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JOHN HOWARD MUELLER

1891—1954

A Biographical Memoir by

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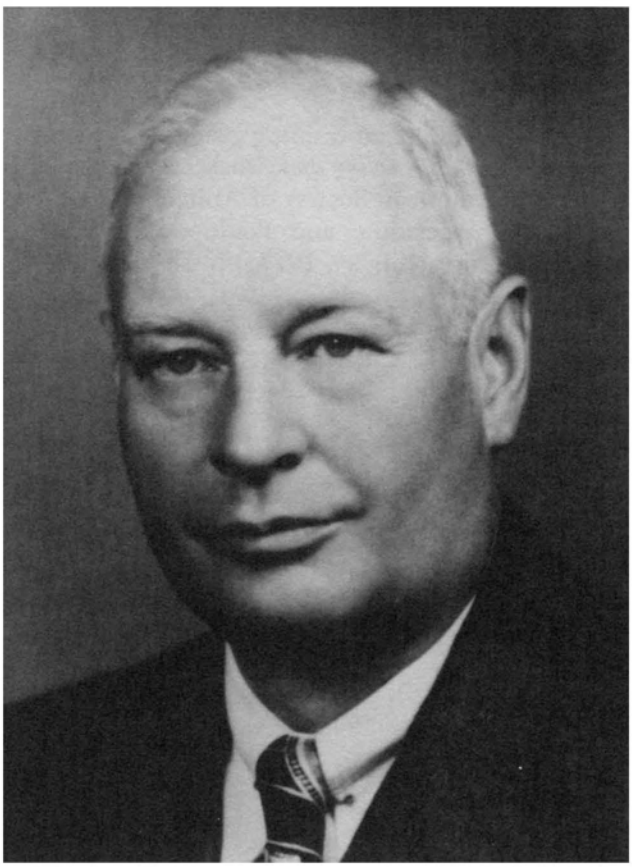

JOHN HOWARD MUELLER

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BY A. M. PAPPENHEIMER, JR.

JOHN HOWARD MUELLER was born June 13, 1891, in Sheffield, Massachusetts, where his father was a Unitarian minister. After a few years, the family moved to Illinois, where young Howard received his secondary schooling. He then attended Illinois Wesleyn University, receiving his B.S. degree with honors in biology in 1912. Two years as an instructor of chemistry at the University of Louisville followed; he was awarded an M.S. degree in 1914.

While at Louisville he became interested in bacteriology and pathology, and in the summer of 1914 he attended a summer course in pathology at the College of Physicians and Surgeons, Columbia University. The instructors in this course encouraged him to remain as a graduate student at Columbia, which he did. He was awarded an Alonzo Clark Fellowship in Pathology and received his Ph.D. degree in 1916. He worked as assistant pathologist at the Presbyterian Hospital until war was declared in 1917; he then enlisted as a private and went overseas with the Presbyterian Hospital Unit to Etretat, France, where he actively participated in the work that demonstrated that "trench fever" (like typhus fever, a rickettsial disease) was transmitted by lice. It was doubtless during this period that he became interested in pathogenic bacteria and in their physiology and metabolism. His

talents were recognized by the Army, and he was commissioned a lieutenant in the Sanitary Corps before the war ended.

On his return to civilian life in 1919, Mueller was appointed an instructor in the bacteriology department, chaired by Hans Zinsser, at the College of Physicians and Surgeons. There he began his studies on the cultural requirements of pathogenic bacteria. Papers I and II of this series appeared in 1922. In introducing the series, Mueller wrote:

> Perhaps the most important results to which success in such a piece of work might lead, are the applications of the findings to problems of more general biological importance, particularly to those of animal metabolism. For, whatever may prove to be the nature of these substances which cause growth of bacteria, they are largely or entirely components of animal tissue, and it is probable that they are either needed also by the animal body and supplied by plant or other sources, or else are synthesized by the animal itself to fill some metabolic requirement. When it is possible to catalogue the substances required by pathogenic bacteria for growth, it will probably be found that most of them are either required by, or important in, animal metabolism, and while many of them will surely be compounds at present familiar to the physiological chemist, it is equally probable that some will be new, or at least of hitherto unrecognized importance.

It was not long before his predictions were verified. He soon found that although he could use an acid hydrolysate of animal protein supplemented with tryptophane—instead of commercial "peptones"—as a base for growth of *Streptococcus hemolyticus,* the hydrolysate could not be replaced by a mixture of the then known amino acids. This led to the fractionation of casein hydrolysate and to the discovery of a new, ubiquitously distributed sulfur-containing amino acid: methionine. Mueller's 1923 paper in the *Journal of Biological Chemistry* reporting the isolation of the new amino acid from acid hydrolysates of casein and of ovalbumin and his determina-

tion of its elemental composition is an excellent example of the thoroughness that characterized all of his work. He made a good many derivatives and carried out the elementary analysis of each one himself. This led to the correct empirical formula: $C_5H_{11}NO_2S$. Only two likely structural formulae were possible. An organic chemist from Cambridge University suggested to him that the new amino acid might simply be the ethyl thioether of cysteine. Mueller proceeded to synthesize this thioether and proved that it was *not* identical with his new amino acid, the structure of which he stated remained to be determined.

In the same year as the discovery of methionine, Hans Zinsser was appointed chairman of the Department of Bacteriology and Immunology at the Harvard Medical School and asked Mueller to join him as an assistant professor. After arriving in Boston, Mueller was persuaded to abandon—temporarily—his studies on bacterial nutrition. Instead he joined Zinsser in the study of so-called "residue antigens" extracted from pneumococci, tubercle bacilli, yeast, and other microorganisms. These heat-stable, nonprotein antigens were independently shown to be polysaccharides by Avery and his coworkers, whose subsequent brilliant work revealed their role in the pathogenesis of pneumococcal lobar pneumonia and other bacterial diseases.

At about this time in England, W. E. Gye published a series of papers purporting to show that Rous chicken sarcoma filtrates contained two essential factors, both of which were required for tumour induction. The first was a substance specifically affecting certain chicken cells and thereby rendering them susceptible to transformation to malignancy by a virus. The same virus was supposedly present in many mammalian tumours, including human carcinomas. Because the implications of this work seemed so important at the time, Mueller attempted unsuccessfully to repeat Gye's experiments and

spent several frustrating years working on this problem. In the end, Gye came to work in Mueller's laboratory, and in 1929 a joint paper appeared in the *Journal of Experimental Medicine*—but with two opposing sets of conclusions: one written by Gye, the other by Mueller. Subsequent events proved Mueller's interpretation of the data to be correct.

After these rather disappointing years, Mueller finally returned to the field that had always been his major interest since his early work leading to the discovery of methionine. Papers I and II of the series "Studies on the Culture Requirements of Bacteria" appeared in the *Journal of Bacteriology* in 1922; paper III did not appear until 1933! Nevertheless, in 1930 when Mueller began his classic studies on the nutrition of the diphtheria bacillus, bacterial cells were *still* regarded as lowly forms of life that had little, if any, relationship to the cells of higher animals and plants. Tissue extractives, inspissated serum, "peptones," etc., were regarded as essential for the cultivation of pathogenic microorganisms, and any notion of bacterial genetics was unheard of. It was not until 1929 that the first enzyme (urease) was crystallized and shown to be protein.

The decade that followed must be regarded as the most fruitful of Mueller's career. He selected the diphtheria bacillus as the organism for his intensive studies. From the very outset, he recognized the importance of being able to measure growth quantitatively. Spectrophotometers were not yet on the market, and Mueller decided to use the micro-Kjeldhal method for estimation of growth as bacterial nitrogen. Although this method was tedious, it was accurate. It also gave him a great advantage over others who were beginning to enter the field because it became possible to work out conditions for *maximal* yields of the bacteria and their products.

Within a few years the Mueller laboratory had identified

which amino acids were essential for growth of the diphtheria bacillus and had made the important observation that different strains of the same bacillus varied widely in their amino acid requirements. (For example, if a certain amino acid was not needed for growth of a given strain, that strain possessed the enzymes required for its biosynthesis.) Mueller then went on to isolate and identify what he called "accessory" factors. He isolated nicotinic acid from liver and showed that it or nicotinamide were essential growth factors for all strains of the diphtheria bacillus being tested. With S. Cohen he isolated a second factor from liver that proved to be β-alanine. He then showed that pantothenic acid would also satisfy the β-alanine requirement, and others found diphtherial strains that were dependent on pantothenic acid itself for growth. Soon afterward, both nicotinamide and pantothenic acid were shown to be part of the vitamin B complex required for animal nutrition. Finally, in paper X of the series that appeared in 1937, Mueller described the isolation and identification of pimelic acid from cow urine, which was required in trace amounts for growth. Later, after the discovery of biotin, pimelic acid was shown by du Vigneaud to be an intermediate in biotin biosynthesis.

Thus, by 1940 the prediction Mueller had made in 1922 had been fully realized, and the universality of biochemistry had become accepted by everyone. The importance of Mueller's research in helping to bring about this recognition should not be forgotten. It was his work on bacterial nutrition that paved the way for the rapid identification of coenzymes and for the elucidation of the pathways of intermediary metabolism and biosynthesis that took place in the 1940s and early 1950s. The work on nutrition of the diphtheria bacillus also had important practical applications in improving the yield and quality of the diphtheria toxin used in production of toxoid for human immunization. By 1941 the diphtherial

studies were essentially complete, and Mueller turned his major attention to the tetanus bacillus and to production of its toxin. These studies were still in progress at the time of his death in 1954.

Hans Zinsser died in 1940 and shortly thereafter Howard Mueller was appointed to succeed him as head of the department. Mueller was one of those rare individuals who continued to work with his own hands even after becoming chairman of a large department at a major university. He was an early riser, and daybreak usually found him at the bench. Several hours later, soon after his devoted research associate and friend Pauline Miller had arrived, Mueller was ready to leave the laboratory for his office to pursue his administrative duties. He took his obligations toward the department, toward the teaching of medical students, and toward his clinical associates very seriously. He was interested in all aspects of infectious disease and felt that his own research should be medically oriented with ultimate practical applications. And indeed, in addition to its fundamental scientific importance, much of his work did have important practical application in immunization and in diagnosis.

Mueller was a man of great generosity who had impeccable integrity—particularly with regard to scientific accuracy—and an unusual capacity for brushing aside all that was irrelevant in order to get at the core of the matter. He had no use for the sham, the half-truth, or the pretentious. Those who knew him well were always impressed by his modesty—indeed, he used to refer to himself as only "a high school chemist." Yet as pointed out in the "Minute" on his life that was read to the faculty of medicine of Harvard University following his death: "Howard Mueller belongs among the scientific 'élite' as Kirtley Mather has recently defined them—that is among those who actively seek insight and meaning, whose minds are constantly on the alert to the possibility of

new generalizations and new relationships as distinguished from those who merely know how to do that which they have been trained to do." No better example of his remarkable insight can be given than his reaction to the 1944 discovery by Avery, Macleod, and McCarty that the rough-to-smooth transformation could be induced by DNA. In his chapter on the chemistry and metabolism of bacteria, which was written in that same year for the *Annual Review of Biochemistry*, Mueller wrote (the italics are mine):

> In other words it appears that a polymer of a nucleic acid may be incorporated into a living, degraded cell, and will endow the cells with a property never previously possessed, namely, the ability to produce a capsule composed of a complex polysaccharide entirely different in structure from that produced by the smooth organism from which the degraded form was originally derived. When thus induced the function is permanent, and the nucleic acid itself is also reproduced in cell division. The importance of these observations can scarcely be overestimated and stimulates speculation concerning such matters as the chemical basis for specificity in nucleic acids, and the *genetic* implications presented by the ability to induce permanent *mutation* in a cell by the introduction of a chemical substance. Such speculation may well include considerations of the relation of this phenomenon to the sequence of events following the introduction of a filterable virus (or a bacteriophage particle) into a susceptible cell.

Mueller wrote those words at a time when recombination in bacteria was unknown, microbial genetics did not exist, and no one had previously spoken of mutations in connection with bacteria.

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