A Career Begins at the National Institutes of Health

Marshall W. Nirenberg is best known for his work on deciphering the genetic code by discovering the unique code words for the twenty major amino acids that make-up DNA, for which he won the Nobel Prize in Medicine or Physiology in 1968.

Nirenberg was the first government scientist to win the Nobel Prize. The National Library of Medicine and the Office of NIH History has amassed a collection of correspondence, laboratory administrative and research materials, and publications that documents Nirenberg's career as a researcher in biochemical genetics at the National Institutes of Health.

Dr. Nirenberg is featured in The Profiles in Science web site of the National Library of Medicine celebrates twentieth-century leaders in biomedical research and public health. Students appreciate the history, and share some of the excitement of early scientific discoveries in molecular biology. The National Library of Medicine is digitizing and making available over the World Wide Web a selection of the Marshall W. Nirenberg Papers, for use by educators and researchers.

In 2007, the Archives and Modern Manuscripts Program, History of Medicine Division completed a Finding Aid to the Marshall W. Nirenberg Papers, 1937-2003 (bulk 1957-1997). Individuals interested in conducting research in the Marshall W. Nirenberg Papers are invited to contact the National Library of Medicine.

The NLM digital materials and references provide the background for the series of six interviews conducted with Marshall W. Nirenberg, Ph.D., by Ruth Roy Harris, Ph.D., between September 20, 1995 and January 24, 1996.

The "Harris Interviews" took place in Nirenberg's laboratory on the campus of the National Institutes of Health (NIH) in Bethesda, Maryland. Harris also conducted several supplemental interviews, both by telephone and in person, with individuals either involved in the breaking of the genetic code or personally acquainted with Nirenberg: James Pittman, Joan Geiger, Philip Leder, Thomas Caskey, Sidney Udenfriend, and Perola Nirenberg. Interviews with Pittman and Geiger are now in the Marshall Nirenberg Collection at the National Library of Medicine (NLM). Notes from other interviews are held at the Office of NIH History.

A number of individuals and institutions worked on editing the interviews for clarity and content: Sarah Leavitt, Victoria Harden, Caroline Hannaway, Alan Schechter, Robert Balaban, and Alan Peterkofsky. Caroline Leake, Katrina Blair, and Mary Alvarez provided administrative and technical assistance. In 2008, Deborah Kraut edited and formatted the interviews to correspond to the NLM digital materials.

Each Section begins with the NLM digital summaries summaries and references. Additional references, when appropriate are added:

FROM Nirenberg, Marshall W. Marshall W. Nirenberg Papers. 1937-2003. Located in: Modern Manuscripts Collection, History of Medicine Division, National Library of Medicine, Bethesda, MD; MS C 566.
http://www.nlm.nih.gov/hmd/manuscripts/ead/nirenberg566.html

In 1957, the American Cancer Society awarded Nirenberg a two-year postdoctoral fellowship to the laboratory of DeWitt Stetten Jr. at the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD), a part of the National Institutes of Health (NIH) in Bethesda, Maryland.

He continued his work as a postdoctoral fellow of the Public Health Service's Section on Metabolic Enzymes at NIAMDD before joining the staff as a research biochemist in 1960.

***

Additional Sources: 1961 NIH Data book, prepared by the Office of the Director, NIH, located Office of NIH History. NIH Almanac
http://www.nih.gov/about/almanac/index.html
The Clinical Center 50th Anniversary
http://clinicalcenter.nih.gov/about/news/anniver50/opening.shtml

Federal expenditures for medical research had increased dramatically during this post-war period. By the summer of 1957, when Dr. Marshall Nirenberg began his career at the National Institutes of Health (NIH) as a post-doctoral fellowship, the agency, then a part of DHEW, was in the midst of a tremendous surge in funding appropriations. In 1947, the agency had received $8.4M and in the next decade would increase to $125.2M. NIH would receive a 10 percent increase in funding in 1958 and rise to nearly $285M by 1960. The dramatic increase in funding was directed to the extramural program, which by 1957 was nearly 138,212,000. The intramural component of NIH received $44,795,000, of which $34,142,000 was allocated for direct research.

NIH employed 8,000 full-time employees; Nirenberg would join the approximately 2,000 were classified as professional and scientific researchers.

In 1957, NIH consisted of seven institutes: National Cancer Institute.,National Heart Institute, National Institute of Mental Health, National Institute of Neurology, Diseases and Blindness, National Institute of Allergy and Infectious Diseases, National Institute of Dental Research, and the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD). In 1956, the Army Library had been renamed the National Library of Medicine and would move to a new building at the south end of the NIH campus, a Federal reservation.

Dr. Nirenberg would work in the massive red brick building called the “Clinical Center.’’ In November 1948, construction had begun on the 14-story research

hospital with 500 research beds surrounded by twice that number of scientific laboratories. The opening of the NIH Clinical Center on July 2, 1953, "was the culmination of the NIH's transformation from a small federal agency into the powerhouse that has since propelled a large part of all biomedical research in this country." Along one corridor and in the wings of the huge red brick building were laboratories; along the south corridor were patient rooms and a hospital. The hospital occupied less than half the space of the Clinical Center. The idea was to bring both basic and clinical science to the patient's bedside. The Clinical Center's innovative physical—and philosophical—structure encouraged informal interactions in the corridors between clinicians and basic scientists. The physical set-up of the Clinical Center encouraged a cross-fertilization of ideas.

When Nirenberg began to retrain himself in the field of molecular genetics, he enrolled in courses given by the Foundation for Advanced Education In the Sciences (FAES). The FAES graduate school offered courses taught by NIH scientists.

1995-1996 Harris Interviews

Ruth Harris (RH): ***How did you begin your career at NIH?***

Marshall Nirenberg (MN): I had been offered a job as an assistant professor **WHERE?** without a postdoc, which I turned down because I thought I needed more training. Things were easier then for starting Ph.D.s. than they are now.

I was awarded a postdoctoral fellowship from the American Cancer Society. I was a Minnie Kukla Fellow in cancer research. That fellowship paid the stupendous amount of something like $3,000 or $3,500 a year. But that was certainly enough to live on at that time, and it allowed me to do whatever I wanted. I had this fellowship between 1957 and 1959.

Dr. Hogg suggested that I use the fellowship to take a position with Dr. DeWitt Stetten, Jr., also known as "Hans," at the National Institutes of Health (NIH). I had met Dr. Stetten at a meeting of the Federation of American Societies for Experimental Biology (FASEB) in Atlantic City when I was a graduate student, and we had a cup of coffee together. So, because Hogg suggested it, I wrote to Dr. Stetten and he accepted me as a postdoctoral fellow.[1]as

RH: Can you tell me a little about your arrival at NIH?

I first drove ***from Michigan to Florida*** to see my parents, and then I drove from Florida to Bethesda. When I came here, I came about 9:00 o'clock at night and I wanted to see what the NIH looked like, so I drove on the grounds and I saw Building 10 (the Clinical Center). I saw all the lights were on, and I thought to myself, "Wow." I said, "This is the place where I belong." I thought that all the investigators were working at night. What I didn't know is that they leave the lights on for the cleaning people. But I thought that that was a good sign, that this was the real McCoy.

When I first arrived in 1957, Hans Stetten was away for the summer. I was surprised, but every summer he and his wife went to Wood's Hole[2] and apparently he forgot that I was coming. Yale Topper, who was Stetten's lieutenant in the lab, wondered whether I would like to work with somebody else in the laboratory at the time, but I welcomed the opportunity to do my own thing.[3] There are pros and cons to doing that, but I thought it was highly advantageous. I wanted to do my own thing. It was quite unusual.

But, since Stetten was away for the summer, I continued the work that I had been doing as a graduate student for my doctoral thesis. I changed some of the objectives, but it was based on that work. I had problems identified that I wanted to work on. I knew how to do it. It is unusual for somebody to do that, to do basically independent work as a postdoctoral fellow. But I enjoyed the opportunity to do that. Hans Stetten was extremely generous and good about allowing me to do this.

Hans Stetten was the director of intramural research of the National Institute of Arthritis and Metabolic Diseases, and once he got back to town, he was occupied most of the day with administrative matters.[4] He still had a laboratory in the institute, but he wasn't active in research directly at the time. But every day he made the rounds. He came in and would see everybody. I liked him very much as a person, and I talked to him quite a bit.

I kept working every day, but my fellowship stipend didn't come. Finally, several months after I came to NIH, I ran out of money. I went to a loan office in downtown Bethesda. It was a terrible experience. They made me fill out all sorts of papers and I felt like a deadbeat. So, when he stopped in, I told Stetten about this and he immediately straightened out the fellowship.

At one time, later on during my postdoctoral fellowship, Stetten suggested a problem for me to do which had to do with gout. I don't even remember the exact problem he posed, but it had to do with rats in activity cages and feeding them different diets. For about a

month or so I worked on it, but then nothing came of it. The answers that we were looking for were not found. The evidence suggested that the problem was just not working, and so I dropped it. I worked with him for a couple of months, but I wasn't very enthusiastic about that problem—not at all.

Stetten was a wonderful person—a wise, intelligent, literate individual, and extremely articulate. He always could say the right thing at the right time. He had a wonderful sense of humor. Both he and his wife were extremely kind to me. I liked both of them very much. They had parties and invited lots of people to their house, including visitors to the NIH. I met lots of people there. His wife, Marjorie Stetten, worked in the lab just across the corridor. She was a wonderful woman, very nice. I enjoyed talking with her. Actually, I had more of an opportunity to talk to her than I did to Hans because she was in the lab all the time.[5]

RH: And you soon moved on from your dissertation work? Did your studies at Michigan prepare you for your next work?

MN: My graduate work was much different from what I ended up doing later. The education I got really didn't prepare me for my subsequent work. I must say that the research that I did at Michigan, the techniques that I used for my research, were older techniques. They were not at the cutting edge of biochemistry at the time. I was measuring anaerobic sugar metabolism in a Warburg apparatus.[6] After I left the University of Michigan, I never used a Warburg apparatus ever again in any experiment.

So I only used some of the techniques later on. But I learned a little enzymology there. And the cutting edge was enzymology.

So, I was totally unprepared for the work that I did later. As a matter of fact, I have found that to be true in almost everything I have done for my entire professional life—that I am always working in an area that I don't know anything about, that I am a student basically, and that I have to learn the field as I am working in it. Later in my career, when I switched from the genetic code into neurobiology, I didn't know the first thing about neurobiology, and, again, I really started from the beginning.

It ***[NIH]*** is a very entrepreneurial atmosphere. You can do anything you want to do in research and work on any problem that you decide is a truly worthy, important problem to work on. You are competing with all other people who are working in that area then.

First, I picked the area to work in, which was regulation of gene expression. In molecular biology, this was of great interest. Some of the best people in the world were working on this problem. I asked myself, how can one person, working alone, who has never worked in the field before, make a contribution? How could such a researcher be compared with all of the really bright people who are very experienced, who have big labs, and who are working in this field? As I said, it was one of the most exciting and most competitive fields in biochemistry at the time.

But one of the postdocs in Stetten's lab, [John] Jack Bryant, had just come back from a postdoctoral fellowship in Germany where he worked with a very famous German biochemist, Feodor Lynen, in Munich. Jack told me that he asked Lynen, "If you were a young man, just starting out in biochemistry, how would you pick a problem?" Lynen told Jack that he would pick a problem that interested him, that he was really excited about, and that other people were interested in. He wouldn't worry that much about competition from other people because even if somebody publishes results before you, you will do it in a different way, and it will be publishable. So that is the philosophy that I used.[7] ***Years later***, I met Lynen and I told him about ***our talk***, and I said that he had influenced my work. When he heard the story, he didn't think very much of his statement of how to pick a problem. He thought it would be dangerous. I also would never recommend to a young postdoctoral fellow who is working for me to pick a problem like this. It is too dangerous, especially since times have changed so much. Now it is so much more difficult to get a position than it was then.

An Independent Investigator at NIH

MN: Usually at the end of a postdoctoral fellowship a person is supposed to find a position elsewhere. A postdoctoral position is almost always a transient position, a training period, and a person goes elsewhere afterwards. Actually, I was offered a few jobs which I didn't take. I think it was around that time that Jim Pittman referred me for a position. I can tell you what he told me about that. Every now and then he brings it up to

me that he tried to get me a position at the University of Alabama, but the chairman of the department supposedly wouldn't consider hiring me.

But, during my postdoctoral fellowship in Stetten's lab, I had met Gordon Tomkins and I decided to stay at the NIH and work with him.

We had a lunchtime seminar, a "journal club," in which we discussed the recent literature that was published. Through this, I got to know Gordon Tomkins, who was a member of the seminar. He was extraordinarily bright. He was a young M.D., and it was clear that he was brilliant. His associations were so unusual and so original that usually what he said was very funny. He had a marvelous personality. He was a Renaissance man. He was not only brilliant in science, but he also could have been a professional jazz musician. And, he was a wonderful comic. He was as good as (the comedian) Robin Williams —none of it rehearsed and all spontaneous, yet directed. It was kind of a stream of consciousness. That is the way he was all the time. It was quite clear that he was an exceptional individual. He had become the head of a section, and I got to know him in these lunch club seminars which were held two or three times a week.[8]

I think that near the end of my two post doc years, probably 1959 to 1960, Gordon offered me a position as an independent investigator in his section. He had just become a section chief. I very gratefully accepted because I thought that his section would provide a wonderful atmosphere and environment. After I had accepted his offer, he told me that there was some difficulty in getting the position at that time. He wondered if I would

accept a fellowship from the Public Health Service for the first year and of course I accepted. That was perfectly okay. During the first year of my work in Gordon's lab, I was classified as a Public Health Service postdoctoral fellow. But I was really an independent investigator.

I wanted to do something completely different from what I had done before. I felt competent and ready to try to discover. So I cast around for good fields to work on—first to pick the field, then to select the specific problem in the field. I was interested in both molecular genetics, understanding how genes were regulated, as well as the nervous system, neurobiology. But, I thought that neurobiology was too primitive at that time for me to be able to make much headway. So, I totally changed my area of research, and plunged into an area that I had absolutely no experience in, when I started to work as an independent investigator. That is the last thing in the world that one should do now.

I never had a course in nucleic acids: it was an act of faith to jump into these fields. That was probably the most important decision that I made, because after you take a postdoctoral fellowship and after you take a first job, a position, you are supposed to prove yourself and do it fast, too, and be productive. That is what a postdoctoral fellow wants to do. He wants to get all of the knowledge that will fit him for getting a position in a university or some department.

I started from zero. That was what I did on my first job. You have to learn the field first. I changed fields, and it was a very dangerous thing to do. To switch fields at the

beginning of your first job is the wrong way to go. I understood this very well, and I was worried about it. But I didn't really care. I wasn't trying to make a living. All I wanted to do was to explore and discover. Those were the major factors that drove me, and this was the time to do it. I just wanted to play with the “big boys,” with the best people in the field.

I wanted to set up a cell-free protein synthesizing system and get the cell-free synthesis of an enzyme and then determine how gene expression was regulated. I thought that my best bet was protein synthesis because at the rate that the mechanism of protein synthesis was being unraveled, by the time I was ready to do it, it [the mechanism] probably would be solved. Then, I could use the techniques and the tools that people had found, unraveled, and turned up. I could apply the tools to the problem of how genes are regulated at the molecular level, the biochemical level. That is basically an enzymological problem.

The field I went into was one of the hottest fields in biochemistry: protein synthesis. At first, Gordon put me in the instrument room because there was no other space in his section. I had a bench, one half of the room actually, in the instrument room, but the noise of the ultracentrifuge that would be running all the time drove me crazy, so we finally got the ultracentrifuge moved out. Later on another person came in that room and worked on another bench, so then it became a lab instead of an instrument room.

Working in Gordon’s laboratory was a marvelous opportunity for me. I have never found anybody else that I could talk to in my entire life who really understood me. We weren't

working together. He was working on his own project, but he knew everything about my experiments. He knew the literature, he knew the details, and he was very much interested in what I was doing. At lunchtime, we would talk about the results of my experiments and about the exciting results that had just been published by others. That was a wonderful experience. I really enjoyed it.

I think that the opportunity that Gordon offered me as an investigator at the NIH was absolutely superb. Gordon was a live wire. He knew everything that was happening. He was probably the most articulate person I have ever met in my life. His detailed knowledge of basic science was tremendous. He had a remarkable ability to synthesize information from different fields, be it physical chemistry or genetics. What he basically did best was to talk to people. I found it wonderful to be able to talk to him because, even though he wasn't working on what I was, he followed every experiment, and he knew the details of what I was doing and what other people had done, as well.

What he gave me was his time, the most valuable thing that he had, basically, and that is a rare thing. He would come in around lunchtime. I can see him slouching up against the refrigerator with a sandwich in one hand, and we would talk. We developed something like shorthand in talking so that he would say something and I would know what he was going to say. Before he could finish it, I would cut it off and give him an answer. He also would know what I was going to say and would cut me off before I finished. It was a rapid-fire talk. He would pin you to the wall if you said something illogical or wrong. Out of all this exchange, ideas came and flowed and were batted around. At night what I do

is go home and think about the work and write down notes and get ideas. To be able to bat it back and forth with somebody else is just wonderful. I didn't realize how important it was until I didn't have it anymore.

Just a few months before I went into a protein synthesis problem, as a matter of fact, I heard Fritz Lipmann at a seminar. [9] This was when I was still in Stetten's laboratory. Somebody from the laboratory just around the corner on the same floor came and told me to slip into the noon seminar. Stetten's laboratory had a seminar every day at noon where people ate lunch, and Lipmann was going to give a seminar.

Lipmann's name probably doesn't mean much to you. He was a hero of mine. He was a giant, the best biochemist of his day, and did many, many things. He had come down to the NIH to work with Leon Heppel for a day or so to find out how to make an enzyme. Heppel was a real authority in nucleic acid biochemistry, and this was a nuclease of some kind that Heppel would teach him how to make.[10]

His work concerned the characterizing of an amino acid activating enzyme, that is, an enzyme that is involved in the way proteins are made. These are ribonucleic acid (RNA) adaptors; for each amino acid, there's a small RNA, and the amino acid, the appropriate species of amino acid, is covalently attached to the RNA. This reaction is catalyzed by an amino acid activating enzyme. Just to show you what the feeling was like at the time: it was known then that there were two classes of RNA—ribosomal RNA and small soluble RNA of unknown function, but that was it. Lipmann's work was the discovery of the first

amino acid activating enzyme that catalyzes the synthesis of an intermediate protein synthesis. This whole class of intermediates wasn't known at the time.

As I said, Lipmann was a tremendous figure. In the early 1940s, he was the one who came up with the idea that energy can be stored in phosphate bonds. He was a Nobel Prize winner and, as I said, the best biochemist of the day, of his generation. He was a very gentle individual with a hesitant manner. Lipmann had agreed—they had really badgered him—to tell the people what he was working on, so this was his first report. This was before it was published. Actually, this was the first time I heard about his work.

I remember that he presented this discovery in that seminar, and I was appalled because that particular seminar group was a very tough group. They asked for the controls. “Did you do this control? Did you do that control?” The answer was “no” in all cases because this was a very early stage in the work. Lipmann didn't come prepared to give a seminar. They talked him into giving a seminar, so it was unrealistic for anybody to expect these controls to have been done at the time. The evidence that he had was enough to convince me, but it sure wasn't enough to satisfy others. I never saw anything like it. He was virtually crucified. Of course, he was correct in everything that he said. That was a remarkable seminar.

Transfer RNA was not known, or had just been discovered, at that time, and the amount of cell-free protein synthesis that people had been able to get was extremely tiny. You

would look at radioactive amino acids being incorporated into protein, and you would record a small number of counts going into protein. That was the extent of it. It wasn't known how the process occurred.

Many years later, I met Alexander Dounce. He got his Ph.D. in the 1930s with James Sumner, who was ***also*** a giant in biochemistry at the time. Sumner won the Nobel Prize for showing for the first time that an enzyme is a protein.[11] He crystallized urease, the enzyme, and characterized it and for the first time showed it was a protein. Dounce said that during his final doctoral exam when his doctoral committee got together to ask him questions after he had finished his thesis research, his mentor, Sumner, asked him the question, "How do proteins synthesize other proteins?" He said that question remained in his mind ever since then. So in the early 1950s—by then he was chairman of the department of biochemistry, I think, at Rochester—he wrote a beautiful speculative review on how proteins were synthesized. I didn't know anything about this. I didn't read it until after the code breaking, or towards the end of the code work, ~~in the 1960—this was when I first found out about it.~~

In the review he was far ahead of everybody else in that he predicted that amino acids were activated by RNA, or that if they were activated, that ATP would be required for the activation. He predicted that the code would be a triplet code, and that, as I recall, RNA was the template for protein synthesis. But he buried this article in the proceedings of an Oak Ridge symposium that nobody read. Aside from that, it was detailed in terms of biochemical mechanisms and protein synthesis. By and large, the ideas were good,

although he was wrong on minutiae. I was amazed when I finally read it. But there was really very little known about protein synthesis at the time.

RH: How did you go about getting trained in your new field?

MN: I jumped in at the beginning and just learned. I had estimated it would take me two years to prepare myself, and that is exactly how long it took. It was a very accurate estimate. But after about a year and a half of working by myself, what I did was to systematically vary conditions looking for an increase in enzyme activity. I learned how to make the preps. I learned how to make cell-free protein synthesizing systems. I set up and worked out an assay for penicillinase. I made mutants of penicillinase, of the *B. cereus* cysteine mutants.

RH: ***How did you learn the literature of an entirely new field?*** **A**re you a speed reader?

MN Actually, I took a course in the District of Columbia, Evelyn Woods' Speed Reading Course, and I got the Heart Institute to pay for this. I thought that this was something that was worthwhile, so both Perola and I took it together on Saturday mornings. We got up early and went down to the District. I think that speed reading is a real skill. It is a new kind of skill to develop because I think you have to establish new neural pathways in order to comprehend the material. They give you these books designed for about 12-year-old children to read with little content. So you can read them

and maybe get up to 3,000, 5,000, or even 10,000 words per minute. I was just at the point where I was breaking about 3,000 when we stopped going. Perola said the course gave her headaches, so because of that we stopped going. I didn't do it long enough to solidify the gains that perhaps I had made. I don't think I could read the *Journal of Biological Chemistry* at that speed. You have to look at things much more carefully. But for scanning the newspaper and for scanning journals sometimes, I think it can be a useful skill to have. It takes training, and practice, to acquire it. Actually, I read pretty fast. I read about 700 or 800 words a minute, which is not a speed reader, but it is fast reading.

I started going to professional meetings and learning more about biochemistry. It was very thrilling because you could hear some of the hottest work that was going on in biochemistry at the time. The people who were doing the work would be presenting or discussing the research, so some of the sessions were exciting sessions. The atmosphere was virtually electrically charged. I remember hearing Earl Stadtman, for example, present his work on the characterization of the compound that eventually came to be known as acetyl-CoA (acetyl-coenzyme A). [12] Fritz Lipmann was there as were all the other people who were working on the problem at the time. It was a very exciting central problem in biochemistry then.

RH: Was taking science courses also part of your training?

MN: Yes. I took a lot of courses when I first came to the NIH. I took a number of courses that I thought were really excellent. The number of courses offered in the NIH night school (the Foundation for Advanced Education in the Sciences, Inc.) is tremendous, and there is a wide variety of courses offered.[13] They now include many courses in languages and even the arts. It is completely open to people in and out of NIH. I think the NIH is wonderful in that respect because it is almost like a college campus. I took advantage of these courses.

It is interesting that at a research institution such as this, compared to a university, the major advantage is that you don't have to teach. But lots of people want to teach, and so they spontaneously organize courses and teach in the evening to anyone who wishes to take them. There are some wonderful lecturers here. I took lots of courses, and I found them to be extremely interesting. Some of them were outstanding, actually.

I took a course on molecular biology—on regulation of gene expression in bacteria. It was a terrific course. I didn't learn molecular biology when I was a graduate student. There were no courses in molecular biology that I recall that were given in the biochemistry department at that time. I took a wonderful course in the evening here at the NIH that was given by [Robert] Bob DeMars. He was a terrific lecturer. I took it in the late 1950s while I was a postdoc. I don't remember if it was before, or concurrently when, I worked with Bill Jakoby. But that course, given in a time when what was known about enzyme induction and repression in bacteria was, I think, the most exciting area of research at that time. It was one of the best courses I have ever taken in my life.[14]

I also took an excellent course that was given by Earl Stadtman on biochemical pathways of metabolism. I don't remember exactly what he called it, but Earl Stadtman is still working here at the NIH. He is a superb enzymologist and lecturer. It was a really interesting course that went into the metabolism of bacteria that could live on unusual substances, such as hydrogen sulfide, methane-producing bacteria, and things of this sort. It was mostly microbial metabolism. He went through so much material that it was all the students could do to keep up. Each lecture lasted two hours, I remember. We would be writing and taking notes as fast as we possibly could.

I took a course in enzyme mechanisms taught by [Louis] Lou Cohen here at the NIH. [15] I took a number of additional courses as well. Taking the courses gave me information in some cases in fields that I knew very little about. I took a course in enzyme mechanisms, basically, mechanistic organic chemistry. I took a course in crystallography. I didn't get too much out of that course, but it was my first real experience with crystallography. I think those courses were excellent. I was invited to give lectures in courses on some occasions, and I did, but I never took responsibility for an entire course, usually because it took a lot of time to prepare the lectures.

RH: You also took courses at Cold Spring Harbor Laboratory[16], didn't you?

MN: I think it was in the summer, so it must have been right at the beginning of moving to Gordon Tompkin's lab. I enjoyed it because it was total immersion for about a

month. Cold Spring Harbor Laboratory is famous for its summer courses. They give excellent courses. It is a very nice, rustic place, relatively primitive, right on the bay, very wooded and very pretty. We worked from 8:30 in the morning until midnight every day, seven days a week. There was a table piled high with references covering the various experiments and interesting things that you could read at any time and during the day you did experiments. I thought it was the best course I have ever taken in my life because it was so intensive.

The course was on bacterial genetics and we learned how to make mutants. Gordon took the course also. One of the instructors was [Philip Emil] Hartman, I think. There were a number of instructors.[17] One of the first things I did was to make cysteine mutants of *Bacillus cereus,* which I then used in that work. **I started to prepare DNA to use as a template RNA to use as templates,** and I had to work out all the techniques for making the RNA and DNA preparations and the cell-free extracts of bacteria. I also worked out a very sensitive assay for penicillinase.

I decided to work on penicillinase specifically to find out how the penicillinase gene was regulated. Penicillinase is the enzyme that inactivates penicillin. There were two microbial systems that were being used to study the mechanism of gene regulation. One was turning on or off the gene for ß-galactosidase. The other was turning on or off the gene for penicillinase, and that was done in *B. cereus* instead of *E. coli*. Martin Pollock in England was responsible for working out many of the details of the penicillinase system.[18] He had been studying the induction of penicillin in a bacterium called *B.*

cereus. He had many mutants—inducible mutants, non-inducible mutants, non-repressible mutants, various kinds. I wrote to him [in 1959] and asked him for mutants.[19] I decided to try to get the cell-free synthesis of this enzyme. Those were the conditions which would allow the synthesis of penicillinase to proceed. This was my plan of action. I had to devise a highly sensitive assay for penicillinase as part of that project, and one of the attractive features of the system was that penicillinase is a very small protein and it contains nineteen of the 20 amino acids. It lacks cysteine, and I thought that if I ran the cell-free reaction in the absence of cysteine, that maybe the only protein that could be synthesized would be penicillinase because very few proteins lacked cysteine. So I thought that would reduce background protein synthesis.

I thought at first that I would use enzyme activity as the assay, and initially I did this. I had an opportunity to publish the assay for penicillinase, but I didn't want to take the time to write up the paper. While I was doing this work, I also discovered that another amino acid, leucine, was incorporated into protein N-terminal positions, really the beginning of the protein. I think it was N-terminal position in proteins, which was an unexpected reaction. This would have made a good paper also, but I didn't want to take the two or three weeks that it would take to clean up the work and to redo the experiments for publication.

At this time, I had the opportunity of publishing two papers: one paper describing a sensitive colorimetric assay that I worked out for penicillinase. The other paper was on the incorporation of leucine into an unusual position in protein in cell-free extracts of *E.*

coli. On both of them I could have written papers. But, I decided that my time was too valuable to waste to write a paper on what I thought were dead-end research projects, that weren't really important. I decided it was more important for me to continue to explore.

One thing I will be forever grateful to Gordon Tomkins for is that he made me feel secure, and so I didn't publish. I published nothing in this period of almost two years. Toward the end of the two-year period we got out our first publication. I never felt threatened or bothered by my lack of publications. Now the climate is so totally different that you just have to publish. As I have said, my feeling was that it was a waste of my time to publish those two papers. One was a blind alley and the other wasn't important enough even though it was a new phenomenon. About five years later an excellent investigator in cell-free protein synthesis found the same incorporation of leucine that I found in *E. coli* and wrote and published a paper on it. So it was very publishable stuff.

I thought the whole theme of my work in biochemistry was that I wanted to explore and discover. I didn't care about being successful. I didn't care about getting a job. I didn't care about any of the conventional things. I thought that this was an excellent time to do this. I felt that once I had gotten my feet wet in the field, I ***would feel*** competent in enzymology and in biochemistry.

I thought I needed training in enzymology because I had never done much enzymology. Enzymology is a basic tool of biochemistry. It can be used in any way. It can be used in molecular biology, in genetics. It is very useful, and it is applicable to all of these fields.

It is one of the very basic tools that is used in molecular biology, for example. I needed training, and [William] Bill Jakoby was a good enzymologist. [20] He was just around the corner from me in Building 10. When I worked with Bill Jakoby, I wanted to learn enzymology, induction, and how to purify enzymes, all very useful things. I liked working with Bill. He is a very nice guy. Bill showed me how to get enrichment cultures of bacteria that would metabolize various compounds. We purified three or four enzymes and worked out a pathway of metabolism.

Bill Jakoby and I had a noxious compound, γ-butyrolactone, for which we wanted to find the enzymes that metabolized this compound. You scoop up dirt from different places and look for bacteria in dirt that will grow on a Petri dish that contains γ-butyrolactone as the sole carbon source. Then you clone the bacterium that is able to metabolize this compound. I tried, but I couldn't find a bacterium. I went all over Bethesda taking samples of dirt. So, I called my father and asked him to collect some samples of dirt and mud from the swamps and streams and lakes around Orlando. He sent me a whole collection of black, rich soil. Sure enough, I found a bacterium in one of the samples that he had sent me that would metabolize γ- butyrolactone, and we isolated a pathway of enzymes that was able to metabolize it.

Our work on α-hydroxybutyric acid turned into a very interesting problem. When we purified the enzymes, we found that there were two different enzymes, and we separated them from one another. One used DPN and the other used TPN and they metabolized the same α-hydroxybutyric acid substrate. So I wondered at the time whether they had shared

information, whether one gene encoded two separate but quite related enzymes or whether each enzyme was encoded by a separate gene. That was interesting.

Our next paper was on the sites of attachment in reaction of aldehyde dehydrogenase. The constraints in the determination of active center topography came out of a discussion I had with Bill Jakoby. According to the discussion, binding of a substrate to an enzyme would alter the conformation of the enzyme. Thus, it was not possible to study the original shape of the enzyme because it would be deformed by interaction with the substrate. I am not sure if I am saying that correctly. But it was just an idea that we published, and we flipped a coin to see who would be the first author of this paper. It was very interesting doing this work with Bill Jakoby. I learned a lot.[21]

My training in enzymology was very valuable for my wanting to work on finding out how cell-free proteins really were synthesized. The hottest thing in molecular biology at the time was being done in bacteria, in intact cells. Nobody knew how the genes were regulated. They inferred how they were regulated by very fancy experiments that were done with bacteria. Every month new papers would come out. It was an exciting, fast-moving field of research. We called it the latest Parisian fashion because the Pasteur Institute was the center of this research, and there they did some extremely ingenious, extremely interesting experiments on gene regulation, ß-galactosidase regulation.

The only thing wrong with having a good biochemical system for studying the mechanisms of gene regulation was that protein synthesis was one of the hottest fields in

biochemistry at the time. How could somebody who knew nothing about protein synthesis, possibly compete with large groups of such people? By “large groups” I mean ten people, five people—in that range—who were the best, most experienced in that field.

That bothered me because it was the worst way—the wrong way—to begin when you start a job. I knew it would take two years to get my work set up, to learn the field, to get the systems going, to just set up to be ready, to be prepared for the challenge. You are supposed to hit the ground running and have everything all set up and know exactly what you want to do. It should be productive because, in a sense, you have to prove yourself. You have to show what you are capable of and that involves productivity and publications.

The way I was doing it was absolutely backward, and I fully realized how dangerous it was. But I liked the field and I thought: “Maybe I won't find what I'm looking for, but I'll find something that will be publishable and interesting. If I publish it, a lot of people will be interested in it because it's in a hot field.”

In retrospect, that was the biggest factor—that was the key decision. For everything else that flowed from the system I had to have the guts to go ahead and do it with the knowledge that I was just starting from scratch.

I would not advise anybody to do this that way in today's climate of short money and the extreme difficulty of getting grants. On the average only one out of ten of the grants approved today for funding is actually funded.

Everything else flowed from that.

It was the biggest decision that I made, and it set the stage for everything else.

The footnotes that appear below will be placed in a separate digital file for linkage to this file.

[1] DeWitt Stetten, Jr. (1909-1990) earned his M.D. and Ph.D. in biochemistry from Columbia University where he later taught biochemistry. He joined the NIH in 1954 and until 1962 he served as director of the Intramural Research Program of the National Institute of Arthritis and Metabolic Diseases and also as chief of that institute's Section on Intermediary Metabolism. In 1962 he became the first dean of the Rutgers Medical School. Dr. Stetten returned to the NIH in 1970 as director of the National Institute of General Medical Sciences. In 1974 he was appointed deputy director for science of the NIH.

[2] http://www.whoi.edu/page.do?pid=7016 need identify what Wood's Hole is

[3] Yale Topper (1916-1995) earned a Ph.D. in chemistry from Harvard University in 1947. After conducting research at Harvard Medical School and the Massachusetts General Hospital, he served as an associate at the Public Health Research Institute of the City of New York from 1948 to 1953. He joined the National Institute of Arthritis and Metabolic Diseases in 1954. In 1963 he became chief of that institute's Section on Intermediary Metabolism in the Laboratory of Biological Modeling. He was known for his research on the influence of hormones on the mammary gland's protection of milk proteins.

[4] The National Institute of Arthritis and Metabolic Diseases (NIAMD) was founded in 1950. It was the predecessor to the current National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS).

[5] Marjorie Stetten (1915-1983) received a Ph.D. in biochemistry at Columbia University in 1944 and worked for the Public Health Research Institute in New York from 1948 to 1954. She then joined the National Institute of Arthritis and Metabolic Diseases.

[6] http://history.nih.gov/exhibits/stadtman/lab.htm wonderful – in ONH!

[7] John Bryant (1925-) was a research fellow at the NIH between 1956 and 1959. Feodor Lynen (1911-1979), a German biochemist, shared the 1964 Nobel Prize in Medicine or Physiology with Konrad Emil Bloch for his work on cholesterol.

[8] Gordon M. Tomkins (1926-1975) earned an M.D. from Harvard Medical School in 1949 and a Ph.D. in 1953 from the University of California, Berkeley. From 1962 to 1969 he was chief of the Laboratory of Molecular Biology, National Institute of Arthritis and Metabolic Diseases, NIH.

[9] Fritz Lipmann (1899-1986) shared the Nobel Prize in Physiology or Medicine in 1953 with H. A. Krebs for the discovery of coenzyme A.

[10] Leon Heppel (1912-) received a Ph.D. from the University of California, Berkeley, in 1937 and an M.D. in 1941 from the University of Rochester. He served as a biochemist for the Public Health Service from 1942 to 1967, at which time he became a professor of biochemistry at Cornell University. In 1960 he received the Hillenbrand Award from the American Chemical Society.

[11] Alexander Dounce (1920-) received a Ph.D. in organic chemistry from Cornell University. He became a professor at the University of Rochester School of Medicine and Dentistry. James B. Sumner (1887-1955) earned a Ph.D. in chemistry at Harvard University in 1914. He proved that an enzyme was a protein by crystallizing an enzyme, work that won him a share of the Nobel Prize in Chemistry in 1946. He spent his academic career at Cornell University.

[12] Earl Reece Stadtman (1919-), received his Ph.D. at the University of California, Berkeley. He joined the National Heart Institute in 1950 and in1958 became chief of the Section on Enzymes. From 1962 to 1994 he served as chief of the Laboratory of Biochemistry. He continued after 1994 in his position as chief of the Section on Enzymes.

[13] The courses were part of a graduate evening program begun in 1954 at the NIH to provide supplemental laboratory training for the NIH scientific and medical communities. The program later became part of the Foundation for Advanced Education in the Sciences (FAES), which was incorporated by 1959 as a nonprofit organization to encourage scientific research and education and to ease communication among scientists.

[14] Robert Ivan DeMars (1928-) received a Ph.D. in bacteriology at the University of Illinois in 1953. He worked as a microbiologist at the NIH from 1956 to 1959. In 1959 he joined the Department of Medical Genetics at the University of Wisconsin, Madison, where he became a full professor in 1969.

[15] Louis A. Cohen (1926-1996) earned his Ph.D. in 1952 at the Massachusetts Institute of Technology and came to the NIH in 1954. He served as dean of the FAES graduate school and was an authority on amino acid and peptide chemistry and enzyme mechanisms.
[16] http://www.cshl.edu/

[17] Probably Philip Emil Hartman (1926-2003), who received a Ph.D. in 1949 at the University of Pennsylvania. An NIH fellow at the Carnegie Institute from 1954 to 1955, he later joined the Johns Hopkins University faculty where he became the William B. Gill professor of biology.
[18] Martin Rivers Pollock (1914-1991) concentrated on enzyme induction in bacteria, particularly on the development of bacterial resistance to antibiotics especially involving penicillinase. In 1962 the Royal Society elected him to its fellowship for that contribution.
[19] Letter from Nirenberg to Martin Pollock, 22 October 1959, Binder VIIA p. nineteen, MN Collection, NLM.
[20] William Jakoby (1928-) was a biochemist from 1957 to 1967 in the Laboratory of Biochemistry and Metabolism, National Institute of Arthritis and Metabolic Diseases. In 1967 he became chief of the Section on Enzymes and Cellular Biochemistry in that institute.
[21] M.N. Nirenberg and W.B. Jakoby,"Enzymatic Utilization of Ÿ- hydroxybutyric Acid," *Journal of Biological Chemistry*, 235:954-60 (1960).