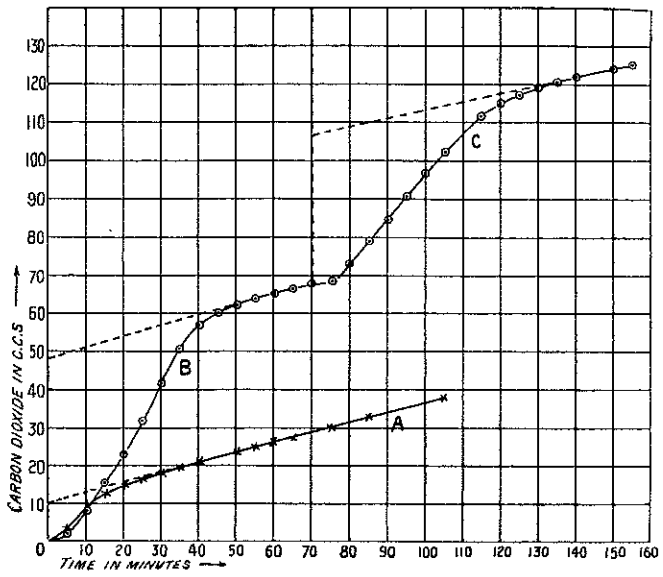


FIG. 1. **Effect of inorganic phosphate on production of CO_2 during fermentation of sugar by yeast juice.** For details see Ref. 4.

preservative. They were surprised to observe a vigorous production of CO_2 and the formation of ethanol. Announcement of the cell-free fermentation, by "zymase," was astonishing. In many ways, this discovery was the opening wedge leading to modern biochemistry [3].

Soon after the serendipitous discovery of cell-free yeast juice fermentation, Arthur Harden (United Kingdom) began a systematic analysis of the system. Eduard Buchner had observed that the production of CO_2 by yeast juice declined rapidly with time, long before the sugar was exhausted. Harden looked into this and found that addition of inorganic phosphate resulted in an immediate increase in the rate of fermentative CO_2 production. This is illustrated in Fig. 1 [4]. Curve A is a control with no added phosphate. In B, 0.03 M phosphate was added, and after 70 min another addition of phosphate was made. Harden and his colleagues found that during the course of fermentation, the inorganic phosphate was esterified into organic forms, and they isolated three esters, glucose 6-phosphate, fructose 6-phosphate, and fructose 1,6-bisphosphate. The isolation of the sugar phosphates eventually led to the unraveling of the molecular details of the intermediary steps of sugar fermentation and glycolysis.

By 1924, it became apparent that the sequence of reactions in fermentation of glucose by yeast was virtually identical to the sequence of reactions in glycolysis, the conversion of glucose to lactic acid in mammalian muscle. It also became clear that the purpose of these anaerobic sequences was the regeneration of ATP, the universal energy currency used by all types of cells for biosynthesis and other energy-requiring processes. ATP was first isolated in 1929, from muscle, by Fiske and Subbarow, and in the same year Sir Arthur Harden was awarded a Nobel Prize in Chemistry.

It is reasonable to believe that fermentation and glycolysis were very ancient mechanisms in the evolution of biological energy conversion systems. The anaerobic mechanisms are widespread, even in contemporary organisms, from microbes to muscle tissue of humans. An example of an organism that obtains all of its energy by anaerobic glycolysis of sugar is the bacterium *Streptococcus pneumoniae*, formerly known as the pneumococcus. This bacterium is responsible for many millions of cases of pneumonia and bronchitis, and antibiotic-resistant strains are now of great concern. The search is on for new kinds of effective antibiotics.

S. pneumoniae holds a unique place in the history of biochemical research. During the 1940s, studies by Oswald Avery and his colleagues at the Rockefeller Institute on the genetics of polysaccharide formation by this bacterium showed for the first time that the genetic material is DNA. This was clearly demonstrated in 1944 when the conventional wisdom was that genes are made of protein [5]. It is remarkable to recall that between 1944 and 1950, the Avery's conclusions were considered very dubious. I remember hearing a seminar in 1949 that seriously questioned Avery's identification of DNA as the genetic material.

Going back a bit in the chronology, the 1930s was an important decade in the development of the fundamental background of cell biochemistry. There was a renewal of interest in the mechanism of aerobic respiration. Otto Warburg, in Berlin, isolated and characterized flavoproteins and the pyridine nucleotide electron carriers NAD and NADP. He also developed manometric methods for assay of metabolic gases, and the so-called Warburg manometric apparatus soon became a standard fixture in biochemistry and microbiology laboratories. Equally important was Warburg's development of the methodology of spectrophotometric assays of coenzymes and enzyme activities.

During the same period, Albert Szent-Györgyi in Hungary found that fumarate and succinate played an important role in respiration and proposed that triose phosphate from sugar breakdown was oxidized aerobically by a C-4 dicarboxylic acid cycle. The methods that Warburg developed and Szent-Györgyi's observations provided Hans Krebs in Sheffield, United Kingdom with some tools and clues he needed to develop and prove a brilliant conception, namely the citric acid cycle, otherwise known as the tricarboxylic or Krebs cycle. This is the primary metabolic machinery that furnishes the reducing power for energy-yielding respiration of aerobic organisms.

In 1937, *Nature* magazine rejected the brief paper in which Krebs first described the cycle; the form rejection letter is reproduced in an autobiographical memoir published by Krebs in 1981 [6]. When we learned years later that this paper had been rejected it greatly encouraged the hopes of many young scientists who had negative dealings with *Nature*. Fig. 2 (from Ref. 6) shows the Krebs cycle, which generates CO_2 and hydrogen atoms from major foodstuffs. The cycle occurs in virtually every kind of animal and plant and in the majority of ordinary bacteria. The hydrogen atoms derived from reactions of the cycle constitute the fuel for regeneration of ATP by the aerobic respiratory system. One question Krebs was unable to answer was the exact nature of the C_2 compound that is funneled into the cycle from carbohydrates, fats, and proteins. It was obviously similar to acetate, but what was it really? The answer was mainly provided by Fritz Lipmann,

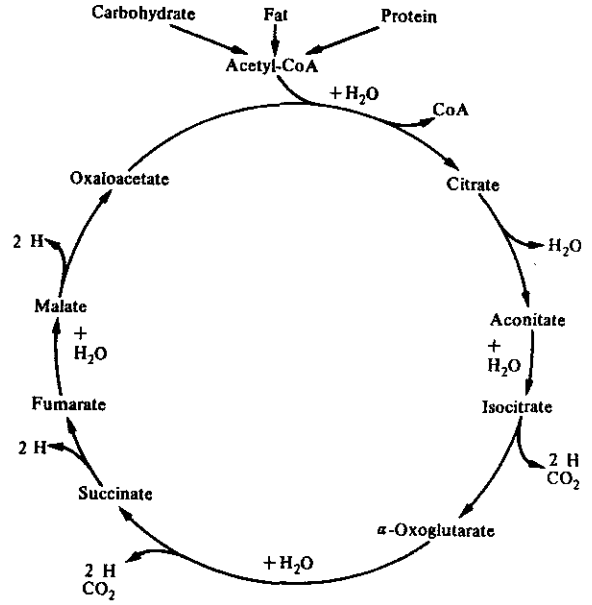


FIG. 2. **The Krebs tricarboxylic acid cycle (also known as the citric acid cycle).** Carbohydrate, fat, and protein all form acetic acid attached to CoA. CoA is regenerated when citrate is formed; thus it acts as a catalyst, and only small amounts are needed. For details see Ref. 6.

who discovered coenzyme A, the coenzyme of acetylation, in 1947. Acetyl-CoA is the actual energy source for the cycle. Lipmann also introduced the concept of energy-rich phosphoryl groups and made other important contributions to biochemistry, and he shared the 1953 Nobel Prize in Physiology or Medicine with Krebs.

INTERMEDIARY METABOLISM

When research accelerated after the end of World War II, many biochemists began exploring metabolic pathways of carbon and nitrogen in animal and plant tissues and in microorganisms. ^{14}C was first produced in the Berkeley cyclotron by Sam Ruben and Martin Kamen in 1940 and started to become commercially obtainable about 1950. The availability of this isotope had a profound effect on research in intermediary metabolism. Among many other applications, ^{14}C provided the means for elucidation of the Calvin/Benson cycle of photosynthetic CO_2 reduction [7].

Biosynthesis of amino acids and proteins became "hot" topics, and studies on properties of enzymes led to important discoveries on the regulation of enzyme activity. An example of the latter was negative feedback inhibition of aspartokinase activity [8]; the kinase is the first enzyme of the branched pathway leading to isoleucine synthesis. Horizons of biochemistry were expanding rapidly, and there were spectacular advances in our knowledge of the complexity of metabolic pathways.

The explosion of new information on cell biochemistry condensed in the metabolic pathways map was greatly facilitated by the study of biochemical mutants of bacteria and *Neurospora*. Mutational interruption of individual steps in complex pathways led to accumulation of transient intermediates that could be isolated and identified. There was another very important development during the 1940s. Namely, the establishment of the Sigma Chemical Company in St. Louis, MO. Sigma began producing valuable biochemicals, sparing investigators arduous, time-consuming labor needed to make the reagents for experiments. Before Sigma came into existence, I personally recall spending weeks isolating NAD or NADP from a large mass of yeast cells, ending up with preparations that were no more than 30% pure. Later, when I was a faculty member at Washington University (St. Louis, MO) in the 1960s, I could get the pure compounds delivered from Sigma by taxi within an hour. Incidentally, Sigma now makes available the latest metabolic pathways map on an impressive chart, 33 × 50 inches, for the bargain price of $6.95 (product M 3907).

BIOENERGETICS

In the background of the flood of new biochemical information being generated during the 1940s to 1960s, another great research current was forming. I am referring to investigation of the details of how electron flow in aerobic respiration and photosynthesis drives regeneration of ATP from adenosine diphosphate and inorganic phosphate. This was a persistent topic of symposia at biochemistry meetings for some time.

The involvement of cytochromes and the terminal electron-transporting heme proteins in energy conversion had a curious history. The cytochromes were first discovered in a great variety of animal tissues in 1884 by Charles MacMunn, a practicing Irish physician. MacMunn had a small laboratory in a hay loft over his stables and did research in his spare time. Later he built a small laboratory in his garden and had a horizontal iron pipe built into the wall. Through the pipe he could see which patients were coming up the path. If he didn't want to be interrupted, he would warn the maid to say that he was out. MacMunn's research publications were strongly criticized by some eminent chemists, notably Felix Hoppe-Seyler. The main criticism, which was completely erroneous, was that MacMunn was simply observing breakdown products of hemoglobin (see Ref. 9 for details of the early history).

For the next 40 years, MacMunn's findings were ignored. Then, in 1925, the cytochromes were rediscovered by David Keilin, who spent his entire career working on the redox behavior and distribution of cytochromes in cells of many kinds. Most of Keilin's observations were made with the naked eye using a microspectroscope. Fig. 3, from Keilin's classic book [9], shows the appearance of reduced cytochrome absorption bands in various organisms as he observed them. Keilin examined only a few microorganisms, including a strictly anaerobic *Clostridium*. He could not detect any cytochrome in the latter, and this led him to generalize that cytochromes are absent from anaerobes. Keilin's conclusion seemed reasonable at the time, but he was outwitted by the diversity of metabolic types in the microbial world. In 1954, John Postgate in the United Kingdom discovered a *c*-type cytochrome in a strictly anaerobic sulfate-reducing bacterium, and in 1953, Leo Vernon found *c*-type cytochromes in anaerobic purple photosynthetic bacteria. In the purple bacteria, the cytochromes function as carriers in the electron flow that drives photophosphorylation. Since 1954, both *b*- and *c*-

132

BAMBED, Vol. 30, No. 1, pp. 9–13, 2002

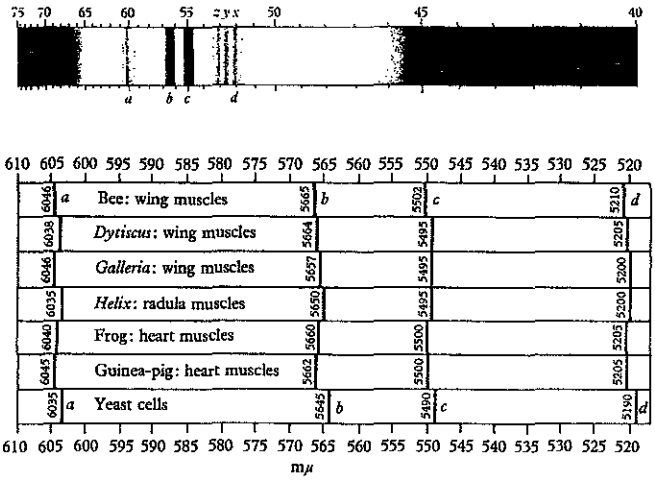


FIG. 3. **Absorption bands of reduced cytochromes in various organisms, as observed with a spectroscope.** For details see Ref. 9.

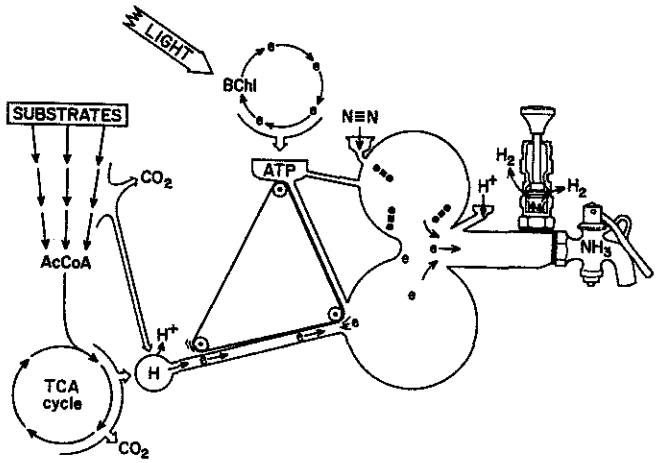


FIG. 5. **A representation of metabolic conversions occurring in photosynthetic bacteria during photoproduction of H_2 catalyzed by nitrogenase.** If N_2 becomes available H_2 is not produced, and reducing power is used instead for reduction of N_2 to ammonia.

type cytochromes have been observed in a variety of anaerobes where they function as electron carriers in anaerobic energy conversion processes that are analogous to aerobic respiration.

The actual mechanism of the energy-requiring coupling of ADP and inorganic phosphate baffled investigators for a long time. It remained for Peter Mitchell, in the United Kingdom, to develop a totally new concept of how this occurs. This was the chemiosmotic theory. Mitchell endured a long battle with what can be called the entrenched "Oxidative Phosphorylation Establishment." The Establishment consisted of about six prominent investigators who dominated almost all symposia of the biochemical societies for years. In my own laboratory, I referred to them as the Traveling Vaudeville Show. In 1963, Mitchell resigned his position at the University of Edinburgh, bought a very old manor house in the remote moors of Cornwall, United Kingdom, and remodeled half of it as a laboratory. Eventually, Mitchell's "protonmotive force" toppled all the old ideas. Mitchell's persistence and insights were recognized by the 1978 Nobel Prize in Chemistry.

Efraim Racker [10] described the history of research on oxidative phosphorylation with the clever cartoon shown in Fig. 4. His summary was that "The history of oxidative phosphorylation had its beginning in Europe. David Keilin (1925) in England deserves credit for the concept of the respiratory chain. Single-handed, literally, with a hand spectroscope, he deciphered the *a-b-c* of the cytochrome chain. In Russia, Vladimir Engelhardt (1930) conceived the idea that phosphorylation is linked to oxidative processes. But oxidative phosphorylation became a respectable field only when Severo Ochoa (1943) established that for each atom of oxygen three molecules of phosphate are esterified. Lehninger (1951) made the crowning discovery that NADH is the primary hydrogen donor for the respiratory chain in isolated mitochondria."

In the cartoon, Edward Slater (1953) represents part of the Establishment that kept busy, for years, searching for the mythical "A squiggle X," the supposed precursor of "X squiggle P," which supposedly gave rise to ATP. Finally, Mitchell is dumping "A squiggle X" into a waste basket. The mechanism of ATP formation by ATP synthase is still under active study, and we now know that the enzyme is an exquisite "machine" consisting of two interconnected molecular motors that are driven by protonmotive force (see Ref. 11).

N_2 FIXATION AND PHOTOSYNTHESIS

Before 1949, only three kinds of free-living bacteria were known to have the capacity to use N_2 as a nitrogen source for growth. These were identified before 1900, and it was generally believed that this metabolic ability was very restricted in nature. Serendipity, however, proved otherwise. While I was a graduate student at Washington University (St. Louis, MO), I spent the summer of 1948 doing research at the Hopkins Marine Station of Stanford University, located in Pacific Grove, CA. I was testing the growth of purple photosynthetic bacteria in various media, as a preliminary to research on the possible involvement of ATP and phosphorylation in photosynthetic energy conversion. One morning, I was astonished to observe that in one particular medium, the experimental organism, *Rhodospirillum rubrum*, had produced copious amounts of molecular hydrogen during photosynthetic growth.

After I returned to St. Louis, I conducted many experiments trying to demonstrate H_2 formation by illuminated resting cells of *R. rubrum* derived from H_2-producing cultures. The cells were resuspended in a dilute solution of buffered mineral salts under an atmosphere of N_2. In experiment after experiment, not a trace of H_2 was produced. After many trials, one day in January, 1949 I performed an experiment in which I deliberately replaced the supposedly inert gas phase of 100% N_2 with 100% H_2 or 100% helium, and for the first time observed light-dependent production of H_2. It was evident that N_2 was not inert in the metabolism of *R. rubrum.* The obvious conclusion that *R. rubrum* could fix N_2 was quickly substantiated [12, 13], and subsequent research has shown that virtually all anoxygenic phototrophs have the same capacity.

A key fact leading to the discovery of N_2 fixation by *R. rubrum* was that the medium in the culture showing H_2 production contained glutamate as the sole nitrogen

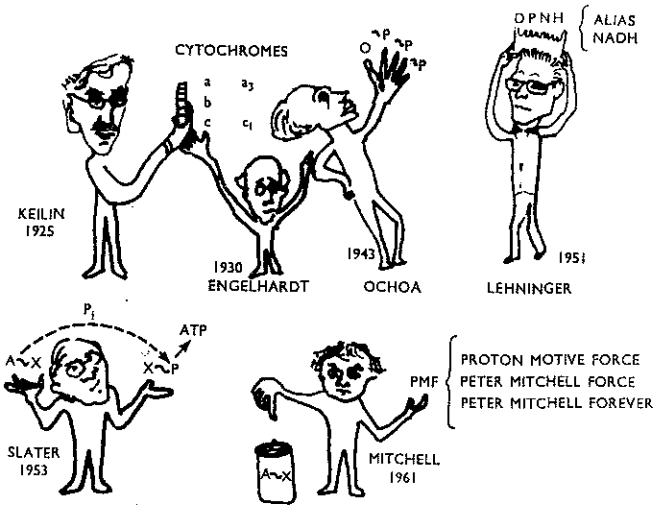


FIG. 4. **Highlights in the history of research on oxidative phosphorylation.** For details see Ref. 10.

source, rather than an ammonium salt. In all previous research with photosynthetic bacteria, NH_4^+ was routinely provided as the nitrogen source. It has since been found that ammonium salts repress synthesis, as well as activity of the nitrogenase complex. In my glutamate medium, the nitrogenase complex was derepressed. This set the stage for hydrogen production, because in the absence of N_2 and NH_4^+, all nitrogenases show the alternative activity of energy-dependent reduction of protons to H_2. Experiments with *R. rubrum*, in fact, gave the first indications that the nitrogenase complex can catalyze H_2 formation [14, 15].

Fig. 5 depicts the flow of carbon and electrons in purple photosynthetic bacteria producing H_2 during photoheterotrophic growth. The double-bulbed device on the right represents the nitrogenase complex. In the absence of ammonia, the nitrogenase is derepressed, and when N_2 is also absent, the complex functions as a hydrogen-evolving catalyst. With glutamate as the nitrogen source, the supplies of ATP from photophosphorylation, and electrons from organic substrates, are evidently in excess relative to the requirements of the biosynthetic machinery. Under these conditions, protons are reduced yielding molecular hydrogen, which is shown here as being discarded by a "hydrogen relief valve." If molecular nitrogen is added, hydrogen evolution stops, because ATP and the electron supply are used for the formation of ammonia, which is rapidly consumed for the production of amino acids and other nitrogenous compounds. The hydrogen relief valve is construed as a control device that permits "energy idling" when this is required by the balance between generation of ATP and reducing power on one hand and overall biosynthetic rate on the other [16, 17].

EPILOGUE

In reviewing the history of research in biochemistry from 1876 to approximately 1960, it soon became clear to me that it would be very difficult to present a comprehensive view of the highlights in a short article. The 80-odd-year period witnessed a cornucopia of advances that was equivalent to erection of the main girders of the skyscraper of biochemistry and also to the design and validation of its basic operating machinery. The metaphor of constructing a grand edifice to depict the development of a scientific discipline was deftly used by Lewis and Randall in their classic book on thermodynamics [1],

"There are ancient cathedrals which, apart from their consecrated purpose, inspire solemnity and awe. Even the curious visitor speaks of serious things, with hushed voice, and as each whisper reverberates through the vaulted nave, the returning echo seems to bear a message of mystery. The labors of generations of architects and artisans has been forgotten, the scaffolding erected for their toil has long since been removed, their mistakes have been erased, or have become hidden by the dust of centuries. Seeing only the perfection of the completed whole, we are impressed as by some superhuman agency. . . . Science has its cathedrals, built by the efforts of a few architects and of many workers."

I have noted some of the chief architects. There were many more. Their achievements, summarized here, are described in historical accounts by Florkin and Stotz [3] and Fruton [18, 19].

REFERENCES

[1] G. N. Lewis, M. Randall (1923) Thermodynamics and the Free Energy of Chemical Substances, McGraw-Hill Book Co., New York, Dedication, vii.
[2] H. Gest (1988) Sunbeams, cucumbers, and purple bacteria. Historical milestones in early studies of photosynthesis revisited, *Persp. Biol. Med.* **19,** 287–308.
[3] M. Florkin and E. H. Stotz, Eds. (1975) Comprehensive Biochemistry, Vol. 31, Elsevier Science Publishers B.V., Amsterdam.
[4] A. Harden (1914) Alcoholic Fermentation, Longmans Green, London.
[5] M. McCarty (1985) The Transforming Principle, Discovering that Genes Are Made of DNA, Norton, New York.
[6] H. Krebs (1981) Reminiscences and Reflections, Clarendon Press, Oxford.
[7] J. A. Bassham, A. A. Benson, L. D. Kay, A. Z. Harris, A. T. Wilson, M. Calvin (1954) The path of carbon in photosynthesis. XXI. The cyclic regeneration of carbon dioxide acceptor, *J. Am. Chem. Soc.* **76,** 1760–1770.
[8] H. E. Umbarger (1956) Evidence for a negative-feedback mechanism in the biosynthesis of isoleucine, *Science* **123,** 848.
[9] D. Keilin (1966) The History of Cell Respiration and Cytochrome, Cambridge University Press, Cambridge, UK.
[10] E. Racker (1975) Reconstitution, mechanism of action and control of ion pumps, *Biochem. Soc. Trans.* **3,** 785–802.
[11] M. J. Schnitzer (2001) Doing a rotary two-step, *Nature* **410,** 878–881.
[12] H. Gest, M. D. Kamen (1949) Photoproduction of molecular hydrogen by Rhodospirillum rubrum, *Science* **109,** 558–559.
[13] M. D. Kamen, H. Gest (1949) Evidence for a nitrogenase system in the photosynthetic bacterium Rhodospirillum rubrum, *Science* **109,** 560.
[14] J. G. Ormerod, K. S. Ormerod, H. Gest (1961) Light-dependent utilization of organic compounds and photoproduction of molecular hydrogen by photosynthetic bacteria; Relationships with nitrogen metabolism. *Arch. Biochem. Biophys.* **94,** 449–463.
[15] J. G. Ormerod, H. Gest (1962) Hydrogen photosynthesis and alternative metabolic pathways in photosynthetic bacteria, *Bacteriol. Rev.* **26,** 51–66.
[16] P. Hillmer, H. Gest (1977) H_2 metabolism in the photosynthetic bacterium *Rhodopseudomonas capsulata*, H_2 production by growing cultures, *J. Bacteriol.* **129,** 724–731.
[17] P. Hillmer, H. Gest (1977) H_2 metabolism in the photosynthetic bacterium *Rhodopseudomonas capsulata*, production and utilization of H_2 by resting cells, *J. Bacteriol.* **129,** 732–739.
[18] J. S. Fruton (1972) Molecules and Life, Historical Essays on the Interplay of Chemistry and Biology, Wiley InterScience, New York.
[19] J. S. Fruton (1999) Proteins, Enzymes, Genes, The Interplay of Chemistry and Biology, Yale University Press, New Haven, CT.

STEPHENSON, Marjory (1885-1948)

In 1913, Stephenson was awarded a Beit Memorial Fellowship for Medical Research, but relinquished it when World War I began. From 1914 to 1918 she worked with the British Red Cross in France and Salonika. Returning to science in 1919, she joined the staff of the Biochemical Department of Cambridge University, which was headed by the famed biochemist Sir Frederick G. Hopkins. In 1936, Stephenson was awarded the degree Doctor of Science (Cambridge) and in 1945 she became the first woman elected to Fellowship in the Biological Sciences Division of the Royal Society.

Stephenson's research focused, at first, on the use of washed bacterial cells ("resting cells") for study of metabolic reactions. She apparently was the first to prepare a bacterial enzyme preparation in the cell-free state (lactic dehydrogenase of *E. coli*). Her major interests focused on hydrogen transfer reactions and she published a notable paper on this subject in a Jubilee Volume of *Antonie van Leeuwenhock*, issued in honor of A.J. Kluyver [Some aspects of hydrogen transfer: 12:33-48, 1947].

She wrote an influential book on *Bacterial Metabolism* (Longmans Green), which went through three editions. Coverage of the original experimental literature was impressive. The following excerpts from the prefaces of the three editions gives a good "kinetic" view of progress in understanding bacterial metabolism during the formative period, 1929-1948.

1929: "The place of bacteria in evolution is a question very difficult of approach; we have, for example, no idea whether the forms familiar to us resemble primitive bacterial types or whether, like modern animals and plants, they are the successful competitors of the ages. Perhaps bacteria may tentatively be regarded as biochemical experimenters; owing to their relatively small size and rapid growth variations must arise very much more frequently than in more differentiated forms of life, and they can in addition afford to occupy more precarious positions in natural economy than larger organisms with more exacting requirements. No large animal or plant, for example, could hope to survive if obliged to depend solely on the oxidation of ammonia or sulphur for its energy. The profitable sources of energy have been seized upon and probably fully exploited by the green plant and the animal respectively; the autotrophic bacteria lead a hard and precarious existence due to the adoption of a type of metabolism ill-adapted to life on this planet, and only possible to organisms whose demands are small. Apart from their importance in soil economy, however, the autotrophic bacteria are of intense interest as suggesting courses which physiological evolution might have taken had a slightly different equilibrium established itself in the inorganic world."

1938: In the field of bacterial fermentations, facts have accumulated fast largely owing to the stimulus gained from the great advances made in our knowledge of alcoholic and muscle fermentation; the main check to

advance has been the difficulty of making active cell-free extracts of bacteria comparable to those obtainable from certain strains of yeast.

The study of bacterial enzymes shows that though they conform to the same laws as do those belonging to the animal and vegetable world their range of action is incomparably wider. Numerous systems completely missing in animals and plants flourish in different groups of bacteria, as, for example, the enzymes concerned with the utilization of molecular hydrogen and with the reduction and oxidation of many inorganic molecules. It would not be unsafe to predict that all the enzymes found in the animal will ultimately turn up in some bacterium or other whilst those found only in bacteria will be far more numerous.

1948: Since 1939 the progress of bacterial metabolism has been startlingly rapid. This is due partly to the greater number of workers in the field, partly to the introduction of new techniques, but most of all to the evolution of new concepts involving fresh experimental approaches.

One of the most important technical innovations has been the use of isotopes; so far the subjects most favourable affected have been fermentation, oxido-reductions involving CO_2, and nitrogen fixation. By the use of labeled carbon it has been possible to clear up many obscure patches in the intermediary processes of bacterial fermentations which could not otherwise have been settled with certainty. The same technique has led to the recognition that CO_2 is one of the principal oxidising agents of anaerobic life; its reduction to methane and to acetic acid has now been established together with the fact that such reductions

supply not only the energy but also the carbon compounds for cell synthesis. In the case of nitrogen fixation the use of N^{15} has enabled the course of nitrogen both fixed and free to be followed within the cell through the mechanism of fixation itself is still obscure....

The advances so far mentioned have been along lines laid down long ago but during the last few years a fresh view of bacterial metabolism has been opened up. Information is now being rapidly gained on the course of the biochemical processes leading to cell synthesis; such studies are peculiar to microbiology though certainly of wider application; they owe their success to the use of biological material which is prone to biochemical variation and tolerant of interference with its normal biochemical habit. This new stream of knowledge has its origin in several sources: microbial genetics, nucleic acid metabolism, adaptive enzyme formation, function of growth factors, the intracellular changes resulting from chemotherapeutic agents, antibiotics and other cell poisons, and interference with metabolism resulting from the introduction into the cell of chemical analogues of essential cell metabolites. All these are contributing to produce a picture – at present incomplete and patchy – of the biochemical machinery of growth. We seem, in fact, to be witnessing a transition from katabolic to anabolic studies, made possible only by the use of the microbe as experimental material."

WOOD, Harland G. (1907-1991)

In 1931, Wood became a graduate student of C. H. Werkman at Iowa State College (Ames), where Werkman was beginning studies on the chemistry of bacterial fermentations. Wood pursued research on fermentations by propionic acid bacteria and made the stunning discovery in 1935 that these organisms utilize CO_2 during fermentation of glycerol. Soon after Wood's discovery, A. J. Kluyver visited Ames to give a lecture, and Werkman informed him about Wood's findings. In his public lecture, Kluyver ridiculed the idea that a heterotrophic bacterium could use CO_2 in a major metabolic role. It is odd that Kluyver, the foremost spokesman for "unity in biochemistry" could not entertain the possibility that Woods was right.

Wood became chairman of the Biochemistry Department of Western Reserve University School of Medicine in 1946, and I joined the faculty of the Microbiology Department in 1949. We shared common interests in microbial metabolism and published a joint paper (1957) on chemical determination of formic acid, a frequent product in fermentations by coliform bacteria. I soon learned that the mention of Kluyver's name in Wood's presence raised his temperature very quickly.

During his 45 years at Western Reserve, Wood developed an outstanding biochemistry department strongly oriented to the use of isotopic tracers in analyzing metabolic pathways. Prior to coming to Western Reserve, Wood was at the University of Minnesota, where he built a water-cooled thermal diffusion column in a five-story elevator shaft for the separation of

^{13}C isotopic carbon, He was fond of describing the day that he found the column warped and distorted due to a temporary drop in the water pressure. This drop, he finally discovered, occurred when the home economic class let out and three toilets were flushed simultaneously! Wood also showed great versatility by constructing his own mass spectrometer for measurements of ^{13}C in metabolic products.

The overall thrust of Wood's research over 60 years was centered on CO_2 fixation in animal and bacterial metabolism. This was pursued in various ways including enzymology and molecular biology. During the last 30 years of his life, Wood concentrated his efforts on the reaction mechanism of the transcarboxylase of propionibacteria and a new pathway of autotrophic growth he discovered in *Clostridium thermoaceticum*. This bacterium ferments one mole of glucose to ca. 3 moles of acetic acid. One of the 3 moles of acetate is synthesized from CO_2 by the "acetyl-CoA pathway" in which the enzyme carbon monoxide dehydrogenase plays a major role. Details of the pathway are described in White, D.: *The Physiology and Biochemistry of Prokaryotes*, 3rd ed., Oxford University Press, Oxford (2007).

Wood received many honors and awards, including the U.S. National Medal of Science (1989). One obituary notes:

"He was remarkable for several reasons. First, one could always feel the sense of excitement and drive that he brought to the experimental aspect of science. The focus of the excitement was always on discovery. Second, he continually developed and applied the latest technology to his experimental problem. There were many jumps from fermentation balances all the way to gene sequencing."

LEDERBERG, Joshua (1925-2008)

ASM

1946: Joshua Lederberg and **Edward L. Tatum** publish the first paper on conjugation in bacteria. The proof is based on the generation of daughter cells able to grow in media that cannot support growth of either of the parent cells. Their experiments showed that this type of gene exchange requires direct contact between bacteria. From the work of Beadle and Tatum, Lederberg knew that fungi reproduced sexually, and he suspected that bacteria did as well.

1952: Joshua Lederberg and **Norton Zinder** report on transduction, or transfer of genetic information by viruses. They show that a phage of *Salmonella typhimurium* can carry DNA from one bacterium to another.

Gest, 2003 Lederberg, Joshua. 1925- . American pioneer in the field of bacterial genetics who received the 1958 Nobel Prize in Physiology of Medicine. Lederberg demonstrated that bacterial strains can be crossed to produce offspring containing new combinations of genetic factors. This seminal discovery was of basic importance in the development of molecular genetics and molecular biology.

The short ASM timeline entries on Lederberg give only a glimpse of his major contributions to microbiology, genetics and molecular biology. He shared the 1958 Nobel Prize in Physiology or Medicine

with G. W. Beadle and E. L. Tatum, and eventually became President of Rockefeller University. In 1986, Lederberg published, "A fortieth anniversary reminiscence" of the discovery that genetic recombination occurs in *E. coli*. [Nature 324: 627-628, 1986]. He recounts that "In September, 1941, when I started as an undergraduate at Columbia University, the genetics of bacteria was still a no-man's-land between the disciplines of genetics and (medical) bacteriology. The question whether "bacteria have genes, like all other organisms" was still unanswered, indeed rarely asked. My own thoughts at that moment lay elsewhere. I looked forward to a career in medical research applying chemical analysis to problems like cancer and the malfunctions of the brain. Cytotoxicology then appeared to be the most promising approach to cell biochemistry....My notes dated 8 July 1945 detail hypothetical experiments both to search for mating among *Monilia* (medically important yeast-like fungi) and to seek genetic recombination in bacteria (by the protocol that later proved to be successful). These notes coincide with the beginning of my course in medical bacteriology. They were provoked by the contrast of the traditional teaching that bacteria were *Schizomycetes*, asexual primitive plants, with an appreciation of sexuality in yeast."

A much more detailed history of the discovery is given in J. Lederberg: Genetic recombination in bacteria – a discovery account (Ann. Rev. Genet. 21:23-46, 1987). In this, he described books, courses, and faculty at Columbia University that guided his thinking and development:

"September, 1947 was the next deadline of personal history; I was to return to New York and continue my interrupted medical studies. [Francis] Ryan also offered me laboratory facilities, and he and Tatum looked hard and partly successfully for some financial support to make all that possible. Meanwhile, Tatum had negotiated with Yale my retroactive registration as a graduate student and had obtained assent from other professors that I had de facto enrolled in a number of their lecture courses and seminars. The work of 1946-1947 became my dissertation, which I had already defended before an international panel of experts. A more serious personal obstacle was obligatory retroactive payment of tuition to Yale University; but the happy result was to qualify for a PhD degree that would, as it turned out, widen my career options. I spent the summer of 1947 at Woods Hole (and the magnificent library of the Marine Biological Laboratory), completing the dissertation. The stacks gave a wonderful opportunity to explore the history of microbiology; how its pioneers had sought to cope with the perplexities of bacterial variability, totally isolated from the intellectual apparatus of modern genetics.

In mid-August, days before the resumption of medical school, I learned from Ed Tatum that the University of Wisconsin had contacted him about an opening in genetics. In a fashion revolutionary for the time, they were seeking a microbial geneticist! He had recommended my name, and as a Wisconsin graduate his word carried great weight there. I have since learned of the controversy that this proposal evoked.

Understandably, the appointment of a 22–year old as an assistant professor warranted close examination."

He was offered the position "and when I did come to Madison I was given no inkling of what a struggle I had engendered."

Lederberg concluded his memoir with: "I have never encountered the extremities that Jim Watson painted in his self-caricature of ruthless competition in *The Double Helix*, which is hardly to argue that they do not exist. Side by side with competition, science offers a frame of personal friendships and institutionalized cooperation that still qualify it as a higher calling. The shared interests of scientists in the pursuit of a universal truth remain among the rare bonds that can transcend bitter personal, national, ethnic, and sectarian rivalries."

Replica plating

A new technique or invention can sometimes advance a research area markedly. This was no doubt the case when the method of replica plating was developed by Joshua and Esther Lederberg [J. Bacteriol. 63:399-406, 1952]. They were able to perfect the germ of an idea suggested by physicists Leo Szilard and Aron Novick to a simple and practical procedure of great value. This is how it happened: [From J. Lederberg: Genetics 121:395-399, 1989]

"Penicillin could exert positive selection in favor of auxotrophic mutants. But many bacterial strains were relatively recalcitrant to the

feeble mutagens then available, and even after penicillin selection there was still the tedious task of screening thousands of colonies for the 1% or so that might be growth-factor dependent. In addition, the escalation of recombination studies imposed the equally tedious task of classifying vast numbers of individual recombinant colonies to score them on a series of growth factors, sugars, drugs and bacteriophages. For the first several years of my work at the University of Wisconsin, starting in the fall of 1947, I was deeply preoccupied with these technical and doctrinal issues and eager to follow other leads.

L. Szilard and A. Novick, at the University of Chicago, faced similar problems in scoring the phenotypes of an abundance of colonies. In February, 1951, at one of the monthly phage seminars that Szilard had organized, they remarked that they had been using multipronged inoculators, even a wire brush, for a primitive kind of what I later called replica plating (Novick, 1972). This was not very satisfactory owing to the poor resolution available with that material....

Perhaps the multipoint sampling technology of Novick and Szilard could be applied to the broader problem as well as to the tedium of colony scoring! But: how to improve upon the poor resolution and handling properties of the wire brush? Ed Tatum had taught me to use a beakerful of sterilized toothpicks, one by one, for colony picking; that saved the time needed to flame a platinum loop between picks. The brush was conceptually an ordered array of toothpicks. What might be a functional equivalent?

Paper was unsatisfactory: its lateral capillarity and its compression of the colonies distorted and broke up the original growth pattern. It occurred to me that some fabric with a vertical pile would be an analog of the paper on one hand and the wire brush on the other, and I soon collected a wide variety of remnants from the local dry goods shops to put them to empirical tests. (The predictable myth that I invaded my wife's wardrobe for this purpose is pure fantasy.) Also helpful were books on fabric structure which helped me to focus on cotton velveteen as the most desirable material."

BACTERIOPHAGE

Reflections on: the discovery of phage by Frederick Twort, the re-discovery of phage by Felix d'Herelle, the novel Arrowsmith and how it influenced the course of Gest's research career, and some aspects of phage research in molecular biology.

Twort's discovery of "an acute infectious disease" of bacteria (1915)[1]

In his book *Bacteriophages*, Mark Adams (1959)[2] summarized the first discovery of phage as follows:

"There is no doubt that numerous early bacteriologists saw and described signs of phage action in bacterial cultures. However, no intensive investigation of these phenomena was undertaken prior to the appearance of a brief but provocative paper by F. W. Twort (1915). This British bacteriologist described an acute infectious disease of staphylococci that produced marked changes in colonial morphology. The infective agent was filterable and could be passed indefinitely in series from colony to colony. Twort considered various hypotheses to explain this phenomenon; among others that it was a filterable virus analogous to the virus pathogens of animals and plants. Twort's remarkable paper contained in essence the present concept of the nature of bacteriophage, yet the paper remained unnoticed by scientists and Twort failed to pur-

sue the matter further, perhaps because of his wartime duties in the British Army."

Who was Twort?

Frederick Twort (1877 –1950) qualified as a physician in 1900, and acquired expertise in bacteriology as an assistant to William Bulloch, bacteriologist to the London Hospital. He then became superintendent of the Brown Animal and Sanatory Institution. The latter was an animal pathology research center (in London); Twort remained there for most of his career.

The 1915 paper (Lancet, December 4, 1915)

Twort speculated that non-pathogenic varieties of viruses probably exist in nature and "should be more easily cultivated than the pathogenic varieties; accordingly, attempts to cultivate these from such materials as soil, dung, grass, hay, straw, and water from ponds were made on specially prepared media. Several hundred media were tested....Inoculated agar tubes, after 24 hours at 37C, often showed watery-looking areas, and in cultures that grew micrococci it was found that some of these colonies could not be subcultured, but if kept they became glassy and transparent. On examination of these glassy areas nothing but minute granules, staining red with Giemsa. could be seen.

Further experiments showed that when a pure culture of the white or the yellow micrococcus isolated from vaccinia is touched with a small

portion of one of the glassy colonies, the growth at the point touched soon starts to become transparent or glassy, and this gradually spreads over the whole growth, sometimes killing out all the micrococci and replacing these by fine granules."

Twort further observed that the "action" is more rapid and complete with vigorous-growing young cultures than with old ones. Also: "The transparent material when diluted (one in a million) with water or saline was found to pass the finest porcelain filters (Pasteur-Chamberland F. and B. and Doulton White) with ease, and one drop of the filtrate pipetted over an agar tube was sufficient to make that tube unsuitable for the growth of the micrococcus.... This condition or disease of the micrococcus when transmitted to pure cultures of the micrococcus can be conveyed to fresh cultures for an indefinite number of generations; but the transparent material will not grow by itself on any medium."

The forgoing exemplifies the "flavor" of Twort's remarkable paper. I include the following paragraph because it shows Twort's critical thinking in the background of knowledge available in 1915.

"From these results it is difficult to draw definite conclusions. In the first place, we do not know for certain the nature of an ultramicroscopic virus. It may be a minute bacterium that will only grow on living material, or it may be a tiny amœba which, like ordinary amoebae,

thrives on living microorganisms. On the other hand, it must be remembered that if the living organic world has been slowly built up in accordance with the theories of evolution, then an amoeba and a bacterium must be recognised as highly developed organisms in comparison with much more primitive forms which once existed, and probably still exist at the present day. It is quite possible that an ultra-microscopic virus belongs somewhere in this vast field of life more lowly organized than the bacterium or amœba. It may be living protoplasm that forms no definite individuals, or an enzyme with power of growth."

It is important that Twort also noted he observed similar phenomena with a "member of the coli-typhoid group of bacilli."

Enter Felix d'Herelle (1873-1949)

In 1917, two years after Twort's report appeared, Felix d'Herelle -- a Canadian working at the Pasteur Institute -- published a short paper announcing discovery of "An invisible microbe that is antagonistic to the dysentery bacillus." The paper appeared in *Comptes rendus des séances de l'Académie des Sciences* (Séance du 3 Septembre 1917). The original, in French, and an English translation are included in Tom Brock's *Milestones in Microbiology*[3].

d'Herelle describes isolation of "an invisible microbe endowed with an antagonistic property against the bacillus of Shiga" from feces and urine of patients convalescing from infection with the "dysentery

bacillus." The "anti-Shiga microbe" passes through a Chamberland filter and lyses Shiga bacillus cells in broth culture. d'Herelle was able to carry "the first isolated strain through 50 successive transfers." He observed "clear areas" on agar slant cultures (i.e., plaques) and states "I have been able to show that a lysed culture of Shiga bacillus contains around 5 to 6 billion *filterable germs* per cubic centimeter. (my italics)....The antagonistic microbe can never be cultivated in media in the absence of the dysentery bacillus. It does not attack heat-killed dysentery bacilli, but is cultivated perfectly in a suspension of washed cells in physiological saline. This indicates that the anti-dysentery microbe is an obligate bacteriophage" [this is the first use of this word].

d'Herelle also states that he "isolated a filterable microbe able the lyse nicely the para-typhoid A bacillus." His paper has no references, and he later claimed that he was unaware of Twort's 1915 communication in Lancet.

Moreover, d'Herelle persisted in insisting that "Twort's phenomenon" was not the same as his own discovery. In his 1926 book,[4] d'Herelle begins his discussion of this matter as follows:

"In 1915, almost two years before my first communication upon the subject of bacteriophagy, Twort described a phenomenon which possesses a character in common with that which I have described, namely, it is reproducible in series. Aside from this common character, it offers

other characteristics, not merely different but which preclude all possibility of identity, for the characteristics of the two phenomena are mutually exclusive. But inasmuch as some authors have tried, despite this, to attribute the two phenomena to a single cause, quite without any experimental demonstration it is true, it seems necessary to consider this subject at some length."

Who was d'Herelle ?

Despite his fame, it is not easy to find an authoritative account of his life. A number of aspects of his history are unclear, and also controversial. According to Bulloch,[5] d'Herelle graduated M.D. from the University of Leiden. Summers[6] states, however, that d'Herelle "was awarded honorary M.D. degrees from Laval, Montreal, and Leiden but there is no evidence that he held an earned doctorate. Whatever the case, his writings exhibit considerable medical knowledge, and he seems to have practiced medicine because he cites case reports of patients in his care." In a later publication, Summers[7] describes d'Herelle's academic career as follows: "He held a permanent position in the scientific establishment only during his five years as a professor at Yale, He had no graduate students and only a handful of collaborators, and he was dismissed from key temporary positions and even declared persona non grata at the place where he did his most famous work, the Pasteur Institute." It is said that he became a self-taught microbiologist, and his only work focused on practical microbiology (notably fermentation proc-

esses). Many more details of d'Herelle's checkered history, idiosyncracies, and combative personality are given in W.C. Sommers book *Felix d'Herelle and the Origins of Molecular Biology* (Yale University Press, New Haven 1999).

There is no doubt that d'Herelle was an assertive entrepreneur, with a combative personality. His forte was promoting phages for treatment of various animal and human diseases, and in this connection he traveled widely pursuing projects in Mexico, Argentina, Guatemala, Indochina, India, Tunisia, Egypt, the East Indies and Russia. Intermittently, he was in Paris at the Pasteur Institute (1909 - 1921?) where he was at times either an unpaid assistant or was "promoted from an assistant in the vaccinotherapy service to a 'chef de laboratoire'." According to Dr. Antony Twort,[8] d'Herelle "was appointed Professor of Protobiology at Yale University in 1928 and apparently left under something of a cloud five years later." The Dictionary of Scientific Biography[9] notes that d'Herelle received many honors, including the prestigious Leeuwenhoek Medal (1925).

Contrasting Twort and d'Herelle

Dr. Antony Twort, son of Frederick Twort, wrote a comprehensive biography of his father.[8] The dispute over priority for discovery of phage is discussed in detail in a chapter of 25 pages, and I believe it is even-handed. Despite obfuscation by d'Herelle, the facts are clear.

Twort's 1915 paper described the basic features of the overall phenomenon of bacteriophage reproduction. In fact, the scientific sophistication of his report is far greater than that of d'Herelle's 1917 note. Antony Twort gives d'Herelle credit where it is due:

"Even if Fèlix d'Herelle was not the first to recognize and describe the phenomenon of transmissible bacteriolysis, or bacteriophage as he named it, he certainly must be given credit for fostering the discovery and bringing it to the attention of the world at large. This he did by means of his own continued researches, his several books, and numerous papers on the subject. He also travelled widely to promote further investigation into bacteriophage and its practical use in the treatment of bacterial infections, and he played a significant part in the establishment of special institutes in Egypt, India and Russia. At times his zeal reached missionary intensity. As Donna Duckworth (see ref. 10) says: '... he made research into bacteriophage one of the most exciting fields of work in the 1920s' ".

Antony concludes: "Perhaps Pierre Nicolle should be allowed to have the last word in this account of the bacteriophage controversy. In his paper, *Le Bacteriophage* [Biologie Medicale, October 1949], he wrote: 'Nearly everything subsequently learnt about the bacteriolytic agent was already contained 'en germe' in the admirable article by Twort in *The Lancet* of 1915." Nevertheless, as the years passed, d'Herelle was increasingly identified as the discoverer of phage. How

did this happen? In large part, it resulted from sharp differences in the personalities and working styles of the two investigators. Twort was a "loner" with a number of eccentricities. He was well-known for conflicts with the (British) Medical Research Council relating to diminishing research funds. In contrast, d'Herelle had a vigorous aggressive personality and was very ambitious for recognition. Thus, it was inevitable that Twort's priority would gradually become submerged, while d'Herelle would reap increasing fame. Writing in 2009, I think there is little more to be gained from all the details given in the references cited. For those who wish to go further, I recommend the article by D.H. Duckworth[10] and my paper on *"Dr. Martin Arrowsmith: Scientist and Medical Hero"* (see below). In sum, d'Herelle's fame strikes me as an early example of "hype in science", now a familiar phenomenon.

An unexpected aggravation

Priority controversy revived! In 1925, Sinclair Lewis published his famous and popular novel Arrowsmith, in which bacteriophage played a central role.

The storyline in Arrowsmith

In medical school, Martin Arrowsmith comes under the sway of Professor Max Gottlieb, the professor of bacteriology. Gottlieb, an immigrant from Germany, is described as having worked with both Louis Pasteur and Robert Koch, two of the giants of infectious disease re-

search. Following graduation, Arrowsmith makes a number of false starts in his career. Eventually he accepts a position as an independent researcher at the McGurk Institute of Biology in New York, where Gottlieb is a revered staff member. Working with a strain of staphylococcus from a carbuncle of a patient at the "Lower Manhattan Hospital," Arrowsmith makes his first exciting, and serendipitous, discovery, recording in his notebook, "I have observed a principle, which I shall temporarily call the X Principle, in pus from a staphylococcus infection, which checks the growth of several strains of staphylococcus, and which dissolves the staphylococci from the pus in question."

He establishes that the X Principle reproduces itself indefinitely in living staphylococci–but it is not clear whether the principle is a virus or an enzyme of some sort. In any event, the idea that the X Principle may cure "germ diseases" quickly surfaces. The discovery causes considerable excitement at the institute, but then the roof falls in. Gottlieb has the unpleasant duty to inform Arrowsmith:

"It iss a pity, Martin, but you are not the discoveror of the X Principle."

"Wh-what—"

"Some one else has done it."

"They have not! I've searched all the literature, and except for Twort, not one person has even hinted at anticipating—Why, good Lord,

Dr. Gottlieb, it would mean that all I've done, all these weeks, has just been waste, and I'm a fool—"

"Vell. Anyvay. D'Herelle of the Pasteur Institute has just now published in the *Comptes Rendus, Académie des Sciences*, a report–it is your X Principle, absolute. Only he calls it 'bacteriophage.' So."

"Then I'm—"

"In his mind Martin finished it, 'Then I'm not going to be a department-head or famous or anything else."

After recovering from this blow, Arrowsmith spends more than a year doing basic research on bacteriophage before the idea of curing plague with "phage" is put into his mind. As luck would have it, a plague epidemic is raging on the island of "St. Hubert" in the West Indies. At hand was the opportunity for Arrowsmith and the McGurk Institute to achieve world fame. Martin prepares antiplague phage on a large scale, seals the agent in tiny ampoules, and is off to St. Hubert with his devoted wife Leora. He is empowered to conduct the experiment in a St. Hubert parish in which the plague has just begun to appear, and the results look very promising. "The pest attacked the unphaged half of the parish much more heavily than those who had been treated."

Six months later the epidemic comes to an end, evidently from the use of the phage coupled with a vigorous program of eradicating the rat and ground squirrel vectors. On return to New York Martin is given a

hero's welcome and becomes head of the institute's new Department of Microbiology. But trouble is brewing. The institute publishes a report on the new plague "cure" in which Arrowsmith's qualifications regarding the need for a statistical evaluation of the results are altered to read "while statistical analysis would seem desirable, it is evident that this new treatment has accomplished all that had been hoped." This leads to various complications in Arrowsmith's professional and personal life which are summarized in ref. 11. The latter also discusses the important role of microbiologist Paul de Kruif as Lewis's collaborator in development of the novel. Quotes from a recent review[12] of *Arrowsmith*: "The overarching thesis of this story is the powerful and some-times damaging influence of business over medicine and science....de Kruif [who had once worked at the Rockefeller Institute for Medical Research] was fired from the institute after publishing a book chapter that was highly critical of US medicine. Its gist: too much business and mindless ritual, too little science.... Interestingly, infectious-disease specialists today are once more studying phage therapy owing to widespread bacterial resistance to antibiotics. Unfortunately, the mistakes of the early days of phage research are still felt. Many researchers who are unfamiliar with the modern work still think of it as an obsolete method. If only the early phage therapists had read *Arrowsmith*."

Twort and Arrowsmith

Twort was understandably annoyed by what he considered still another distortion of the true history of his discovery of bacteriophage. He sent Lewis a letter of complaint, and received the following answer[8]:

"I have read your letter with extraordinary interest, and regret that your name was not in *Arrowsmith* given the credit it, of course, deserves. It happened thus. Not only did I know through de Kruif and others of your relation to bacteriophage, but I had actually in my first version of the book, a paragraph stating Martin's tardy discovery of your work. Then I found I was getting into controversy entirely unsuitable to a work of fiction…hundreds of paragraphs had to be sacrificed, often regretfully, to keep the main themes of the book from being clogged. Among these was the small section in which I tried to give suitable credit to yourself…

Let me with the utmost earnestness send you my regrets and my greetings."

Twort also sent an urgent letter to the Deputy Director of the Pasteur Institute asking for help:

"… Although Mr. Sinclair Lewis knows the facts… In the film version Dr. Gottlieb again announces Dr. d'Hérelle as the discoverer of bacteriophage.

I think you will agree that it is very unfair to British science that Mr. Lewis should use his great influence in literature and by means of

the cinema to uphold the claims of d'Hérelle. I greatly appreciate the support you have given me in the past, but as the claim is coupled with the name of the Pasteur Institute I have been wondering if by any means it would be possible for you or Dr. Roux to take any steps to make the position clear to the general public. The film will probably be seen by several million people and it cannot fail to do me some harm..."

These and other attempts evoked only consolatory responses, and he eventually gave up. He informed some of his correspondents[8]: "I have no desire to write anything myself to the press and I do not propose to take any further action."

From Arrowsmith to Photosynthesis

[Based largely on refs. 13-16]

During my high school years in Los Angeles, I became firmly committed to a career in science and vividly recall journeys to the California Institute of Technology in Pasadena on Fridays to observe dramatic lecture demonstrations by famous scientists, mostly physicists. Later, the novel *Arrowsmith* influenced me to become a bacteriologist, and after two years at Los Angeles City College I became (at age 19) a junior majoring in bacteriology at the University of California at Los Angeles (UCLA). From the Arrowsmith story line, I became particularly interested in bacteriophages and somehow learned that such viruses might be research subjects at the Cold Spring Harbor Laboratory

(CSHL) in New York. I wrote to the then director Milislav Demerec, asking if this was so and he responded that a physicist named Max Delbrück agreed that I could come to his laboratory at Cold Spring Harbor for the summer of 1941 (at my own expense) and assist in his collaborative research with Salvador Luria. My fondest dreams were about to be realized. The only way I could afford to get to Cold Spring Harbor was a lengthy and arduous bus trip – the fare was $39, pillow and all meals included! The summer was inspiring and very busy for me – working in the lab at all hours except at meals times, when I waited on tables in the dining hall to earn free board and room.

During the summer, the CSH symposium was on 'Genes and Chromosomes/Structure and Organization'. It was attended by about 120 scientists. Demerec had expected 35 - 50 attendees. At first, he was disturbed by the large number, but soon found that the large audience actually stimulated discussion. I was dazzled by contact with famous scientists, and as a waiter was amused to observe their idiosyncrasies. After graduating from UCLA in 1942, I spent the summer at CSHL again, assisting Delbrück and Luria. My wife and I had a small apartment adjoining the Delbrück's (shared bathroom), and we paid no rent in return for my tending the house furnace. Max paid me $7.50 per week as a research assistant, and my wife worked as one of Demerec's secretaries.

Before coming to CSHL in 1942, I met John Spizizen at the California Institute of Technology. As part of his PhD work, Spizizen did some of the earliest research on biochemical aspects of bacteriophage replication with Emory Ellis, who was responsible for introducing Delbrück to phage. John was awarded one of the few National Research Council's Fellowships in the Medical Sciences for work with Delbrück and in 1942, he joined our small group at CSHL (see Figure 1).

During the spring of 1942, I wrote Max asking if he would accept me as a graduate student. The following was his reply:

VANDERBILT UNIVERSITY

DEPARTMENT OF PHYSICS

NASHVILLE, TENNESSEE

April 1st, 1942

Dear H. G.

It is correct that you would have to pay tuition (of $200) from the stipend. Living expenses here are fairly low, $300 are supposed to be enough for a single student. I am afraid I cannot offer you more now.

I am glad to hear that you would like to come here. We will also have Luria and Spizizen here next year. But I must warn you that this is a very poor place to come to where you have your chances for the future in mind. You will do here very specialized work - phages - which may

have a future, but not a very immediate one. And the Southerners are very conservative and narrow-minded when it comes to appointments. Also they are self satisfied, you will not meet here people from the north or make the contacts that might help you on. I believe all this would be better in Wisconsin. I, of course, would be very glad to have you, but don't think because you like phages and like me it is the best thing to come here. You cannot afford to follow your liking. It may well be that in another year or two I will be in a stronger position, so that I can take the responsibility of advising you to come here.

With best regards,

M. D.

P.S. There is a lurking possibility of trouble here because I am technically an enemy-alien.

These were, of course, desperate times. Max's brother, Justus Delbrück (a lawyer), his sister Emmi Bonhoeffer, and his brother-in-law Klaus Bonhoeffer (brother of the theologian Dietrich Bonhoeffer) were in the German Resistance against the Nazi regime. Klaus and Dietrich were executed in the last days of Hitler's Germany.

At the end of the summer of 1942, we proceeded to Vanderbilt University in Nashville, where Max was an instructor in the Physics Department. I began my graduate thesis research under his supervision (while formally enrolled in the Biology Department) on the effects of

inorganic salts on phage replication, and also assisted Max and Salvador in their other research. [My thesis research with Max was summarized in my earliest paper; H. Gest: The effects of inorganic salts on the multiplication of bacterial viruses, *Journal of Infectious Diseases* 73: 158-166, 1943).

Research on radioactive isotopes in the Manhattan Project

The rapid escalation of World War II during the fall of 1942 made continuation in graduate school problematic and very uncertain. This led me to accept an invitation from Charles D. Coryell (one of my chemistry instructors at UCLA) to join his research group at the University of Chicago (and later at Oak Ridge, Tennessee). Coryell was a brilliant physical chemist who had coined the terms 'exergonic' and 'endergonic', and he also had interests in biochemistry. Our group, a unit of the Manhattan Atomic Bomb Project, was responsible for characterization of radioactive isotopes that are created by the fission of uranium, and we also investigated chemical processes associated with the spontaneous disintegration of radioactive elements.

Graduate research on photosynthesis at Washington University

Several months after World War II ended (summer 1945) Coryell accepted a professorship in the chemistry department of Massachusetts Institute of Technology, and a number of the young members of our

group accompanied him to complete PhD work. Although I had this option, I decided to return to biology. In view of my experience with radiochemistry, Coryell suggested that I resume graduate study with Martin Kamen at Washington University (St. Louis). Kamen was the co-discoverer of radioactive ^{14}C and in 1946 was using the isotope for studying photosynthetic CO_2 reduction by unicellular green algae. With Coryell's recommendation, Kamen accepted me promptly as his first graduate student.

The major aim of my thesis research was to test the hypothesis that in photosynthesis, light energy is converted to chemical energy in the form of "high energy phosphate compounds". The research plan was based on using ^{32}P as a tracer. Although this isotope was not commercially available when I began my thesis research, we were able to prepare it ourselves. Kamen and I intermittently made large quantities of ^{32}P in the Washington University cyclotron, for use by Institute of Radiology clinicians in treating certain blood diseases.

Discovery of ^{32}P suicide of bacteriophage

During '47/'48, several reports appeared in the literature on the origin of the P found in the DNA of T-even phages. Because of my previous work with Delbrück and my then current research on P metabolism in photosynthesis using ^{32}P, I became interested in exploring phosphorus metabolism during phage infection of *E. coli*. This led to discus-

sions with Alfred Hershey, who was then an associate professor in the Washington University Department of Bacteriology and Immunology. Since we had $^{32}P_i$ available, I convinced Hershey and Kamen that we should start collaborative research. At the time, Hershey was not biochemically inclined but did express an interest in determining the P content of phage.

Eventually we decided on a novel experiment in which we would infect the bacterial host cells with viruses that contained extremely radioactive P, and follow the fate of the phosphorus in the virus progeny. Joseph Kennedy, chairman of the Washington University Chemistry Division, was enlisted in the research because of his wide expertise in radioactivity measurements (he had been head of the Chemistry Division at the Los Alamos laboratory of the Manhattan Project).

We never did the intended experiment because of unexpected events. Hershey and I had gone so far as to make the radioactive phage preparation, which was stored in a refrigerator. The phage radioactivity was so 'hot' that the only feasible way I could assay the preparation was with an electroscope! But it turned out that Hershey was then occupied for about a month with teaching duties, and I was busy with course work and other matters. When we were ready to set up the actual experiment we first checked the virus titer again, and were surprised to find that had decreased significantly. This was quite bothersome, and had the effect of causing another delay in doing the "big" experiment. Several weeks

later we found that the virus titer was still declining rapidly. Finally, it dawned on us that the virus titer was declining at the same rate that the ^{32}P was undergoing radioactive decay. We had accidentally discovered the phenomenon of "^{32}P suicide". (Hershey, Kamen, Kennedy and Gest, 1951, ref.16). Thus, as ^{32}P in the phage DNA disintegrated spontaneously, the transmutation of P to S sheared the DNA chains causing inactivation of the virus. In retrospect, I regard this as a curious episode of serendipity since one of the main problems I had worked on at Oak Ridge was the chemical effects that occur when radioactive elements in inorganic compounds undergo transmutation by beta decay to other elements.

For further details on the mechanism by which the nuclear conversion of ^{32}P to ^{32}S kills radioactive phage [^{32}P "suicide"], see ref. 15. The latter also discusses reasons to believe that the research for our 1951 paper must have played a large role in Hershey's thinking when he designed the famous "Hershey-Chase experiment". This celebrated experiment, in which phage was labeled with ^{32}P in DNA and with ^{35}S in protein, was generally interpreted as further evidence supporting the earlier conclusion of Avery et al (1944) that DNA is the "true carrier of heredity".

A continuing interest in bacteriophage

The course of a scientist's career is typically influenced by many kinds of events, large and small, usually peppered with episodes of ser-

endipity. This was certainly true for me, as detailed in my article "*A microbiologist's odyssey: Bacterial viruses to photosynthetic bacteria* (13). The latter traces my various involvements with bacteriophage, and lists my publications in this connection. Even after the main emphasis of my research shifted to photosynthetic bacteria, I continued to follow the constantly escalating phage literature. It became evident that phage was a remarkable "tool" for studying genetic processes. A major step forward in respect to photosynthetic bacteria was made by Barry Marrs. He spent a year in my laboratory and soon thereafter he discovered a "phage-like" entity called the Gene Transfer Agent (GTA). Barry exploited GTA to construct the first map of photosynthesis genes using *Rhodopseudomonas* [renamed *Rhodobactor*] *capsulata* as the test system. He wrote a personal memoir describing the history of this research milestone, part of which is reproduced below. In related work, Judy Wall, Paul Weaver and I (ref. 19) examined 33 wild-type strains of *R. capsulata* for ability to engage in genetic recombination through mediation by GTA. A majority of the strains could either produce or receive GTA. Sixteen types of virulent phages were isolated and their host ranges determined. There was no apparent correlation between capacity of the *R. capsulata* strains to donate or receive GTA and susceptibility to the phages. At the time (1975) it seemed possible that "GTA particles" are defective phages, but there was no definitive evidence to support this interpretation.

Phage and bioenergetics

Bioenergetics has always been of particular interest to me (see ref. 17), and was the stimulus for a study on "Bioenergetic aspects of bacteriophage replication in the photosynthetic bacterium *Rhodopseudomonas* [*Rhodobactor*] *capsulata* (18). We isolated, from sewage, a virulent phage active on *R. capsulata* and examined its replication under various conditions. In photosynthetically grown cells, phage replication was supported by anaerobic photophosphorylation, and virus growth in illuminated infected cells was markedly suppressed by inhibitors in photophosphorylation. When the cells were cultivated anaerobically in darkness, synthesis of the photopigments was greatly diminished, as expected. In the 'aerobic cells' energy for phage replication was provided by respiratory (oxidative) phosphorylation, but the low *anaerobic* photophosphorylation capacity of the pigment-depleted cells did not suffice for virus multiplication. Our results suggested that in vivo phosphorylation rate below some critical point may be adequate to support (uninfected) cell growth, but not phage replication. In other words, it appears that there is a relatively high threshold value of ATP regeneration rate necessary for orderly synthesis of phage components. We suggested that photosynthetic bacteria-phage systems may offer unique advantage for further study of the bioenergetics of phage growth and the *in vivo* development of energy-converting membranes. As far as I know, no one has followed up on this suggestion.

Advancing knowledge of phage molecular biology

Before 1945, no one foresaw that eventually bacteriophages would become extremely powerful tools for understanding the mechanisms of reproduction of plant and animal viruses and that phages would also be of crucial importance in establishing what we now call molecular biology.

Phage and the origins of molecular biology (J. Cairns, G.S. Stent, and J.D. Watson (eds), 1966).

Over the past six decades, there has been considerable expansion of our understanding of phage biology and biochemistry. Much of this knowledge is capably summarized in Madigan and Martinko/Brocks *Biology of Microorganisms* (11th ed.; Prentice Hall, 2006). Those interested in the "kinetics of discovery" of phage biology and biochemistry can find the dates of major new findings in Raymond W. Beck *A Chronology of Microbiology*/in Historical Context (ASM Press, 2000). Some examples:

1948 Alfred Hershey and R. Rotman develop the first genetic map of a bacteriophage. They analyze genetic recombinants obtained by dual infection of the host bacterium with host range and rapid lysis mutants.

1952 Norton D. Zinder and J. Lederberg report the discovery of a genetic transfer mechanism that they give the name "transduction".

Their experiments with *S. typhimurium* prove that bacteriophage P22 carries small sections of DNA from a donor bacterial cell to a recipient cell, thereby conferring a genetic trait to the recipient derived from the donor.

1955 Seymour Benzer introduces the term "cistron" to describe the shortest sequence of DNA that functions as a gene. He bases the terminology on fine-structure analysis of the gene by means of genetic complementation (*cis-trans* test) of the rII mutants of bacteriophage T2 that cannot bring about the lysis of strains of *E. coli* that carry prophage lambda.

1984 A.D. O'Brien discovers that two different bacteriophages isolated from highly toxigenic strains of *E. coli* 0157:H7 causing hemorrhagic colitis convert *E. coli* K12 to produce high-titer Shiga-like toxin.

Note also: In 1966, on the occasion of Delbrück's sixtieth birthday, an important survey of phage research was published by the Cold Spring Harbor Laboratory Press,...*Phage and the origins of molecular biology* [J. Cairns, G.S. Stent, and J.D. Watson (eds).

The Gene Transfer Agent

Discovery of the GTA by Barry Marrs is a particularly interesting example of how discoveries are made. The history illustrates how the

threads of personal interactions and serendipity are woven into a fabric of discovery. Marrs wrote a personal account (ref. 20), parts of which are reproduced here. His abstract: "The development of genetics as a tool for the study of photosynthesis is recounted, beginning in the period when no genetic exchange mechanism was known for any photosynthetic microorganism, and ending with the sequencing of the key genes for photosynthesis."

From reference 20:

"I went from the University of Illinois at Urbana to Stanford University to do a postdoc with Charlie Yanofsky, and I set aside the photosynthetic bacterial system to look at the regulation of mRNA synthesis and degradation in the *trp* operon in *E. coli*. As my time with Charlie drew to a close, I was having no success in finding a faculty position. I wanted to start my own research into the regulation of gene expression in photosynthetic bacteria, and I cold-called Howard Gest at Indiana University, since he was a major figure in the field. Howard quickly suggested that I come to his lab and start developing biochemical genetic studies there until I could find a permanent home of my own. It is typical of Howard that he would reach out to help someone, and this was important mentoring for me. Even when my household possessions arrived in Bloomington ahead of me, and Howard was unexpectedly pressed into advancing money for my move without really knowing where I was, his support never faltered. When I finally appeared in

Howard's lab, I expected to be asked to work on the biochemical physiology of photosynthetic microbes. Instead, Howard guided me to use my know-how of microbial genetics on problems related to respiratory and photosynthetic electron transport. *Rhodobacter capsulatus* was the organism of choice in Howard's lab, and it was very similar to *R. sphaeroides* with which I had worked under Kaplan's tutelage. Howard's guidance opened up a fruitful field at the interface between microbial genetics and electron transport physiology and biochemistry (Marrs et al. 1972; Marrs and Gest 1973a, b).

The breakthrough

Although much can be learned from the study of mutants without the tools of genetic exchange, when I set up my own laboratory at Saint Louis University School of Medicine in 1972, high on my list of priorities was the development of true genetic tools for any nonsulfur purple photosynthetic bacterial (NSPPB) species. I was a new faculty member without grant support, so I was free to try any approach, as long as it did not cost much. I decided that it was needlessly limiting to try and guess how photosynthetic bacteria exchanged genes in nature. What we should do is set up a screen so that we could tell when genes were being exchanged, and then figure out the mechanism later. Sandy Bilyeu, Nien-Tai Hu, and I went to Forest Park in Saint Louis and collected soil and pond samples for the isolation of new strains of NSPPB. We isolated one NSPPB from each enrichment. We did not bother to determine

which species we had isolated, because we could work with whichever one showed genetic exchange. From each isolate we selected one rifampicin- and one streptomycin-resistant mutant. We then mixed the antibiotic-resistant derivatives pairwise, one strep-resistant strain A with one rif-resistant strain B and so forth, and allowed them to grow together into stationary phase. These mixed cultures were plated on nutrient agar containing the two antibiotics simultaneously. If any pair of strains could exchange genes, we would find more double-resistant mutants than the spontaneous mutation controls, which had been grown without mates. Once this design was set up, it was Sandy's responsibility to plow through the many pair-wise combinations - many weeks of repetitive work. After about a month of negative results, I was ready to move on to something else. I feared that my new technician would tire of doing the same thing over and over. But Sandy said she enjoyed the predictability of these well-defined experiments, and she wanted to stick it out until we had done all the combinations. Within the week we found our first hit, the first genetic exchange system to be described for any NSPPB (Marrs 1974).

What ensued was one of those all-too-brief periods of ecstatic research, where almost every day brought new revelations. Did the cells need to be in contact for gene exchange to occur? Did Dnase block the transfer? No. Did the genetic material get shed into the medium by the bacteria? Yes. Did it behave like discrete particles? Yes, it sedimented

in a tight band at 70S in sucrose density gradients. 70S? That is too small for known transducing phages. Yes, 70S, the same size as a ribosome. OK, so it was small, but let's see the plaques. No plaques. Try again, there must be plaques. No plaques, no plaques, no plaques. And no lysis upon production of the particles, and all genes seemed to move at the same frequency (Marc Solioz did most of this work). This was truly a strange, new genetic exchange mechanism. And by the way, the species that was doing this was my old friend, *R. capsulatus* (Solioz et al. 1975) .... So now there was a genetic exchange system for *R. capsulatus*, and we could begin analyzing how the genes for photosynthesis were arranged on the chromosome, as a first step toward understanding the regulation of gene expression. One of the beauties of working with NSPPBs was that one could isolate mutants in pigment synthesis, recognized by their colors, and these mutants could be easily propagated by aerobic growth, even if the mutation blocked photosynthesis. We quickly established that there was genetic linkage between the genes for bacteriochlorophyll synthesis and those for carotenoid synthesis (Yen and Marrs 1976). In fact, we were able to argue that these genes were located very close together on the chromosome, because we had laboriously determined the amount of DNA carried by the GTA particles to be about 5000 base repairs. If two genetic markers were separated by more than the amount of DNA carried by a single GTA particle, no genetic

linkage would be seen, and the average gene is about 1000 base pairs long (Solioz and Marrs 1977).

We were better able to determine the DNA capacity of GTA particles through the clever work and persistence of Bill Yen, a postdoc who had done his PhD work with Howard Gest. When Bill started in the lab, we only had bioassays as a tool for characterizing GTA. We could not see it in the electron microscope or directly visualize its protein or nucleic acid. This is really a tribute to the sensitivity of genetics, where one detects single molecules, but it was quite a hurdle for understanding the nature of the GTA mechanism. Bill decided that we needed more GTA to resolve these questions, and he set out to discover 'over-producer' mutants of *R. capsulatus* that would make more GTA. This involved developing an intricate and precise plating system in which we could visualize by bioassay the productivity of each clone of mutagenized *R. capsulatus* cells. Bill continued to optimize the assay and screen with it, unti one day he placed in front of me, without a word, a plate containing about a thousand tiny colonies of normal type, and one colony that was clearly pumping out hundreds of times more GTA than the rest. With the over-producer in hand, we quickly cranked out the complete characterization of the GTA particle....

The sequence of things

Teaming up with Dean Taylor in Stan Cohen's lab, we were able to do restriction mapping of the photosynthetic gene cluster, and we could assign genetic markers to different restriction fragments by complementation studies. We could align the the genetic and physical maps of the region (Taylor et al. 1983). After I described this work at a seminar at the University of California at Berkeley, Doug Youvan and his mentor John Hearst proposed that we team up to study and sequence the genes for photosynthesis (Youvan and Marrs 184). I was not much interested in sequencing, so I readily agreed to supply the clones for them to sequence, including some clones that might carry reaction center protein genes. It turned out that Hartmut Michael , at the Max Planck Institute in Martinsreid, Germany, was working on crystallizing the photosynthetic reaction centers from a related phototroph, and sequence information was needed to complete the work. Hearst's group, which I was supporting, got into a kind of race with another group, Mel Simon and JoAnn Williams working at Cal Tech, who were going after the sequence of *R. sphaeroides* reaction centers, using a very different approach to cloning the genes. I believe both sequences were completed within a few months of each other, and this information enabled Michael to solve the crystal structure for which he, along with J. Deisenhofer and R. Huber, won the Nobel Prize in Chemistry in 1988. This was a turning point for work with the genetics of NSPPB, launching it from a little-known backwater

of microbiology into the spotlight, and bringing to a close, to my mind, the 'early days' in this field."

Some of Marrs' references:

Mars B and Gest H (1973a) Genetic mutations affecting the respiratory electron-transport system of the photosynthetic bacterium *Rhodopseudomonas capsulata*. J. Bacteriol. 114: 1045-1051

Marrs, B and Gest H (1973b) Regulation of bacterio-chlorophyll synthesis by oxygen in respiratory mutants of *Rhodopseudomonas capsulata.* J. Bacteriol. 114:1052-1057.

Solioz M and Marrs B (1977) The gene transfer agent of *Rhodopseudomonas capsulata*: purification and characterization of its nucleic acid. Arch Biochem Biophys 181: 300-307

Solioz M, Yen H-C and Marrs B (1975) The gene transfer system of *Rhodopseudomonas capsulata.* II. The release and uptake of gene transfer agents. J. Bacteriol 123: 651-672

Taylor DP, Cohen SN, Clark WG and Marrs BL (1983) Alignment of genetic and restriction maps of the photosynthesis region of the *Rhodopseudomonas capsulata* chromosome by a conjugation-mediated marker rescue technique. J Bacteriol 154: 580-590

Yen H-C, and Marrs B (1976) Map of genes for carotenoid and bacteriochlorophyll biosynthesis in *Rhodopseudomonas capsulata.* J Bacteriol 126: 619-624.

GTA genealogy

J.T. Beatty and his colleagues have made important contributions to our knowledge of how GTA is related to phages (see ref. 21). Their conclusions: "The gene transfer agent (GTA) of the α-proteobacterium *Rhodobacter capsulatus* is a cell-controlled genetic exchange vector. Genes that encode the GTA structure are clustered in a 15-kb region of the *R. capsulatus* chromosome, and some of these genes show sequence similarity to known bacteriophage head and tail genes. However, the production of GTA is controlled at the level of transcription by a cellular two-component signal transduction system....Some GTA proteins share a common ancestry with genuine phage proteins, but the pattern of these relationships is a mosaic, and so gene exchanges between GTA and one or more phages appear to have taken place. However, based on the relatively exclusive clustering of the GTA-like sequences, such gene exchanges with (pro)phages were rare over the time since these bacteria diverged from the last shared ancestor."

YES, there is more to the history of bacteriophage discovery than a typical textbook entry:

1915/1917: F. Twort/F. d'Herelle Discovery of bacterial viruses (bacteriophages)

References

1. Twort, F.W. 1915. An investigation on the nature of ultra-microscopic viruses . The Lancet, December 4, pp.331-334.

2. Adams, M.H. 1959. *Bacteriophages*. Interscience Publishers, New York

3. D'Herelle, F. 1917. Sur un microbe invisible antagoniste des bacillus dysenteriques. Comptes rendus Acad. Sciences 165: 373-375. English translation in Brock, T.D. (Ed.), *Milestones in Microbiology* (1961). Prentice-Hall, Englewood Cliffs.

4. D'Herelle, F. 1926 *The Bacteriophage and its Behavior.* Williams and Wilkins, Baltimore.

5. Bulloch, W. 1938. The History of Bacteriology. Oxford University Press, Oxford. (Dover Edition published in 1979).

6. Summers, W.C. 1991. On the origins of the science in *Arrowsmith*: Paul de Kruif , Felix d'Herelle. and phage. J. Hist. Med. & Alllied Sci. 46: 315-332.

7. Summers, W.C. 1999. *Felix d'Herelle and the Origins of Molecular Biology.* Yale University Press, New Haven.

8. Twort, A. 1993. *In Focus, Out of Step.* A biography of F.W. Twort F.R,S. 1877-1950. Allan Sutton, Dover, NH 03820. A perceptive review of this book was published by polymath N.W. Pirie in Nature 365: 703-704 (1993). Pirie notes the pioneering research of Twort in 1911 on vitamin requirements of bacteria; more on this research can be found in my paper at http://hdl.handl.net/2022/3149

9. Theodorides, J. 1972. Herelle, Felix D'. In: *Dictionary of Scientific Biography*, vol. VI, ed. by J. Hachette and J. Hyrtl, pp. 297-299. C. Scribner's Sons, New York.

10. Duckworth, D.H. 1976. Who discovered bacteriophage? Bacteriol. Revs. 40: 793-802.

11. Gest, H. 1991. Dr. Martin Arrowsmith: Scientist and medical hero. Persp. Biol. Med. 35: 116-124.

12. Hausler, T. 2008. When business became biology's plague/A 1920's best-seller about risky campus capitalism and early phage therapy still resonates today. Nature 453:38.

13. Gest, H. 1994. A microbiologist's odyssey: Bacterial viruses to photosynthetic bacteria. Photosyn. Res. 40: 129-146.

14. Gest, H. 2002. Photosynthesis and phage: early studies on phosphorus metabolism in microorganisms with ^{32}P and how they led to the serendipic discovery of ^{32}P-decay "suicide" of bacteriophage. Photosyn. Res, 74: 331-339.

15.Gest, H. 2005. The early history of ^{32}P as a radioactive tracer in biochemical research. Biochem. Molec. Biol. Education 33: 159-164.

16. Hershey, A.D., Kamen, M.D., Kennedy, J.W. and Gest, H. 1951. The mortality of bacteriophage containing assimilated radioactive phosphorus. J. Gen. Physiol. 34: 305-319.

17. Gest, H. 2002. Landmark discoveries in the trail from chemistry to cellular biochemistry; with particular reference to mileposts in research on bioenergetics. Biochem. Molec. Biol. Education 30: 9-13.

18. Schmidt, L.S. Yen, H-C., and Gest, H. 1974. Bioenergetic aspects of bacteriophage replication in the photosynthetic bacterium *Rhodopseudomonas capsulata*. Arch. Biochem. Biophys. 165: 229-239.

19. Wall, J.D., Weaver, P.F., and Gest, H. 1975. Gene transfer agents, bacteriophages, and bacteriocins of *Rhodopseudomonas capsulata*. Arch. Microbiol. 105; 217-224.

20. Marrs, B. L. 2005. The early history of the genetics of photosynthetic bacteria: a personal account. In: *Discoveries in Photosnythesis,*

Govindjee, J.T. Beatty, H. Gest, and J.F. Allen, Eds. Springer, Dordrecht.

21. Lang, A.S., Taylor, T.A., and Beatty, J.T. 2002. Evolutionary implications of phylogenetic analyses of the Gene Transfer Agent (GTA) of *Rhodobacter capsulatus*. J. Mol. Evol. 55: 534-543. See also: Lang, A.S. and Beatty, J.T. 2007. Importance of widespread gene transfer in α-proteobacteria. Trends in Microbiol. 15: 54-62.

Photosynthesis Research **74:** 331–339, 2002.

Historical corner

Photosynthesis and phage: early studies on phosphorus metabolism in photosynthetic microorganisms with ^{32}P, and how they led to the serendipic discovery of ^{32}P-decay 'suicide' of bacteriophage

Howard Gest
Department of Biology and Department of History and Philosophy of Science, Indiana University, Bloomington, IN 47405, USA (e-mail: hgest@bio.indiana.edu)

Received 16 May 2002; accepted in revised form 19 August 2002

Key words: ATP, bacteriophage 'suicide', *Chlorella*, *E. coli*, P metabolism in photosynthesis, *Rhodospirillum rubrum*, *Scenedesmus*, ^{32}P

Abstract

During my PhD thesis research (1946–1949), I explored the effects of light on the uptake of ^{32}P-labeled inorganic phosphate (P_i) by cells of photosynthetic bacteria and microalgae, and the dynamics of P turnover between low and high molecular weight cell constituents. The results were interpreted as evidence for the conversion of light energy to the chemical energy of phosphorylated compounds. The experimental results also suggested to me that the precursors of the P in DNA bacteriophages of *Escherichia coli* must be low molecular weight phosphorylated compounds present within the host cells and led to the design of an experiment to determine the conservation of ^{32}P of an infecting phage particle in its numerous progeny. The experiment envisaged was never conducted because phage labeled with ^{32}P of high specific activity showed unexpected loss of viability. Thus, by serendipity, 'suicide' of phage due to ^{32}P-β decay was discovered. ^{32}P-decay 'suicide' provided a technique that was useful for analysis of phage genetic structure and replication. This memoir describes the unusual circumstances leading to the decisive role of serendipity in revealing an extraordinary phenomenon.

'Expect the unexpected, or you won't find it'
–Heraclitus, ca. 500 B.C.

'It is an essential part of scientific method to expect the unexpected'
–Haldane 1968

Biological research at Cold Spring Harbor Laboratory and Vanderbilt University

While I was an undergraduate majoring in bacteriology at University of California, Los Angeles (UCLA) in 1940, I became particularly interested in bacteriophage through reading D'Herelle's book on the subject (D'Herelle 1926). From Milislav Demerec, Director of the Cold Spring Harbor Laboratory (CSHL), I learned that Max Delbrück, a faculty member at Vanderbilt University, did research on bacteriophages at CSHL during the summer months. Max invited me to come to CSHL for the summer of 1941 (at my own expense) and assist in his collaborative research with Salvador Luria on *Escherichia coli* phages. To defray the cost of my room and board at the laboratory, I waited on tables in the dining room. During the summer, the CSH symposium was on 'Genes and Chromosomes/Structure and Organization'. It was attended by about 120 scientists. Demerec had expected 35–50 attendees. At first, he was disturbed by the large number, but soon found that the large audience actually stimulated discussion. I was dazzled by contact with famous scientists, and as a waiter was amused to observe their idiosyncracies. After graduating from UCLA in 1942, I spent the summer at

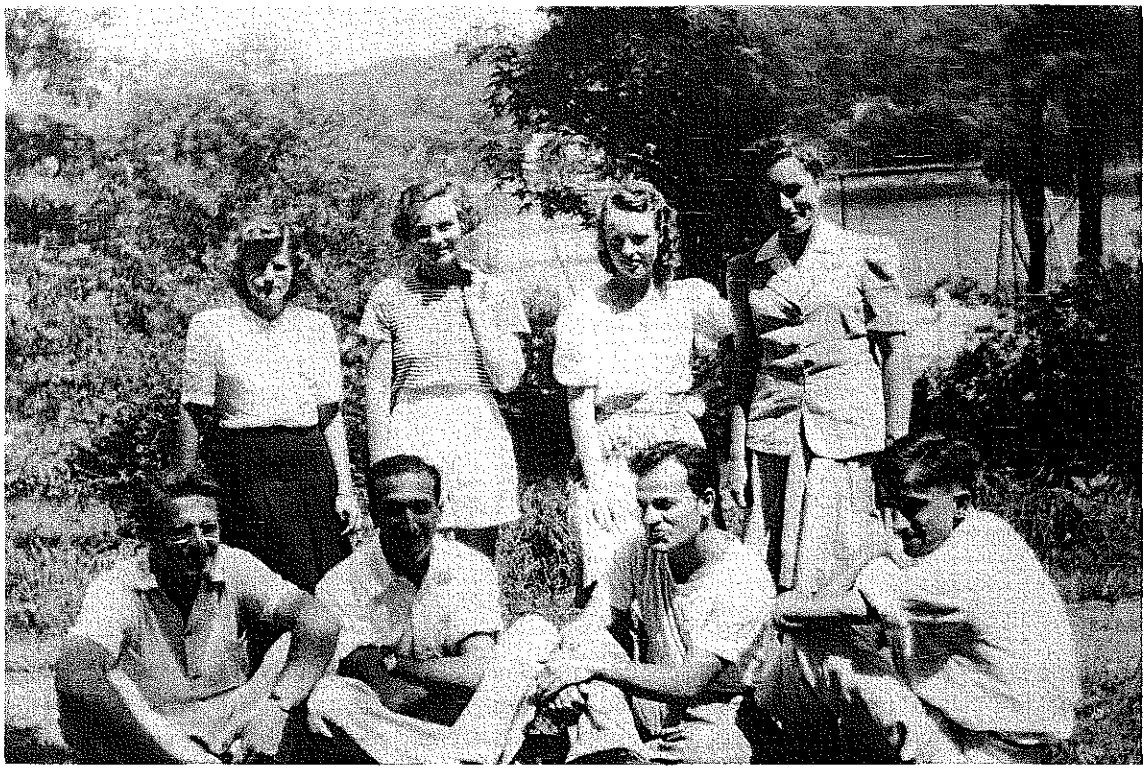


Figure 1. Bacteriophage researchers at Cold Spring Harbor Laboratory, summer 1942. Bottom row, left to right: Howard Gest, Salvador Luria, John Spizizen (see text), Max Delbrück. Top row, left to right: Edna Cordts (a graduate student from Vanderbilt University), and wives: Manny Delbrück, Evelyn Spizizen, Janet Gest*. (*Deceased, 1994.)

CSHL again, assisting Delbrück and Luria. My wife and I had a small apartment adjoining the Delbrück's (shared bathroom), and we paid no rent in return for my tending the house furnace. Max paid me $7.50 per week as a research assistant, and my wife worked as one of Demerec's secretaries.

Before coming to CSHL in 1942, I met John Spizizen at the California Institute of Technology. As part of his PhD work, Spizizen did some of the earliest research on biochemical aspects of bacteriophage replication with Emory Ellis, who was responsible for introducing Delbrück to phage (see Ellis and Spizizen 1940, 1941 and Spizizen 1943). John was awarded one of the few National Research Council's Fellowships in the Medical Sciences for work with Delbrück and in 1942, he joined our small group at CSHL (see Figure 1). Unfortunately, Delbrück was then (and later) 'famously' uninterested in biochemistry. Figure 2 shows Spizizen and Gest discussing the present memoir in 2002.

At the end of the summer of 1942, I proceeded to Vanderbilt University in Nashville, where Max was an instructor in the Physics Department. I began my graduate thesis research under his supervision (while formally enrolled in the Biology Department) on the effects of inorganic salts on phage replication (Gest 1943), and also assisted Max and Salvador in their other research.

Research on radioactive isotopes in the Manhattan Project

The rapid escalation of World War II during the fall of 1942 made continuation in graduate school problematic and very uncertain. This led me to accept an invitation from Charles D. Coryell (one of my chemistry instructors at UCLA) to join his research group at the University of Chicago (and later at Oak Ridge, Tennessee). Coryell was a brilliant physical chemist who had coined the terms 'exergonic' and 'endergonic', and he also had interests in biochemistry. Our group, a unit of the Manhattan Atomic Bomb Project, was responsible for characterization of radioactive isotopes that are created by the fission of uranium, and we also investigated chemical processes associated with

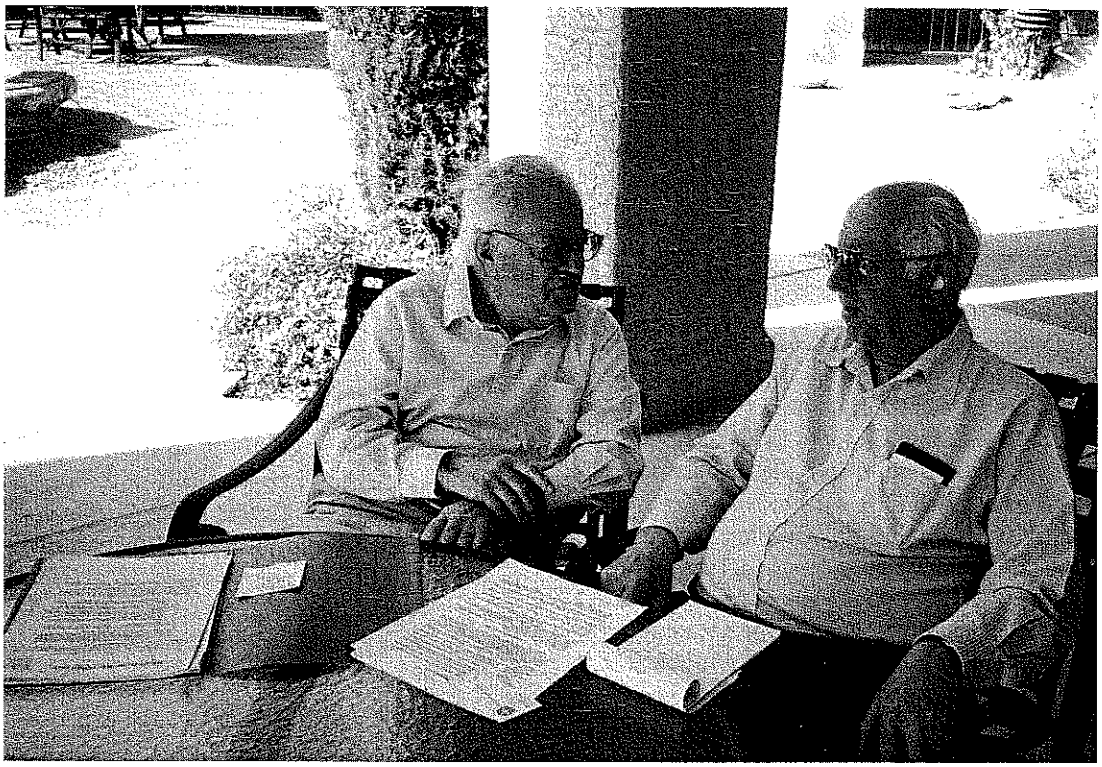


Figure 2. John Spizizen and Howard Gest (Tucson, Arizona, 2002). During the 1950s, Spizizen and Gest were faculty members in the Department of Microbiology of Western Reserve University School of Medicine. In 1958, Spizizen published his pioneering paper on DNA transformation of *Bacillus subtilis* (Spizizen 1958) which became a 'Citation Classic'.

the spontaneous disintegration of radioactive elements (some of these researches were eventually declassified and published; e.g., Burgus et al. 1948; Edwards et al. 1951; Gest and Edwards 1951; Gest et al. 1951; Gest and Glendenin 1951). As a special assignment, my colleagues and I prepared gigantic quantities of a radioactive isotope of barium produced in uranium fission (Gest 2001). The barium isotope was needed by Los Alamos physicists as a radiation source for testing atomic bomb detonation characteristics.

Graduate research on photosynthesis at Washington University

Several months after World War II ended (summer 1945), Coryell accepted a professorship in the chemistry department of the Massachusetts Institute of Technology, and a number of the young members of our group accompanied him to complete PhD work. Although I had this option, I decided to return to biology. In view of my experience with radiochemistry, Coryell suggested that I resume graduate study with Martin Kamen at Washington University (St. Louis). Kamen at the time was using ^{14}C for studying photosynthetic CO_2 reduction by unicellular green algae. With Coryell's recommendation, Kamen accepted me promptly as his first graduate student.

My situation at Washington University was somewhat jumbled in that Kamen's appointment was in the Chemistry Department, our laboratory was in the Mallinckrodt Institute of Radiology at the School of Medicine, and I was formally registered as a graduate student in the Department of Bacteriology & Immunology in the School of Medicine. I soon felt that the requirements for the PhD degree in the department were too restrictive, and I prevailed on Kamen to arrange a special interdepartmental 'microbiology graduate committee' that would administer the final examination for my PhD degree.

The major aim of my thesis research, was to test the hypothesis suggested by Ruben (1943) that in photosynthesis, light energy is converted to chemical energy in the form of 'high energy phosphate compounds'. The research plan was based on using ^{32}P as a tracer. Although this isotope was not commercially

available when I began my thesis research, we were able to prepare it ourselves. Kamen and I intermittently made large quantities of ^{32}P in the Washington University cyclotron, for use by Institute of Radiology clinicians in treating certain blood diseases.

Using ^{32}P-labeled P_i, I studied the uptake of P_i by cells, and turnover of P between cell fractions in three organisms: *Rhodospirillum rubrum, Chlorella pyrenoidosa,* and *Scenedesmus D3 (Gaffron).* With all three organisms, the P_i uptake by illuminated cells was considerably greater than in cells incubated in darkness. The experimental plan for detecting conversion of light energy to 'phosphoryl chemical energy' was based on the following assumptions. P_i taken up by the cell is converted to low molecular weight organic phosphoryl compounds. The latter are in the trichloroacetic acid (TCA)-soluble cell fraction, and are the precursors of 'energy rich' phosphoryl compounds such as ATP. Light-dependent transfer of ^{32}P from the low molecular weight componds to macromolecules in the TCA-insoluble cell fraction ('turnover') was interpreted as supporting the concept that light energy is converted to energy-rich phosphoryl compounds used for biosynthesis. It should be noted that the TCA-insoluble cell components were hydrolyzed with KOH to bring phosphoproteins and nucleic acids into solution. In a typical experiment with *R. rubrum,* the ratios of ^{32}P in cell fractions of bacteria incubated in light and dark were:

	Light/Dark
TCA extract	2.04
Lipid fraction	2.75
KOH extract	13.0

The main experimental data of my thesis were summarized in the first paragraph of the abstract presented to the nine-membered Committee of Examiners (which included two representatives from the Bacteriology & Immunology Department, namely, Professor Jacques Bronfenbrenner, who was well known for his early studies on bacteriophage biology (see Bronfenbrenner 1928) and Associate Professor Alfred Hershey):

> The participation of phosphorylated compounds as energy carriers in photosynthesis and chemosynthesis has been previously postulated on the basis of comparative biochemistry. Using radioactive phosphorus, P^{32}, as a tracer, it has been found that illumination significantly accelerates the turnover of phosphorus compounds in photosynthetic algae and bacteria (*Chlorella, Scenedesmus, Rhodospirillum).* The results are considered as evidence for the conversion and storage of light energy as chemical energy in phosphorylated compounds, by direct or indirect means.

Details of the experiments were published in Gest and Kamen (1948). While our paper was in press, another investigation designed to test the phosphorylation hypothesis using ^{32}P appeared. Aronoff and Calvin (1948) found no effect of light on phosphate uptake or P turnover in grana, leaves or *Chlorella vulgaris.* They concluded: 'If an organic phosphorus fraction constituting less than 10% of the total organic phosphorus were involved in the photochemical interchange with inorganic phosphorus, the present method would not have been able to detect an acceleration of this interchange by light.' In fact, their experimental plan was faulty, and we added the following addendum to our 1948 paper:

> A detailed description of the experiments of Aronoff and Calvin has appeared recently (Plant Physiol (1948)23: 351). These workers used an indirect method for determination of inorganic and organic fractions of phosphate (P^{32}) taken up. Apparently the P^{31} contents of these fractions were not measured in any instance and consequently no data on specific activities are available. Since P^{31} contents were assumed from values given in the literature by other workers using different algae, the significance of the calculations of Aronoff and Calvin relating phosphate uptake and turnover to light and dark metabolism cannot be readily assessed.

It is worth noting that at the time, the 'conventional wisdom' cast doubt on the likelihood that light energy could possibly be efficiently converted to 'ATP energy'. This was the opinion of James Franck, who shared the 1925 Nobel Prize in Physics with Gustav Hertz for research on energy changes that occur when atoms collide with electrons. After World War II, Franck collaborated with Hans Gaffron on photosynthesis research in connection with chlorophyll fluorescence. My correspondence with Franck on this topic in 1949 is reproduced in Gest 1993, which gives a clear picture of how this important aspect of photosynthesis was being discussed before 1950.

In Gest and Kamen (1948), we stated that 'The results obtained in this investigation indicate either that

phosphorylation is an integral feature of the photosynthetic process proper or that non-related "dark" phosphorylation reactions are greatly stimulated in some way as a consequence of illumination.' Even though the experiments with *R. rubrum* were conducted using anaerobic conditions, eliminating the possibility of oxidative phosphorylation, our caveat about possible stimulation of 'dark' reactions was no doubt a reflection of the 'theoretical' doubts of Franck and others. Later, it was satisfying to me that Fritz Lipmann suggested to Al Frenkel that he study our paper before undertaking the experimental work that demonstrated photophosphorylation by *R. rubrum* membranes (see Frenkel 1993).

^{32}P experiments with phage at Washington University; an unlikely convergence of backgrounds and interests

While my thesis work on photosynthesis was in progress during 1947/1948, I continued to read new papers on phage research, especially several reports in which ^{32}P was used as a tracer. Some of these papers claimed that the DNA of the virus 'was built in the main from the inorganic P of the medium' or 'wholly from the environment', rather than from preformed compounds of phosphorus existing within bacterial host cells prior to infection. From my research on the intracellular turnover of P in photosynthetic microorganisms, I concluded that such statements suggesting some kind of assimilation of $^{32}P_i$ directly from the medium into virus DNA were questionable. Because of my previous experience with phage and radiochemistry, I became interested in exploring the dynamics of intracellular phosphorus metabolism during phage infection of *E. coli* using ^{32}P. I discussed my ideas with Hershey who was at the time disinterested in biochemistry *(à la* Delbrück).

Hershey, Kamen and I discussed possible avenues of research, and eventually decided on a novel experiment to trace the fate of radioactive phosphorus in a single phage particle during its multiplication in a single *E. coli* cell. Would all the phage progeny contain ^{32}P or would all the tracer remain in the parental phage particle? To answer this question, we planned to infect the bacterial host cells with radioactive phage containing ^{32}P of high specific radioactivity (this had become available from the Oak Ridge National Laboratory), and determine the fate of the ^{32}P in the new phage progeny. We intended to spread the progeny over a solid, fairly large area of some kind, and scan the area systematically for radioactive 'spots' using a miniature Geiger counter. To help with the scan, Joseph W. Kennedy, chairman of the Washington University Chemistry Department was enlisted as a colleague in the research because of his great expertise in radioactivity detection and measurements.

Kennedy (1916–1957) was an accomplished radiochemist who played an important role in discovery of the element plutonium. He also designed and built novel radiation detectors that were crucial to the early success of the Berkeley (University of California) radiochemistry group. At the age of 27, in 1943, Kennedy organized the Chemistry-Metallurgy Division at the Los Alamos Laboratory of the Manhattan Project, and served as its leader through 1945 (see Wahl 1957).

Kamen (deceased, August 31, 2002), the co-discoverer of ^{14}C, was a pioneer in the production and use of radioactive isotopes. In 1947, he published a seminal book on radioactive tracers in biology (Kamen 1947). I helped him to compile tabular data for the book, which included a lengthy analysis of isotope dilution theory developed by Gest et al. (1947).

Serendipity changes the research plans

The experiment we planned was never done because of unexpected events. Hershey and I had gone so far as to prepare the radioactive phage, which was stored in a refrigerator. The ^{32}P-labeled phage was so 'hot' that the only feasible way that I could assay the radioactivity was with an electroscope (a device that measures ionizations produced by radiations from strong radioactive sources). The proposed experiment, however, was delayed for some weeks, mainly because Hershey was occupied with teaching duties. When we were ready to set up the actual experiment, we reassayed the radioactivity and redetermined the phage titer. To our surprise, the phage titer had decreased significantly, and this perturbing and unexpected finding had the effect of causing another delay. Several weeks later, we again found that the virus titer was declining rapidly. Finally, it dawned on us that a certain number of ^{32}P disintegrations *within* a phage particle leads to biological inactivation. We had accidentally discovered the phenomenon of phage 'suicide' caused by ^{32}P β-decay.

To verify and extend our initial interpretations, phages containing various initial specific radioactiv-

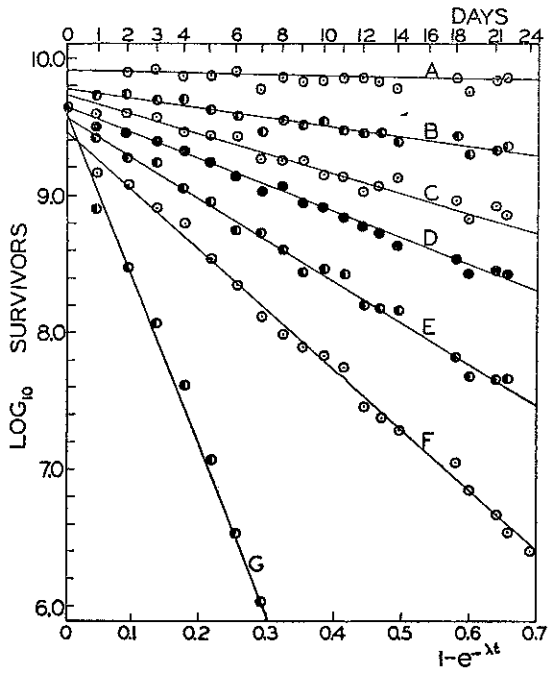


Figure 3. Survival of phage T2Hr1 containing assimilated P^{32}. From Hershey et al. (1951). Initial specific radioactivities of the phage preparations (millicuries ^{32}P/mg P): A, 0; B, 10.5; C, 19; D, 30; E, 45; F, 58; G, > 93. *E. coli* strain B cells were infected with phage in media with different initial specific radioactivities of ^{32}P (as inorganic phosphate). After lysis of the cultures, the lysates were centrifuged to remove debris when this was necessary. Dilutions of the lysates were stored at 5 °C for periodic titration.

ities (millicuries ^{32}P/mg P) were prepared for determination of death rates. Figure 3, from Hershey et al. (1951) shows the results, namely, a proportionality between specific ^{32}P radioactivity and rate of death of the phage.

In our paper, we noted:

> ...it is clear that the efficiency of killing by beta radiation is too low to account on this basis for the deaths from assimilated P^{32}; according to our data 34 times too low. We conclude that the inactivation of phage containing assimilated P^{32} is rarely due to beta radiation, but is usually a direct consequence of the nuclear reaction, which kills with an efficiency of 1/11.6 or a little greater. *The finding that the nuclear reaction $P^{32} \rightarrow S^{32}$ kills radioactive phage with an efficiency of about 0.086 per atom transformed presumably means that at least this fraction of the phosphorus atoms of the phage particle is situated in vital structures, and therefore that the vital structures contain nucleic acid.* [italics added]

Evidently, the transmutation of radioactive phosphorus to sulfur sheared the DNA chains causing inactivation of the virus. Although we never did experiments to directly answer the question that initiated our research, namely, 'whether all or only one of the progeny of a labelled phage particle contains isotopic phosphorus' (Hershey et al. 1951) we were able to say that 'Our present data exclude the possibility that the conserved atoms appear in only one of the phage progeny.'

Our only paper on the suicide phenomenon was still not complete when Hershey left St. Louis in 1950 to assume his new position in the Department of Genetics of the Cold Spring Harbor Laboratory. Thus, it had to be finished by mail correspondence. The manuscript was received by the Journal of General Physiology on June 23, 1950, and publication was delayed until 1951. It should be noted that in 1950, identification of the phage genetic material as DNA was still not generally accepted, and conflicting notions of P metabolism in phage replication were still being discussed in 1952 (see Price 1952).

Serendipity compounded

For serendipitous events to lead to significant discoveries, the investigators obviously must have the background and expertise to exploit the unexpected. Serendipity has played an important role in my research career (Gest 1992, 1997) and in the present instance it emerged in several ways: Hershey had extensive knowledge of contemporary phage biology; Kamen and Kennedy had great experience with physical and nuclear chemistry; and I was in familiar territory because I had a mixture of experience and interests that connected relevant areas of the research. In retrospect, and in the present context I find it remarkable that while I was in Oak Ridge during World War II, one of the main problems I worked on was the *chemical* effects that occur when radioactive elements in inorganic compounds undergo β decay to other elements [β decay of ^{57}La to ^{58}Ce, and β decay of ^{83}Se to ^{83}Br (references noted earlier); these and other studies were summarized and elaborated in Burgus et al. 1948]. Also, I was very experienced in techniques for determining the characteristics of radiations and the half-lives of radioactive isotopes.

Summing up

I left St. Louis in the summer of 1949 to join the faculty of the Department of Microbiology of Western Reserve University Medical School in Cleveland. During February 1950, Hershey and I corresponded about the manuscript of the ^{32}P-decay 'suicide' paper. In one letter I asked him if he happened to see a paper by Robert Goldwasser in the October 1949 issue of The Yale Journal of Biology and Medicine (Goldwasser 1949). Goldwasser reported, as I had suspected, that 'the source of phosphorus of the bacteriophage described in this work is to be found in phosphorus compounds existing in bacterial cells prior to their infection with bacteriophage.' I do not believe Hershey ever answered my question.

Two years after Hershey left St. Louis, the famous 'Hershey–Chase experiment' using phage labeled with ^{32}P in DNA and ^{35}S in protein was reported (Hershey and Chase 1952). I believe that the ^{32}P-decay 'suicide' experiments probably played a significant role in Hershey's planning for the 'double tracer' Hershey–Chase experiment. Although the research of Avery and his colleagues in 1944 with the pneumococcus 'transforming principle' clearly indicated that genes are composed of DNA (see McCarty 1985), this concept was not widely accepted in 1950/1951. The Hershey–Chase experiment of 1952 was considered by most of the skeptics to be the definitive proof that DNA, not protein, is the 'genetic material of life.' In 1969, Delbrück (1906–1981), Luria (1912–1991), and Hershey (1908–1997) were awarded a Nobel Prize for their contributions to molecular biology.

It should be noted that as late as 1951, some investigators still argued that minute amounts of protein associated with the DNA 'transforming principle' might be the active agent. In reviewing the Hershey–Chase paper, Fruton (1999) recently commented:

> Indeed, the chemical evidence for the cautiously worded conclusion that the genetic material of the phage is associated with its DNA and not its protein was subject to greater doubt than that expressed in regard to the pneumococcal transforming factor. In the case of the latter, Avery's cautious reservation that the activity might be "due to minute amounts of some other substance" was emphasized by other investigators. For example, Alfred Mirsky wrote in 1951 "There is accordingly some doubt whether DNA is itself the transforming agent, although it can be regarded as established that DNA is at least part of the active principle."

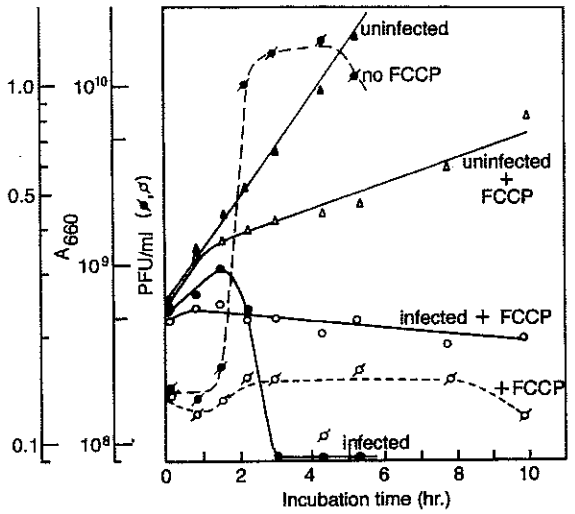


Figure 4. Effect of the phosphorylation uncoupler FCCP on replication of RC1 phage in photosynthetically grown cells of *R. capsulata Z-1* (from Schmidt et al. 1974). FCCP, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone. Cell growth was measured in units of absorbancy at 660 nm (A_{660}): solid triangles, uninfected control, no inhibitor; open triangles, uninfected control + inhibitor; solid circles, infected culture, no inhibitor; open circles, infected culture + inhibitor. Phage titers were assayed as plaque forming units/ml (PFU/ml): slashed solid circles, infected culture, no inhibitor; slashed open circles, infected culture + inhibitor.

Fruton's remarks and comments of McCarty (1985) provide a temporal framework for ideas prevalent when our paper on ^{32}P-decay 'suicide' was published. Many later accounts have obscured the actual historical record, as often happens with the passage of time. The connection between studies on phosphorus metabolism in photosynthesis and bacteriophage replication was an event of serendipity.

Connecting photosynthesis and phage in 1974

During the early 1970s, we isolated (from sewage) a virulent phage active on the photosynthetic bacterium *Rhodobacter capsulatus,* and examined its replication under various conditions (Schmidt et al. 1974). In photosynthetically grown cells, phage replication proceeded rapidly when infected cells were incubated under anaerobic conditions in the light. Virus growth in illuminated infected cells was markedly suppressed by inhibitors of photophosphorylation (see Figure 4). In *Rb. capsulatus* cells grown aerobically in darkness, energy for phage replication was provided by respiratory

(oxidative) phosphorylation, but the low photophosphorylation capacity of such pigment-depleted cells did not suffice for virus multiplication when infected cells were incubated anaerobically in the light; phage did not replicate in cells with a bacteriochlorophyll content less than ca. 0.6 μg/mg dry weight.

Our results indicated that the energy requirement for phage replication is more stringent than that for growth of the uninfected host cells. In other words, it appears that there is a relatively high *threshold* value of ATP regeneration rate necessary for orderly synthesis of phage components. Phage should be exploitable for research on photosynthetic membrane biosynthesis and function, but as far as I know further studies with this approach have not yet been undertaken.

Serendipity in scientific discovery

Many discoveries in science were made by serendipity and have revealed important phenomena or relationships. Roberts (1989) has compiled an impressive collection of accounts of such discoveries. In my own research career, serendipity has struck many times. In fact, this happened to the extent that the direction of my research efforts changed a number of times as the result of sequential serendipic observations (see Gest 1992). There is no doubt that serendipity will continue to play important roles in biological research, even though such events are usually not acknowledged in technical publications. A recent book on 'Medicines 10 Greatest Discoveries' (Friedman and Friedland 1998) shows that at least four of the 10 resulted from serendipity. The frequency of significant serendipic discoveries is obviously a reflection of how completely a particular subject is understood. Because I believe that cell biology and biochemistry are far more complicated than generally assumed, I confidently predict that there will be many more serendipic discoveries in the future.

Acknowledgements

I thank Prof. Charles Yanofsky (Stanford University) and Prof. John Spizizen (University of Arizona) for reviewing this memoir and for recent discussions in which we recalled the sequence of events between 1948 and 1953, when DNA was belatedly and finally recognized as the genetic material. Research of the author on photosynthetic bacteria is supported by National Institutes of Health Grant GM 58050.

References

Aronoff S and Calvin M (1948) Phosphorus turnover and photosynthesis. Plant Physiol 23: 351–358

Bronfenbrenner J (1928) The bacteriophage: present status of the question of its nature and mode of action. In: Jordan DO and Falk IS (eds) The Newer Knowledge of Bacteriology and Immunology, pp 525–556. University of Chicago Press, Chicago

Burgus WH, Davies TH, Edwards RR, Gest H, Stanley CW, Williams RR and Coryell CD (1948) Etude des états de valence des produits formés par désintegration bêta et transition isomérique. Jour de Chim Phys 45: 165–176

D'Herelle F (1926) The Bacteriophage and Its Behavior. Williams and Wilkins, Baltimore, Maryland

Edwards RR, Gest H and Davies TH (1951) Chemical effects of nuclear transformations. II β decay of selenium to bromine. In: National Nuclear Energy Series, Radiochemical Studies: the Fission Products, pp 237–254. McGraw-Hill, New York

Ellis EL and Spizizen J (1940) Glycine-an essential factor for growth of bacteriophage. Science 92: 91

Ellis EL and Spizizen J (1941) Rate of bacteriophage inactivation by filtrates of *Escherichia coli* cultures. J Gen Physiol 24: 437–445

Frenkel AW (1993) Recollections. Photosynth Res 35: 103–116

Friedman F and Friedland GW (1998) Medicines 10 Greatest Discoveries. Yale University Press, New Haven, Connecticut

Fruton JS (1999) Proteins, Enzymes, Genes; the Interplay of Chemistry and Biology, pp 440–441. Yale University Press, New Haven, Connecticutt

Gest H (1943) The effects of inorganic salts on the multiplication of bacterial viruses. J Inf Dis 73: 158–166.

Gest H (1992) A long trail of serendipity-directed research on photosynthetic bacteria. FEMS Microbiol Lett 100: 417–422

Gest H (1993) History of concepts of the comparative biochemistry of oxygenic and anoxygenic photosyntheses. Photosyn Res 35: 87–96

Gest H (1997) Serendipity in scientific discovery: a closer look. Persp Biol Med 41: 21–28

Gest H (2001) The July 1945 Szilard Petition on the Atomic Bomb; Memoir by a signer in Oak Ridge. http://bio.indiana.edu/Gest/

Gest H and Edwards RR (1951) Discovery of 19 minute La^{143}. In: National Nuclear Energy Series, Radiochemical Studies: the Fission Products, pp 1144–1146. McGraw-Hill, New York

Gest H and Glendenin LE (1951) Half-life and radiations of Se^{75}. pp 1924–1930. McGraw-Hill, New York

Gest H and Kamen MD (1948) Studies on the phosphorus metabolism of green algae and purple bacteria in relation to photosynthesis. J Biol Chem 176: 299–318

Gest H, Kamen MD and Reiner JM (1947) The theory of isotope dilution. Arch Biochem 12: 273–281

Gest H, Edwards RR and Davies TH (1951) Chemical effects of nuclear transformations I. β decay of lanthanum to cerium of mass 143. pp 232–236. McGraw-Hill, New York

Goldwasser RA (1949) The source of phosphorus in bacteriophage. Yale J Biol Med 22: 1–22

Haldane JBS (1968) On expecting the unexpected. In: Science and Life; Essays of a Rationalist, pp 135–144. Pemberton Publishing Co, London

Hershey AD and Chase M (1952) Independent functions of viral protein and nucleic acid in growth of bacteriophage. J Gen Physiol 36: 39–56

Hershey AD, Kamen MD, Kennedy JW and Gest H (1951) The mortality of bacteriophage containing assimilated radioactive phosphorus. J Gen Physiol 34: 305–319

Kamen MD (1947) Radioactive Tracers in Biology. Academic Press, New York

McCarty M (1985) The Transforming Principle/Discovering That Genes Are Made of DNA. WW Norton, New York

Price WH (1952) Bacterial viruses. Ann Rev Microbiol 6: 333–348

Roberts RM (1989) Serendipity/Accidental Discoveries in Science. Wiley, New York

Ruben S (1943) Photosynthesis and phosphorylation. J Am Chem Soc 65: 279–282

Schmidt LS, Yen H and Gest H (1974) Bioenergetic aspects of bacteriophage replication in the photosynthetic bacterium *Rhodopseudomonas capsulata*. Arch Biochem Biophys 165: 229–239

Spizizen J (1943) Some preliminary studies on mechanisms of viral multiplication. Proc Natl Acad Sci USA 29: 109–114

Spizizen J (1958) Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. Proc Nat Sci USA 44: 1072–1078

Wahl AC (1957) J.W. Kennedy, scientist, teacher, leader. Science 126: 65–66

192

Howard Gest **Photosynthetic Bacteria Group**

Office:(812)855-9612
Home:(812)339-5888
Fax: (812)855-6705
E-mail: gest@indiana.edu

Department of Biology
Jordan Hall
Indiana University
Bloomington, IN 47405

Reflections on Scientific Lives

Howard Gest surveys the changing scene

(June 2009)

Historical Events in Microbiology(Biochemistry);
current textbook style

Leading textbooks of microbiology are now heavy encyclopedic tomes of ca. 1,000 pages; in an early chapter, only about 25 pages deal with the history of the subject. Typically, some of pioneering researches of several 19th century "giants" (e.g., Pasteur and Koch) are described and later events are summarized in a long table of one-line entries, telegraphic style.

I have compiled part of an "example table" by combining entries from several recent texts (see below). They are given verbatim, except for minor editing to improve the English. In tables of this kind, the name of the investigator is sometimes not included. The first historical observation and depiction of microorganisms is almost always given erroneously. In 1665, Robert Hooke described the microfungus *Mucor*; about eleven years later, Antoni van Leeuwenhoek observed bacteria (see Gest 2004 and 2009: The discovery of microorganisms by Robert Hooke and Antoni van Leeuwenhoek, Fellows of the Royal Society, Notes and Records of the Royal Society vol. 58, pp. 187-201, 2004; Homage to Robert Hooke (1635-1703): New Insights from the Recently Discovered "Hooke Folio." Perspectives in Biology and Medicine 52(3): 392-399, 2009.

A typical textbook table of notable events in microbiological (biochemical) research

1835 A. Bassi discovers that a silkworm disease is caused by a fungus

1881 L. Pasteur developed the first artificial vaccine (vs anthrax)

1885 T. Escherich discovers *Bacterium coli*, a cause of infant diarrhea (later renamed *Escherichia coli*)

1899 M. Beijerinck The concept of a virus is proposed to explain tobacco mosaic disease

1929 A. Fleming discovers penicillin

1937 H. Krebs discovers the tricarboxylic acid cycle

H. Wood working with propionic acid bacteria is the first to discover that heterotrophs utilize CO_2 in metabolism

1944 O. Avery, C. Macleod and M. McCarty DNA is genetic material

1945 Max Delbruck and S. Luria Bacteriophage replication mechanism is elucidated

1946 E. Tatum and J. Lederberg Bacteria transfer DNA by conjugation

1952 J. Lederberg and N. Zinder Bacterial transduction

A. Hershey and M. Chase show that bacteriophages inject DNA into host cells

1953 J. Watson and F. Crick propose the double helix structure for DNA

1964 C. Yanofsky and colleagues demonstrate linear correspondence between a gene segment and a protein sequence

1966 M. Nirenberg, H. G. Khorana and others decipher the DNA genetic code

1969 T. Brock and H. Freeze Isolation of *Thermus aquaticus*, source of Taq DNA polymerase

1973 S. Cohen, A Chang, R. Helling, and H. Boyer Recombinant DNA

1982 S. Prusiner Prions, infectious agents consisting solely of proteins, are discovered

How would a new generation of young scientists learn how these discoveries were made? Who were these investigators? Where did they work? How long did it take for the discovery to be made – one month? one year? ten years?

Two Snapshots in the History of Microbiology

1665

The bubonic plague is raging in London -- more than 7,000 deaths each week. Pharmacist William Boghurst, a heroic helper, explained the disease as follows:

> "Plague or pestilence is a most subtle, peculiar, insinuating, venomous, deleterious exhalation arising from the maturation of the faeces of the earth extracted in the aire by the heat of the sun and difflated from place to place by the winds and most tymes

gradually but sometymes immediately aggressing apt bodyes."

In the same year, Robert Hooke, Fellow of the Royal Society of London, published his great classic *Micrographia,* which contained the first description and drawing of a microorganism, the microfungus *Mucor.* Hooke, a prolific inventor, described *Mucor's* very thin cylindrical and transparent stalks which had "a white knob that grew on the top of each of them." His observations were made with a microscope of his own construction.

1894

Dr. Alexandre Yersin, an officer in the French Medical Colonial Corps, is sent to Hong Kong at the suggestion of Louis Pasteur. A plague epidemic had developed in the city; people were dying by the hundreds. Yersin is instructed to study the outbreak and try to isolate the causative agent. With help from an English priest, he manages to build a small shack to live in, outside, but adjoining, a hospital. The shack contains a small folding bed and a very small makeshift laboratory. Yersin gave a few dollars to two English sailors who were helping to take care of the hospital morgue. With their connivance he got into the morgue for a few minutes and had access to the corpse of a patient who had just died of plague. Yersin punctured the patient's swollen inguinal lymph node (i.e., bubo) with a sterile pipette, ran to his small laboratory and went to work. He wrote in his notebook: June 20, 1894 "The specimen is full of microbes, all looking alike, with rounded ends, staining very poorly (gram negative); this is without question the microbe of plague."

Yersin then examined blood, lymph nodes and other organs of dead rats lying on the streets and found they were full of the same bacilli that he had observed in the people dying of the plague. He named the organism *Bacterium pestis.* It was later renamed *Yersina pestis.*

The historical record shows that explanations of complex biological phenomena usually required the efforts of many scientists over long periods of time. In a number of instances, there were sudden surges of clarification when a lone investigator or a small group performed a crucial experiment, perhaps using a new technique, or reinterpreted known facts in a new way.

What factors predispose to creativity in scientific research? This question is discussed in the philosophical writings of Max Perutz who was awarded a Nobel Prize in Chemistry (1962) for solving the crystal structure of hemoglobin. His pioneering work paved the way for many later researches of proteins important in the metabolism of microorganisms and other forms of life. Perutz's success was based on persistent and very hard work, inspirations, and a web of interactions with other scientists at just the "right times." In his 2003 book (see Further Reading, at end):

"There is little benefit in following scientists' daily grind but much in tracing the unique combinations of theoretical knowledge and manual skills, the web of personal encounters and accidental observations, the experience, temperament, moods and clashes that go into the making of discoveries, even though the crucial leap of the

mind is often impenetrable. There is also something to be said for finding out why others, seemingly just as able, were too blind to grasp what Nature tried to tell them."

Basic vs applied research

Much has been written comparing basic ("pure") research and applied ("industrial") research. Applications of knowledge from microbiology, biochemistry and molecular biology are now especially prominent in biotechnology (i.e., "biotech"). A very large number of biotech companies have been established, ranging from "start-ups" with less than 10 employees to organizations with thousands of scientific personnel. Many graduate students and post-doctoral fellows are now faced with making a career choice—academic science vs biotech. The choice dictates the kind of life style that ensues, and it is a fundamental decision.

According to a recent book by Steven Shapin, the march of modern events has led to an entity he calls "The Scientific Life" (University of Chicago Press, 2008). This refers to a blend of "relative" freedom to do basic research coupled with working on money-making projects. Shapin states that his book deals "very substantially with American industrial scientists, entrepreneurs, venture capitalists, and Organization Men: research managers at electrical and photographic firms, team-playing organic chemists, Southern California investors in high-tech companies, engineering professors, trying to develop and sell intellectual property and to get ahead in their academic careers." He discusses biotech at some length, but apparently forgot to include it in

his list. Shapin's last chapter is an Epilogue titled "The way we live now."

It begins with a photo of a reception at the University of California San Diego on a beautiful April day in 2004. The reception is essentially an outdoor "networking" cocktail party on a deck attached to an architect-designed building. Who is there?

"They are scientists, engineers, and research physicians; high-tech and biotech entrepreneurs; CEO, CSOs, and CTOs of start-up companies; venture capitalists and angel investors; intellectual property lawyers and service providers to the high-tech community; and academic administrators basking in pleasure—both at the perfection of the day, of course, and, especially, at the sight of all of these people assembled on the premises of a major pubic research university. It is a visible sign that the university is fulfilling one of its major acknowledged functions in a late modern economy, building bridges between knowledge making and wealth making, doing the sort of things that make political and business leaders happy."

That says it all, in a nutshell.

A review of Shapin's book by William Deresiewicz appeared in *The Nation*, March 16, 2009 ("Lab Test," pp. 23-28). Following are some excerpts:

> Shapin's narrative begins with the shift from science as a calling to science as a job. . . .Does the profit motive distort and degrade the unpredictable path of scientific discovery? Through the early decades of the twentieth century, the old notions came

under still greater pressure. The allegiance to knowledge for the sake of knowledge and consequent elevation of "pure" over applied research, the principled disdain for self-enrichment and corollary belief in the scientist's moral superiority, the commitment to investigative autonomy and free exploration as essential to the scientific project—all these were challenged by the explosive growth of industrial science. . . .

"By mid-century [20th], the typical scientist was no longer an independent investigator toiling in splendid isolation or an unworldly academic ensconced in an upper floor of the ivory tower but an increasingly well-paid company man working project to project on tasks selected by superiors with the goal of enhancing the bottom line. . . .

"Some scientists, including those with experience in the private sector, deplore what they see as the conformity, hierarchy and materialism of the corporate environment. Others though, are equally disillusioned by academia—not only the burden of teaching duties and the constant scrabble for grant money but the paradoxical fact that universities, having absorbed a great deal of managerial philosophy of late, have created environments that are often more hierarchical, tightly controlled and inimical to intellectual autonomy, especially for the young scientist, than corporations. Still others welcome the integration of the two spheres. . . .

"Does injection of the profit motive into scientific research

distort the kind of questions that get investigated and degrade the quality of the results that get produced? There are strong reasons to believe that it does."

Shapin's book was also reviewed by H. Allen Orr in *The New York Review of Books*, March 26, 2009 (p. 34, "Which Scientist Can You Trust?"). From his review, the essence of the book emerges quite clearly. Much is devoted to the question of whether or not scientists are "priests of nature, endowed with exceptional moral competence, or ordinary people who have acquired esoteric technical knowledge. . . . And to what extent do personal virtues matter in the practice of science."

Another topic: Is, or was, research in industrial companies "more regimented than in academia"? Again, the answer is obvious despite the few examples Shapin gives of companies where a few exceptional senior scientists had free time to pursue their own research interests. To be sure, the present difficulties of young scientists in obtaining research grant funds is a serious problem --"the game of grants" -- but stringent review is certainly a mechanism for filtering out research plans that are not of basic significance or well designed. Some of the examples Shapin cites for *entrepreneurial science* (as contrasted with academia) are almost entirely based on previous basic research in universities, the National Institutes of Health, or independent non-profit institutes. Orr notes: "There can be little doubt that, at least in some areas of science, particularly biology and information technology, entrepreneurial science will grow in size and, possibly, significance."

It is abundantly clear that in contrast to Shapin's perception, research by individuals or small groups in academia in the decades between 1940 and 1990 gave us a Golden Age of Basic Discovery in the biological and medical sciences. . . .general biology, biochemistry, general and medical microbiology, virology, genetics, molecular biology, etc. This led to important advances in medicine, agriculture and public health, and biotechnology, now exploited by commercial companies. To cite only one of numerous examples, the discovery and development of the first antibiotic, penicillin, came from a handful of scientists; one in a hospital research laboratory in London and a small group of investigators at the University of Oxford. But the *basic* advances did not come from industry to a significant extent.

The correct time frame of great strides in understanding major aspects of biology was given by Susan Hockfield, President of the Massachusetts Institute of Technology (*Science*, 27 April, 2009; p. 1147). She refers to "the convergence of the life sciences with the physical sciences and engineering" as follows:

> "The next convergence follows from the elucidation of the structure of DNA in the 1950s and from subsequent fundamental discoveries in molecular and cellular biology. These discoveries created a revolution in the life sciences and drove the development of recombinant DNA technology and the launch of the biotechnology industry. By the mid-1980s, the explosion of data from genomics and proteomics brought about a second revolution, further accelerating life science innovation."

There is no doubt that the contributions of the basic ideas and experimental studies contributed by industry to these revolutions were minor.

Further comments by Orr:

> "Though generally sound, Shapin's discussion is in some ways unsatisfying, For one thing, he draws his main evidence for the role of the personal from the interaction of venture capitalists with entrepreneurs, an interaction that has more to do with investing than with science. . . . Also, Shapin's discussion of industrial science mostly breaks off around the middle of the twentieth century. . . . Is research at Pfizer really shaped by the personal virtues of it scientists?"....While there can be no doubt that the figure of the independent academic scientist has been overly romanticized, when it comes to truly transformational science, it is at least possible that the lone wolf mythology isn't entirely mythological."

What history tells us

Modern biotech originated almost entirely from basic research in academia and nonprofit institutes, from research aimed at explaining the mechanisms of cell (organism) growth and development. A very large number of investigators contributed to the solution of complex questions, using diverse experimental systems. Two outstanding academic scientists merit particular attention in connection with the emergence of biotech, Ernst B. Chain and Joshua Lederberg.

Ernst Chain (1906 – 1979)

In 1928, the microbiologist Alexander Fleming was working at St. Mary's Hospital and Medical School in London. His research centered on ways of killing pathogenic bacteria with antiseptics, and he frequently used *staphylococci* as the test organism. One day, by accident, he noted a strange phenomenon on a discarded Petri dish culture. In a circular zone around a contaminant mold colony, all colonies of *staphylococci* had been destroyed. Evidently, the mold must have secreted a lethal substance of some kind. The mold was later identified as belonging to the genus *Penicillium*, and Fleming named the mysterious secretion penicillin.

Fleming published his observations in 1929, and it is clear that he did not realize the potential value of penicillin for treatment of infectious diseases. Nine years later (1938), the scene shifts to the University of Oxford and Ernst Chain, a refugee from Hitler's Germany. He was a researcher in the Sir William Dunn School of Pathology headed by pathologist Howard Florey. Chain came across Fleming's 1929 report and convinced Florey that research on penicillin would be of interest and scientific value. Chain collected about 200 references on growth inhibitions caused by the action of bacteria, streptomycetes, fungi and yeasts on one another. It was evident that in many cases the growth inhibition was caused by specific metabolites produced by the various microorganisms. In Chain's own words: "However, next to nothing was known about the chemical or biological nature of the inhibitory substances, and it seemed an interesting and rewarding field of exploration."

Chain was determined to isolate and characterize the chemistry of penicillin and this led to an extraordinary effort under difficult conditions in wartime England. The first problem of Chain's small team was to grow *Penicillium* in substantial quantity on agar surfaces. Because limited supplies were available, they had to use an astonishing assortment of sterilized trays, pie dishes, gasoline cans, flat bottles, biscuit tins, and porcelain bedpans. Chain was successful in determining the thiazolidine-beta-lactam structure of penicillin, and was the lead author on the first paper showing the therapeutic effects of purified penicillin on infected rats and mice [E. Chain, H.W. Florey, A.D. Gardner, N.G. Heatley, M.A. Jennings, J. Orr-Ewing and A.G. Sanders: Penicillin as a Chemotherapeutic Agent, *Lancet*, Aug. 24: 226 (1940)].

Fleming, Florey and Chain shared the 1945 Nobel Prize in Physiology and Medicine. Perutz's account of the penicillin story[*Is Science Necessary?* E. Dutton, New York, 1980] notes that "only Fleming made the headlines, and mentions of Florey and Chain appeared in small print. Fleming became a world hero, while the names of Florey and Chain and their colleagues have remained unknown outside the world of science. . . . Fleming spent the remaining ten years of his life collecting twenty-five honorary degrees, twenty-six medals, eighteen prizes, thirteen decorations, the freedom of fifteen cities, and honorary membership in eighty-nine scientific academies and societies. . . . Effusive admirers soon hailed him as the greatest scientific genius of all time, and he became the subject of several "hero-worshiping biographies."

The original strain of *Pencillium notatum* studied by Fleming produced relatively small amounts of penicillin. A related organism, *P. chrysogenum,* isolated in 1951, was more useful; it produced about 60 mg of the antibiotic per liter of growth medium. However, this was still too small a yield to form the basis of an industrial isolation process. Over a number of years, several groups of scientists systematically investigated *P. chrysogenum* with the aim of isolating mutant strains that secreted more of the antibiotic. Strain E-15.1, the "final strain," produced 7000 mg of penicillin per liter, and after other improvements, the yield reached 20,000 mg per liter.

In 1948, Chain left Oxford to organize the International Centre for Chemical Microbiology at the Istituto Superiore di Sanita in Rome. There, he and his colleagues pursued research in a number of fields. A new strong interest was development of industrial-scale fermentation pilot plants as research tools. This continued when Chain moved back to England in 1964 to become head of the Department of Biochemistry at the Imperial College of Science and Technology (London); Chain's activities in Rome and at Imperial College were reviewed in a 1991 article in *Nature* [by his son, B. Chain; vol. 353; pp. 492-494]. The keynote caption of the article is "The discovery of penicillin remains one of the greatest advances in medical science. From the success of the discovery the biotechnology industry became established."

"Influenced by his experiences during the first frustrating attempts at scaling-up penicillin production in the Oxford laboratories using antiquated and inappropriate technologies, Chain was convinced that progress in isolation and characterization of biologically active

substances (not only antibiotics, but vitamins, hormones, growth factors and other biological molecules active at very low concentrations) absolutely required large scale production of biological material. . . .Chain's own career also predisposed him to an interdisciplinary approach to scientific problems. He trained as an organic chemist, turned later to biochemistry, and ultimately became interested in bioengineering. . . .Both in Rome and later in London, Chain's ambitions to work on a scale unprecedented within an academic biochemistry department were fulfilled."

(Sir Ernst) Chain published a detailed history of the penicillin story, from Fleming's observations of penicillin action on the famous Petri dish, to the status of penicillin therapy in 1971 [*Thirty years of penicillin therapy*; Proc. Roy. Soc. London B 179: 293-319]. It is an outstanding paper in the annals of scientific discovery. He notes:

> "I started to work on penicillin in 1938, long before the outbreak of the war. The frequently repeated statement that the work was started as a contribution to the war effort, to find a chemotherapeutic agent suitable for the treatment of infected war wounds, has no basis. The only reason which motivated me to start the work on penicillin was scientific interest. I very much doubt, in fact, whether I would have been allowed to study this problem at that time in one of the so-called 'mission oriented' practically minded industrial laboratories. The research on penicillin which was started as a problem of purely scientific interest, but had consequences of very great practical importance is a good example of how difficult it is to

demonstrate sharp limits between pure and applied research."

Chain discusses the problems of antibiotic-resistant pathogenic bacteria and reviews the immense efforts expended in searching for new antibiotics of clinical usefulness. There are probably lessons to be learned even today from Chain's comments on the complex relations between academia and industry.

Joshua Lederberg (1925-2008)

During the mid-1940s, Lederberg and I were in graduate school (at different universities) and we met at annual meetings of the American Society for Microbiology. He was already giving spectacular talks and clearly was destined to become an outstanding luminary. A retrospective in *ASBMB Today* (American Society of Biochemistry and Molecular Biology) gives an excellent succinct survey of his academic career (April 2008, p. 15). Part of the retrospective summarizes his famous experiments demonstrating basic features of bacterial genetics, which paved the way for making bacteria and bacteriophages model systems in the development of molecular biology and later, of applications in biotech.

"Lederberg was born in Montclair, New Jersey, in 1925 and was raised in New York City. He enrolled at Columbia University where he met Francis J. Ryan, who introduced him to the red bread mold, *Neurospora*. Lederberg received his bachelor's degree in 1944 and began working toward an M.D. at Columbia University's College of Physicians and Surgeons. Although medical students were not

encouraged to do research, Lederberg continued to do experiments under Ryan's supervision, investigating the genetics of bacteria.

"In 1946, Lederberg took a leave of absence from medical school to carry out experiments on *Escherichia coli* in collaboration with Edward L. Tatum at Yale University. He demonstrated that certain strains of bacteria undergo a sexual stage during which they mate and exchange genes. At the time, scientists believed that bacteria reproduced asexually, so Lederberg's discovery of bacterial recombination was a radical one. He and Tatum were also able to map the *E. coli* chromosome, showing the locations of several of its genes. With Tatum's support Lederberg submitted this research as his doctoral thesis and received his Ph.D. from Yale in 1947.

"Rather than go back to medical school, Lederberg decided to accept the offer of an assistant professorship in genetics at the University of Wisconsin at Madison. There, he continued to study bacterial genetics and produced a steady stream of techniques and results that became the basis of genetic engineering in the 1970s. His most important discoveries at the time were that of transduction, the transfer of genetic fragments from one cell to another by a virus, and of the extra-chromosomal genetic particles called plasmids. . . .

"In 1957, Lederberg helped found and became chairman of a new Department of Medical Genetics at the University of Wisconsin. One year later, he accepted an offer to become the first chairman of the newly established Department of Genetics at Stanford University's School of Medicine. Later that year, he was awarded the 1958 Nobel

Prize in Physiology or Medicine, along with Tatum and George W. Beadle."

A more detailed description of Lederberg's accomplishments by Gerald Weissmann can be found in *The FASEB Journal* 22:3411-3414, 2008 [Science as Oath and Testimony: Joshua Lederberg (1925-2008)].

Lederberg's experimental work was only one aspect of his erudition and wide knowledge of the sciences, medicine, and human affairs. I valued his judgment on controversial questions in microbiology and we maintained a relevant correspondence over many years. We exchanged reprints, and even a partial list of the papers he sent me shows his extraordinary intelligence and knowledge. He was really in a class all by himself.

Titles of some of Lederberg's papers in my file:

Forty years of genetic recombination in bacteria. *Nature* 324: 627-628, 1986

Genetic recombination in bacteria: A discovery account. *Ann. Rev. Genet.* 21: 23-46, 1987

How DENDRAL was conceived and born. In: ACM Conference on the History of Medical Informatics, pp. 5-24. Association for Computing Machinery, N.Y., 1987.

The second century of Louis Pasteur: A global agenda for biomedical research. *Molecular Biology and Infectious Diseases,* Elsevier, Paris, pp. 19-30, 1988.

Pandemic as a natural evolutionary phenomenon. *Social Research* 55: 343-359, 1988.

Ontogeny of the clonal selection theory of antibody formation. Reflections on Darwin and Ehrlich. *Molecular Basis of the Immune Response*, vol 546 of the Annals of the New York Academy of Sciences, pp. 175-187, 1988.

The Gene (H.J. Muller 1947). In: Anecdotal, Historical and Critical Commentaries on Genetics. *Genetics* 129: 313-316, 1991.

The interface of science and medicine. *Mount Sinai J. of Med.* 59: 380-383, 1992.

Bacterial variation since Pasteur / Rummaging in the attic: Antiquarian ideas of transmissible heredity, 1880-1940. *Amer. Soc. Microbiol. News* 58: 261-265, 1992.

What the double helix (1953) has meant for basic biomedical science /A personal commentary. *J. Am. Med. Assoc.* 269: 1981-1985, 1993.

Smaller fleas. . . *ad infinitum*: Therapeutic bacteriophage redux. *Proc. Natl. Acad. Sci.* 93: 3167-3168, 1996. Inscribed: *"I should have recalled your discussion on this in Perspectives 1993." JL.*

Some early stirrings (1950 ff.) of concern about environmental mutagens. *Environ. & Molec. Mutagenesis* 30: 3-10, 1997.

In the 1992 paper in *American Society for Microbiology News*, there is a box, based on an interview with Lederberg. It notes that from 1978 to 1990, he served as President of Rockefeller University. Then, as a University Professor, he continued research in the field of

transcriptional specificities in mutagenesis in bacteria. Some of his remarks are still cogent:

"Even though we've seen some dimming of unblinking support for scientific research, and molecular biology is of course much more crowded, any of my students still has a crack at revolutionary discovery if they will but seize the day," he said.

"Although public scrutiny of scientific research and standards of accountability are more stringent, perhaps more hostile than in the recent past, Lederberg doesn't see a recrudescence of scientific McCarthyism. "Yes, the screws are a little bit tighter, and people are going to look more closely at marginal research, including plagiarism as well as imputed fraud. However, anyone who exercises a modicum of common sense and integrity has no rational basis for being deterred.

"Unfortunately, social vigilance about the integrity of scientific research may create the impression that the discipline is loaded with crooks and predators," Lederberg said. *"We urgently need to dispel the idea that the that the primary motivation of researchers is to beat their competitors. I firmly believe that idealism and the excitement* ***of*** *discovery are necessary parts of science."* (Italics added)

More on history

Lederberg, like Perutz, had a strong interest in the history and sociology of scientific research. He noted that "Missing from most primary literature in science are all but the faintest clues about the social context of discovery—how the scientific community is shaped by its operating norms

and institutions, as well as by its fraternal and intergenerational networks....Biography depicts directly the personal relationships among scientists, their mutual debts, their etiquettes, sometimes their jealousies and transgressions." [see his introduction to *The Excitement and Fascination of Science: Reflections by Eminent Scientists*, Vol.3, Part 1 (Ann. Reviews, Palo Alto, CA (1990). Unusual insights into this aspect of scientific life were provided by bacteriologist Dr. Claude Dolman, who was a professor at the University of British Columbia for many years.

Dolman received his medical education at St. Mary's Hospital Medical School (London), where Alexander Fleming was one of his teachers. During the early 1960s he renounced "the lure of the laboratory and the comforts of home" for 8 months "in order to go around the world gleaning a few bundles of historical straws." He traveled to 4 continents and 15 countries where he interviewed "many scores of distinguished persons." The journey was summarized in his interesting and informative paper "Tidbits of Bacteriological History" [Canad. J. Public Health 53: 269-278, 1962]. Dolman describes an episode of unexpected value when he paid a courtesy call to the widow of William Bulloch, who wrote the most authoritative history of bacteriology up to the 20th century (see Further Reading). She offered him a trunkful of Bulloch's papers, which contained working notes, illustrations and other material relating to his classic book.

A few of Dolman's remarks: "Too many younger scientists nowadays find it tempting to clarify an issue by doing an experiment rather than by first seeking the answer in the literature. Though I found no evidence that our predecessors were on the whole significantly more ascetic, less clay-footed, pleasanter personalities or better world-citizens than their counterparts today, yet still their achievements merit our homage. For they

were heirs to centuries of wishful thinking, of groping speculation, of controversial dogmas, of voices crying in the wilderness; and they dedicated diversities of gifts and dauntless courage to prophesying, unraveling, demonstrating and harnessing for the good of mankind those invisible agents of previously unimagined complexity, to whose mastery most of us owe our survival, and by manipulating which we as a group earn our livelihood . To deny them honour by arguing that if they had not done their work so well others soon enough would have found the way, is to make a mockery of history and a plaything of science."

Dr. Dolman (1906-1994) assembled a large and priceless collection of rare books on many aspects of microbiology and immunology, some dating back to the 16th century. The Dolman Collection is in the I.K. Barber Learning Centre of the Point Grey Campus of the University of British Columbia, Vancouver.

Summing Up

Obviously, there is no such thing as *THE* Scientific Life. We can certainly expect that an increasing variety of "9-to-5" technoscience jobs will develop in the future. Many, perhaps most, of them will not include basic research as it is usually understood. Academic science careers will continue in the usual pattern despite the chronic problems of obtaining financial support for research at the frontiers. I expect that the top universities will manage to preserve older academic traditions, while many others will gradually tailor graduate studies to train technoscientists.

Despite all its problems, academic basic research is still a long way ahead of Shapin's brave new world of technoscience/finance development. Research at the frontiers of basic knowledge is not the real subject of his book, whose title is misleading. *The Technoscience/Financial Vocation* would have been more descriptive. Essentially, it is all about making money from the scientific fruits grown mainly in academia and non-profit institutes. Nowadays, leading science magazines (*Nature, Science*) frequently include special sections about careers in biotech, biopharmaceutical corporations, etc. They discuss the ups and downs of "corporate culture." The business sections of major newspapers routinely publish relevant articles. A long report in the *New York Times* of March 10, 2009 is illustrative—key words and phrases:

Drug investors, losing patience, demand cash from companies; unsuccessful biotech company's quest for the next blockbuster; mega-mergers; second quarter dividends; controversial reverse mergers; "zombies" – companies that lurch from product to product, surviving years or even decades without ever achieving success; tender offers. One searches such reports in vain for news on fundamental research advances.

There are some attempts to modify the typical so-called "entrepreneurial model" in biotech. Genentech has 11,000 employees and about 100 billion dollars in market capitalization and boasts having 120 postdocs (trained in academia) in a "relaxed culture." According to their new executive vice president for "research and early development," Genentech plans to make sure that their scientists

"continue to have time to work on their own projects that aren't translational [i.e., *to products*], that aren't governed in any specific way, and that scientists have time to think and imagine and invent, not just do routine things." Incidentally, the laid back work environment features Friday night keg parties. [See *Science* 324: p. 583 (1 May 2009).

The oasis of academia at Genentech apparently does not include the time-consuming burden of teaching basic subjects to the next generation of scientists. We can expect that sooner or later, Genentech will have to develop a new generation of blockbuster drugs to keep up with the competition (such as from Roche, which has 80,000 employees). At the other end of the spectrum, multitudes of small start-up biotechs are teetering on the brink, praying that someone will buy them out.

Yes, many aspects of "scientific lives" involve gambles and, sometime, sacrifices. You have to decide what drives you and provides the most satisfactions; which, of course, is really not news.

Further Reading

More about the lives and research of eminent scientists can be found in the following:

Bernal, J.D. (1965; 3rd ed.) *Science in History.* Hawthorn Books. New York.

Bloch, K. (1994) *Blondes in Venetian Paintings, the Nine-Banded Armadillo, and Other Essays in Biochemistry.* Yale University Press, New Haven.

Brock, T.D., translator and editor. (1961) *Milestones in Microbiology.* Prentice-Hall. Englewood Cliffs, NJ.

Bulloch, W. (1938) *The History of Bacteriology.* Oxford University Press. Oxford. A classic.

Florkin, M. (1975) *A History of Biochemistry. Part III. History of the Identification of the Sources of Free Energy in Organisms.* Elsevier. Amsterdam.

Fruton, J.S. (1972) *Molecules and Life. Historical Essays on the Interplay of Chemistry and Biology.* Wiley-Interscience. New York.

Gest, H. (2002) Landmark discoveries in the trail from chemistry to cellular biochemistry, with particular reference to mileposts in research in bioenergetics. *Biochem. & Molec. Biol. Educ.* 30: 9-13.

Gest, H. (2003) *Microbes / An Invisible Universe.* American Society for Microbiology Press. Washington, D.C.

Gest, H. (2006) *Associations with distinguished scientists during an academic career of over 60 years.* Special Collection, Lilly Library,
Indiana University (Bloomington).
https://scholarworks.iu.edu/dspace/bitstream/2022/1083/1/Gestfinal.pdf

Gest, H. (2009) *Historical Adventures in Scientific Discovery: Microbiology/Biochemistry*
https://scholarworks.iu.edu/dspace/handle/2022/3358

Joklik, W.K., Ljungdahl, L.G., O'Brien, A.D., von Gravenitz, A., and

C. Yanofsky, Eds. (1999) *Microbiology / A Century Perspective.* ASM Press, Washington, D.C. A foreword by Joshua Lederberg entitled "Microbiology Past, Present, and Future" notes:

"Because students will often exploit any excuse not to read, particularly not to read works more than 5 years old, not to mention those that predate their own lives, the ready reaccessibility of these historic documents will be of some assistance in connecting 21st century-researchers with their 20th century roots."

Kornberg, A., Horecker, B.L., Cornudella, L. and J. Oro, Eds. (1976) *Reflections on Biochemistry / In Honour of Severo Ochoa.* Pergamon Press, Oxford. The numerous authors, including ten Nobel Prize-winners, provide a fascinating autobiographical and historical perspective of the development of their own subjects, and include a summary of present research and indications for future study.

Lechevalier, H.A. and Solotorovsky, M. (1965) *Three Centuries of Microbiology.* McGraw-Hill. New York.

Medawar, P. (1991) *The Threat and the Glory / Reflections on Science and Scientists.* Oxford University Press. Oxford. Medawar (Nobel Laureate 1960) is generally considered to be one of the most erudite biological scientists of the past 50 years.

Perutz, M.F. (1989) *Is Science Necessary? Essays on Science and Scientists.* E.P. Dutton. New York.

Perutz, M.F. (2003) *I Wish I'd Made You Angry Earlier. Essays on Science, Scientists, and Humanity.* Cold Spring Harbor, NY.

Wolpert, L. and Richards, A., eds. (1988) *A Passion for Science.* Oxford University Press. Oxford. BBC Radio 3; Interviews with noted scientists, including Sydney Brenner and Francis Crick.

Acknowledgments

The author thanks Roger Beckman, Head of the Life Sciences and Chemistry Libraries of Indiana University (Bloomington), for help in retrieving historic scientific publications. I am also indebted to my former student Prof. J. Thomas Beatty, Microbiology and Immunology, University of British Columbia for introducing me to the world of Claude Dolman.

Valuable References; history of microbiology and biochemistry; essays on science and scientists

Beck, R. W. (2000) *A Chronology of Microbiology in Historical Context*. American Society for Microbiology Press. Washington, D.C.

Bernal, J. D. (1965; 3rd ed.) *Science in History*. Hawthorn Books. New York.

Brock, T. D., translator and editor. (1961) *Milestones in Microbiology.* Prentice-Hall. Englewood Cliffs, NJ.

Bulloch, W. (1938) *The History of Bacteriology.* Oxford University Press. Oxford. A classic.

Crick, F. (1988) *What Mad Pursuit/ A Personal View of Scientific Discovery.* Basic Books. New York.
A fascinating account of how the structure of DNA and related problems were solved. Plus some of Crick's off-beat ideas (e.g., on "panspermia"). He comments on contributions by a number of scientists noted in these "Adventures."

Florkin, M. *A History of Biochemistry. Part III. History of the Identification of the Sources of Free Energy in Organisms.* (1975). Elsevier. Amsterdam.

Fruton, J. S. (1972). *Molecules and Life. Historical*

Essays on the Interplay of Chemistry and Biology. Wiley-Interscience. New York.

Gest, H. (2003) *Microbes/An Invisible Universe.* American Society for Microbiology Press. Washington, D.C.

Gest, H. (2006) *Associations with distinguished scientists during an academic career of over 60 years,* Special Collection, Lilly Library, Indiana University (Bloomington). https://scholarworks.i.u.edu/dspace/bitstream/2022/1083/1/Gestfinal.pdf

Kluyver, A.J. and van Niel, C.B. (1956) *The Microbe's Contribution to Biology*. Harvard University Press. Cambridge, MA.

Krebs, H. A. and Kornberg, H. L. (1957) *Energy Transformations In Living Matter, A Survey.* Springer Verlag. Berlin, Gottingen, Heidelberg. Extensive references to basic research articles.

Lechevalier, H. A. and Solotorovsky, M. (1965) *Three Centuries of Microbiology.* McGraw-Hill. New York.

Medawar, P. (1991) *The Threat and the Glory/Reflections On Science and Scientists.* Oxford University Press. Oxford. Medawar (Nobel Laureate 1960) is generally

considered to be one of the most erudite biological scientists of the past 50 years.

Perutz, M. F. (1989) *Is Science Necessary? Essays on Science and Scientists.* E. P. Dutton. New York.

Perutz, M. F. (2003) *I Wish I'd Made You Angry Earlier. Essays on Science, Scientists, and Humanity.* Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY.

Schlegel, H. G. (1999). *Geschichte der Mikrobiologie.* Deutsche Akademie der Naturforscher Leopoldina. Halle (Saale). A detailed history, many references to the literature, and excellent photographs of investigators.

Stephenson, M. (1949) *Bacterial Metabolism* (3rd Ed.) Longman's Green. London.

Thimann, K. V. (1955) *The Life of Bacteria.* Macmillan. New York. A book of outstanding scholarship; extensive references to the original literature. Outstanding indices: author index, 18 pages; organism index, 14 pages; subject index, 26 pages.

Ullmann, A. (2007) Pasteur-Koch: Distinctive Ways of Thinking about Infectious Diseases. *Microbe* 2: 383

-387. "Linguistic misunderstandings along with genuine scientific differences over virulence and and immunity drove the two geniuses apart."

Wolpert, L. and Richards, A., eds. (1988) *A Passion for Science.* Oxford University Press. Oxford. BBC Radio 3; interviews with noted scientists, including Sydney Brenner and Francis Crick.

IN ADDITION:

Two important publications provide basic accounts of major advances in the history of microbiology/biochemistry during the 20th century.

A. *Microbiology/A Centenary Perspective.* Edited by W.K. Joklik, L.G. Ljungdahl, A.D. O'Brien, A. von Graevenitz, and C. Yanofsky. ASM Press, 1999. The book is divided into five sections, which deal with major branches of microbiology. Each section contains reproductions of classic papers, which are introduced by a Preface, written by one of the editors. A foreword by Joshua Lederberg entitled "*Microbiology Past, Present, and Future*" notes: "Because students will often exploit any excuse not to read, particularly not to read works more than 5 years old, not to mention those that predate their own lives, the ready reaccessibility of these historic documents will be of some assistance in connecting 21st

century-researchers with their 20th century roots."

B. *Reflections on Biochemistry/In Honour of Severo Ochoa.* Edited by A. Kornberg, B.L. Horecker, L. Cornudella, and J.Oro. Pergamon Press, Oxford, 1976. From the back cover: "This book illustrates many of the major advances in biochemistry during the past 50 years. It is written by some of the distinguished students and collaborators of Severo Ochoa and covers the main research areas to which he has contributed. The authors, including ten Nobel Prize-winners, provide a fascinating autobiographical and historical perspective of the development of their own subjects, and include a summary of present research and indications for future study."

The personal accounts by leading investigators give insights into how important problems were approached and solved. This book is a veritable "Who's Who" of modern biochemistry; the Author Index lists hundreds of scientists.

American Society for Microbiology News
June, 2004 (vol.70, p. 271)

Valuing Impact of History on Science, Witnessing Science's Impact on History

Howard Gest feels very strongly about the impact of history on science. "I believe that many young scientists know little about the early history of their own research areas, and I think this limits their understanding of how major discoveries are made," he says, adding that too often they assume that contemporary biological knowledge is nearly complete. "History clearly shows that biology is far more complicated than each generation thinks it is."

Gest came to this realization years ago, influenced by the late J. H. "Jack" Hexter, a noted historian and professor of history at Washington University, St. Louis, Mo., where Gest knew him, and Yale University, New Haven, Conn. "Hexter once said: 'History with a capital H deals with major trends, large movements, deep running tides, portentous rumbles. When a "small h" historian fixes his attention on a fragment of the past washed up on the littered beach of the present, he is likely to ask simple questions about it: How did it get there? What the devil is it? What was it for? Where is it from? What happened to it?'," Gest says. "Hexter's remarks made me realize that I had become a 'small h' historian of microbiology and biochemistry."

Gest, 82, is distinguished professor emeritus of microbiology and adjunct professor of history and philosophy of science at Indiana University, Bloomington. His research over many years focused on microbial physiology and metabolism, especially with photosynthetic bacteria.

Gest was born in London, and emigrated in infancy with his family to America. He received a B.A. in bacteriology from the University of California, Los Angeles (UCLA) in 1942, and his Ph.D. from Washington University in 1949. While attending UCLA, he spent the summers of 1941 and 1942 assisting Max Delbrück and Salvador Luria, who were studying bacterial viruses. Although Gest began graduate work with Delbrück at Vanderbilt University, World War II intervened. Later in St. Louis, he did research with Alfred Hershey, using the radioactive isotope P_{32} to examine what happens to phosphorus-containing cell components when bacterial viruses replicate. These studies culminated with their discovery of P_{32} "suicide" of bacteriophage.

During World War II, Gest was involved in the Manhattan Project with physical chemist Charles Coryell—one of Gest's teachers at UCLA—first at the University of Chicago, and later at Oak Ridge, Tenn. His role was to conduct basic research on the radioactive elements formed in uranium fission. He looks back at that period with conflicted emotions. While proud of his contribution toward developing the atomic bomb, he was troubled by the real possibility that its use would result in the needless loss of life—a fear that ultimately became a reality. Gest was among those scientists who signed a petition that urged President Truman to consider the moral implications of dropping the bomb, and asked that the Japanese first be offered an opportunity to surrender. Truman apparently never saw the document (see http://www.bio.Indiana.edu/Gest/).

"When this huge project was successful, we felt great pride in our work; we felt it was very important for the security of the United States," Gest says, recalling the Manhattan Project. "But we were very disappointed in how the bombs were used. We'd hoped they would be used in such a way that would not lead to the death of many innocent civilians. And we were very perturbed that the petition never reached President Truman."


Howard Gest

Gest has served on the faculties of Case Western Reserve University in Cleveland, Ohio, Washington University, and Indiana University, and has been a visiting researcher at the California Institute of Technology, Dartmouth Medical School, Stanford University, Oxford University, Tokyo University, and UCLA. He was twice named a Guggenheim Fellow and has served on a number of advisory committees of the U.S. government. During his second Guggenheim fellowship, he studied problems of biochemical evolution as a member of the Precambrian Paleobiology Group.

Gest no longer engages in experimental research. Instead he spends much of his time writing about the history of microbiology and biochemistry. He also is a continuing and familiar presence among graduate students and postdoctoral fellows, with whom he continues to work. "I go to the university every single day," he says.

Marlene Cimons
Marlene Cimons is a freelance writer in Bethesda, Md.

For a survey of Gest's research up to 1994 see: "A microbiologist's odyssey: Bacterial viruses to photosynthetic bacteria"; a Personal Perspective, in Photosynthesis Research 40: 129-146, 1994