The Isolation of the Vitamin B_{12} Coenzyme and the Role of the Vitamin in Methionine Synthesis

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The invitation to contribute a "Reflections" article has brought back many memories as I recall the events early in my career that were most significant in determining the path I have taken for more than 50 years. I could reflect on several periods in my career, such as the early work on serotonin biosynthesis and metabolism or later studies on the role of GTP and elongation factors in protein synthesis, but the period when I worked on vitamin B_{12} in the late 1950s and early 1960s especially stands out in my mind, and I will focus mainly on these studies. Of the known B vitamins at that time, only the coenzyme form of vitamin B_{12} had not been identified, and its role in metabolism was not known. The identification of the first B_{12} coenzyme in studies on glutamate metabolism in Clostridium tetanomorphum opened up this field, leading to an understanding of how this vitamin functions in other systems, such as methionine synthesis and deoxyribose formation. It also helped to explain the important metabolic interrelationship among vitamin B_{12}, folic acid, and one-carbon metabolism. On a personal note, it was during this time that I made the transition from a postdoctoral fellow to an independent scientist, and as I will discuss, these studies on vitamin B_{12}, directly or indirectly, provided the foundation for much of the later research I have been involved in.

I am taking the liberty throughout this article to include some biographical information and personal thoughts since it is hard for me to reflect on my early years in science without providing some personal history. I was raised in the Bronx, the middle of three children with a brother, Arthur, 5 years older, and a sister, Carol, 7 years younger. I mention this because my brother, unknowingly, had a significant impact on my career. My parents barely had a high school education, and our family was financially close to the poverty line, but they were determined that their children obtain a good education. I followed my brother through the superb public school system in New York City, including the Bronx High School of Science, which was close enough to our apartment in the West Bronx that I could walk to and save the cost of public transportation. Perhaps even more important, Bronx Science had a mediocre baseball team that I felt I could easily make. My hidden dream was to be a professional baseball player, a dream that came to an abrupt end after playing baseball in my freshman year in college. However, selecting Bronx Science for my high school training was one of my fortunate decisions. It started me on the path that I have followed until now.

The College Years

The only hope for college was to go to The City College of New York (CCNY). It was a free college when I entered in 1949, after passing an entrance exam, and had an excellent reputation. My brother had graduated earlier from CCNY and had started his Ph.D. studies in biochemistry at
Columbia University under the guidance of David Sprin-
son. I would occasionally stop off at his laboratory on my
way home from CCNY since most students at this City
College lived at home and commuted every day to the
college. The trips to the laboratories at Columbia were my
first real exposure to research and had a lasting impres-
sion. However, since I was determined not to follow in my
brother’s footsteps, I majored in organic chemistry until
my senior year. At that time, I had room for some electives
and decided to take a year of biochemistry (called physio-
logical chemistry) using a textbook written by a CCNY
professor, Benjamin Harrow. Abraham Mazur and Ernest
Borek were two of the professors who taught the course.
Mazur, or Abe, as he was known to his colleagues, had a
significant influence on my career and on those of many
other students both before and after me, including my
brother. In fact, most of the students, in a class of about a
dozen, who took the course in the spring of 1953 decided
on careers in research. Those I remember and with whom
I have had some contact are Ted Breitman, Nathan Brot,
Howard Goldfine, Alan Peterkofsky, and Jack Preiss.
Mazur was a short man, a chain smoker, and, in thinking
back, the most dynamic teacher I ever had. His enthusiasm
for this relatively new field called biochemistry was infec-
tious. I knew by the winter of 1952 that I was headed for a
career in biochemistry, and early in 1953, Mazur arranged
for me to meet Sidney Udenfriend, also a CCNY alumnus,
to discuss doing my graduate studies at the National Insti-
tutes of Health (NIH) under a new program with George
Washington University (GWU) Medical School. I believe I
had already received an acceptance from Western Reserve
University, but Udenfriend made the NIH sound so attrac-
tive that I was not sure what to do. I asked Mazur for
advice, and he had two concerns. First, this was an unor-
thodox, untested Ph.D. program where I would carry out
my research at the NIH, but do my course work and obtain
my degree at GWU. Second, the NIH was just at the begin-
nning of a growth spurt and had not yet obtained the repu-
tation as one of the premiere biomedical research insti-
tutes in the world, despite the depth of talent that was
already there. However, Mazur thought it was worth the risk,
and when my brother later told me that he had accepted a
postdoctoral fellowship with Bernard Horecker at the NIH,
many of my doubts were erased. Without planning it, the
Weissbach brothers were both heading to the NIH.

The NIH Early Years: Graduate Studies on
Serotonin Metabolism

Several important events occurred in 1953, including
my graduation from CCNY, the start of my graduate stud-
ies at the NIH, and my marriage to Renee. In June of 1953,
I left for Bethesda and became Udenfriend’s graduate stu-
dent in the laboratory headed by Bernard (Steve) Brodie in
the National Heart Institute. Our laboratories were on the
second floor of Building 3. In the late summer of 1953, Art
joined the Horecker laboratory, and his laboratory was
located on the first floor of Building 3. For most of the next
35 years, Art and I worked at the same institutions,
namely, the NIH and the Roche Institute of Molecular
Biology, but we never published together until rather late
in our careers. We then decided we should have at least
one joint publication, and in 1986, we edited a volume of
Methods in Enzymology on Plant Molecular Biology (1).

The Udenfriend laboratory had just begun working on a
newly discovered indoleamine, 5-hydroxytryptamine
(serotonin). My initial project was to help purify the
enzyme that converted 5-hydroxytryptophan to serotonin
and develop a sensitive assay for serotonin in tissues. In
September of 1953, I began graduate school at GWU,
which meant having to organize my 45-minute trips to the
GWU Medical School in downtown Washington, D. C.
for course work so that I could still carry out experiments
at the NIH each day. It was a challenge, to say the least, but
I quickly learned to make the best use of my time, which
has proved to be valuable throughout my career. The Bro-
die laboratory was at the cutting edge of the newly devel-
oping field of biochemical pharmacology, and I could not
help but be cognizant of the concentration of biochemical
talent in Building 3. In addition to the scientists in the
Brodie laboratory, which included Julie Axelrod, Elwood
Titus, and Chozo Mitoma, there was the Horecker labora-
tory on the first floor, which had attracted young scien-
tists, such as Bruce Ames, Jerry Hurwitz, Paul Marks, and
my brother. Leon Heppel and Sy Black were also partici-
pants in the daily journal club that the Horecker laboratory
held at noon time. Earl and Terry Stadtman were also in
Building 3, along with Chris Anfinson and his group. Herb
and Celia Tabor would come over from another building,
and I remember taking an evening course at the NIH on
nucleic acids from Herman Kalckar. In those first few
years, I met other bright young scientists, such as Bob
Bowman, Gordon Tomkins, Marvin Siperstein, and Bern-
hard Witkop, an organic chemist who worked closely with
Udenfriend over the years. There were many more I have
not mentioned. I did not appreciate at first how fortunate
I was to be in the midst of this exceptional group of scien-
tists, but I soon realized that the exposure to research that
I received while working in Building 3 was as important, or
even more important, than my graduate studies at GWU.
In 1955, the Brodie laboratory moved out of Building 3 to
the seventh floor of the Clinical Center, called Building 10.
The facilities, space, and support were superb in this new building, and there were many more scientists to interact with, but the unique environment, one could say a culture, that we had in Building 3 was in large part lost.

As mentioned, my graduate studies were mainly on the biosynthesis and metabolism of serotonin (2–5). Udenfriend was an impatient but wonderful mentor. Two important lessons that I learned from him have remained with me to this day. The first was the value of developing a good assay, and the second was to, whenever possible, do simple, quick experiments to get a feel of how to proceed. Sid was superb in devising what we often referred to as “quickie” experiments. Just as important was his support of my career over a span of 40 years, as a student and as a colleague at both the NIH and the Roche Institute of Molecular Biology.

The Period at Berkeley with Barker: Discovery of the Vitamin B12 Coenzyme

By the summer of 1956, I was writing my thesis and planning to graduate in February of 1957. It was time to think about getting postdoctoral training. I had spent my graduate years working with animals, and the advice I received was to learn a new field to broaden my knowledge as well as to provide flexibility for approaching scientific problems in the future. Thanks to Udenfriend’s efforts, the NIH agreed to cover my postdoctoral training, with the unwritten agreement that I would return after a year. I had never worked with bacteria, but from the journal clubs and lectures I attended, it appeared that procaryotes had distinct advantages compared with animal tissues for both biochemical and genetic studies. The name Horace Barker had come up on several occasions since he had spent time at the NIH in Arthur Kornberg’s laboratory in Building 3, before Kornberg moved to Washington University in St. Louis. In addition, the Stadtmans had done their graduate work under Barker in Berkeley. Early in 1957, I spoke to Earl and Terry Stadtman about doing a postdoctoral fellowship with Barker, and they strongly recommended that I contact him. Udenfriend was also enthusiastic about my doing postdoctoral training with Barker.

I contacted Barker in early 1957 and inquired about doing a postdoctoral stay in his laboratory, and we arranged to meet at the FASEB meeting in Chicago in 1957. Barker made it clear that if I came to his laboratory, I would work on the metabolism of glutamate acid in an organism that I had never heard of, C. tetanomorphum. To be honest, it seemed like a rather uninteresting research problem since this was a unique pathway of metabolism limited to relatively few organisms and not found in mammals. However, I was going to Barker’s laboratory to learn techniques, so the research problem was secondary. How wrong I was, for the year with Barker turned out to be one of the most exciting times of my career. The lesson I learned was that if you dig deep enough, all science can be exciting. In December of 1957, we arrived in Berkeley, and I could not have predicted when I walked into Barker’s laboratory how that decision would impact my future scientific career.

What a difference in venue. The laboratories in Berkeley were so “uncrowded” compared with those in Buildings 3 and 10 at the NIH, and it seemed like such a quiet, calm environment. Some of the individuals in the Barker laboratory that year whom I remember were Bob Smythe, a technician; John Toohey and Ed Vaskievich, graduate students; and Ben Volcani, a visiting professor. I immediately became familiar with C. tetanomorphum, which had to be grown under strict anaerobic conditions in large 20-liter carboys with glutamate as a carbon source. At that time, sodium glutamate was a meat flavor enhancer sold commercially under the name Accent, and we had huge containers of this compound. When I arrived, it was already known that a major intermediate in the fermentation of glutamate by C. tetanomorphum was mesaconic acid (6), and there was evidence that this conversion was a two-step reaction involving the formation of β-methylaspartic acid, which was then deaminated to form mesaconate. The initial conversion of glutamate to β-methylaspartate was not easy to imagine chemically. It involved rearranging the carbon atoms in the glutamate by breaking the bond between carbon atoms 2 and 3 and connecting carbon atom 2 to carbon atom 4. It was known that this reaction required a cofactor that could be removed by treating the extracts with charcoal. A similar type reaction in mammalian cells turned out to be the conversion of succinyl-CoA to methylmalonyl-CoA. The second step, the reversible conversion of β-methylaspartate to mesaconate, was catalyzed by an enzyme called β-methylaspartase, and some preliminary studies had been done on this reaction before I arrived. Bob Smythe was mainly involved with the first reaction, and my initial project was to purify β-methylaspartase and try to identify one of the products of the reaction when mesaconate was used as the substrate. The main product that had been identified by paper chromatography, using relatively crude enzyme preparations, was threo-1-β-methylaspartate, but there was another minor product that had not been identified. It took a couple of months to purify the enzyme more than 50-fold and to identify the minor product as erythro-1-β-methylaspartate, the other possible epimer since β-methylaspartate has two asymmetric carbons, the α- and β-carbons (7).
was somewhat disappointed since I had hoped that this minor product might be a new intermediate in the metabolic pathway. Barker then suggested that I work more closely with Smythe and try to isolate the cofactor for the first reaction. It was easy to prepare extracts that were lacking the cofactor by treating the extracts with charcoal, and the enzymatic photometric assay was quite simple, but the purification of the factor was difficult because of its liability. When freshly prepared, it was stable for long periods in the freezer, but once an aliquot was thawed and tested during the day, it would slowly lose activity. Recovery during the purification steps was very low, and I was getting desperate to find ways to stabilize the cofactor. We tried a variety of obvious changes, such as altering the pH and salt concentration and adding reducing agents, etc., with no success. One day in the early spring, Barker, in his usual soft manner, suggested that we see whether the loss of activity I observed each day with fresh cofactor preparations might be due to light sensitivity of the active component. The laboratory I was in was not that well lit, but the bright California sun would often illuminate my bench. Sure enough, a brief exposure to a tungsten lamp inactivated the cofactor, and from that time on, I learned to work in a dark laboratory, and the tubes containing the cofactor were always kept protected from light. The light sensitivity put us on the road to identifying the cofactor. I must admit I doubt whether I would have ever thought that light was causing the stability problem. This was a turning point in the problem, thanks to the wisdom of Barker. As the purification proceeded, it became apparent that the active fractions were yellow orange in color. We had considered early on that the cofactor might be related to vitamin B₁₂ but the spectrum of partially purified preparations did not show the typical large peak in the 350 nm area. However, once there was enough material to do spectral studies, it was possible to show that, after light exposure, the spectrum changed to one similar to that of hydroxy-B₁₂ except in the UV region. The normal vitamin contains cobalt with six ligands, four to the pyrrole groups, one to 5,6-dimethylbenzimidazole, and one to a CN group. Hydroxy-B₁₂ has the CN replaced with a OH group. It was attached to the cobalt in place of cyanide by a bond that was both light- and cyanide-sensitive. By growing the organism on 5,6-dimethylbenzimidazole, we could replace the adenine in pseudovitamin B₁₂ and produce the coenzyme form of the vitamin, containing one residue of 5,6-dimethylbenzimidazole and one adenine derivative (9).

I remember the day I literally ran into Barker’s office, with the spectrum in hand, to tell him that our cofactor was a derivative of vitamin B₁₂. It was early summer, and Barker was preparing to take his yearly scheduled vacation and go to his summer home near Lake Tahoe. I thought we should immediately write up our results, and I was taken aback when he said he was leaving for vacation and that we would work on the paper after he returned. I spent the next few weeks getting the data for the paper, and when Barker returned from vacation, the paper was quickly completed and sent to the Proceedings of the National Academy of Sciences of the United States of America. I saw another side of Barker from that incident. He knew exactly what his priorities were and could put life’s events into proper perspective.

By the time I left Berkeley to return to the NIH, we knew that the adenine was attached to a sugar derivative, which we called X (10), but despite help from David Rammler and investigators in Clint Ballou’s laboratory, we could not elucidate the structure of adenine-X or determine how it was attached to the cobalt. Once crystals of the coenzyme were prepared (11), the structure was elucidated by Lenhart and Hodgkin (12), who showed that adenine-X was a 5-deoxyadenosyl group that was linked to the cobalt via carbon 5 of the deoxyadenosyl group (Fig. 1).

There was another event during the year in Berkeley that should be mentioned. As my postdoctoral stay was coming to a close, I was offered a faculty position in the Biochemistry Department at Davis, which was being established by Paul Stumpf. Renee loved California; in fact we both did, but I had agreed to return to the NIH and did not want to break my promise to Udenfriend. I contacted Udenfriend and told him about the offer. He wrote back that there would be no problem if I wanted to take another position, but he thought that my career would be best served if I returned to the NIH. He assured me that I would be an independent investigator, and I knew that the NIH had become a premier place to do research. I will never know what would have happened if I had gone to the University of California at Davis, for in December of 1958, after an unbelievable stay in Barker’s laboratory, we returned to Bethesda.

**Back to NIH: Role of Vitamin B₁₂ in Methionine Synthesis**

As promised, I was now an independent investigator when I returned in 1959 to Udenfriend’s laboratory at the
NIH. Betty Redfield, who had joined the laboratory in 1956 as a technician and worked with me for about a year before I left for Berkeley, was back in my laboratory. I knew I wanted to continue working in the B12 area, but also had the luxury and freedom to work on other projects, and I would like to say a few words about those studies. I thought that the synthesis of polypeptide antibiotics might be a good model system for how proteins were synthesized and initiated studies with Ed Katz, a Professor of Microbiology at Georgetown University, on the biosynthesis of actinomycin (13, 14). Although we were able to find the enzyme that catalyzed the synthesis of the phenoxazinone moiety, to which the 5-amino acid polypeptide chains were attached, we never succeeded in elucidating how the amino acids were added. As it soon turned out, due to the studies of Marshall Nirenberg, the peptide antibiotic approach to understand the mechanism of protein synthesis was wrong. I said that there was a new metabolite of serotonin, 5-methoxy-N-acetylserotonin, that was found in the pineal gland, called melatonin, and wondered whether I would collaborate with him on the acetylation reaction and elucidate the biosynthetic pathway from serotonin to melatonin. In about a year’s time, we worked out the initial acetylation reaction yielding N-acetylserotonin, the subsequent methylation of the hydroxyl group to form melatonin, and the major route of melatonin metabolism (15–17). It was a productive time, and I enjoyed working closely with Julie, but when our collaboration ended, my interest in serotonin also came to an end. I was thrilled when he received the Nobel Prize in 1970.

I had not forgotten about vitamin B12. The structure of the coenzyme, 5’-deoxyadenosyl-B12 was known, but the conversion of the vitamin to the coenzyme had not been elucidated. A college friend from Mazur’s class at CCNY, Alan Peterkofsky, was working in the Tabor laboratory at that time. Alan and I decided to work on the biosynthesis of the B12 coenzyme in C. tetanomorphum. We were unaware that Roscoe Brady, who had taken leave from the NIH to spend time in Barker’s laboratory, was also working on the biosynthesis of the B12 coenzyme in Propionibacterium shermanii. Both laboratories were able to identify enzymes that transferred the deoxyadenosyl moiety from ATP to hydroxy-B12 with the release of three phosphates (18–21). We had evidence that the initial product was tripolyphosphate (20), although Brady et al. (21) reported that inorganic phosphate and pyrophosphate were the products of their reaction.

At the time I was in Barker’s laboratory, vitamin B12 had been implicated in both deoxyribonucleoside (22) and methionine (23) synthesis. The methionine story was intriguing. D. D. Woods had initially isolated a mutant of Escherichia coli that required either methionine or vita-
min B₁₂ for growth and showed that the vitamin was needed for methionine synthesis using an *E. coli* cell-free extract (23). It was concluded that *E. coli* had two methionine biosynthetic pathways, one of which required vitamin B₁₂. Studies by Buchanan and co-workers (24) also demonstrated that the vitamin and a reducing system were required for methionine synthesis, and there was evidence that three methyl donors might be involved in the reaction: S-adenosylmethionine (SAM), methyl-B₁₂ (a B₁₂ derivative in which the 5′-deoxyadenosyl group is replaced by a methyl group), and N⁵-methyltetrahydrofolic acid (5-MeTHF). I decided that there was still much to learn about this reaction, especially if methyl-B₁₂ was a new coenzyme form of the vitamin. In 1962, Herb Dickerman joined our group, and together with Alan Peterkofsky and Betty Redfield, we were able to demonstrate the cell-free conversion of homocysteine to methionine in both *E. coli* and liver extracts using either 5-MeTHF or methyl-B₁₂ as methyl donor (25). As had been reported by others, the reaction using 5-MeTHF as methyl donor required a reducing system and SAM. In contrast, with methyl-B₁₂ as methyl donor, both the reducing system and SAM were not required. We proposed a scheme in which a reduced cobamide on the enzyme accepted a methyl group from 5-MeTHF to form a methyl-B₁₂ holoenzyme in a reaction that required SAM. However, the data suggested that the transfer of the methyl group from exogenous methyl-B₁₂ appeared to involve a different site on the enzyme. Dickerman also carried out studies on the formation of alkyl cobamide derivatives of the enzyme and their light reactivity (26–28), while Nat Brot, a postdoctoral fellow who had also been a classmate of mine at CCNY, studied the role of different cobamide derivatives on the activity of the enzyme, the reducing requirement, and the formation of holoenzyme (29, 30). Another postdoctoral fellow, Bob Taylor, made major contributions to our understanding of the mechanism of this reaction. Taylor purified the enzyme from *E. coli* and clarified the role of SAM as a priming agent for the enzyme, transferring the first methyl group to homocysteine, which allowed the enzyme to catalyze the subsequent transfer of a few hundred methyl groups from 5-MeTHF to homocysteine before the enzyme had to be primed again by SAM (31–34). Having substrate levels of enzyme made it possible to identify methyl-B₁₂ on the enzyme as an intermediate in the methyl transfer (33). It was also established that exogenous methyl-B₁₂ transferred its methyl group through another site on the enzyme, and this methyl transfer did not involve SAM or a reducing system (32). In recent years, elegant studies by the Matthews’ laboratory on the structure of the B₁₂-dependent transmethylase have elucidated many of the fine details of this complex reaction (35). As mentioned, the vitamin B₁₂-dependent terminal reaction in methionine synthesis, involving a methyl transfer from a folate derivative, helps to explain the known metabolic interrelationship between folic acid and vitamin B₁₂. I should also point out that a graduate student, Carolyn Whitfield, purified the *E. coli non*-B₁₂ transmethylase, which catalyzes a much less complex reaction (36).

**How Studies on Methionine Synthesis Led to Research on Protein Synthesis**

The studies on the vitamin B₁₂ coenzyme not only led to my interest in methionine biosynthesis, but, in an unexpected way, opened up new avenues of research. In the mid-1960s, thanks to the efforts of Udenfriend, the Nirenberg laboratory had moved from the National Institute of Arthritis and Metabolic Diseases at the NIH to the National Heart Institute, into space contiguous with our laboratories. It was shortly after Nirenberg had cracked the genetic code, and his laboratory was actively engaged in trying to elucidate the complete genetic code and understand how the information in the mRNA was used to make proteins. During that period, Nirenberg had attracted a large number of talented physicians and Ph.D.s, including Tom Caskey, Jorge Allende, Joel Trupin, and Dick Marshall, with whom I collaborated. They brought to my attention a report that N-formylmethionine might be involved in the initiation reaction (37). Based on our studies on methionine synthesis, it was likely that the formation of N-formylmethionine involved a transfer of the formyl group from either N⁵,10-methenyl-THF or N⁵,10-formyl-THF to a species of Met-tRNA. Our experience with folate derivatives from our methionine studies made it easy for us to prepare these folate one-carbon donors. Dickerman took on the task of purifying the transformylase from *E. coli*, with the help of Ed Steers from the Anfinsen laboratory, and showed that the formyl donor was N⁵,10-formyl-THF (38). In addition, Caskey was able to show that the *E. coli* enzyme could formylate a mammalian Met-tRNA (39), although the transformylase was not present in soluble extracts of mammalian tissues. Having the ability to make fMet-tRNA led to studies on the role of GTP in the initiation of protein synthesis. Allende was interested in seeing whether the binding of fMet-tRNA to ribosomes during the initiation reaction required GTP and whether labeled GTP could be found bound to ribosomes in a reaction mixture that also included the initiator tRNA, AUG, and a crude ribosomal wash, which contained the initiation factors required for the binding. A Millipore filter was used since ribosomes, but not the radioactive GTP, were
retained on the filter, which provided a simple and rapid assay. The binding of fMet-tRNA to ribosomes did require GTP, and there was also significant binding of GTP to the filter. However, the binding of GTP to the filter did not require ribosomes, but was dependent only on the crude ribosomal wash (40). There appeared to be a protein(s) in the ribosomal wash, presumably one of the initiation factors, that bound GTP and was retained on the Millipore filter. However, further studies demonstrated that the GTP-binding protein was one of the elongation factors, referred to as EF-Tu (41).

**The Roche Institute of Molecular Biology and Florida Atlantic University: Methionine Oxidation, Oxidative Damage, and Aging**

By the late 1960s, protein synthesis was the major research area in my laboratory and continued to be so when Sid Udenfriend, my brother, and I with about a dozen more scientists from the NIH, including Aaron Shatkin, Sid Pestka, Ron Kaback, Syd Spector, and Nat Brot, made the move to Roche in the late 1960s to establish the Roche Institute of Molecular Biology. My laboratory continued to work on the initiation, elongation, and termination steps in protein synthesis, with special emphasis on the role of GTP in the elongation step (42–50). I was fortunate that several of my colleagues from the NIH made the move to Roche, including Brot, Betty Redfield, David Miller, Robert Ertel, and John Hachmann. Ertel and Brot worked on EF-G (42, 47), and Miller did elegant studies on EF-Tu and its reaction with both GTP and GDP (42, 43). Hachmann studied EF-Ts (48, 49), and Miller showed that EF-Tu and its reaction with both GTP and GDP (42, 43). Miller, Robert Ertel, and John Hachmann. Ertel and Brot, made the move to Roche in the late 1960s to establish the Roche Institute of Molecular Biology. My laboratory continued to work on the initiation, elongation, and termination steps in protein synthesis, with special emphasis on the role of GTP in the elongation step (42–50). I was fortunate that several of my colleagues from the NIH made the move to Roche, including Brot, Betty Redfield, David Miller, Robert Ertel, and John Hachmann. Ertel and Brot worked on EF-G (42, 47), and Miller did elegant studies on EF-Tu and its reaction with both GTP and GDP (42, 43). Hachmann studied EF-Ts (48, 49), and Miller showed that this protein catalyzed a GTP/GDP exchange on EF-Tu (50). By the mid-1970s, our laboratory had worked with many of the *E. coli* translation factors, and thanks to a major effort by Hsiang-Fu Kung, we were able to obtain the DNA-directed *in vitro* synthesis of β-galactosidase in a system that was reconstituted with more than 25 highly purified factors (51).

By 1980, we also became interested in the 50 S ribosomal subunit protein L12 and its acetylated form, L7, which appeared to be involved in recognizing the various protein synthesis factors that interacted with GTP (52). During these studies, Brot showed that these proteins, which could be readily released from the 50 S ribosomal subunit, were easily oxidized and lost activity. This was due to the oxidation of methionine residues on L12 to methionine sulfoxide (53), and we were able to identify and purify an enzyme, now called methionine sulfoxide reductase A (MsrA), in *E. coli* that could reduce methionine sulfoxide in L12 and other proteins back to methionine (54). In the past 10 years at Florida Atlantic University, we have concentrated on the role of MsrA and other members of the Msr family in protecting cells against oxidative damage (55), especially with regard to age-related diseases and the aging process (56). More than 40 years after I first started studies on the vitamin B12-dependent biosynthesis of methionine, we are still, to this day, interested in methionine chemistry and biochemistry.

**Conclusion**

Life’s decisions, unlike experiments in the laboratory, which can always be repeated, are not so forgiving. Only in hindsight can one evaluate the decisions that were made, never really knowing whether the choice made was, in fact, the best one. Having said this, I am pleased to say that overall, thanks to a great deal of luck and superb mentors and colleagues, I have few, if any, regrets. Science has been good to me. There is no question in my mind that the year with Barker, in which I was involved in the discovery of the first coenzyme form of vitamin B12, was instrumental in my career. In that postdoctoral year, I grew up scientifically and realized that I could carry out an independent research program. In addition, the work on vitamin B12 led directly to the studies on methionine biosynthesis, which brought me into the protein synthesis field and to my current interest in methionine oxidation, oxidative stress, and aging.

I was fortunate that over a 40-year period, both at the NIH and the Roche Institute of Molecular Biology, I had the freedom to carry out research in scientific areas that I selected, without the need to obtain external funding, and in scientific environments that were stimulating. I was also fortunate to have superb colleagues in my group, two of whom, Betty Redfield and Nat Brot, were colleagues for more than 40 years, and Brot has recently joined our group at Florida Atlantic University, where we still work together. I apologize to the numerous other students, postdoctoral fellows, visiting scientists, and colleagues that I did not mention who made important contributions to the research programs over the past five decades. I also realize that I have not referred to the studies of a large number of investigators in the fields I have discussed since I attempted to focus primarily on the work from my laboratory. I hope they understand. Finally, in reflecting on those early years in my career, there is sadness that three of the individuals who were my mentors and had great influence on my career, Abe Mazur, Sid Udenfriend, and Horace Barker, have all passed on. I am happy to report, however, that my brother Arthur is alive and well.

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**REFERENCES**

1. Weissbach, A., and Weissbach, H. (eds) (1986) *Methods in Enzymology: Plant Molecular Biology*, Volume 118, Academic Press, New York
REFLECTIONS:

Isolation of Vitamin B₁₂ Coenzyme

2. Clark, C. T., Weissbach, H., and Udenfriend, S. (1954) 5-Hydroxytryptophan decarboxylase. Preparation and properties of the enzyme. J. Biol. Chem. 210, 139–148

3. Udenfriend, S., Weissbach, H., and Clark, C. T. (1955) The estimation of 5-hydroxytryptamine (serotonin) in biological tissues. J. Biol. Chem. 215, 337–344

4. Weissbach, H., Bogdanski, D. F., Redfield, B. G., and Udenfriend, S. (1957) Studies on the effect of vitamin B₁₂ on 5-hydroxytryptamine (serotonin) formation. J. Biol. Chem. 227, 637–624

5. Weissbach, H., Redfield, B. G., and Udenfriend, S. (1957) Soluble monoamine oxidase: its properties and actions on serotonin. J. Biol. Chem. 229, 953–963

6. Wachsman, J. T. (1956) The role of vitamin B₁₂ in nucleic acid synthesis. Enzymatic formation of holoenzyme. Proc. Natl. Acad. Sci. U. S. A. 44, 1093–1097

7. Weissbach, H., Tootey, J., and Barker, H. A. (1959) Isolation and properties of B₁₂ coenzymes containing benzimidazolylcobamide. Proc. Natl. Acad. Sci. U. S. A. 45, 521–525

8. Barker, H. A., Smith, R. D., Wilson, M. R., and Weissbach, H. (1959) The purification and properties of ß-methylaspartase. J. Biol. Chem. 234, 320–328

9. Weissbach, H., Tootey, J., and Barker, H. A. (1959) Isolation and properties of B₁₂ coenzymes containing benzimidazolylcobamide. J. Biol. Chem. 234, 320–328

10. Miller, D. L., and Weissbach, H. (1970) Interactions between the elongation factor Tu, E. coli, and the ribosome. Arch. Biochem. Biophys. 139, 141–152

11. Miller, D. L., and Weissbach, H. (1970) Studies on the purification and properties of factor Tu from E. coli. Arch. Biochem. Biophys. 139, 141–152

12. Ruan, H., Tang, X. D., Chen, M.-L., Joiner, M. A., Sun, G., Brot, N., Weissbach, H., Heinemann, S. H., Iverson, L., Wu, C.-F., and Hoshi, T. (2002) High-quality protein crystals of factor Tu from E. coli. J. Biol. Chem. 277, 882–886

13. Brot, N., Weissbach, L., Werth, J., and Weissbach, H. (1981) Enzymatic reduction of protein-bound methionine sulfoxide. J. Biol. Chem. 256, 337, 331–338

14. Ertel, R., Redfield, B., and Weissbach, H. (1968) Role of GTP in protein synthesis: interaction of GTP with soluble transfer factors from E. coli. Arch. Biochem. Biophys. 128, 331–338

15. Miller, D. L., and Weissbach, H. (1969) An interaction between the transfer factors required for protein synthesis. Arch. Biochem. Biophys. 132, 146–150

16. Miller, D. L., and Weissbach, H. (1970) Studies on the purification and properties of factor Ts from E. coli. Arch. Biochem. Biophys. 141, 26–37

17. Brot, N., Redfield, B., and Weissbach, H. (1968) Binding of GTP by soluble factors required for polypeptide synthesis. Proc. Natl. Acad. Sci. U. S. A. 59, 861–868

18. Brot, N., Redfield, B., and Weissbach, H. (1970) Studies on the reaction of the aminoacyl-tRNA-Tu-GTP complex with ribosomal subunits. Biochem. Biophys. Res. Commun. 41, 1388–1393

19. Brot, N., Spears, C., and Weissbach, H. (1969) The formation of a complex containing ribosomes, transfer factor G, and a guanosine nucleotide. Biochem. Biophys. Res. Commun. 34, 843–848

20. Weissbach, H., Miller, D. L., and Hachmann, J. (1970) Studies on the role of factor Ts in polypeptide synthesis. Arch. Biochem. Biophys. 137, 262–269

21. Brot, N., Weissbach, H., and Hachmann, J. (1971) Purification of factor Ts: studies on the formation and stability of nucleotide complexes containing transfer factor Tu. Arch. Biochem. Biophys. 147, 457–466

22. Miller, D. L., and Weissbach, H. (1970) Interactions between the elongation factors: the displacement of GDP from Tu-GDP complex by factor Ts. Biochem. Biophys. Res. Commun. 38, 1014–1022

23. Kung, H.-F., Redfield, B., Treadwell, B. V., Eskin, B., Spears, C., and Weissbach, H. (1977) DNA-directed in vitro synthesis of ß-galactosidase. Studies with purified factors. J. Biol. Chem. 252, 6889–6894

24. Brot, N., Yamashita, E., Redfield, B., and Weissbach, H. (1972) The properties of an E. coli ribosomal protein required for the function of factor G. Arch. Biochem. Biophys. 148, 148–155

25. Caldwell, P., Luk, D. C., Weissbach, H., and Brot, N. (1978) The effect of the oxidation of the methionine residues on ribosomal protein L12 on its biological activity. Proc. Natl. Acad. Sci. U. S. A. 75, 5349–5352

26. Brot, N., Weissbach, L., Werth, J., and Weissbach, H. (1981) Enzymatic reduction of protein-bound methionine sulfone. Proc. Natl. Acad. Sci. U. S. A. 78, 2155–2158

27. Weissbach, H., Resnick, L., and Brot, N. (2005) Methionine sulfide reductase: history and cellular role in protecting against oxidative damage. Biochem. Biophys. Acta 1703, 203–210

28. Huang, Y., Tang, Y. T., Chen, M.-L., Joiner, M. A., Sun, G., Brot, N., Weissbach, H., Heinemann, S. H., Iverson, L., Wu, C.-F., and Hoshi, T. (2002) High-quality life extension by the enzyme peptide methionine sulfone reductase. Proc. Natl. Acad. Sci. U. S. A. 99, 2748–2753