

Chapter 19

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Berkeley, California

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VM = Vivian Moses; BB = Bob Buchanan; SM = Sheila Moses

VM: This is a conversation with Bob Buchanan in Koshland Hall, Berkeley, on June 18th, 1996.

Bob, as I mentioned a little while ago, I saw the Calvin group from the inside, you saw it from the outside. When was that? When did you first become associated with photosynthesis here?

BB: I first came to Berkeley to work with Jesse Rabinowitz as a postdoc in the Department of Biochemistry. I had worked with fermentative bacteria. I joined Daniel Arnon's group about a year later, this was 1962 I came. So, in 1963, after he (*Arnon*) heard me give a lecture at the Federation Meetings on ferredoxin, which was a contemporary topic, timely topic, and he asked me to join his group. That was in September 1963. I guess I came in kind of at what was the second phase of his photosynthesis career, when he started working with proteins, ferredoxin in particular. The earlier part would have been with the discoveries with isolated chloroplasts. Bob Whatley would have been the one. Bob Whatley and I overlapped by a year. He went back to England, to London in 1964, I believe.

VM: Did you overlap with David Hall?

BB: No, but I know David. David had left already.

VM: He's a colleague of mine at Kings College; I have an office at King's.

BB: You have an office at King's? Kunyo Tagawa (*correct spelling?*) was here; we overlapped one month but David had gone.

VM: When you were working with Jesse you had nothing to do with photosynthesis?

BB: Nothing. I just heard tales of Arnon and Calvin, really from the outside (*laughter*).

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VM: What tales did you hear?

BB: That there were these two groups that were pretty isolated...

VM: From one another?

BB: ...from one another. Each worked in its own kind of territory. They didn't interact much. Occasionally I would see one or the other at a seminar, and I was a young postdoc., and that was very interesting. I never thought I'd be involved in any way with either.

VM: There was, of course, contact at the lower level, but at the upper level — for reasons I haven't totally discovered — there was considerable antipathy. I haven't yet quite run it down. When you got into Arnon's group, what did you start working on, what did you start doing?

BB: He heard me lecture about ferredoxin in *Clostridia*, in fermentation. He had found, with Tagawa, that ferredoxin is important. The protein that San Pietro had earlier identified as PPNR is actually two proteins: ferredoxin and the enzyme, ferredoxin-NADP reductase. He was interested in ferredoxins and I provided just a new expertise in that area. He saw, I think, an opportunity to do things that he hadn't been able to do before. I also had a strong microbiology background.

VM: How long did it take you to become suffused with the photosynthesis story?

BB: Quite a while. As a matter of fact I'm still learning things even though I'm not working in photosynthesis any more. He had the idea, Arnon had the idea that the PGA, the 3- phosphoglycerate which Calvin and associates had identified as the first stable product of photosynthesis under their conditions, might not be the real first product. He had thought that maybe one could use the reducing power of ferredoxin to bypass the ATP step needed for the formation of phosphoglyceraldehyde. One would start out with RuDP and get phosphoglyceraldehyde directly and circumvent this step. That was one of the things he had in mind when he invited me to join his group. Overall, he was thinking, perhaps a little more generally than that, that ferredoxin might be involved in CO₂ assimilation.

And so we started to work on that aspect in bacteria and it has been known for many years that fermentative bacteria break down pyruvate to CO₂, hydrogen and acetyl CoA and ultimately acetate. Ferredoxin then proved to be a carrier in the electrons from the pyruvate dehydrogenase to hydrogenase. Our first discussion was: can that reaction be reversed? It had been reversed *in vitro* by Wolf, at Illinois, using hydrosulphide or dithionite, and it was very, very marginal, but it happened; the extent was low. Would ferredoxin do that under physiological conditions?

I worked with Reinhard Bachoven (*spelling?*) who was a postdoc. from Zürich; he was here for about a year. We overlapped for about a year and then he went back and

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like many of these people from Berkeley he lived happily ever after with a chair in Europe!

VM: Some of us do — yes!

BB: (*Laughter*) Though he's still living happily ever after he retired a few years. We were able to show that ferredoxin can indeed reverse this reaction so one can get pyruvate synthesis from acetyl CoA and hydrogen and CO₂. We found this first with the *Clostridium* in which the reaction usually goes in the breakdown direction. But, it was an experimental achievement so we published that. Bachoven left and we began to look at photosynthetic bacteria: so this was my first entrée into photosynthesis was with photosynthetic bacteria. I had started some work to look for this reaction in photosynthetic bacteria and we were joined by Mike Evans, who is now a professor, again one of these who rode back to a chair in England, at University College I believe. He spent two years here. He had a very strong microbiology background, especially with photosynthetic bacteria since he had worked with Elsdon (Sidney Elsdon) in England and Davenport; he knew Davenport very well, they were buddies. Evans came and we looked for this more seriously, this reaction, which we called it the "pyruvate synthase reaction".

We found it in green bacteria. The green bacteria at that time were very neglected. Roger Stanier was here at the time and he gave us a culture of *Chlorobium thiosulfidatum* str. Tassajara because it had been isolated in the Tassajara Valley here. Stanier came over and talked about growing it and so forth, and we learned how to do that. It turns out that it was great at assimilating acetate, the whole cells, but they required CO₂ to do that. Van Niel had done some work on this earlier. It was a very interesting organism and seemed to be different from the other photosynthetic bacteria which had been worked with much more extensively at that time — the purple bacteria, such as *Rhodospirillum rubrum* (purple non-sulphur) and the purple sulphur such as *Chromatium*. And so we looked and we found the reaction was very active in this organism. The evidence that Elsdon and collaborators had obtained earlier with acetate showed that acetate seemed to enter by this group and the alanine was labelled where it should be labelled from CO₂ and acetate if this reaction operated. It looked like it really worked *in vivo*.

VM: Were you using chromatographic techniques for identifying compounds?

BB: Yes we did, at that time paper chromatography. So that was the way we identified the pyruvate, with acetyl CoA, ferredoxin which we reduced with chloroplast in the light and crude preparations of the hydrogenase. We would run the reaction under carbon monoxide to inhibit the hydrogenase so that all the electrons didn't go off as hydrogenase. It wasn't such a poison in those days. (*Laughs*) Now you would have to take great precautions. We found evidence for it. Then we said: "is this the only place that ferredoxin can act in this capacity?" Because again, there was labelling evidence in the literature that the α -ketoglutarate dehydrogenase reaction could be reversed in photosynthetic bacteria. We looked for that, and we found it. We had an α -ketoglutarate synthase reaction.

Then we said: "Does this really mean that there is some kind of cycle operative that would not be the reductive pentose phosphate, or Calvin-Benson, cycle?" So we looked and we obtained evidence that indeed there was. What this organism was able to do is to reverse the citric acid cycle and incorporate CO₂. It uses reduced ferredoxin to do this, and it runs it backwards: instead of the condensing enzyme it

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has an enzyme that cleaves citrate and all the other steps were reversible. So, it seemed to work this way. We used a lot of chromatography. We did short exposure experiments. We used the “lollipop” that had arrived in the drawer at some juncture and carried out photosynthetic bacterial experiments in the lollipop and did short exposure experiments that showed that the first product was not PGA, we didn’t even see any PGA, but was amino acid and glutamate was a very early product as one would expect if the citric acid cycle were operating in the reverse direction.

VM: Can I ask you some questions before I forget them all because you’re going very fast? The question that immediately arises is: were these techniques already developed in Arnon’s lab. or did he go to Calvin to get them?

BB: The chromatography techniques had long been in place. When they discovered — Arnon, Whatley and Allen — CO₂ assimilation by isolated chloroplasts, they looked at the products. I’m not sure who brought the technique.

VM: Whatley might know that

BB: Whatley would know that, where they came from. They were indeed in place and Losada in Spain had done quite a bit of chromatography work. Arnon used to be in LSB but then, by the time I came, he had moved into Hilgard Hall for about one year. We had a deluxe chromatography room with many cabinets. Actually, chromatography kind of went out after we got there. (*Laughs*)

VM: There were one or two stalwarts who kept it going but its time had gone.

BB: Its time was very limited but we did use it a lot with the *Chlorobium* so it was all in place. And Evans had experience with that, too.

VM: The other question I wanted to ask you which was some minutes ago: You mention that Arnon had postulated that the first product was already in a reduced form of glyceraldehyde. Was this something which arose while you were with him or had he thought about it earlier?

BB: No, he had thought about that earlier.

VM: Because I wonder whether this might have been a cause of some of the tension between the two groups.

BB: Yes, it may be.

VM: Calvin was, of course, very proprietary about PGA.

BB: About PGA — right.

VM: And then Arnon came along and said “perhaps not”.

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BB: But that was one of the things that he thought. It's still PGA, of course. What it did it provided us an avenue to discover other things.

VM: And, of course, the other systems, the Hatch-Slack system which came later, I think, when Calvin was already well established, caused less of a trauma than a possible undermining at a rather earlier stage — when, perhaps, a certain recognition had taken place!

BB: That's true. We did find evidence for this cycle in *Chlorobium* and I don't think that was received with open arms by a lot of people. Then we looked to see if it had the Calvin cycle and it turns out it doesn't. The genes aren't there, the enzymes aren't there, nothing is there. To get this cycle into textbooks took 20-25 years because there was so much resistance to it that this was the only cycle in autotrophic cells.

VM: When you say, so much resistance, how did you perceive resistance? Who were the resistors?

BB: There were reviewers, whom one never identifies, they were people who spoke in reviews that Rubisco was a marker of autotrophic life. That has since been shown not to be the case, not only with our systems but with others. I don't think there's anything peculiar to this particular group of people — cast of characters, one might say, were it a play — but it happens with others. Once an idea is accepted, and I am sure Calvin had his own problems in getting the cycle accepted, then science tends to think that's it and that there is nothing new.

VM: So the resistance didn't just come from Calvin's group? It came from...

BB: From other people. There may have been some from there, of course.

VM: There was perceived wisdom by that time and you were, in a sense, counter-attacking and that's what was causing the trouble.

BB: He might remember that, I don't know. I wouldn't say he was the major force. There were others. Finally, the opposition admitted in print in 1988 — we published it in 1966 — that it seems to go that way; it took that long. Now it's in textbooks and so on. It's a good lesson for me because we did it again in other things.

VM: If I can come back to your earliest days here in Berkeley with Arnon and focusing on the Calvin group, what did you see of the Calvin group, what was your understanding of it, or reaction to it?

BB: I just know that there wasn't any contact.

VM: You never had any yourself?

BB: No, I didn't. I did my job. I had plenty to do and I never went up to see them. We had everything we needed. In those days, money was no question, no problem anywhere with quality groups. I guess the first thing, and I didn't know the people — I met Al

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Bassham much later — the first kind of move to get the groups together was John Olson, who was a professor in Denmark, I believe; this was some years later, this may have been as late as, I believe this was in the seventies. He came to spend a sabbatical year with Calvin or with someone in the group. I don't know whether you remember him, or not.

VM: I don't think I knew him. I'd left by '71.

BB: It must have been a little after that. What he did was to organise a weekly seminar in the Round House; they had a beautiful seminar room. That actually, people started going. It became a fixture on the campus for many years when photosynthesis research was continued very actively. So the two groups did get together.

VM: Did Arnon and Calvin come?

BB: Arnon came a number (*of times*). Calvin didn't come very often because I think his mind was on other things., It had shifted from photosynthesis. I don't remember seeing him there; maybe once. Arnon was a little reluctant at first but then he started to going to all of them. Then, I began to meet the people and so forth. We were in a College of Agriculture and my job wasn't an instruction job, it was a research job. I was an assistant microbiologist which was 100% in the Agricultural Experiment Station. It's a tenure-track job but you don't teach.

VM: When you say "assistant microbiologist": what does that lead to in promotional terms?

BB: If I had not become a professor, if life hadn't unfolded in that direction, it's the equivalent of assistant professor in salary and everything, except one doesn't teach.

VM: You could have become an associate microbiologist?

BB: That's correct, and a microbiologist at the same level as a professor without the teaching duties. As I look back on it, that was a pretty fortunate appointment in some ways. It lacked stature but you don't need stature at that stage. I was able to get a lot done. With time, I started teaching.

VM: How big was Arnon's group at the time you were there?

BB: I think at its peak it probably got up to 15, maybe as high as 20.

VM: Everybody included?

BB: Everybody; 15 to 20.

VM: That was a sizeable group for an academic department.

BB: I think I came pretty much at the peak of its size.

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VM: Was Arnon himself very hands-on?

BB: He had been earlier but at that stage he wasn't really. He did try to visit everybody every day.

VM: To discuss nuts and bolts?

BB: Yes, right.

VM: So he was very much a part of the activity.

BB: He knew everything that was going on and he had his own way of doing things that was different from most Americans.

VM: How do you mean?

BB: He ran a very tight ship, I guess you might say. Notes had to be kept a certain way and he was really very on top of things. He spent a year with Warburg, or six months with Warburg, and he really saw many faults with Warburg but he saw many, many virtues. I think deep down he liked the way the institute ran, which was very Germanic. Some of that, as American science permitted, was translated here. He ran it with a kind of Germanic style. Bob Whatley would, I think, confirm that. And I'm not saying it in a derogatory way; it worked.

VM: Yes. It's just that one somehow feels that that style of management doesn't sit very well in the American psyche.

BB: That's right. But he was successful. It worked very well for me. I came up from the south and we're pretty much used to anything, and it didn't bother me. But it would have bothered some people.

VM: What was he like in the lab.: was he a relaxed person, was he a formalist?

BB: He was pretty formal in the lab. and things were quiet.

VM: What did you call him? How did you address him?

BB: Dr. Arnon.

VM: Never Dan?

BB: Not for some years, no one did.

VM: Everybody else was first names in the group, were they?

BB: That's right. Bob Whatley did, I think.

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VM: It was a very parallel situation with the Calvin group. It was a long time before people addressed him by his first name, although his wife always referred to him as Melvin talking to other people.

BB: There was a formality. When Bob Whatley left, it became pretty formal.

VM: Is this characteristic of the relationship between professors in groups generally? I would have thought they all would be first names.

BB: It was not. For example, when I was with Jesse Rabinowitz everybody was on a first-name basis. There may have been situations in Biochemistry, with more senior people, that was a little different but not quite the same as here.

VM: At that time in Arnon's group did he get all the money to support the group?

BB: Yes, he got all the money.

VM: So all you did was to support him in whatever he needed.

BB: Yes. It was very effectively organised. People had different obligations and so there was only a secretary and a repair person who was very good, a retired Navy petty officer who could fix anything. Arnon told me that if you are on a ship you have to be able to make do with what you have and that's why he hired him. That paid off. I was in charge of all the ordering for the laboratory, for example. Someone wanted an order, I would...

VM: So you divided up the housekeeping duties?

BB: The housekeeping duties; we didn't hire people. And since there was no teaching — we can't get by like that now; everybody has to teach. Those days are all gone. But it worked well in this time. Some people didn't find it as comfortable as others.

VM: Because of his dominance of the group, or what?

BB: And direction, overall direction.

VM: He really did direct?

BB: Oh yeah. The ideas. He had a lot of ideas.

VM: The ideas predominantly came from him?

BB: Not all, of course, but many did.

VM: Was he receptive to other people's suggestions?

BB: Not at once. That wasn't his style. We had a seminar, a group meeting once a week, these could go on for hours, and he said it was like a kitchen where you try the dishes

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before they are served. That's where there were great discussions and debates about ideas. I think sometimes, he was a genius it seemed, of course he knew what was going on, he knew there was an idea that would supplant his. But he would hold out, I think, because it would bring even newer ideas and he was a genius at getting people to express their creativity.

VM: Well, it also has the advantage that it would force people to support their ideas with evidence and arguments.

BB: That's right but he would hold on to his (*ideas*) forever until it became kind of ludicrous sometimes. In the meantime, of course, something would surface that was worthwhile.

VM: Did he stop people following their own initiative in the lab. in experimentation?

BB: No, not that. I think you could do that. You would always be put in the light of a certain idea.

VM: Did his name go on every paper?

BB: Pretty much until I came, that's true, and then it began to change. For the first two or three years his name went on all of our papers. Then Mike Evans and I published a paper, the α -ketoglutarate synthase paper, by ourselves. We also published a paper on the presence of a non-cyclic electron transport system in bacteria which is the only one that has really held up. That was on our own. About the time I came it began to change a little because he could see that people had to develop careers independently.

VM: What was the style of authorship of papers? If his name was on all the early papers, did he contribute, did he at least read the manuscripts of everything?

BB: He wrote them.

VM: He wrote them?

BB: Absolutely. He was a scholar to the nth degree. Every reference had been looked up by him, oh yes, and written. Kunyo Tagawa describes it in one of the write-ups for the special issue of *Photosynthesis Research* and I saw it but I guess Kunyo Tagawa saw it even more so. When a paper was in the works....First of all, there's always a deadline, a PNAS deadline, or some deadline to meet. There were no word processors in those days and so everything had to be typed fresh. He would have the secretary type everything and make carbon copies initially (but ultimately Xerox copies) and make ten of those and give it to everybody in the lab. to read to get it to perfection. (We may have to leave; maybe not [*the room in which we were talking had been booked by another group*]). He would say once it's published you can't use an eraser. He was very much a part of every paper. There was no perfunctory name-adding. He knew everything.

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VM: But he wasn't necessarily the original drafter of every manuscript, was he? Presumably the guys at the bench...

BB: They would write the first draft. But they didn't bear much resemblance to the final draft. He taught me how to write. He taught me so many things, writing being one of them.

VM: That was rather different from the Calvin style where other people wrote the papers in the first draft and he certainly went through them, and there were endless arguments over commas and other factors. The style was the guy who did the work and wrote the paper came first in the author list. He (*Calvin*) usually came last and in between they were alphabetical.

BB: It was usually obvious who should be the author. Arnon was always last except in the early days when he was actually participating in the work with Arnon, Allen and Whatley, this team and then sometimes he was first. I learned a lot with this system...

VM: I can imagine.

BB: ...and I look back, and there are several people I quote when I tell students things, and my mother is one, for wisdom, and Dan Arnon is another. You never forget some of those things.

VM: It's very interesting, the comparison of these two leaders of photosynthesis, working within a few hundred yards of each other, at arms length, more or less ignoring one another's existence, at least for a long time. I hope to get some more insight into that.

BB: I think one interesting distinction between the two groups is the habitat. Calvin was in a very strong Department of Chemistry with many luminaries. Arnon was in a College of Agriculture in which he was doing most, if not all, the fundamental work that was being done. There were not a lot of colleagues around him who even appreciated what he was doing. It was a very different type of situation. No kind of stars to help him along. We probably have to...Come in!

VM: Obviously, we are being thrown out of the room. Thank you very much.

BB: If anything comes up...