## Chapter 34

# WILLIAM STEPKA

**Richmond, Virginia**

**July 28th, 1996**

**VM = Vivian Moses; WS = William Stepka; SM = Sheila Moses**

**VM:** This is a conversation with Bill Stepka in Richmond, Virginia on July 28th, 1996.

Bill, you had a rather complex path that led you to Calvin involving your early scientific career. Why don't you tell us about it?

**WS:** Well, the story starts when I was separated from the Air Force and returned to the University of Rochester where I had been doing undergraduate work before I went into the Air Force. I served in the Air Force for a little over three years. At the University of Rochester I ran into an Englishman who was in a laboratory with Bob Dout (*spelling?*) in London and he came back to visit this country and was being groomed for the chairmanship of the Biology Department at the University of Rochester. His name was F.C. Steward and he was interested in some amino acid metabolism in potato slices and we were going to do research on the metabolism of the potato slices as they healed. So, since I had been out of the academic environment for over three years, I had a lot of library research to do and so I spent a good bit of time in the library looking over the journals. I came across an article by Gordon (*Editor: this should be "Consden"*), Martin and Synge describing a new method of analysis called "paper chromatography". That really struck my fancy because it was a method that seemed very easy and simple to me in contrast to the very difficult analytical schemes that the analytical chemists had prescribed prior to that.

**VM:** Can I ask you a question — what was your own scientific background? Were you yourself a chemist?

**WS:** No. I was a major in biology in my pre-war years.

**VM:** You had been at Rochester before you went into the Air Force?

**WS:** Yes.

**VM:** How long had you spent there?

**WS:** Actually, I joined the reserves in my junior year with the promise that juniors and seniors who joined the reserves would be permitted to finish their degree. But something happened and I got called a month early. At the beginning of my senior year at the University of Rochester, they passed some sort of a resolution recognising the fact that some of the students might be leaving so they decreed that any student, any senior, who was in good standing on February 20th or some such date in February would automatically would get his degree. I got called one month earlier. All of my professors and the powers at the University of Rochester said "no, this is a mistake, just keep on going to classes. Pay no attention; we'll get this straightened out". But it didn't happen so I had to leave the University on the 20th of January and proceeded to Boca Raton, Florida, for basic training for the Army Air Force Technical Training Command.

**SM:** What year was that?

**WS:** That was in January of 1943.

**VM:** So you didn't get your degree at that time?

**WS:** No, I had to finish a semester.

**VM:** And that was the immediate reason for going back to Rochester to finish off?

**WS:** That's right. And I got back to Rochester on February 6th, 1946.

**VM:** And so in what capacity did you meet Steward?

**WS:** Well, I had been a Teaching Assistant before I left for the service and I guess they needed Teaching Assistants. It was actually David R. Goddard, who was the chairman who had hired Steward to come to Rochester, with whom I was getting my degree and who hired me as a Teaching Assistant for the balance of the semester.

**VM:** Sorry, I left you in the library looking up...

**WS:** So we got to paper chromatography, the article by Gordon (*Consden*), Martin and Synge, and then I immediately set up some equipment to test this method and, indeed, ...this was just unidimensional because what I used was a large, inverted bell jar with a microscope dish (a slide dish) for holding the paper and the solvent. Indeed, we found amino acids from the potato slices using phenol as the solvent. Then we got some collidine, which was the solvent described by Gordon (*Consden*), Martin and Synge and that didn't work. There was no separation; it was absolute disaster. Then one day — at the Medical School...at the Medical School every week there was sort of a list of seminars which was published — and I saw a seminar listed at the Medical School by a Charles E. Dent and the title of his talk was "Paper Chromatography as a Diagnostic Tool in Rare Liver and Kidney Diseases".

In the meantime the only other person I knew who had tried paper chromatography was a friend of mine, Dr. Patton at Cornell University who was an insect physiologist and he worked on...he was analysing insect blood and therefore microtechniques were of great value to him. So he tried. He also...we had been friends before I went into the service and we were in contact and we discussed our experiences with paper chromatography and both of us agreed that phenol worked fine but collidine didn't. So we were unable to get 2-dimensional chromatograms as described by Gordon (*Consden*), Martin and Synge. So I called him and invited him to come to hear Dent at the seminar. He and his students started out from Ithaca but this was in April and they got stuck in a snow storm and didn't get there until the seminar was over. Dent had these beautiful chromatograms showing nice purple spots with amino acids on the sheet so after the seminar I went up and I talked to him and told him about my experiences so he said, "Oh, you must be doing something wrong." I said, "Yes, but what is it?" He gave me some advice and I went back and I tried whatever he suggested and this went back and forth several times none of the stuff worked. And one day I was talking to him in his office and we were discussing the possibilities and then he said, "Would you mind coming with me? I have some chromatograms to take out of the cabinet." He had sort of an inside room for his chromatography room where the temperature was a little more stable. And I followed him and he was saving his — he had a large separatory funnel and he was saving the residue of the solvent in the troughs and poured it back into the separatory funnel. I looked at this and I noticed that his collidine was coloured, it was quite yellowish, and mine was clear. I mentioned this to him so he said, "bring me some of your collidine." So I brought him some of the collidine and he tested it on samples he had been getting good results with the collidine he brought with him from England and that's why he was saving it. He didn't use any American collidine. So I gave him some of the collidine that we had and he tried it and he got the same results that I did — no separation.

When we discussed that, we decided that his collidine he brought from England must be contaminated with something and that I had a purer sample than he did. The only source of collidine at that time in the United States was Riley Tar Chemical Corporation — I think they were in St. Louis — and so we wrote to them and asked them what possible contaminants there might be in a fraction called collidine and they wrote back and said there were several suggestions...and could we have samples. They wrote back and said the possibilities were quinolines and lutidines and they sent about eight samples, I think. When we lined up the samples that we got, we immediately rejected the quinolines because they were too orange. The lutidines looked about right.

**VM:** In yellow colour?

**WS:** Yes, the yellow colour. So we took the collidine that I had and kept on adding lutidine and immediately the results improved as we added but we still weren't getting the same $R_f$ values that he was getting with his British collidine. So it wasn't until we had 50% lutidine and 50% collidine that we were able to reproduce the map of amino acids that he had.

**VM:** This was using phenol in one direction and collidine/lutidine in the other?

**WS:** Yes. The disadvantage of the collidine and lutidine mixtures is that they have a very offensive smell. They are almost like pyridine. So we were using and doing some work in Steward's laboratory with these things and I immediately thought at the time, "gee what a wonderful tool this would be for photosynthesis" because $C^{14}$, you see, had recently been discovered and that there would be a great possibility of using this and using radioautography along with it.

**VM:** Can I ask you some questions now? What did you know about the state of photosynthesis research at that time?

**WS:** Very little. I didn't even know much about Calvin's work because I hadn't caught up with that literature. I didn't learn about Calvin's work until I got to Berkeley.

**VM:** You didn't even know about it?

**WS:** No. This was in '46.

**VM:** This was a thought you had quite independently of Calvin about how you could use it?

**WS:** Oh, yes, yes.

**VM:** And the second question I have: was radioautography a technique which was in use at that time?

**WS:** Chargaff had used some phosphorus-labelled compounds. He was looking at phosphorus-labelled compounds and unidimensional photographs. There was a paper published in *Science* but the pictures of the chromatograms didn't look very good, to me at any rate...separations...But the idea that you could use radioautography and expose the chromatogram to a film was certainly there.

**VM:** And the last question I have to ask is what sort of paper did you use at that time because presumably...?

**WS:** Whatman No. 1.

**VM:** Was that available in large sheets?

**WS:** Yes, it was, by special order. It was rather expensive at the time; I forget how much we paid for a sheet.

**VM:** Because if that was before general use of chromatography. I wonder why Whatman produced large sheets of the paper? What they did it for.

**WS:** Well, Gordon (*Consden*), Martin and Synge already had used the Whatman No. 1 paper.

**VM:** Oh, had they?

**WS:** Yes. That was prescribed in the original article.

**VM:** I see. So it pre-dated all of chromatography. It had been made for some other purpose.

**WS:** I don't know why they manufactured it.

**VM:** Well, it might be interesting to talk to Whatman to find out. OK.

**WS:** To find what they were doing.

**VM:** Sorry. I interrupted you when you had just realised the value of this for photosynthesis.

**WS:** The offensive odour of the collidine/lutidine mixtures made me look into some other types of solvents and I noticed that the solvents that had been published in the literature — by now people were chromatographing sugars, amino acids and other things and there were some other solvents prescribed — and there was one that was based on butanol and acetic acid. But as published, it didn't provide very good separations of amino acids and it looked as if the proportion of water was wrong. So I set up a phase diagram of butanol, acetic acid and water and did the phase diagram and I chose a spot of the three components on the diagram which provided about 12% water. I don't remember the exact formula at the moment. But anyway, that was a substitute for the collidine and gave $R_f$ values very similar to collidine and lutidine except for the basic amino acids which, of course, were affected by the acetic acid in the components. So that was a better solution.

Then while I was there at the University of Rochester, a professor from the University of California came for a visit. He had known F.C. Steward in his student days in Hoagland's laboratory and he was on a sabbatical and was sort of travelling around the country and he came to the University of Rochester. His name was Professor Roy Overstreet. He lived in a dormitory on campus and we used to have dinner together and then in the evenings he was also a connoisseur of wines and we used to test the state wines in my office and lab. He was there maybe two or three months. At the time that he was leaving he said, "You know, Bill, I've gotten to know you pretty well. There may come a time when you and F.C. Steward are not going to get along and if you have any kind of trouble, call me. I think I'll have a place for you at the University of California." And, indeed, I thought that if I stayed much longer I might be mowing the professor's lawn eventually. So I thought that the best thing might be to leave so at the end of the semester I decided to go to California and wrote him. Indeed, they had a fellowship for me and I went to Hilgard Hall.

**VM:** As a graduate student?

**WS:** As a graduate student in Berkeley.

**SM:** Which year was that?

**WS:** That was in 1947, I guess, fall of 1947. I had already ordered solvents and things for the lab. where I was going to be working. I was working with Professor Lewis Jacobson and Dr. Roy Overstreet who were both interested in ion uptake by plant roots. We were actually working on the ion uptake by plant roots and their effect on the metabolism of the roots and I was using paper chromatography to see if there were any changes in the component extracts, in the extracts. While we were doing this I kept egging both of them, Jacobson and Overstreet, to see if they could get some carbon-14 as we had an ideal situation here for analysing the early components of photosynthesis, metabolites of photosynthesis. Eventually Dr. Overstreet, who frequently ate lunch at the Faculty Club, spoke to Dr. Calvin.

I have to backtrack a little bit. In the meantime I had seen, I had heard two seminars, one given by Calvin and one given by Andy Benson, about photosynthesis and in the question period after the seminars I inquired about using paper chromatography and they had no inkling of paper chromatography as a method of separation. So Professor Overstreet eventually spoke to Dr. Calvin at the Faculty Club and arranged a meeting. So I brought along some chromatograms that we had. By then I knew the positions; I had a map of amino acids, sugars and some of the phosphorylated compounds. I brought this along and Professor Calvin was very impressed with this technique and he wanted me to take some samples that they had prepared and had been analysing and see what might happen if I chromatographed the samples. So I took them back to Hilgard Hall, took the samples, and I had great difficulty in applying the sample to the origin of the chromatogram. It looked as if the paper was waterproofed by the sample.

**VM:** What sort of samples were these that they gave you?

**WS:** These were samples that they had prepared. They were samples of *Chlorella* that had been exposed to carbon-14 in the lollipop for one minute and then they fractionated them and gave me a nice lot of clear...

**VM:** They fractionated them on ion exchange?

**WS:** Yes. I don't know what was the entire technique that they used but they had gone through ion exchange resins. At that time Dr. Calvin was a consultant to Dow Chemical Corporation and they were just developing ion exchange resins so he brought back samples and they were using them. I guess in the early days there were different degrees of cross linkages in the resins and I guess they used some of the resins that were of the lower cross linkages so that they were somewhat soluble and starting with the initial fraction because what they did when they exposed the *Chlorella* cells to carbon-14 to stop the metabolism quickly, they dumped the exposed *Chlorella* into what they called the "hemlock mixture" which was a concoction of trichloroacetic acid, hydrochloric acid and alcohol, boiling, so there was a lot of fractionation to do...see, they had to get rid of all the trichloroacetic acid and the hydrochloric acid. The result was...in trying to apply the sample to the origin

the result was that...because I had to guess how much I should put on because I had no idea what volume of cells, *Chlorella* cells this represented at the end and it was sort of by-guess-and-by-gosh but I tried to get on as much as I could.

The separations were not satisfactory at all because apparently this waterproofing material held...Most of the radioactivity stayed at the origin and a little bit of it smeared off in both directions but not much. So after several such attempts without any success, I decided there wasn't any point in running any more of these samples. We were discussing these results with Dr. Calvin and I said, "Why don't you use the simpler methods? Why don't you just kill the *Chlorella* in hot alcohol, which is easy to get rid of, and then concentrate the material and give it to me as is and let the paper do the separation instead of ion exchange resins and other chemical procedures?" So he apparently thought that was a good idea so he said, "Why don't you tell Andy how to do the experiment and then do it your way." So we did. About four o'clock in the afternoon we started the experiment and did what was called a "1-second exposure"...just virtually dumped a lollipop into hot alcohol quickly and then concentrated it very quickly and I washed the green gunk out of the *Chlorella* with alcohol, with maybe 25% alcohol or so, and took it back and ran some chromatograms, made some radioautographs a week later, looked at the radioautographs and lo and behold I counted 30-some compounds. Many of them I could identify — all the amino acids, of course, that were there I could identify right away. I should say also the organic acids I had already mapped and some of the phosphates, sugars — and brought the results to Calvin and he was absolutely amazed at what he saw. As a matter of fact, he was scheduled to give a Sigma Xi lecture that evening and so he kept the chromatogram and had a slide made. There were no labels on the spots at the time and, indeed, in the evening, when we went to hear him, he showed the slide and he said something to the effect "We have been working for 3-4 years", I think he said, "and we had discovered radioactivity". I think the only compounds they had identified until then was malic acid, which was indestructible in the "hemlock mixture", you see. And then he said, "And then we discovered paper chromatography," and showed the slide.

**VM:** Did he acknowledge how the discovery was made?

**WS:** No, no. "Then we discovered paper chromatography" and he was pointing to spots and I was sitting in the audience and he was asking me to identify the spots as he was pointing with his pointer. In the back of the room Michael Doudoroff had been there and he had received his copy of the *Journal of Biochemistry*, I think it was, where Loewer (*spelling?*) and Gardini had discovered glucose-1-6-diphosphate. It was that week that this publication came out. We didn't have our (*copy*) because that was being catalogued in the library so none of us knew about glucose-1-6-diphosphate. Doudoroff asked, "Is there any evidence of glucose-1-6-diphosphate in the...?" and Calvin took his pointer and pointed and said, "Well, we think it might be this one".

**VM:** But he made that up on the spot, did he? (*Laughter*)

**WS:** I guess so. So I turned to John Weigl who was sitting next to me and I said, "We have our work cut out for us for the next two years to catch up with the seminar".

**VM:** But you weren't formally an affiliate of Calvin's group at that time, were you?

**WS:** Not at that time, not yet. See, all the chromatography was still being done in plant nutrition, Dr. Jacobson's laboratory.

**VM:** Was he your thesis supervisor or research director at the time?

**WS:** No. Not at the time.

**VM:** Who was?

**WS:** All of my thesis committee was composed of people from Plant Nutrition and Biochemistry, all people from Hilgard Hall and the Life Science Building.

**VM:** And you were notionally working on a problem of plant nutrition?

**WS:** That's right, yes. I also identified...confirmed the presence of sucrose not only by co-chromatography but I took some invertase...I eluted a spot, a radioactive spot, treated with some invertase solution and then ran a chromatogram and, indeed, I got glucose and fructose. Now. the interesting thing was that when the radioactivity was compared between the two, one of them had more (*radio*)activity than the other (I don't remember which was which) which was, of course, some indication as to what the pathway might be. It was at that point, I think...well, quickly Calvin had cabinets made and a chromatography room set up. I think it was Al Bassham who first utilised paper chromatography to a great extent and he did a great job at analysing because he did the same thing with the sucrose and separated it and then actually broke it up atom by atom and was able to quantitate the radioactivity in each one of the six carbons for each sugar. That was a very great...By then, let's see, I had an AEC Pre-doctoral Fellowship and I guess Calvin invited me to join the lab. and that's when I moved over to the Radiation Lab. and worked at the Radiation Lab.

**VM:** Sam Aronoff remembered you coming and telling him and Vicky Lynch about paper chromatography.

**WS:** Yes, yes, yes.

**VM:** And that was before you told Calvin or afterwards do you think?

**WS:** No, yes, that was before, yes, because I was trying to...I was always trying to get my hands on some $C^{14}$ and I thought that maybe through them, since they had access to $C^{14}$ and I explained what I would like to use it for. Yes; that was true.

**VM:** So then when you came into that lab. you worked in the Old Radiation Lab., the old wooden building?

**WS:** Yes.

**VM:** In the big room?

**WS:** In the big room right on the spot where the floor had been replaced because of radioactivity where the old cyclotron used to sit.

**VM:** Who was there, who was in the building when you went in?

**WS:** Let's see. Al Bassham worked on the other side of the bench from where I was working. Dick Lemmon was there, Murray Goodman and, of course, Vicky Haas Lynch (she was then still Vicky Haas). Let me see who else. There was some medical student who went to Harvard; his name was Gordo. He worked there in the summers, that I remember. There was an engineer who was working full time designing equipment. He put together the first $CO_2$ analyser, built it from scratch. I don't remember his name. He went to work for Bechtel Corporation eventually.

**VM:** Was John Weigl there?

**WS:** Yes, John Weigl was also there.

**VM:** Of the graduate students, you were one and I recognise that Murray Goodman was a graduate student. Was John Weigl a graduate student?

**WS:** John Weigl was a graduate student at the time. I think Al, Al Bassham was still a graduate student. His thesis was on quantitating the radioactivity in the individual carbon atoms of the sugars. That was the substance of his thesis.

**VM:** And I guess Andy was there?

**WS:** Andy, of course, Andy Benson was there. He was sort of the major-domo as he was Calvin's right-hand man.

**VM:** Did you see Calvin often in the building?

**WS:** Oh, yes. I think he was in every day. Once a week we had a seminar reporting on what we had accomplished during the week and most of those results got published very quickly. The group was very intellectually stimulating. It was a Mecca for scientists from all over the world, actually. I met all sorts of people there: people like Linus Pauling used to come in for seminars, Glintz (*spelling?*) from Stanford. It was really intellectually a very stimulating group. I remember we used to eat in some student cafeteria some place and the discussions ranged from all sorts of topics. And there were also some of the physics people. I was sort of interested in physics because a graduate student of Lawrence's, E.O. Lawrence's, was E.O. Lawrence's first graduate student, and he was my classmate at the University of Rochester and he eventually succeeded Lawrence as Director of the Radiation Laboratory (*Editor: of the Lawrence Livermore National Laboratory*) and was President Eisenhower's Scientific Advisor.

**VM:** What is his name?

**WS:** Herbert York. I guess he made several trips to Geneva negotiating with the Russians on nuclear affairs. He recently wrote a very interesting book called, *Making Weapons, Talking Peace* which is very interesting. It gives a nice history of the Radiation Lab. there. So I often used to attend the physics seminars up on the Hill at the Radiation Lab.

**VM:** And that have been the building in which Lawrence had his original 37" cyclotron, I think? The ORL, Old Radiation Lab.

**WS:** Yes, the Old Radiation Lab.

**VM:** Did Lawrence come in there at all?

**WS:** Yes, Lawrence had a laboratory up on the second floor of the Old Radiation Building and he used to come in at the time. He was working on a colour TV tube and was running some experiments. Apparently the tube worked but it wasn't commercially successful. I think he succeeded in developing a tube that showed colour. Yes, he was quite an interesting man, I thought.

**VM:** Did he come into Calvin's lab and talk to you people about what you were doing?

**WS:** Not very much. I think he got most of his information from Calvin himself. Occasionally he would come in from time to time. Usually he would come in when he had some visiting firemen and he would show them the whole operation and this was, of course, a part of his operation so he brought the visitors in from time to time.

**VM:** So he was regarded as a great man at the time, was he, and someone to be in awe of?

**WS:** Oh, yes. You know that group up on the hill...there was Seaborg there.

**SM:** Last night you described the atmosphere in the seminars and the arrival of E.O. Lawrence. Would you like to do it again?

**WS:** All right. Well, E.O. was a very revered man and the physics seminars which were held in a sort of an army type barracks up on the hill. People would assemble sort of before the appointed time — I think the appointed time was four o'clock in the afternoon — and everybody would be sitting down quietly and E.O. would stride in and walk down the centre of the aisle and he had a leather chair sitting up front and he would walk up to the front of the room, turn around and bow and sit down and then whoever was introducing the speaker would get up and introduce the speaker for the afternoon. The seminar could begin when E.O. was sitting in his leather chair. That was a weekly ritual.

**VM:** Did he interrupt the seminar speakers as they spoke?

**WS:** I don't recall that. I don't recall that he was a great participant in the discussion.

**VM:** But you recall that Calvin was a participant in the discussion?

**WS:** Oh, yes. Calvin was a participant in any seminar and on any topic. You know he had a

**VM:** Coming back to the development of chromatography, paper chromatography, in ORL you said that Calvin had cabinets made and so on. Did you participate in the design?

**WS:** Yes, I designed them, yes.

**VM:** And you designed the troughs?

**WS:** Yes, they were made by the glassblower next door. I don't know if you remember that there was a glassblowing shop right next door. I don't remember the glassblower's name anymore — was it Mr. Powell?

**VM:** I don't remember.

**WS:** I don't remember his name. But he devised a way of making the troughs. He took a glass tube of the proper diameter (I don't remember what diameter that was), sealed both ends and flattened the ends. And he had a diamond saw and he ran the diamond saw and made two troughs in one pass.

**VM:** And these were long ones.

**WS:** Yes, enough to take the 24"...they were about 26". There was an inch to spare on each side after the long dimension of the paper was immersed in the trough.

**VM:** All of this happened before my time. But when I got there they were using stainless steel and it was rather different. That was a later design. But in the early days when you had...

**WS:** I think maybe we had some stainless steel made while I was still there.

**VM:** Right. And ones which had a sleeve over a rod and you clipped the paper to the sleeve so you could take the whole thing out to dry.

**WS:** Right, right. They had little indentations...the ends had little indentations for the rods over which the paper passed. I think I may have participated in the design of those. I had written a chapter for *Methods in Medical Research* on paper chromatography. I was asked to do that by Professor Cho Hao Li, do you remember him in Biochemistry, the chromosome man? That was while I was still...that was my first year there when he learned I was doing paper chromatography. He wanted me to...he was co-editor, I think it was for Volume II of *Methods in Medical Research* and he had asked me to write a chapter on paper chromatography for that volume.

**VM:** Do you still have a copy?

**WS:** No, that copy doesn't exist but that's not the end of the story. I was very reluctant to do this but he eventually talked me into it. He wanted the article to cover amino acids and proteins and I felt very uncomfortable about the protein part so he said, "Well, I'll ask my graduate student, George Hess, to do the protein part if you do the amino acid part". So I did the amino acid part and George didn't get around to doing the protein part so eventually I wound up doing the protein part also. Then Li went off to Sweden for a sabbatical. I remember ...the editor of that volume was Gerard from the University of Chicago and I remember getting a frantic call from Gerard wanting to know where this manuscript was. He had sent it back for condensation; it was too long in the original form and he had sent it to Li for condensation. And I said I had no idea ...I didn't have a copy of this and Li was in Sweden. And he said, "Go see if you can find it on his desk somewhere". So I went to Li's secretary and she found this thing.

So I cancelled a Christmas vacation trip down to Pebble Beach, I think it was, and I was editing and trying to shorten this article. In the meantime, apparently, another copy got to Li in Sweden, who was doing the same thing. But Li, of course, having no experience at all with paper chromatography, used scissors and glue type of editing. That was the copy that was actually returned to Gerard and that was printed and I got a page proof copy of this. I looked at it and I was absolutely horrified to see what had been done to the paper and I didn't want it published. Even the photographs were upside down — they didn't know enough to know which way the photographs went.

*Tape turned over*

So I insisted that the chapter be pulled from the book and eventually that is what happened and the paper was not published. Subsequently, in two or three years, under the editorship of Corker and for Volume V of *Methods in Medical Research* Lyman Craig invited me to brush off the old paper and add some new information and submit it again so I did that and the paper was published in Volume V of *Methods in Medical Research*.

**VM:** Were you actually a graduate student when you wrote the paper?

**WS:** Yes, yes.

**VM:** Did Calvin know about it, did Calvin know you were doing it?

**WS:** I don't think so because I started it before I joined Calvin's laboratory. The paper was almost finished before I joined Calvin's laboratory

**VM:** And it was just published under your name?

**WS:** It was submitted under my name, yes. But when I got it back in this horrible page proof, Li had added his name as co-author. I guess he felt he was entitled to do this because of the editing he had done!

**VM:** Can we talk a bit about the research work that you did for own thesis. You spent a long time developing techniques and methodologies and then you applied them to the problem?

**WS:** Yes. The question at that time, because Calvin's thesis was that photosynthesis was a reversal of the fermentation (the Embden-Meyerhoff cycle) and driven by the energy from light and that carbon dioxide passed through all these intermediates on its way to sugar. There was a body of evidence in the literature which showed that green plants, although they have the capacity for fermentation reactions, often lost that capacity through time. Mostly the tissues that were capable of fermentations were the thick tissues like the cotyledons of peas and others and as the seedlings developed the leaves, for instance, would lose the capacity for fermentation which, of course, made me suspicious about the theory of a reversal of the fermentation process.

So I set out to design experiments to test this theory. One of the tools that was used by the early people who worked with muscle and yeast fermentation was to poison enzymes and in that way find out something about the nature of the process. And one of the enzymes that was, of course, in the fermentative course of reactions was triosephosphate dehydrogenase which is susceptible to inhibition by iodoacetamide. So it occurred to me that the reason that if carbon dioxide had to pass from phosphoglyceric acid through triosephosphate dehydrogenase through the triosephosphate *en route* to the hexose sugars that, if the cells were poisoned by iodoacetamide, that the phosphoglyceric acid should accumulate and the sugars should decrease. An experiment was designed to do this and the results showed that phosphoglyceric acid did not, indeed, accumulate but the amount of sucrose increased with the increase in time of exposure of the cells to iodoacetamide prior to feeding the carbon dioxide. So that, of course, cast doubt on the possibilities so I checked the possibility in another way. This was going from carbon dioxide to sugar. Then I tested the cells' ability to convert sugar to carbon dioxide and water again, feeding them labelled sucrose and labelled glucose.

**VM:** In a respiratory sense?

**WS:** Yes. I did it aerobically and anaerobically and compared the results and again used iodoacetamide to inhibit the triosephosphate dehydrogenase. In this case we would have expected sugar...yes, an increase in the triosephosphates on the chromatograms and a decrease in the phosphoglyceric acid and again we didn't obtain this result; we obtained the opposite result. So the conclusion was, in the thesis, that photosynthesis cannot be a reversal of an unmodified path reversal of the glycolytic sequence of reactions. The thesis was submitted and that was in line with some of the results by an Egyptian student in Biochemistry who was Paul Stumpf's graduate student, who tested a large number of plants for triosephosphate dehydrogenase and found that, indeed, some of the plants lacked this capability of doing this.

**VM:** Did you specifically test whether the triosephosphate dehydrogenase was susceptible to iodoacetamide?

**WS:** Well, that had been established...

**VM:** In the plant enzymes?

**WS:** Yes. That had been well established in the literature. And I used the same concentrations that were suggested in the literature. There was a good value of evidence that triosephosphate dehydrogenase, indeed, is inhibited by iodoacetamide in plants.

**VM:** Who was on your thesis committee?

**WS:** Roy Overstreet was the chairman, Paul Stumpf was on my thesis committee...let's see, who else?...A.C. Krafts, and I guess Calvin was on my committee also.

**VM:** How did he react to your conclusions?

**WS:** He accepted them but he thought that iodoacetamide might do other things besides inhibiting triosephosphate dehydrogenase. As a matter of fact, when he saw the great increase, the 12-fold increase, in sucrose after treatment with iodoacetamide he actually persuaded a sugar beet company (in Idaho, I think it was) to spray the sugar beets with iodoacetamide to increase the sugar concentration. Obviously this was a failure because of the exposure with time, the other reactions and the uptake of $CO_2$ eventually decreases and declines anyway.

**VM:** With hindsight how would you now interpret what you found with the subsequent understanding of the system?

**WS:** Well, I really don't know. I haven't discussed this problem with anybody for years. At the time there were many people — Martin Gibbs — who thought maybe that there was something to this and maybe it deserved further investigation. As a matter of fact, I sent Martin Gibbs a copy of my thesis as he asked to see it. I think maybe he had done some experiments but I have never heard the results.

**VM:** So you submitted your thesis in about '52 did you?

**WS:** Yes, I think about '52.

**VM:** During your stay in Berkeley you'd been married, hadn't you?

**WS:** I got married in...on July 15, 1948 to a wonderful lady, Bonnie Jean Thomas, whom I met as a graduate student at the University of Rochester when I returned from the service.

**VM:** And she worked in Berkeley too?

**WS:** She worked in Donner Lab. She worked for Dr. Tobias and irradiated thousands of mice with the small cyclotron next door to the Old Radiation Laboratory.

**VM:** That was the one in Crocker?

**WS:** Yes.

**VM:** So you presumably, both through her and through your ORL connection, knew the Calvin Donner people as well?

**WS:** Yes.

**VM:** Was there, in your experience, a lot of mixing of people between the two locations in Calvin's group or were they rather separate?

**WS:** Yes, once a week we used to meet — Calvin's group, that is the people who were at ORL proper used to hold a seminar in one of the rooms in Donner and many of the people in Donner used to come to our seminars so there was a good deal of interaction.

**VM:** What were the social relationships like among the people in the group? Did you spend a lot of your spare time together?

**WS:** Well, yes, there were a lot of...most of the people used to spend a lot of time going up to the mountains on weekends although, unfortunately, I used the weekends for work because it was the only time that the counters down in the basement were available and free so there was no competition for the counters so I used my weekends generally and I didn't participate in these excursions to Big Meadow and various other places that they liked to go. But Dick Lemmon, Al Bassham and Andy Benson all liked to go. But we used to have lunch together and there were often...in some cafeteria down on campus, not very far. I don't remember exactly where it was but I seem to recall that it was near a girls' hockey field somewhere, sort of towards town from the ORL and past the Faculty Club somewhere, and those were very fine and stimulating luncheons.

**VM:** So there was a lot of technical discussions within the group between people?

**WS:** Oh, yes.

**VM:** Was the big white table there when you were there on which people would lay out chromatograms? Many people have spoken of it and, indeed, the table still exists. It is a large table, probably bigger than your dining table, with a white Formica top and drawers underneath. People used to stand around and lay these chromatograms out. There was enough space to do this.

**WS:** I don't think so. I used to use some table in the chromatography room upstairs, mostly, I think, to examine my chromatograms.

**VM:** So what was the social focus in the building like? What did people do at coffee time? Did they gather together at coffee time?

**WS:** I don't remember spending a great deal of time with coffee. If we had coffee, I think we just took it to our benches and drank it there.

**VM:** Many people to whom we have spoken have been very fond, very impressed with the structure of ORL as a building for facilitating interaction between people because it was relatively open and...

**WS:** Yes, that's right. You could talk to anybody in the room that you wanted to, for instance. I remember...I think there were what?...three or four of benches...

**VM:** Yes, something like that.

**WS:** ...with a desk at each end and they were sort of islands. There was a graduate student working at each side of the...

**VM:** With a chemical rack behind you.

**WS:** Yes, yes.

**VM:** So you were always chatting with...

**WS:** Right. There was always somebody just across the table from you, the bench from you with whom you could converse. And I happened to have the good fortune of being across from Al Bassham.

**VM:** And then there was this underground counting room which was already there when you were there?

**WS:** Oh yes. It was some three feet, six feet of concrete. It was shielded by six feet of concrete, I guess. But even so, on some weekends when the cyclotron had full power, the background was about 200-500 counts per minute.

**VM:** That counting room was designed as I...was used, anyway, specifically to count chromatograms and so as you were the one who introduced the group to paper chromatography they must have built it while you were in Berkeley whether you were a member of the group then I don't know. But when you joined the group was it already there?

**WS:** Yes, it was already there. But there weren't that many counters there while I was there. Maybe there were two or at the most three counters and then eventually, I think, we had eight or nine counters.

**VM:** Was there a considerable time lapse between your first suggesting chromatography to them and the time you actually joined them? Because you talked about the things you did in Hilgard.

**WS:** Yes, I would say that it was almost...maybe almost a year before I actually joined the laboratory.

**VM:** And it was during that time, presumably, that they were building their own facilities.

**WS:** They didn't build their own facilities until I actually joined the laboratory because I designed the cabinets and the troughs and the equipment.

**VM:** I see. So the counting room in the early days was used for counting not paper samples, but was used for counting planchette samples and things of that sort. The counting of the papers presumably came with your own development of the chromatography.

**WS:** That's right, yes.

**VM:** And you fitted in, then, in that underground room.

**WS:** Right.

**VM:** And the chromatography room was actually built on the second floor, wasn't it?

**WS:** Yes. You had to go upstairs right next door to E.O. Lawrence's private little laboratory there.

**VM:** Oh, he still had a lab. in the building?

**WS:** Yes, he still had a lab. in the building.

**VM:** Did you have contact with him and did he with you?

**WS:** Yes. We had some contact but not a great deal. E.O. was not the kind of a person you talked socially with. He was strictly business and always in a hurry.

**VM:** When you left Berkeley, briefly what happened to you after then, between then and now?

**WS:** Well, let's see: we already talked about my thesis. After I finished my thesis, I had an offer to join the faculty at the University of Pennsylvania in Philadelphia so I left Berkeley and my wife and I drove across country, she doing the navigating mostly and we had a splendid time driving across the country. We did it slowly and stopped to visit my folks in Minnesota, which was the first time they had met Bonnie, my wife, and then we went on to Philadelphia.

**SM:** Which year was that? '52?

**WS:** I think it was '51, 1951.

**VM:** So you took a faculty job in Philadelphia?

**WS:** Yes. Let's see, I was an Assistant Professor of Botany at the University of Pennsylvania. And I stayed there four years. There I worked on sulphur metabolism. I had a graduate student who worked out the details of the reduction of sulphur. The plants take up sulphate ion and then obviously you don't find any sulphate compounds in plants. They are all reduced sulphur, sulphydryl groups. So he worked out the pathway on that.

**VM:** Was that using the same sort of technique you used...

**WS:** Yes. We used pretty much the same technique. Also we used some *in vitro* experiments. We grew *Chlorella* cells in sulphur-deficient media and then we tried to see what supplements would enable the cells to grow. Of course, they were unable to grow in the sulphate-deficient media and we were trying to see which intermediates we could...

**VM:** Which forms of sulphur would satisfy them?

**WS:** Yes. Let's see. I was at the University of Pennsylvania until 1955 and had an offer, was invited to come to the Medical College of Virginia to run a laboratory that had been donated by the American Tobacco Company. The Laboratory was called the Radiological Nutriculture Laboratory. It was a facility for growing plants in an atmosphere of radioactive carbon dioxide, in other words using plants as chemists to synthesise compounds which were difficult for organic chemists to synthesise. And also the compounds were uniformly labelled so that when they were fed to animals you didn't lose track of the...you could see all the pieces and all the metabolites.

We grew many crops of tobacco, obviously, and the idea was to see what happened to specific compounds when, for instance, a cigarette was spiked with a specific radioactive compound to see what happened to the compound as you smoked the cigarette, artificially, of course. We grew some flavouring agents. One of the flavouring agents used in tobacco comes from a plant called *Liaetris* which has a sort of vanilla-like flavour. So we grew that and we grew squill which was of some interest to farmers at the University of Kentucky and many other compounds.

It was an interesting place because it was a novel concept. It was just the second test facility, I guess, in the world. The Argonne National Laboratory had the first one. This laboratory was sort of designed with the experience of the Argonne people in mind so it was considerably improved over the one at Argonne, particularly in the way of leakage. It is very difficult to build a sort of a miniature greenhouse which is completely sealed so that the radioactivity doesn't escape. But we worked out the technique. It is also quite...it's a challenge to design a small enclosure in which you can grow plants where you have to maintain constant temperature. Lots of things happen because the plants are constantly transpiring, picking up water from the nutrient solution. We grew them in nutrient solution, aerated nutrient solution, so the plants are constantly transpiring water and that has a tendency to condense on the interior surfaces so that reduces the amount of light that the plants get and all sorts of problems. And then you must also remember that after you feed a certain volume of carbon dioxide to the plants in the enclosure, after they use that up, they kick out an

equal volume of oxygen. But the next time you feed them carbon dioxide, and that's assimilated by the plants, you get another volume of oxygen so the pressure keeps on increasing inside. So we devised a way of starting with a reduced pressure to accommodate the increases of the oxygen volume inside the chamber.

We had many visitors who were interested in the facility. There was a group of Japanese who came to inspect the facilities because they were interested in building one and I provided the plans and designs and had suggestions for improvements, and similarly a group from Germany who came. I know that the Germans actually built the thing. I don't know if the Japanese ever followed up and built their facility. So there was a facility at some institute in Germany whose name I no longer remember.

**VM:** Did any of the Berkeley people ever come and visit you?

**WS:** No, I don't recall any, no.

**VM:** Have you maintained contact with them?

**WS:** Not very much. I saw some of them at meetings in subsequent years but we didn't have any intimate contact.

The facility was used...After we isolated the compounds, you see, once we grew the plants now it was necessary to fractionate the thing and isolate specific compounds and we did this by chromatography a good bit and then it was possible to elute the radioactivity from chromatograms and feed the specific compounds to animals and also introduce specific compounds into cigarettes and smoke them and find out what happens to that. One of the things we...there was a laboratory in Sweden — Professor Schmitterlu (*spelling?*) in Sweden who devised a microtome which would take micron slices of whole rats, frozen rats, so what they did was feed some radioactive compounds to a rat, well there were several rats and then sacrifice rats at time intervals, sacrifice the rats in liquid nitrogen very quickly, and then sawed them in half right through the spinal cord and after that you could put the half on the microtome and take thin slices and take radioautographs of the thin slices and know exactly where the compound was, in which organs, and it turned out they used — we gave some of this nicotine to a Dr. McKinnes who was also a member of the Pharmacology Department of the Medical College of Virginia and he took it to Sweden to Schmitterlu's laboratory and they did that with the rats and it turns out that the nicotine very quickly goes into the brain and the liver. It is metabolised in the liver and also stays in the brain for a long time, as long as three weeks. One shot of nicotine to a mammal remains in the brain, traces of it ,remain in the brain for a long period of time. Whether it is still nicotine or not that I can't tell. Some of the work indicated it was also...particularly in the liver because the liver could then be scraped out of the remaining...because you only a used a thin slice of the rat. The rest of the liver was still there and you could take the liver out and analyse it and do other things with it. And then we also did...this was in the days when the Surgeon General declared cigarettes to be unhealthful and cancer causing...so we did an awful lot of work on the effects of smoke on animal tissues. One of the things we did was to study the effects of tobacco smoke on the cilia of cat trachea and it turns out that one puff

of smoke sort of disorganises the nice coordinated activity of cilia and they become disorganised and after maybe ten puffs from some cigarettes the cilia reaction quits completely, stops. They recover but who knows what happens after repeated assaults over many years of smoking for instance?

**VM:** So all of this took you a fair way from photosynthesis...

**WS:** Yes.

**VM:** ... but you spent the rest of your career here in Richmond at the College of Virginia.

**WS:** Yes, I did. I retired in 1982.

**VM:** Looking back, and let's perhaps finish on this point, what did being in Calvin's group mean to you.

**WS:** I think it was very stimulating and very rewarding, particularly the people that I met there. And it was such a Mecca for visitors from all over the world. I met many famous scientists in Calvin's laboratory and benefited from discussions with Calvin's very active mind because he was...that was always an education.

**VM:** Well, I think we might stop there and like to thank you very much for your participation. It was a great pleasure coming to see you and it was very exciting being able to locate you because, as you know, the Berkeley people no longer had your address and so when I actually found you it wasn't too difficult, a couple of letters...It was nice to be able to come and see you.