



**B. L. Horecker**

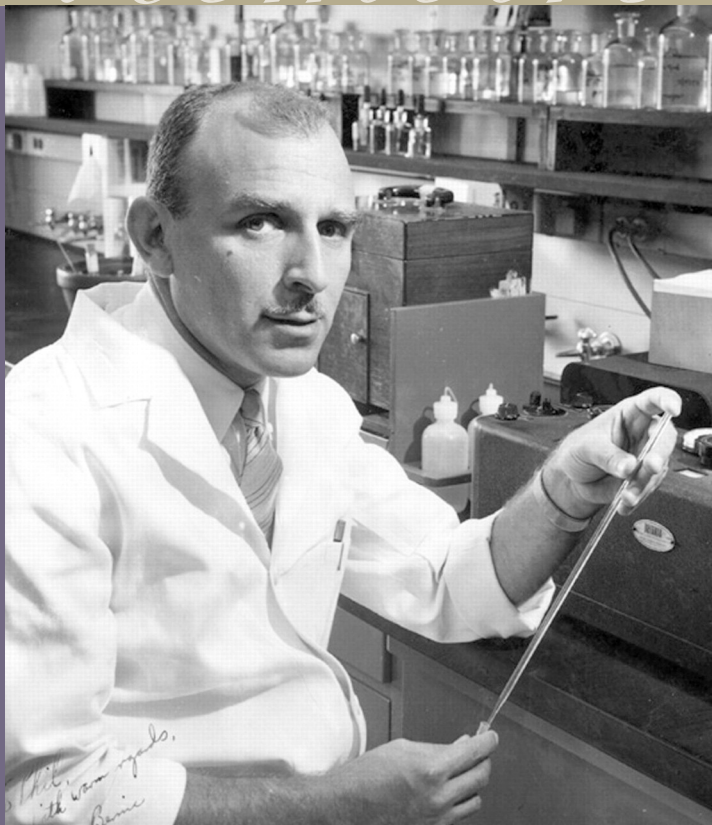
1914 - 2010

BIOGRAPHICAL

*Memoirs*

*A Biographical Memoir  
By Dan G. Fraenkel*

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# BERNARD LEONARD HORECKER

October 31, 1914- October 10, 2010

Elected to the NAS, 1961

Bernard Horecker was an enzymologist and microbiologist. Starting his career at the National Institutes of Health (NIH), and then moving on to the New York University (NYU) School of Medicine, the Albert Einstein College of Medicine, the Hoffman La Roche Research Institute, and finally Cornell University's Weill Cornell Medical College, he made seminal contributions to our understanding of the enzyme-catalyzed reactions in carbohydrate metabolism, especially those of the pentose phosphate pathway. He received many awards and honors, including the 1952 Paul Lewis Award in Enzyme Chemistry (now the Pfizer Award in Enzyme Chemistry) from the Division of Biological Chemistry of the American Chemical Society, served as president of the American Society of Biological Chemists (now the American Society for Biochemistry and Molecular Biology) and was the first recipient of its Merck Award in 1981.



By Dan G. Fraenkel

**B**ernard L. Horecker was born on October 31, 1914, in Chicago, Illinois, to Paul and Bessie Horecker, first-generation Russian-Jewish immigrants who operated a small haberdashery shop. His upbringing as a bookish youth is a familiar story—music (piano), bird watching (it would become a lifelong hobby), chess, butterflies, museums, and so on. He attended the academic Murray F. Tuley High School, and won a half-scholarship to attend the University of Chicago (\$300 a year), where he became a nominal major in zoology in the famous new curriculum of then-President Robert M. Hutchins. This plan of study included two years of generalist courses taught by luminaries, a particular influence being entomologist Alfred E. Emerson Jr., whose subject was termites.

After completing his Bachelor of Science degree in 1936, he began graduate school in biochemistry at the University of Chicago. In the same year, he married Frances Goldstein in Pittsburgh, Pennsylvania, with whom he would have three children (Doris,

Marilyn, and Linda). His thesis advisor was Chemistry Professor T. R. Hogness, who had built the first photoelectric spectrophotometer in the United States. At the time, however, a mainstay instrument for biochemistry was Otto Warburg's adaptation of the Barcroft-Haldane manometer for measuring changes in gas volumes as a general way to follow metabolism. Horecker used it for his doctoral work on respiration of succinate via cytochrome *c* in preparations from beef heart. Those studies resulted in his first paper (1939) on a somewhat arcane finding of activation by trace cations.<sup>1</sup> At the time, the now-familiar respiratory scheme of substrate dehydrogenation, intermediate cytochrome carriers, and reduction of oxygen to water was far from established.

The two pyridine nucleotide coenzymes of dehydrogenases had only recently been identified (circa 1935). One, now called NAD, was discovered through long studies of anaerobic glycolysis, this compound being reduced in the glyceraldehyde-3-P dehydrogenase reaction and reoxidized in the formation of lactic acid or ethanol. The other, NADP, was uncovered in studies of glucose metabolism by red cells as cofactor in the reduction of methylene blue. In the absence of dye, regeneration of the reduced cofactor was thought to use "yellow enzyme," whose bleaching in the process was key to discovery of the flavin cofactors (FAD and FMN). However, purified yellow enzyme did not reduce cytochrome *c*, as might have been expected for a respiratory pathway of NADPH reoxidation. Erwin Haas, one of Warburg's several well-known long-term technical assistants, had joined Hogness's laboratory in 1939 and with Horecker analyzed a different activity found in crude extracts of yeast, an NADPH cytochrome *c* reductase.<sup>2</sup> Because there were no commercial suppliers of biochemical reagents, they had to prepare their own NADP (yeast), glucose-6-P dehydrogenase (yeast), cytochrome *c* (horse heart), and the new enzyme (yeast), which was shown to contain a flavin cofactor as well. Those being the days before chromatographic methods for protein separations were commonplace, the enzyme purification used various fractional precipitations and extractions. Decades later, Horecker would inflict on new postdocs the exercise of preparing muscle aldolase using precipitation from the crude extract with a single narrow cut of ammonium sulfate, which, at least in his hands, yielded crystalline enzyme. Eventually, it would become clear that their NADPH cytochrome *c* reductase had nothing to do with respiration, but rather donates electrons from NADPH to mixed function oxygenases involved in detoxification of xenobiotics, the most important of which are members of the cytochrome P450 family of enzymes. Function of the original old yellow enzymes remains uncertain.

By 1941, Horecker was in the job market, but it was not an easy era in which to find employment in academia; he was one of at least 1,000 unsuccessful applicants for an instructorship at Purdue University. He landed a position in Bethesda, Maryland, at the NIH, probably because the chief of the Division of Industrial Hygiene had an automatic recording spectrophotometer in his laboratory of the sort with which Horecker was familiar from his time with Hogness. With the onset of World War II, four years of wartime work ensued. He remained in the same laboratory but received a commission in the U.S. Navy, being charged with conducting studies on the analysis of hemoglobin in poisoned red cells, the toxicity of ozone in submarines, and, in his own phrase, “a number of other projects.” At the end of the war, he was released for civilian work at the NIH. The next fourteen years, mainly in the Section on Enzymes of the Laboratory of Enzymology, would comprise his deciphering the reactions of what came to be called the pentose phosphate pathway, which is the research achievement for which he is best known and is beautifully recounted in his retrospective of 2002.<sup>3</sup>

Pre-war research on glucose-6-P oxidation had already indicated a second NADP-dependent dehydrogenation with decarboxylation, yielding pentose-P and, in yeast, progression to ethanol (Frank Dickens) as well as, in red cells, interconversions of hexose-6-P and ribose-5-P (Zacharias Dische). The post-war thrust of several laboratories was to work out what was going on. It was, as Horecker would put it, a race, and involved a virtual “Who’s Who” of mid-twentieth-century biochemists, many of whom were born between 1910 and 1915 and of Eastern European Jewish origin. The two pre-eminent figures were Horecker and Efriam Racker, who had astonishingly parallel scientific careers, including time spent at the same institutions, as well as uncovering the same reactions and publishing in the same journals. In the post-World War II decade, manometry would be substantially displaced by spectrophotometric methods, many based on the  $A_{340\text{ nm}}$  absorption band of the pyridine nucleotides, which Haas and Erwin Negelein had first used to assay dehydrogenases in 1935 and for which Horecker and Arthur Kornberg reported the definitive extinction coefficients in their famous 1948 paper.<sup>4</sup> As the result of the characterization of these cofactors, the Beckman DU spectrophotometer, first available in 1941, and its successors became a work-horse of biochemistry.<sup>5,6</sup> There also arose a specialized industry to provide metabolites and enzymes for analytical and other purposes, but much of the work in the 1940s and 1950s still entailed preparing one’s own reagents and chemical analysis still ruled, with paper and ion exchange chromatography coming into use for small molecules but, not yet for proteins.

The pentose-P pathway (Fig. 1) has two parts. For the irreversible oxidative part, glucose-6-P to pentose-P (i.e., 6C → 5C + 1C) a major contribution of Horecker was the first preparation of relatively pure 6-P- gluconate dehydrogenase (from yeast) and its chemical and physical characterization, including use of X-ray diffraction of the 5C product showing that it was D-ribulose-5-P.<sup>7</sup>

The other branch is the non-oxidative and reversible interconversion between pentose-P and, overall, hexose-P. The reactions are accomplished with three enzymes and four intermediates discovered or co-discovered or proven by Horecker: (i) an epimerase for interconversion of ribulose-5-P and xylulose-5-P;<sup>8</sup> (ii) a “transketolase,” transferring (with thiamine-pyrophosphate as the cofactor) a 2-carbon glycoaldehyde moiety from the ketopentose-P donor (xylulose-5-P) onto an aldopentose-P acceptor (ribose-5-P), yielding sedoheptulose-7-P and glyceraldehyde-3-P;<sup>9</sup> (iii) a “transaldolase” reaction (no cofactor) for transfer of the upper 3-carbons of sedoheptulose-7-P to glyceraldehyde-3-P leaving erythrose-4-P and fructose-6-P<sup>10</sup> and (iv), completing the usually written stoichiometry, a second reaction catalyzed by transketolase with xylulose-5-P as donor and erythrose-4-P as acceptor, for an overall 3 x pentose-5-P to → 2 ½ x fructose-6-P.<sup>11</sup>

That work was substantially completed by 1956. It would entrain with a different area of research that arose from recognition in the 1930s that, in photosynthesis in plants or autotrophy in general, the energetics and the assimilation of CO<sub>2</sub> into organic material were conceptually and experimentally separable. Various key developments centered at the University of California, Berkeley, and the Lawrence Berkeley National Laboratory, where Samuel Ruben and Martin Kamen had pioneered the first uses of radioactive tracers in biology, including <sup>32</sup>P, <sup>13</sup>N (10 min half-life), and <sup>11</sup>C (20 min half-life). They used the latter for the first demonstration of carbon dioxide assimilation by plants and then discovered long-lived <sup>14</sup>C, more convenient for such work<sup>12</sup> (and the basis of

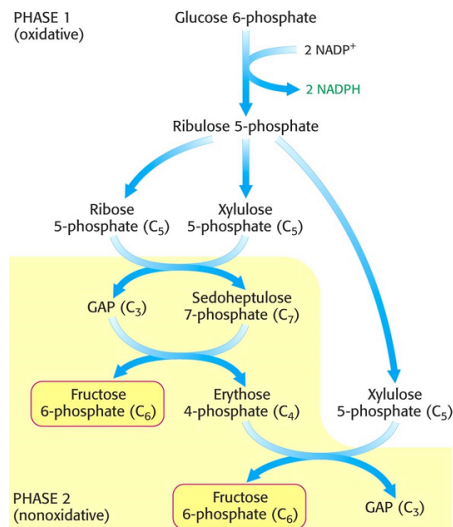


Figure 1. The pentose-P pathway. (In this diagram, the irreversible oxidative branch is abbreviated as a single overall reaction but comprises the glucose-6-P dehydrogenase, lactonase and 6-phosphogluconate dehydrogenase enzymes, and CO<sub>2</sub> is also a product.) (Reprinted with permission, from Berg, Tymoczko & Stryer, *Biochemistry*, 7th Ed.)

yet another new industry, designer radiolabeled metabolites, Geiger and scintillation counters becoming standard equipment.) After the war (Ruben having died in a laboratory accident with phosgene gas and Kamen relegated to shipyard duty as a security risk), the work was continued by Andrew Benson, and others with Melvin Calvin. They labeled intact algae with  $^{14}\text{CO}_2$  for appropriately short sampling times, then extracted and identified the labeled products using chemical degradations and derivatizations, which allowed them to deduce the pathway of photosynthetic  $\text{CO}_2$  assimilation by so-to-speak direct observation. Although logically obvious, this approach to stepwise tracking of intermediates has rarely been done for other pathways.

In that work ( a Nobel Prize in 1961), phosphoglycerate was identified as an early intermediate in 1948, and ribulose-P and sedoheptulose-P were uncovered in 1951. By 1954, the deduced pathway<sup>13</sup> included several of the same intermediates and enzymes as the non-oxidative pentose-P pathway—a mutually informed convergence of two different approaches to apparently quite different subjects. However, the cycle in photosynthesis required assimilatory and reductive steps different from reversal of the oxidative pentose-P pathway. The fundamental contributions of Horecker and his group in this regard would be three papers in 1956 describing purified ribulose-P kinase,<sup>14</sup> ribulose-1,5-P<sub>2</sub>,<sup>15</sup> and the purified carboxylase,<sup>16</sup> later dubbed Rubisco and perhaps the most abundant enzyme in the biosphere.

Returning to the pentose-P pathway in animals and non-autotrophic microbes, it should be noted that at the time its physiological functions were unclear, despite the fact that most of the enzymes were almost universally found in relatively high activities. The original speculation had been that a cyclic version might accomplish the complete oxidative breakdown of hexose-P i.e.,  $1 \text{ glucose-6-P} + 7 \text{ H}_2\text{O} \rightarrow 6 \text{ CO}_2 + 12 \times 2\text{H} + 1 \text{ inorganic phosphate}$ . Although this stoichiometry could be shown in a reconstituted system,<sup>17</sup> other observations indicated that was unlikely to commonly be a major route of hexose catabolism *in vivo*. On the other hand, the non-oxidative branch is a usual route for bringing pentoses into glycolysis for their catabolism. Presently, as Horecker noted,<sup>3</sup> the physiologically important functions of the pentose-P pathway in many organisms with respect to the metabolism of glucose are to provide reduced NADP (by the oxidative branch), ribose-5-P (from either branch) and erythrose-4-P, sedoheptulose-7-P, and their derivatives (by the non-oxidative branch), i.e., it is mainly assimilatory. It should be added that in many microbes, glucose catabolism does proceed via oxidation to pentose-P, or via 6-phosphogluconate followed by the two step Entner-Doudoroff cleavage to pyruvate and glyceraldehyde-3-P.

Horecker would later take interest in such routes, e.g.,<sup>18</sup> but the sustained thrust of his work would be mechanistic enzymology and protein chemistry. One line of investigation was on aldolases and transaldolases. A key clue was the demonstration with transaldolase that a relatively stable apparent enzyme-substrate complex could be obtained by incubation with [<sup>14</sup>C]fructose-6-P in the absence of an acceptor aldehyde substrate; the complex contained the C1-C3 portion of the fructose-6-P, as shown by its transfer onto erythrose-4-P. The enzyme-bound intermediate could be stabilized by borohydride reduction; and upon acid hydrolysis, it yielded- $\beta$ -glyceryllysine, indicating that the original 3C adduct was attached to the enzyme via a Schiff base linkage to the N $\epsilon$  of an active-site lysine residue.<sup>19</sup> These studies were among the first to characterize an enzyme-bound substrate intermediate. The same thing could also be shown with aldolase, establishing Schiff base formation as key in this group of enzymes. It must have been a special satisfaction to present this work in celebration of Otto Warburg's eightieth birthday.<sup>20</sup>

Horecker then engaged in further probing of the mechanism and assignment of likely residues that participate in catalysis, leading to the early determination of the complete amino acid sequence of aldolase.<sup>21</sup> The initial work on aldolase and transaldolase was to a considerable degree a collaboration with a University of Genoa group including Sandro Pontremoli and Enrico Grazi, who would later chair biochemistry in Ferrara and Genoa, and over several decades, there would be more than one hundred joint papers. In Ferrara, the biochemistry institute was first housed in a converted, private bourgeois residence on Via Fossato de Mortara; opening the front door in 1965 was to be greeted by a row of humming Spinco Model E ultracentrifuges, which might have done proud the laboratories headed by Horecker in the United States.

In the mid-1960s, he took up another centrally important enzyme of metabolism, fructose-1,6-P<sub>2</sub> phosphatase, which is critical for gluconeogenesis, key in the liver for conversion of products of fat and protein metabolism to glycogen, required in microbes for the biosynthesis of hexoses and pentoses when grown on substrates like acetate or glycerol, and involved in plants in the nexus of carbon dioxide assimilation. Here again the work was initiated with the Italian group,<sup>22</sup> and a major thrust would become an interest in differences between the liver and muscle enzymes with regard to the degree of limited proteolysis that they were observed to undergo *in vivo* and speculation whether the isoforms so generated might have distinct physiological roles.<sup>23</sup> Later, the line on proteolysis would turn to the thymosins, a variety of pharmacologically active peptides made by the thymus gland, and their biosynthetic origin.<sup>24</sup>

Around 1959, Horecker also undertook work on a rather different subject: biosynthesis of the “O” antigen of Gram-negative bacteria, which comprises the heteropolysaccharide component of their outer membrane lipopolysaccharide. Identification of clinical isolates of *Salmonella* spp. and other enteric bacteria differing in virulence was long based on differences in their

O-antigens. And, from decades of studies in immunochemistry and genetics,<sup>25</sup> it was clear that the antigenicity was based on specific repeating 3- or 4-unit oligosaccharides found on “smooth” strains and that this portion of the molecule was attached to a short “core” oligosaccharide that is still present in “rough” mutants, which lacked O-antigen and was similar between strains; and that core was anchored in the

membrane to toxic lipid A, itself a hexose structure with fatty acids. (Fig. 2).

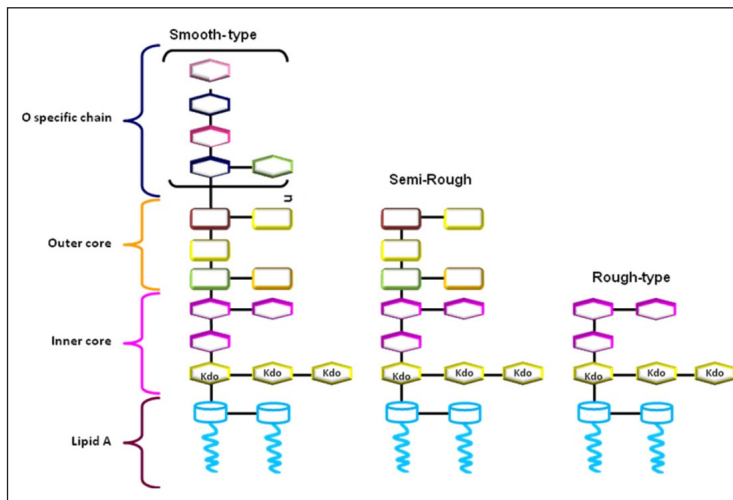


Figure 2. Structure of bacterial lipopolysaccharide. (By R. E. Reyes, C. R. González, R. C. Jiménez, and M. O. Herrera.)

The situation was ripe for discovery of biosynthetic enzymes for these structures. Genetic analysis of galactose metabolism had by 1959 established that UDP-Glu/UDP-Gal epimerase, a product of the *galE* gene in bacteria, was responsible for both galactose catabolism and synthesis. Although most of Horecker’s work had up to then used microbes, the power of combining genetics with biochemistry was not yet high profile at the NIH. For his sabbatical in 1957–58, he chose the laboratory of Jacques Monod at the Institut Pasteur, where he studied galactose transport in *E. coli*,<sup>26</sup> at the height of Monod’s iconic work on bacterial gene expression. (Of course, the fact that Monod’s laboratory was in Paris played no role in his choice, nor in that of so many other American scientists who flocked there on visits and sabbaticals during that decade.) Furthermore, he was soon to leave NIH and take the chair of the Department of Microbiology at the NYU School of Medicine; and, it was studies of bacterial identification,



growth, virulence, and variation, and of bacterial viruses (bacteriophages), from which arose major model systems for what came to be known as molecular biology, that formed the focus of his teaching in the new department. The first *E. coli* paper from his new laboratory was a highly cited one from 1961 on localization of alkaline phosphatase.<sup>27</sup> Just one year later, his group<sup>28</sup> and Hiroshi Nikaido,<sup>29</sup> then in Osaka, published in the same journal essentially the same findings about O-antigen synthesis: that *galE* mutants grown without galactose had a defective O-antigen which could be restored to normality *in vivo* by including galactose in the medium or *in vitro* with UDP-Gal, i.e., the enzymatic incorporation step. The work would continue with the analogous use of phosphomannose isomerase and phosphoglucose isomerase mutants, revealing reactions involving sequential addition of the different sugars from a handful of sugar-nucleotide precursors and bringing to light the steps needed for core and O-antigen synthesis,<sup>30</sup> as well as the first hint of a role for new lipid carriers.<sup>31</sup>

Going back to some of the same enzymes for which Horecker is best known, it is interesting how functional assignments of key residues remain uncertain even for aldolase.<sup>32</sup> Likewise, although issues of enzyme promiscuity had been recognized early on for aldolase and transketolase,<sup>9,11</sup> there would be challenges as to the very setup of the pentose phosphate pathway, particularly from the group of John F. Williams, who forcefully urged consideration of other metabolites, including octulose-1,8-P<sub>2</sub> in the liver and elsewhere,<sup>33</sup> although not without pushback.<sup>34</sup> And on the same line to note how many years later, a non-canonical version of the non-oxidative branch involving sedoheptulose-1-7-P<sub>2</sub> bisphosphate would be shown to contribute to ribose-5-P formation in yeast.<sup>35</sup> There would also be renewed appreciation that enzyme promiscuity can have toxic consequences, such as when glyceraldehyde-3-P dehydrogenase acts on erythrose-4-P (instead of glyceraldehyde-3-P) leading to erythronate-4-P, and the presence of highly evolved, specific enzyme destruction of such otherwise toxic orphan metabolites.<sup>36</sup> Finally, for what such numbers are worth, it is amusing to note that, as of October 30, 2021, total citations in PubMed were 25,798 for glucose-6-P dehydrogenase, 16,749 for Rubisco, and 6,978 for 6-phosphogluconate dehydrogenase, but only 1,027 for 6-phosphogluconolactonase, on which he never worked and whose functions remain uncertain.

The style of doing science then, as now, was to live it, but with certain differences. Everyone was expected to follow the major journals in the field, which, apart from individual subscriptions, could only be done in person in special rooms called libraries, where research reports printed on paper were delivered monthly, then weekly, and even daily. The issues were eventually bound in hard covers and consigned to depositories called the

“stacks.” (Otto Warburg himself was said not to have an office in his institute because he was either at the bench or in the library, where he wrote his papers.) Furthermore, in the 1960s, there was a mania for preprints, which were systematized and delivered by the postal system.<sup>37</sup> In Horecker’s department at NYU, every lunchtime was a journal club, a habit he brought from the NIH where the physical journal was maneuvered under a cumbersome opaque projector, and the data interrogated on a screen. This exercise was for everyone, not just the students and every day meant every day, even moving day to the Bronx where in the to-be library perched on boxes packed with, yes, journals, a hapless postdoc tried to explain about an odd genetic phenomenon called selfing<sup>38</sup> and afterwards Jerard Hurwitz said he thought the talk was about sulfur.

Many in that cohort have fond memories of their time with Horecker, particularly at NYU, where one floor up was biochemistry headed by Severo Ochoa, who was hot on the trail of the genetic code (UUU for phenylalanine, etc.) in competition with J. Heinrich Matthaei and Marshall W. Nirenberg at the NIH. (Francis Crick’s quip that the code war started with the U3 incident was a play on the Cold War and the U2 incident in which a CIA U2 spy plane was shot down during a reconnaissance over the Soviet Union in the summer of 1961.) Further upstairs in pathology was Zoltan Ovary, an immunologist who persuaded Robert White, tenor in New York’s Pro Musica Ensemble, to sing each year to the medical student class Franz Schubert’s “An Silvia,” set to lyrics describing the complement cascade. Also in that department, recruited from Paris by Chair Lewis Thomas, was Baruj Benacerraf, who was analyzing human transplantation antigens (and a 1980 Nobel Prize) while also running the New York City branch of his family’s Venezuela-based bank.

The *raison d’être* of preclinical departments at the time was their courses for medical students, and microbiology under Horecker had a Saturday morning special lecture series on current research given by invited speakers. One week it was Fritz Lipmann, who liked to play the quavering elderly bloke and, after his talk, was set off kindly in the direction of the Rockefeller Institute. Another time it was Jacques Monod, who told the students that he had just got a telegram from Paris saying there were twenty molecules of lac repressor per cell (actually he had misread it; the experiments had failed, suggesting that there had to be fewer than 20 copies per cell).

The department in Manhattan overlooked the East River at 32nd Street. One could walk to lunch at the Philippine Garden Restaurant on Third Avenue and, for special occasions, there was the Mon Paris Restaurant on 30th Street between Park and Lexington Avenues

which served that rarity, potato croquets. So why, wondered certain postdocs, did Horecker decide in 1963 to move to Albert Einstein College of Medicine? Indeed, the reasons for that move, and subsequent ones, are not obvious. Scientists from the NIH seeded the faculties of many universities and medical schools in the post-World War II expansion; Horecker was a star, the world was his oyster, and NYU was an unsurprising choice. By contrast, Einstein was relatively new and, unlike the walk to NYU from his East 64th Street apartment, it was a relatively long commute. But at Einstein, he could head the first department of molecular biology at any medical school, staffed as he wished and with no teaching requirements. Einstein was founded and funded by Jewish donors, and it may be that he had strong feelings for joining on those grounds. And there is an anecdote about his advising a graduate student to think Yiddish but act British, but his generation did not wear their ethnicity on their sleeves. In fact, Horecker's public face was not eccentric but more in the line of a prosperous dentist. Of course, at Einstein there were close professional friendships and colleagues of distinction. In any case, after ten years, he moved to the Roche Institute of Molecular Biology in Nutley, New Jersey, still a long commute, and ten years later to Weill Cornell Medical College as a professor of biochemistry and dean of the Graduate School, back in walking distance to his Manhattan apartment. Retiring in 1992, his later years were spent in Florida on Sanibel Island and in Cypress Cove.

Throughout his career, professional obligations and honors were many. He was president of the American Society of Biological Chemists in 1968, a long-time editor of *Archives of Biochemistry*, and a founding editor of *Biochemical and Biophysical Research Communications*, whose very first paper reported the discovery by Hurwitz et al. of (the real) RNA polymerase.<sup>39</sup> Horecker was what is now called a good mentor. In her own charming memoir,<sup>40</sup> Mary Jane Osborn, who came as a postdoc, cites him as particularly supportive, and of the handful of Ph.D. students, two started as his technicians (Pauline Smyrniotis at the NIH and Orestes Tsolas at NYU), and three others (Michael Malamy, Robert Yuan, and Dan Morse) would make fundamental discoveries in their postdoctoral work in subjects unrelated to their Ph.D. research.<sup>41-43</sup> To be with Horecker was good fortune.

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