

Recollections

How I Became a Biochemist

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Some scientists, like Joshua Lederberg, knew their calling to research from an early age. His most precious Bar Mitzvah gift was the Meyer Bodansky textbook of biochemistry. For me, my first encounter with biochemistry and the Bodansky text was in my course in medical school. The focus of the text and the course was on the analysis of blood, urine, and tissues and I found it utterly boring. I had drifted into medical school after I finished college in 1937 in the midst of the Great Depression. There were no jobs to be had in chemistry, in which I had shown some interest and aptitude. For a conscientious student who got good marks in his subjects, a further 4 years in medical school was a welcome refuge.

Once in medical school, I was eager to become a practicing doctor. I had some interest in research but the avenues for coveted research fellowships in pathology, bacteriology, and physiology were closed to me. I did manage to collect data out of a concern related to having a mild case of jaundice and could show that when I injected myself with bilirubin intravenously, it was eliminated much more slowly than in others. This metabolic defect was much later recognized as Gilbert's Disease, a familial form of jaundice, known from the turn of the century and then completely forgotten. I managed to find and test a few other slightly jaundiced, but otherwise normal, medical students who proved to have the same deficiency. I published these findings during my internship year (1941–1942) in internal medicine in the prestigious *Journal of Clinical Investigation*. The publication with jaundice in its title proved fateful because it appeared at the time of another fateful event, our entry into World War II. [The publication later earned me one of the rare assignments to the National Institute (*sic*) of Health (NIH), the research arm of the U.S. Public Health Service (PHS).] Jaundice had suddenly become a major concern when many thousands of troops vac-

inated with yellow fever virus became mysteriously sick and jaundiced. This catastrophe, much later traced to hepatitis virus in the vaccine, attracted wide attention in the Army Medical Corps and in the PHS, including that of Rolla Dyer, Director of the NIH.

After my internship, I had qualified for a commission in the PHS and was ordered to report for duty on a naval vessel in the Gulf of Mexico. After some months of an idyllic vacation as the ship's doctor came an order, based on a request by Dyer, who had read my jaundice article, to report to the NIH. No protest was raised by the ship's captain, exasperated by my naval ignorance and indifference to his authority.

A novice in research, my incarnation as a biochemist came in two stages. The first was being ordered to do full-time research. The NIH in 1942 was a rather small laboratory, focused on infectious disease, to which a few PHS officers came for a brief tour of duty. There was also a Division of Nutrition and Physiology to which I was assigned. The chief, W. H. Sebrell, directed the laboratory founded by his mentor, the legendary Joseph Goldberger, who showed that epidemics of pellagra affecting hundreds of thousands of people in the southern states was not due to a microbial infection as had been assumed, but rather to the lack of a trace substance in the diet, a vitamin deficiency. Minimal diets that Goldberger fed to dogs produced pellagra ("blacktongue"), which could be prevented or corrected by addition of various nutrients. The responsible vitamin, eventually isolated by others, proved to be niacin.

My project in the Nutrition Laboratory was to find the factor missing in refined rat diets, the absence of which caused severe anemia and white blood cell deficiencies. We were overtaken in this vitamin hunt by others who used a superior bacterial growth assay and tons of spinach to isolate a few milligrams of the pure vitamin they named folic acid (Latin *folium*, leaf). In a variety of related nutritional studies, I found that folic acid deficiency was precipitated by many stresses, such as sulfa drugs, which depleted the gut flora, the source of the vitamin.

After a year of doing full-time research, I felt happier and in more control than treating patients. With my colony of hundreds

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of inbred rats, I could devise month-long experiments and get definitive results. I also realized that even with an opportunity to devote considerable time on focused clinical research, I would still have to deal with a motley assortment of patients and spend years to get any kind of a result. I made the switch from medicine to laboratory research promptly and untroubled, something my colleagues thought rash and adventuresome.

The second stage of becoming a biochemist stemmed from boredom and frustration with feeding rats. For 3 years I had fed them deficient diets under various circumstances and recorded the outcomes: survival, losses in body weight and fertility, depletion of blood cells, and so on. I realized that if I were ever to learn how a vitamin deficiency produced symptoms in a microbe, rat, or human, it would never come from nutritional studies. By contrast, I was becoming aware that a new and dynamic biochemistry was emerging. Enzymes and ATP were being recognized as prime actors in the drama of converting food to energy for muscle contraction and growth. Already, niacin had been identified as the coenzyme in oxido-reductions responsible for the formation of ATP. I had learned nothing of ATP or metabolic enzymes in my 1937 biochemistry course nor of the heroic biochemists who were making these discoveries.

With the end of the war in August 1945, we were no longer committed at the NIH to do studies related to the war effort. Bernard Horecker, trained in enzymology at the University of Chicago, was now freed from studying DDT toxicity in cockroaches and permitted to resume his earlier work on cytochrome reductase. Since we were close friends, I could apprentice myself to him and learn how to separate cytochrome *c* and succinoxidase and to isolate NADP (then known as TPN); we accumulated the world's largest supply at the time. Remarkably, in those few months, we published papers on the discovery of a novel cytochrome *c*-cyanide complex and on the definitive extinction coefficient of the reduced band of the pyridine coenzymes (NAD and NADP).

With advice from Horecker and the late Bernard Davis, I identified Severo Ochoa, a young Spanish biochemist, at the New York University (NYU) College of Medicine, in whose laboratory I might learn enzymology. Ochoa was purifying the enzymes of the citric acid cycle that could hold the key to what seemed to me the holy grail of biochemistry, the mechanism of aerobic phosphorylation. I was fortunate that the Institute approved a few months' assignment away from the NIH, unprecedented for a uniformed PHS officer. The months I was granted in the Ochoa laboratory were stretched to a year and then further extended for 6 months in the Mecca of enzymology, the laboratory of Carl and Gerty Cori at Washington University in St. Louis.

When I joined Ochoa in January 1946, his group consisted of a technician and Alan Mehler, a graduate student, operating in a small space in the NYU Biochemistry Department granted by Isidore Greenwald. (Greenwald had been the first to identify 2,3-diphosphoglycerate and to introduce trichloroacetic acid as a protein precipitant; he was also obsessed with his theory of a viral origin of epidemic goiter.) In the intimate atmosphere of the Ochoa lab, I was introduced to enzymology by trying to separate pig-heart aconitase into the presumed two components that converted citrate in two stages to isocitrate. In this I failed—aconitase was shown much later to be a single polypeptide—but I was overcome with the excitement of getting results within minutes in spectrophotometric assays. I was also converted to enzymology as a means of achieving the molecular resolution and reconstitution of a biological event. After failing with aconitase, I was more successful, collaborating with Alan Mehler, in the isolation of the malic enzyme from pigeon liver.

I fell in love with enzymes from the very first and have never met a dull one, my own or those of others. In my affairs with enzymes, perhaps three dozen, a few like DNA polymerase stand out as a long marriage, but all are memorable and connected to one another. In the Cori lab in St. Louis, I sought the origin of the then mysterious inorganic pyrophosphate in kidney cortex homogenates, during the course of which I stumbled on the discovery of nucleotide pyrophosphatase. It proved to be an enzyme that cleaves nucleotide coenzymes at the pyrophosphate bond and later, on my return to the NIH, I purified it from potato extracts. As a reagent, the enzyme provided me with the substrates for the discovery of coenzyme biosynthesis with the accompanying generation of inorganic pyrophosphate and also the reverse pyrophosphorolysis of coenzymes. This humble enzyme enabled me to determine the location of the third phosphate in NADP and inspired me to tackle the biosynthesis of the phosphodiester bond of phospholipids and then the assembly of nucleotides in the nucleic acids. Along the way, I learned of the novel capacity of ATP to be a pyrophosphoryl donor as in the synthesis of phosphoribosyl pyrophosphate (PRPP) and a nucleotidyl donor as in the synthesis of RNA and DNA.

When I reflect on the more than 50 years of persevering in identifying an enzyme activity in a cell-free system and then proceeding with its purification, I recall each effort as rewarding. As in the stages of climbing an unexplored mountain, attractive views often appeared at plateaus along the way. Upon reaching the summit, even more mysterious and glorious peaks came into sight. With the gift in recent years of reverse genetics, the gene encoding the enzyme can be cloned, mutated, and overexpressed, and with that the vistas are broadened to include the cellular mission of the enzyme and its evolutionary past.