

Earl R. Stadtman, Ph.D.

This is the third interview in a series on the career of Dr. Earl R. Stadtman. It was conducted on February 6, 2001, in his office on the second floor of Building 3, National Institutes of Health, Bethesda, Maryland. The interviewer is Dr. Buhm Soon Park.

Stadtman: [Interview picks up]...lab as postdoctoral fellows. And these are the ones who now are chairmen of a department or have an equivalent. Brady, for example, is here at NIH and Pastan is at NIH. So they have that. And these are the ones who are National Academy of Science members. These are lists of the full professors at various academic institutions.

Park: As of 1997?

Stadtman: No, no. It's an old tablet I have. That has nothing to do with anything. Then I have here is a list of all of the people that have been in the laboratory. It doesn't go back to the very beginning, but it's the best I can do at the moment. And some of these people were postdocs, like Levine, but also I participated in the thing, and here's one, Emilio Garcia. Then, starting here, I put *P* for professor and *C* for chairman. That's all summarized there, so you can ignore that. These are the people who have been in my laboratory since about 1955, something like that. I can't be certain about the time when they first came. Well, maybe it tells here. Yeah, 1983, 1984, 1966. This is 1958.

Park: That's great. Everything is pretty thorough.

Stadtman: Then this is the list of the people that, starting here and going down here, a list of the people who have been through my lab. These are Terry's postdocs, and the list goes on. But I think this is probably as of something like 1998 maybe, something like that. You can tell from the dates here what the latest date was. But that's that. And these are things that I just found that have been in the files. They're really pretty much the same, repeat. That's what that is.

Park: That's great, that's great.

Stadtman: For what it's worth. And then I have here reprints, and, unfortunately, I don't have any reprints of the very early studies that I did on fatty acid. But that's been reviewed in some of the reviews that you'll find in here.

Park: Yes. I think it's collected in a...

Stadtman: Yeah. I've sort of summarized this in a couple of places. And I've broken it down according to areas of investigation. These aren't all of the reprints, but there are a lot. This is regulation of glutamine synthetase, just on the regulatory aspects, not the cascade phenomenon, which is dealt with since then. But that's that. And then I have one here on reactive oxygen species, mediated oxidation of proteins, and so on.

Park: It's kind of you.

Stadtman: With that information, you ought to be able to find out what I was up to over these many years.

Park: Yes.

Stadtman: And this is more of the same.

Park: That's wonderful. I have a couple of more questions, but shall we finish your...

Stadtman: Sure. We can go through that.

Park: We are at almost 1995.

Stadtman: I guess I was telling you that...

Park: I forget what I was, how I introduced the subject to you, but these are, this is in an area when I was--it had been shown by other workers that hydroxynonenal reacts. It's an alpha-beta unsaturated aldehyde. It's a derivative of lipid peroxidation. And so when Uchida came here, I asked him to study 4-hydroxy-2-nonenal, which is one of the major end products of--it's not the ultimate end product, but it is a product of lipid unsaturated fatty acid oxidation by reactive oxygen-mediated mechanisms. And it was shown, as I think I discussed with you last time, by Esterbauer in Austria that the sulfhydryl group of cysteine residues would react across the double bond, and this would leave open a carbonyl group. So, to the extent that this reacted with cysteine residues in proteins, you'd have a carbonyl. So it was another mechanism of generating carbonyl groups in proteins, aside from the direct oxidation of side chains. Uchida found that it reacted not only with cysteine residues but it reacted also with lysine residues and with imidazole residues of histidine. Then he developed antibodies that could be used to detect hydroxynonenal adducts to proteins, and this has been used by a lot of people, in the meantime, to study the effect of or the amount of 4-hydroxynonenal adduct that is

present, especially in some diseases. It's been demonstrated that it's implicated in atherosclerosis, it's implicated in Alzheimer's disease and Parkinson's disease and a few other diseases, where it's been looked for using this technology. Then we got into a study of ozone as a reactive oxygen species, and there we compared the sensitivity of glutamine synthetase and bovine serum albumin to oxidative damage by ozone. Now, the reason that we compared these two--they're very different proteins. One's a dodecamer, one is essentially a monomer. So it was of interest to see what... The reason is that both of these proteins have roughly the same amount of each of the aromatic residues per subunit, so they're comparable in the sense that they have equal amounts of some of the residues that are susceptible to oxidative modification. And we compared them for their sensitivity to ozone and found, in fact, that there was a big difference between them, and that's described in one of these papers that you, that I gave you here. There's one which deals with that. There's also a section I gave you that deals with this nonenal stuff. Cyclic cascades. This was an outgrowth. I think we've discussed this, haven't we? Let me come back to here. This is an important paper. Studies by other workers had shown that nitric oxide is a very important signaling molecule. It does a lot of good things in the cell. But, unfortunately, it reacts with superoxide anion to form peroxynitrite, and peroxynitrite is a very toxic material. It reacts with virtually everything that it comes in contact with, one way or another. And so we were led to

investigate the role of peroxynitrite in modifying proteins. One of the reactions that had been described was the nitration of tyrosine residues in proteins. That's one of the targets for peroxynitrite-mediated modification. And I'll come back to some ramifications of that later. But this study was prompted by a study that I made many years ago, or several years ago at least, and we were studying the cascade control of glutamine synthetase. And I was told by the organic chemists that if you nitrate a tyrosine residue, it would lessen the ability of that residue to be esterified on the hydroxyl group, and so since adenylation of a tyrosine residue was implicated in the regulation of glutamine synthetase, I wanted to see what nitration would do to that system. So I incubated these with tetranitromethane, which is a nice nitrating agent, and it nitrates tyrosine residues. And we found in those studies that nitration of the tyrosine residue converted the enzyme to a form that mimicked the esterification of the enzyme with adeny acid residue. And for that reason, I wondered if peroxynitrite, what it would do to glutamine synthetase, since it had been shown to nitrate tyrosine residues. And, indeed, we found that when you treat glutamine synthetase with peroxynitrite, that you get nitration of tyrosine residues and that this mimics the adenylation reaction. So this called attention to... And also, I might say, it prevents unmodified tyrosine residues from being modified. In other words, if you have a system where you're phosphorylating or adenylylating tyrosine residue, if you get nitration of that tyrosine, it can no longer be phosphorylated or

adenylylated. And so we pointed out that this could be a very important factor in signal transduction pathways, where phosphorylation of tyrosine residues is a very important mechanism. If it could in some cases mimic and in other cases prevent the phosphorylation of those residues, then the cell could be in real trouble. And we found that... We pointed that out in this, and then, subsequently--later on we'll come to it--but Chock and his colleagues looked at peroxynitrite on one of the signaling systems and showed, in fact, that when you nitrate that tyrosine residue, which normally is phosphorylated, that it could not be phosphorylated anymore. These are just... Oh, okay. Now we're coming to an area that I don't know that I introduced earlier. Maybe I did, maybe I didn't. Well, let me continue with the nitration story before we leave and then have to come back to it. We subsequently, in the course of studies, there was a paper that came out describing the fact that peroxynitrite reacts very rapidly with carbon dioxide to form a carboxylate derivative, and it occurred to us that that might be important in the nitration of tyrosine residues. So we carried out experiments where we treated reaction mixtures. We bubbled gas for the reaction mixtures to eliminate all CO<sub>2</sub> that was in the reaction mixture. And much to our surprise, we found that when you did that, you almost eliminated completely the ability of peroxynitrite to nitrate tyrosine residues, but you greatly enhanced the ability of peroxynitrite to oxidize methionine residues in proteins. So then we carried out a study to see what would happen if we deliberately worked in the presence of bicarbonate

buffer, and much to our surprise, we found that if you work in a system where you have the same amount of carbon dioxide as exists in the human body, at the same pH, 7.4 average pH in the body, then you suppress completely the ability of peroxyxynitrite to oxidize methionine residues, but you stimulated its ability to nitrate tyrosine residues. So this called attention to the importance of carbon dioxide in what happens. I mean, it really distinguishes between two pathways of function. And, subsequently, we proposed one mechanism, which now has been confirmed, is that the peroxyxynitrite derivative can undergo cleavage to form a carbonate radical and also a hydroxyl radical, and the two together will account for the ability to nitrate the tyrosine residue, and the schemes are given in the papers. Now I want to come back to one other area of interest that I may not have jogged here, but it has been an important area in the laboratory, and that was an outgrowth of a study that was carried out by Kim working with Sue Goo Rhee when they were both, I mean, really were still working under my control in the laboratory before he was elevated to the level of section head. I don't see the specific reference here. Maybe it comes later, not earlier. Well, there's a series of papers, and one of them here that caught my eye was one that called attention...Kim and Rhee. They were studying glutamine synthetase in yeast to see if it was controlled the same way that it was in *E. coli*. And in the course of purifying the enzyme from yeast, they found that it became inactivated. And then it was discovered that the reason it became

inactivated was because they were putting in dithiothreitol, which is an SH protector. It protects cysteine residues from oxidation. But that was somehow causing the inactivation of the enzyme, and this raised the possibility that maybe sulfhydryl compounds in the presence of metal ions could catalyze reactive oxygen species formation and damage the protein. So they tested this hypothesis, and in fact they found that if you add iron to dithiothreitol, then you got non-enzymic cleavage of the protein by reactive oxygen species that were generated in that system. And that now has become a major project in Rhee's laboratory since he became a section chief. But also, we carried out some studies to follow up on that, and here's the, one of the papers. Purified the specific protector. I should have said that they found that this dithiothreitol didn't have any effect in the crude extract. It was only after it had been purified partially that it became susceptible to this iron-sulfhydryl catalyzed oxidation-inactivation system. And it was subsequently shown that you could replace the dithiothreitol with thioredoxin. So it was a thioredoxin link, could be linked, thioredoxin-linked damage as a phenomenon, where high levels of glutathione would also damage. [But] ascorbic acid, which we routinely use together with iron to generate reactive oxygen, was inactive in this respect. It did not replace the sulfhydryl compounds for deinactivation reaction. But the fact that the enzyme glutamine synthetase in crude extracts was stable in the presence of dithiothreitol, whereas in partially purified preparations it became unstable, suggested that there was an



enzyme there in the crude extract that could protect the system from this kind of radical generation. And, in fact, as was stated here, the isolation and purification of the specific protective protein, which inhibits enzyme inactivation by the thiol mixed-function oxidation system. That was purified, and in the meantime it has been shown that the part of the family of enzymes you find something like, now I think the list is up to around 25 enzymes have been isolated by other workers and also by Rhee and his group, that were isolated because of another function they were looking at, but was found in fact to be able to protect against this kind of damage, and it had a lot of motifs that were very comparable, in the highly conserved motif, that is. Now we're getting about, you'll find a number of papers in this area in the folder there. Then we got involved in amino acid oxidation. Did I talk to you about a free amino acid oxidation before?

Park: No.

Stadtman: I think maybe I did. I thought I did. Anyway, this study was prompted-- call it Fenton chemistry revisited because we... There was a paper by Robert Floyd that was published. He was interested in knowing whether or not he could detect any radicals that are produced when you expose amino acids to the Fenton reagent. Fenton reagent is iron plus hydrogen peroxide, in which iron splits the hydrogen peroxide and generates hydroxyl radical, which is a very damaging species and readily can oxidize amino acids and, for that matter, anything that's around. And he carried out his experiments in bicarbonate buffer because he didn't want to

get radicals of other buffers, such as Tris and Hepes buffer and the other buffers that are routinely used in biological studies to control the pH have been shown to undergo rapid reaction with reactive oxygen species to generate other kinds of radicals, and he didn't want his system to be complicated by that, so he worked in bicarbonate buffer. I saw that paper and I said, "Gee, maybe we can gain some insight into the reaction mechanism of protein oxidation, side chains of protein oxidation, by looking at this system," and so we set up the system that he described and found, in fact, that it was very effective in oxidizing amino acids. But, as you know, it's very difficult to control the pH when you work in a bicarbonate buffer system if you're working in test tubes and things like that. So I tried to explore other buffers. I wasn't so much concerned about generating radicals of the buffer. I was interested in the mechanism of the amino acid oxidation. And when we did, we found that, in fact, bicarbonate was absolutely essential in order to get this oxidation reaction to go. And so then we shifted our studies to Warburgs. Do you know what a Warburg is?

Park: Yes.

Stadtman: Warburg is an apparatus.

Park: Yes, manometers.

Stadtman: Manometers, yes. So we shifted to that because there you can control the pH and bicarbonate buffers very precisely by working with 5 percent CO<sub>2</sub> nitrogen or oxygen in the atmosphere. And varying the bicarbonate

buffer, you can get pHs that are what you calculate, what you want. And so you could study over time. And also, it has the nice advantage that you can use large quantities of amino acids and you have enough material at the end of the experiment to make an analysis and determine what happened both from the gas changes and the products that are formed. And so we carried out a whole series of studies on amino acid oxidation, and you'll find those described in a number of these papers, but particularly this one. And that was a review article. There was one later. I don't know just where it was. But in the course of those experiments, we made a rather interesting observation, and that was that you could replace manganese--I mean you could replace iron with manganese in the amino acid system, and that would lead to a dismutation of hydrogen peroxide, so it just looked like a catalase. It had catalase-like activity. If you had bicarbonate, amino acid, and manganese present, that reaction complex would decompose hydrogen peroxide. Subsequently, that's what this refers to here, this portion. Peter Ward and his co-workers at Michigan, I think he was, took advantage of this observation. They cite our work. And they started out with a cell system and they studied the toxicity of neutrophils. When neutrophils are exposed to conditions that create an oxidative burst, then they will generate reactive oxygen species and hydrogen peroxide. A lot of hydrogen peroxide is formed. And in the presence of metal ions, then that can go on to form more serious oxidants. So he wanted to know whether or not manganese plus an amino acid plus

bicarbonate would protect cells from that kind of damage, and he found, in fact, that it did, that if he grew cells in the presence of a neutrophil system which was generating reactive oxygen species, that he could protect the cells from toxicity simply by working in the presence of an amino acid, bicarbonate, and manganese. And all three he demonstrated were essential in order to get this protective effect. Well, in the meantime, when we got into our studies on signal transduction, I wanted to know if manganese might in fact play a role in the cell's signaling events, because, as you know, cell signaling is initiated by hydrogen peroxide and you can--in many cases, not all cases--and so we thought that maybe this system would have an effect on the cell signaling. And Hammou<sup>1</sup>, who--this is ongoing work at the present time--was able to show, in fact, that when you add manganese to cells, that you get a great enhancement in the amount of caspase activity, which is one of the enzymes that dictates apoptosis on the one hand and also caspase 9 and caspase 12, he's pursuing at the present time. That work hasn't been [published]. But it just shows how the evolution of the problem has gone. Now, I haven't... There are several papers here on this manganese effect, and they're all related to this. But in the course of those studies, they found that if you added a superoxide dismutase to a reaction mixture that had hydrogen peroxide in it, high levels of hydrogen peroxide, that it would in fact generate hydroxyl radicals. So this called attention to the fact that while superoxide

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<sup>1</sup> Hammou Oubrahim, then a post-doctoral fellow

dismutase is considered to be a very protective antioxidant in the cell, that under certain conditions, if the hydrogen peroxide level gets high enough, then it will in fact generate hydroxyl radicals from hydrogen peroxide. So it makes hydrogen peroxide and then, at high levels, it will convert the hydrogen peroxide to hydroxyl radical, and this could lead to some damage to the cell. And Moon Bin Yim has followed up on that work in one of his major activities in the laboratory.

But then it was reported that, in the case of ALS, amyotrophic lateral sclerosis, that the disease, in those individuals where they could show a genetic linkage between one population and another, that it was, that defects in the superoxide dismutase were associated with that disease. And the question was, what do these mutations in superoxide dismutase have to do with the generation of amyotrophic lateral sclerosis? Well, we wondered if maybe it's because you have enhanced the ability of the superoxide dismutase to generate this hydroxyl radical from hydrogen peroxide, and so Moon Bin Yim, together with other colleagues, studied this, and we found that, in fact, the mutations led to an increase in the amount of hydroxyl radical that was formed and that the severity of the mutation with respect to [ALS] correlated with the ability to generate hydroxyl radical in the presence of hydrogen peroxide. And looking at the kinetics of that system, it was demonstrated that these mutations altered the affinity of the superoxide dismutase for hydrogen peroxide and that

this very likely was responsible for the increase in hydroxyl radical formation. Now, to be fair, I have to say that there's a big controversy in the literature over this interpretation. This is just our interpretation.

Park: I see.

Stadtman: And other people don't believe it. But, nevertheless, that's where that stands.

Park: Right.

Stadtman: Many of these are minor. I mean, they're minor activities in the laboratory, and I've got one section here that says miscellaneous studies, and that includes these minor things, which I don't--they didn't amount to a huge effort in the laboratory. They were just one or two studies carried out. Here again is the paper dealing with the modification of histidine residues by 4-hydroxynonenal, and here, selective cleavage of the thio-ester linkage of proteins modified by 4-hydroxynonenal as a means of establishing what fraction of the total damage is due to cysteine versus some other reaction. And then there's a continuation, as you will find that there's a number of papers that deal with this hydroxynonenal reaction. Now, one of the outgrowths of this whole work was... Incidentally, I put a copy of this review. This is the Welch --you knew I got the Welch award<sup>2</sup>.

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<sup>2</sup> The Robert A. Welch Award of 1991 which he shared equally with Edward Krebs. Krebs went on to receive the Nobel prize for his work on regulation by phosphorylation. In part 2, Earl noted that he considered it a little unfortunate that his work on covalent modification involved adenylation and not phosphorylation. The principles of regulation deduced from the two

Park: Yes, I did.

Stadtman: And so I had a summary of the cascade control, and I have a copy of that in there so you can read it. But I want to mention now some work on aging, some studies that were carried out on aging, in collaboration with the group from Kentucky and also from Oklahoma. They were a collaborative group.

Park: How was that collaboration?

Stadtman: Well, Robert Floyd was one of the participants in some of these papers, and he was an expert in EPR and radical trapping and that sort of thing. The group in Kentucky were interested in aging from the standpoint of-- also aging and disease, and they were excited about the possibility that reactive oxygen damage was contributing to the aging process, because we had published this in a number of papers. I think I've discussed that aging [correlates with oxidation of proteins] already. In any case, they carried out some very interesting experiments, which are described here. This is one of them. I think I have an original of that. That's sort of a review article. I don't see it offhand here. But, again, you'll find papers by these people--Smith, Carney, and Floyd and Marksberry --and they all deal with this general problem. But there's one very interesting thing that came out of this. They were studying the--and maybe I talked to you already about it--they were studying the effect of aging in the gerbil, and they confirmed

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systems were identical, but phosphorylation was more widespread so the Stadtman work on adenylylation was often not recognized. With the Welch Award, it received equal recognition to that given phosphorylation.

what we had seen in the rat, that as the gerbil got older, he generated more protein damage as measured by the carbonyl content. And then, because they were experts in the compound is the one that's used as a spin trap and EPR measurements, PBN, phenylbutylnitrone, and because it was a spin trap, it obviously reacted with radicals, and the question was whether or not that would have any protective effect on the aging of the animals. So they added or injected PBN into gerbils and then studied and followed the levels of the reactive oxygen species, I mean of protein damage by the carbonyl measurement, and the levels of the proteases that are involved in degrading oxidized proteins, and also the level of glutamine synthetase, which we had shown goes down [during] aging. And what they found was that if they took an old rat or an old gerbil and injected this into the gerbil, this PBN, that it would immediately cause the characteristics that I described to you to reverse and become equal to what you would find in a young animal. Well, what's that got to do with aging? Of course, the question was whether or not they had still the right function, were they happy or not, and so on and so forth. Well, they found that using a test that is a kind of a maze, you put the gerbil in the middle of the octagonal chamber that had a tunnel off each side of the octagon, and they put the gerbil in the center, and it would go up a tunnel and find it couldn't get out, and it would come back and try going up another tunnel. And if you compare the young animal to the old animal, you find that the old animal makes about 10 errors. They described as an error if the gerbil went up a



tunnel it had already been into before it had exploited all possible eight tunnels. Well, they found that the old animal made about 10 errors and the young animal made about four. But after they treated the old animal with this PBN, it also made four. So there was also an effect on its behavioral aspects of the animal as a consequence of that treatment. And as a consequence of that experiment, a company was founded out in California called Centaur Pharmaceuticals that is exploring the possibility that PBN can be used to treat various diseases, and they've made something like a hundred derivatives of PBN and studied them in various... In fact, there are clinical trials now going on in Europe using some of the products that they have made. But it was all initiated by this one observation. I think we're coming pretty much to the end. These again. This showed that the 4-hydroxynonenal can prevent one form of cancer that's formed in rats. This is this specific antioxidant enzyme that I told you about. Probably the paper is in that mixture. I think now--this is another one on the dithiothreitol system. This is looking at the mechanism of the process, and I won't go into that, but it's here. And we're rapidly coming now to the last topic that I want to discuss with you, and that's the importance of... This paper is about peroxynitrite-disabled tyrosine phosphorylation regulatory mechanisms. This is one of the signal transduction pathway systems, where it found essentially the same thing that we did in the other system. Well, there was one thing. We got involved in the methionine story because it was very clear that methionine residues in proteins are by

far the most susceptible to oxidative damage than any other amino acid residue, with the possible exception of cysteine residues in proteins. But unlike all of the other modifications that occur, oxidative modifications, the oxidation in methionine residues is reversible. Oxidation to methionine sulfoxide can be reversed by an enzyme that was described by Brot and Weisbach some years ago. So we were taken by the fact that methionine residues are the most easily oxidized residue, as I say, with the possible exception of cysteine. And like cysteine residue oxidations, the methionine residue oxidations can be reversed. And that is unique because none of the other amino acid oxidative modifications are reversible. And the only way you can repair those is to degrade the protein and get rid of the modified amino acid residues and build up that protein. But with methionine residue, that's not the case, and so we started a series of studies to examine the enzyme, methionine sulfoxide reductase, that converts the oxidized methionine back to methionine. And we found that the system works very well. It requires thioredoxin as a reducing agent in the overall process, and it... So there's a linkage between the thioredoxin reductase and the methionine sulfoxide reductase processes in making this work in a cyclic manner. And because it could work in a cyclic manner and because the methionine residues are by far the most sensitive, we proposed in a paper by [Rodney L.] Levine and myself that methionine residues might be antioxidants because they would be the first

line<sup>3</sup> of defense against antioxidant damage by virtue of the fact that--I mean of oxidant damage by virtue of the fact that they would react faster with the reactive oxygen species than other amino acid residues, and then it could be reversed. So the net result is that you have gotten rid of the reactive oxygen species.

Park: How smart!

Stadtman: Yeah. And so we proposed that it was the first line of defense against radical damage in proteins. And then, to test that hypothesis, Jacob Moskovitz, who's still here, made mutations in yeast where he could knock out the methionine sulfoxide reductase or could overexpress it, either way, so you had more methionine sulfoxide reductase activity. And what he found was that the toxicity to hydrogen peroxide was greatly reduced in the mutant, which overproduced the methionine sulfoxide reductase, but that in a mutant lacking the enzyme, it was more susceptible to toxicity than the wild type strain, which supported this concept that it might serve as an antioxidant. And also, he was able to show, together with Barbara Berlett, my colleague, that in those cases, the level, that in the case of the mutant lacking methionine sulfoxide reductase, there was a considerable enhancement in the amount of methionine sulfoxide in the proteins exposed to reactive oxygen species compared to the wild type,

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<sup>3</sup> Trivia note: Earl and I had a number of discussions over lunch in which we disagreed as to whether this was the "first line" or the "last line" of defense. He hewed to the former and I to the latter. We both recognized that both terms could be defended, but neither of us ever convinced the other to adopt the other's nomenclature. [Rodney Levine, *Ph.D.*]

and that the overproducing strain, there was less methionine sulfoxide present in the protein. So that looked like a pretty good lead on that. In the meantime, in ongoing studies that are going on at the moment, he prepared a knockout in the mouse, a mouse that does not have methionine sulfoxide reductase, and ongoing studies now have shown that the mutant mice live only about half as long as the wild type mice do. He's shown that if you take a wild type in the mutant mouse and expose them to 100 percent oxygen, that the wild type will live longer than the mutant will in that stress condition, and that as a consequence of the stress, there's an elevation in the level of protein damage as measured by the carbonyl content of the mutant.

But very much to our chagrin, we found that when you have the knockout mouse, you up-regulate another activity that also has methionine sulfoxide reductase activity, and if you measure the amount of methionine sulfoxide reductase activity in the mutant versus the wild type, that it's the same. But there's clearly something else that the mutant enzyme is involved in which dictates the life span, and also it's very clear that the mutants are more sensitive to reactive oxygen generation, oxidative stress. The only thing that we know for sure is that this mutant form of the, this replacement enzyme, if you will, which is upgraded, cannot work as well in the presence of thioredoxin and thioredoxin reductase as did the normal strain. So it may be that there's a combination of things that's involved in this overall thing, but we're working on that area now.

So that's pretty much where we are. You'll find papers by Moskovitz here that deal with this phenomenon that I just discussed with you, and they're ongoing experiments. You'll find this sort of thing listed among the miscellaneous studies that have been carried out. But I think that that gives you a reasonably good idea of what we've been up to over these years. Here's this amyotrophic lateral sclerosis stuff that I was telling you about.

Park: How many people are working with you at this moment?

Stadtman: At this moment I have four postdocs and I have one position that is unfilled, so I'm recruiting for a fifth one.

Park: And the size of your group has been around...

Stadtman: Pretty much constant.

Park: Constant.

Stadtman: Yeah. That's been about it in my own group. But, of course, the way the laboratory has worked is that there's a lot of collaboration among different sections.

Park: Right. I have noticed that.

Stadtman: And so, for example, Levine, who is one of the prominent section heads now, very much involved in almost the same things that we are, he came as a clinical associate in my lab. And after some four or five years, he was made a permanent member of the staff, and then a little later was promoted to a section head, where he has his own group. And [Ann] Ginsburg, who is an expert in physical chemistry of proteins, I hired way

back when we were studying the cascade control of glutamine synthetase because we needed to get some information on molecular weights and things like that of what we were dealing with. She was brought in then as a postdoc, but in the meantime has been elevated to the position of section head. Boon Chock I recruited many years ago now. I forget exactly when. But he was recruited because it became clear to me that we needed someone who was a good kineticist and someone who was familiar with the rapid reaction kinetics, stop-flow type of kinetic studies. And he, after getting his Ph.D. at Chicago, he went to Germany, where he worked with [Feodor Lynen] but there they did a lot of fast-reaction kinetics, and he was considered by his mentor there to be one of the best people he had at the time when I inquired of him. And, incidentally, [Lynen] became a Nobel Laureate. He came because I felt that we needed someone in this general area of expertise. And then, after a number of years, he was promoted to a section head. Then, after another period of time, when I stepped down as head of the laboratory, I suggested that he replace me, and that's what happened.

Park: So it's not surprising that there are a lot of [unintelligible].

Stadtman: A lot. Sue Goo Rhee came initially as a postdoc that worked together with Boon and me on various problems, and then I made him an independent investigator in a tenured position, and then, after a few years, suggested he should be made a section chief. So we have good collaboration between Rhee and Levine and Chock. They're all interested in very similar things

still.

Park: Yeah. Probably the only exception is Dr. Terry Stadtman.

Stadtman: Yeah. Well, she was, of course, assigned to this laboratory. Initially she was up in Building 10 after I moved down here to Building 3. You know, during the construction of Building 10, we were still here. We were all here then. And then when we moved to Building 10, she was working rather closely with Chris Anfinsen<sup>4</sup>, and I was more or less independent then, at that time. And after a few years, I requested permission to come back to this building because I could have more space and freedom to do things. Among other problems in the Clinical Center was that we were studying an organism that created a lot of foul-smelling products, and as chance would have it, our hood had a vent that went up, and the hood opened up close to the air intake that went into the hospital part, the clinical part, and so there were a lot of complaints about the smell. So they were eager to let me come back here. As a consequence, it's been very beneficial for all of us. But then, when Chris Anfinsen left for a period of time--he went back to Harvard for a while--Terry was reassigned to this laboratory down here, so... But I think, as I pointed out to you before, we've never worked closely together on a problem. She's had her area and I had mine.

Park: Another question related to your scientific work is the relationship

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<sup>4</sup> Christian Anfinsen, awarded the Nobel prize for his work at NIH in 1972. At the time Earl is discussing, Anfinsen was the Chief of the Laboratory. When Anfinsen left NIH, Earl succeeded him as Chief.

between biochemistry and microbiology.

Stadtman: Well, we didn't really get into any significant amount of molecular biology until rather recently, and that came about as a consequence of our work on methionine story. It was clear that we needed to take advantage of molecular biology techniques in order to get organisms that had the characteristics that we were interested in. This is officially a section on enzymes, and, historically, most of the work here was on purified enzymes, and that sort of thing went on. But it's very clear that molecular biology together with biochemistry, classical biochemistry, can contribute a lot to programs that neither pure enzymologists nor pure molecular biologists can do. It's really the overlap of the two which can come up with important observations, and that's where I think we'll continue to drift. We'll probably have more and more molecular biological influence in our work, especially when we go to whole organisms for examining what goes on. We need those technologies in order to get at the issues that we're going to be looking at. And in the long run, I think it'll be profitable because we have a lot of biochemical experience, and to the extent that we can gain some insight into molecular biological technologies, we'll be able to complement one with the other.

Park: Arthur Kornberg was in this building?

Stadtman: When I came, when Terry and I came here--I think I may have told you this story; I'm not sure--but I was a postdoc in Fritz Lipmann's laboratory after I left California, so she had to hunt for a position. And when we got



to Boston, she went to Harvard, and Chris Anfinsen gave her an assistantship to work on cholesterol. Then when the Heart Institute started, it was all in this building, this building. And Shannon<sup>5</sup> was head of the Heart Institute at that time, and he brought in Anfinsen to head up a laboratory on cellular physiology, and Anfinsen wanted to bring Terry, but in order to get her, he had to also offer me a job, so that's--we both came. And at that time it was fortunate for both of us because there were laws against nepotism in academic environments, and this, while both of us had been offered jobs elsewhere in academic institutions. This was the first job that was offered where we could both come together in the same place. And so when we came here, Shannon had his office just down the hall here, and he had Steve Brody and... I was trying to think of the name of the other people. It'll come to me. But, strangely, when we arrived here, we were supposed to be in the basement, and there were no labs in the basement at that time. So we were farmed out, and in this building, Kornberg had the first floor. About half of the first floor belonged to Kornberg, who was head of the section in the Arthritis Institute, and he had under him Bernie Horrecker, who was a very outstanding, well-recognized biochemist of his time. You probably know about Horrecker. He did a lot of work on the pentose phosphate shunt mechanism of glycolysis carbohydrate metabolism. [Arthur] Weissbach came a little later into this group. But the senior people were all here. Jack Orloff was

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<sup>5</sup> James Shannon, who went on to become Director of NIH.

here at that time, and the kidney electrolyte metabolism group, and became scientific director [of the Institute]. Again, the names are vague. I have trouble remembering names, especially names. I can see the faces, but I can't recall the specific names. That's something that comes with age. But all of the Heart Institute employees were in this building. Bernie Witkop was here at the time, and he went over to the other Building 2, Building 4, actually, before he moved back again here. And I was trying to think of the name of the guy who succeeded Shannon as the head of the Heart Institute when Shannon was promoted to director. Berliner. Berliner was here at that time, and he became--he was head of the kidney electrolyte group and he became the [scientific] director of the Heart Institute, and then, from there, was promoted to associate director of the whole NIH. But at that time, in this building, there were a lot of very outstanding scientists--Bernie Brody, Berliner, Shannon. I can't think of the name of the guy who was head of the group that was inherited by Hank Fales. But, anyway, we have someplace a list of all of the people that were in this building at that time, and if that's of any interest to you, we can dig it up.

Park: Now, Arthur Kornberg said that he became an enzyme hunter.

Stadtman: Yes. He was really an enzymologist, and he still is. He does mostly enzyme studies. He uses molecular biology to some extent. But when he left here to go to Washington University, he asked Terry and me to go with him, and we considered that, but Terry really didn't want to go

because he was going to be head of microbiology group in St. Louis, and she was the only real honest-to-goodness microbiologist. She felt she'd get stuck with too much of the teaching, so she preferred to stay here, and so we didn't go with Arthur Kornberg, although he wanted us to go with him when he left. We always had very good rapport with Kornberg. He made available a temporary laboratory for us to work in on his floor when they were still building the labs down in the sub-basement. And he lived not too far from where we were living at that time, and so we had a lot of social activities together. But the Heart Institute was small compared to what it is today.

Park: Yeah. Actually, I--next week I want to go back to your very early education, your childhood, and your family background, and your high school education, and then you mentioned your college education, but I'd like to go back to that point. And then, as time goes on, you may talk about the important people around you and kind of have a recollection of them and how you interacted with them in the course of your scientific career. And before that, before leaving today, I'd like to ask you about what kind of instruments did you, have you used most heavily or most dependently. For example, if I want to put one of the instruments you've used on the exhibit, what is it going to be? Not the EPR, but other kinds of [spectrometers].

Stadtman: Well, we used Summerson colorimeter<sup>6</sup> a lot in the early, very early phases of research, and then we had a Beckman spectrophotometer, one of the old types. And, in fact, we were among the first to get the upgrade on the Beckman that had this electronic--it didn't run on batteries, it ran on an electronic-motivated system, and that helped a lot at the time. But we also had a lot of problems with it. It's really curious. When I went to attend a meeting in Japan--and this is 1957--they had an international conference on enzymes in Japan. And while I was there, we went to visit some of the universities' laboratories, and I was rather impressed by the fact that they had instruments that were comparable to what we had here, but they were all Japanese made. In fact, they were all copies of instruments that had been developed in this country. And, among others, they had this Beckman spectrophotometer, and the unit that gave the power to it, they didn't have. And I asked them, "Well, you know, now there's this other instrument," and he said, "Oh, that was a faulty instrument, so we didn't reproduce that one." But, among other things, I was impressed by the fact that in the laboratories at that time, they had a urinal right in the laboratory so that you didn't have to go down to the restroom or anything like that. But I made a lot of very good acquaintances at that time of people in Japan. They've lasted over many years.

Park: Have you devised any instrument for yourself?

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<sup>6</sup> Better known as "The Klett" and formally named, "Klett-Summerson Photoelectric Colorimeter".

Stadtman: Yeah, I did. I'm not very good at electronics, so I haven't done anything with electronics. But I do remember when I was working on the browning of dried fruit in California during the war. We had the problem of visual determination of when fruit had darkened to the point that it was inedible. We had developed a technique where we made extracts, ground up the fruit and made extracts of 50 percent alcohol and then read the concentration of the ground pigment, which was a Maillard reaction product. We read that in the Summerson colorimeter. But then the question was, how can you transfer your measurements in this soluble extract of what the fruit looked like? So I took two cylinders about so big around. Do you know the kind that they wrap cloth around in shopping centers? Fabric. You take fabric and you have these long tubes, cardboard tubes, and they wrap the fabric around that to make a big roll.

Park: Yes.

Stadtman: Well, I got a hold of two of those and I mounted them on a desk and had the end fixed so that I could look down through the thing and see the ends of these tubes. And then I had a slanted area where I crowned up the fruit and packed it into little containers, really jar-top containers, and put it down in the, as an angle which could be, I could see and you could see through the tube. And then I fixed the, took a strip of wood and put a light on the end of it on both these parallel tubes, and I took and, by moving the light away from the fruit, I could move it to a point where even the fresh fruit would look about as dark as the darkened fruit. And so I could get a

visual measurement, how did this vary, and I compared that to the extract measurements so I could get some index of how the fruit looked on an arbitrary scale compared to the normal based on the distance the light was away from the fruit. And so that was something that I just concocted, and it worked very well. You have a reference point on the one side, but the other side you could move and compare. So you could take a darkened sample and put it on a fixed scale and move the normal fruit to a point where it looked like the darkened fruit, and from the distance you would have a--since the light varies as the square of the distance, you could calculate how much light was required in order to mimic this or that. It worked very well, actually. So little things like that. I made distillation systems, glass blowing to collect samples, fatty acids and things like that.

Park: When your lab moved to Building 50 in April, do you have any instruments or that you don't want to carry with you? Anything that you want to donate to the museum?

Stadtman: I think all of the instruments that we didn't want have already been surplus, so that's not a possibility. There were a few instruments that might have been interesting. The Warburg is one of the instruments which we're going to take with us, but we had two of them, and one of them has already been claimed by the people who want to set up something with that. Maybe you're aware of that. I don't know.

Park: \_\_\_\_\_.

Stadtman: It's for the museum.

Park: Yeah, for the museum. Dr. Terry Stadtman's lab. So we... I'm thinking of putting it on the exhibit if possible.

Stadtman: We want one. We're moving one with us because you can do things in the Warburg that are not easily done any other way, and if you're familiar with the technology, which we are, you know when that situation arises<sup>7</sup>. And, unfortunately, you can't buy Warburgs anymore. No one makes them. So if you want one, you've got to have one or know where you can get an old one from somebody. But in terms of instrumentation in the current work, we use an awful lot of sophisticated spectrophotometry. We have a system that allows us to use redox characteristics of compounds coupled with electrophoresis or with separation techniques<sup>8</sup>.

Park: For enzyme purification, do you use any particular instruments?

Stadtman: We use a variety of techniques. We still, I'm still old-fashioned. I always look first with ammonium sulfate fractionation to get crude separations of the enzyme I'm looking for, and then we go and we use other technologies such as affinity chromatography or hydrophobic chromatography, various chain lengths, and whatnot. I mean, we use a lot of column chromatography for purification of proteins. We still do a lot of radioactive...

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<sup>7</sup> He and Barbara Berlett did perform studies in Building 50 with the Warburg. As he states here, they just couldn't have been done otherwise. After his death, his Warburg was donated to the NIH Museum.

<sup>8</sup> He's referring to an electrochemical detector that was interfaced with a high pressure liquid chromatograph (HPLC-EC). [*comments by Rodney Levine, Ph.D.*]