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Molecular hermeneutics and unusual sorbets: An interview with Barbara Ehrlich

Transcript of a conversation between Elizabeth M. Adler and Barbara E. Ehrlich[1,2]

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Transcript condensed and edited by EMA

EMA: This is Liz Adler, the Executive Editor of *The Journal of General Physiology*. I'm in Nassau, Bahamas, at the FASEB Science Research Conference on Calcium and Cell Function, speaking with Barbara Ehrlich of Yale.

BEE: Hi, Liz.

EMA: Hi, Barbara. Thank you so much for agreeing to do this.

BEE: It's nice to sit down and have a chance to chat.

EMA: If I look at your website, it lists you as the head of the Laboratory of Molecular Hermeneutics. So, I have to ask you, what are Hermeneutics, and what are Molecular Hermeneutics?

BEE: Hermeneutics is a philosophical discipline, and it comes from Hermes, who was the Messenger of the Gods. And messengers had to interpret: They would not just deliver the message; they would have to interpret the message. So Hermeneutics became the exegesis of the Bible—so interpreting the Bible—and eventually it became interpretation, and especially of Truth and Beauty. So, that's what Hermeneutics means.

Now, how did I come to Molecular Hermeneutics?

I was at the University of Connecticut in Farmington in a cardiology division; that was my first position as an independent. I had a wonderful Chief of Cardiology, Arnold Katz. And one day I came in and he had put up a sign. It was the Division of Molecular Cardiology.

And I went up to him and I said, "Arnie, you don't know what a molecule is; you're a cardiologist."

And he harumphed around, and he said that he wanted to call it Molecular Cardiology because everyone who was modern had to be molecular.

So I went home and thought about this, and I decided, well, if he's going to have a sign saying "Molecular Cardiology," I'm going to make a sign and I'm going to be "Molecular Hermeneutics." Because we were interpreting Biology, which I consider to be part of Truth.

There are people that come and they want to have a *named* chair, and they come and ask advice and I say, "Does it really matter to you that you have a *named* chair?"

And they sort of look to the left and right and say, "Well, yes," and I say, "Then just give yourself a name."

EMA: [laughs]

BEE: They say, "Can you do that?" and I say, "I'm the Director of the Laboratory of Molecular Hermeneutics!"

These names—they have a value—but they can also be a little silly. Because, really, what we're trying to do is do the science, and the extra parts, like the named chairs, they're nice, but not necessary.

EMA: When I first met you at Woods Hole, if I recall, you were working on a voltage-gated calcium channel in paramecium. Is that right?

BEE: That's correct.

EMA: But most of the work of yours that I know about since then has involved ligand-gated intracellular calcium channels.

BEE: So how did that change?

EMA: Yes. How did it change? How did you get interested in either paramecium or ligand-gated intracellular calcium channels?

BEE: Well, I've been interested in calcium signaling, and paramecium were chosen because they're the most primitive organism that are known to have voltage-gated calcium channels, and that sounded like a great system to work with.

Most paramecium live in fresh water, which makes it very difficult to look at ion channels. But there is *one* paramecium species, *Paramecium calkinsii*, that lives in sea water. And, actually, it's one of the most osmotically tolerant organisms; it can live in brackish water, and it can live in, I think, 4-times sea water. So it's very tolerant. So we were interested in looking at how those voltage-gated calcium channels compared with mammalian.

We were working on that for quite a while, but it turned out that paramecium have an unusual genetic code— protozoa do—and they're also a thousand ploidy, so it's very hard to do anything with genetics, and a lot of the molecular tools and antibodies were not cross-reacting.

EMA: Right.

BEE: So it became very hard, and a colleague across the hall was looking at calcium signaling in smooth muscle, and had the idea that IP_3-gated signaling was with an ion channel that was in the ER. So we started looking at that, and the experiments were easier to do and the students wanted to work on that more than the paramecium, so slowly the paramecium experiments stopped and we worked more and more on intracellular calcium channels.

It was *hard* to make the decision, but I've *never* looked back and regretted that switch.

EMA: So you started looking at the IP_3 receptor channel.

BEE: Yes.

EMA: Can you tell me a little about it?

BEE: I was working with Jim Watras, who was at the University of Connecticut, in the same cardiology division—he's a spectacular biochemist—and he would isolate microsomes, which were basically pinched off ER membranes, from smooth muscle.

Eventually, we switched to brain, because IP_3 receptors are more concentrated in the cerebellum than any other organ that it's known. No one understands why, or at least I have no idea why. The Purkinje cells of the cerebellum are basically calcium machines. They have more of anything that's related to calcium signaling than . . .

EMA: Really? I didn't know that.

BEE: Yes. It's probably 1,000 times more IP_3 receptors than any other cell type.

He would isolate the microsomes. And then, at that point, I was putting channels into planar lipid bilayers, and I would sit at the bilayer, and he'd bring in the microsomes, and we'd look and see if we could see ion channels—and we did. That was a very exciting time.

We still look at IP_3 receptors; or the ryanodine receptor, which is another intracellular channel; or polycystin-2, which is a third intracellular channel. We've been looking at how they all regulate each other.

EMA: Are they all located in the same intracellular compartments?

BEE: As far as we know, in cells that have all three, they're all in the same ER. There's subcellular localization of the different channel types, and even the different isoforms of the IP_3 receptor are localized in different parts of the cell.

So, for example, in a liver cell, at the apical end, it's predominantly the type 2 IP_3 receptor, and throughout the rest of the cell is the type 1 IP_3 receptor. They have small differences in their function, which seem to correlate with their localization. And different cell types have different ratios of the different isoforms and different locations.

EMA: How interesting.

BEE: Yeah, we found that really interesting. Not only is it subcellular, but the hippocampus, for example, is the organ with the second highest concentration of IP_3 receptors. And CA1 and CA3 have totally different concentrations of IP_3 receptors.

EMA: Really?

BEE: We've enjoyed looking at the variations among tissues, and just even in what's in the same cell, and then, in disease, there are changes.

EMA: What kinds of changes?

BEE: There's different ways. You can get increases, you can get decreases, and you can have loss—total loss. Let's see; in bile duct obstruction, all the IP_3 receptors disappear. That's hard to understand.

EMA: From the bile duct?

BEE: From the cells of the bile duct.

EMA: If you have total loss of IP_3 receptors, like in the bile duct, do you have an increase in ryanodine receptors to compensate? Or do the cells just become incompetent at releasing intracellular calcium stores?

BEE: So in the bile duct, we think, because it's an obstruction, the cells are no longer secreting, and you want to stop the signaling. So we think it's a compensatory response to slow things down. In some forms of heart failure, there's a decrease in ryanodine receptor, and in many of those the IP_3 receptor is elevated to compensate. So you get different kinds of compensation depending upon different systems.

EMA: It sounds incredibly complicated, with all the different receptor types.

BEE: Yeah, it actually gets complicated when you try to include all the different receptors. And, I think, to me, that's what makes being a scientist such a great joy; I know that we're both puzzle-doers, and, to me, this is another one of the puzzles that we like to do, is to try to figure out how the different pieces all fit together.

EMA: It's true. It's an interesting puzzle.

BEE: I don't think it's like the puzzles that you get in a magazine; I don't think we'll ever really get to the complete answer. But we make progress in figuring out how things are interacting.

We're very interested in figuring out some of these disease things, but starting from basic principles. There's a decreased amount of polycystin-2 in cells in polycystic kidney disease, and people made the hypothesis that polycystin-2 only *starts* the process, and then you need to propagate calcium throughout the cell, and to get the propagation, you need to have activation of ryanodine receptors.

And my students and I thought, "Well, that's a very interesting idea, and if you think that's true, let's look at a cell that has the most ryanodine receptor you could imagine, because then that would give you an exaggerated response."

So we thought, "OK, let's look at heart cells, because heart cells need ryanodine receptor."

So, based on this idea that polycystin-2 should activate ryanodine receptors, we started the biochemistry: "Do they bind?"

And we found, "Yes, they do."

"Is there a functional effect of this binding?"

"Well, yes, there is—just with looking at the two proteins by themselves."

So then you have to go and see, "Well, does that have any effect on the intact cell?"

And so, we looked, and yes, it *did* have an effect: If you lower polycystin-2 in a *heart* cell, you get a change in the calcium signaling—but you need calcium signaling to get the heart to contract.

EMA: Right.

BEE: So, then we thought, "Gee, that should have an effect on heart function."

So, in fact, we've looked at two models. One is a zebrafish model—people have made a total knockout of polycystin-2 in a zebrafish—and we found that there were actually big changes in the heart function. But, it was a problem, because a zebrafish only has a two-chamber heart.

EMA: Right.

BEE: Now, we like zebrafish because it has a heart rate of about 100, which is more like a human.

EMA: Uh-huh.

BEE: But everyone said, "No, you have to do it . . . first of all, you have to do it in a *mouse*," which has a heart rate of about 500 beats per minute, and "second of all, you have to do it in a heterozygous, because that's more like the human."

EMA: Right.

BEE: So we've gone ahead and now looked at mouse, and found that yes, indeed, there are changes in the heart of heterozygous mice that seem to be similar to what you would expect in humans. So then you ask, well, is this really a problem in polycystic kidney disease, because it's *kidney* disease.

EMA: Right.

BEE: But, it turns out that most people who have polycystic kidney disease die from cardiovascular effects. The cardiologists and nephrologists would say, "Well, of course, that's because the kidney is distorted, and they get renal hypertension, and that causes the heart . . ."

EMA: Oh, of course. Yeah.

BEE: But it turns out we were lucky enough to collaborate with a group of people at the Mayo Clinic who had genotyped patients, and they had patients who had mutations in polycystin-2 that were nonhypertensive; so they were normotensive. They were young, so they had almost no cysts in their kidney. And yet, they had dilated cardiomyopathy [*editor's note*: see Paavola et al., 2013].

EMA: Huh.

BEE: So the incidence of dilated cardiomyopathy in the normal population is about 1 in 2,500; maybe less than that, even less. In polycystic kidney disease, with mutations in polycystin-2, it's about 1 in 11.

EMA: *Wow*.

BEE: So it's a huge effect. So we're trying to figure out why it is that you get this effect. But it's all based on this initial basic science observation.

So, if I were to say something that I thought was really important for young people to think about, it's that you start with the basic science idea, and if you know the system really well, you can then take that into a pathological situation; you can suggest how the system has stopped working. It's very hard to start with the idea, saying, "We're going to discover why people have one ear bigger than the other." I don't know.

EMA: [laughs]

BEE: It's hard to start there, and then go back to the basic science. So it's been rewarding to start with these very clear physiological processes, and know how they're working, and then see that, if you just change a little bit, you can get into a pathological situation. So we try to do that in all of the projects in the lab.

EMA: Did you have a favorite project of all of the things you've worked on? Is there one that was a more interesting puzzle?

BEE: Well, that's really a hard question to answer. I guess you feel like the one that you're working on now is the one that's drawing you the most. We have two in the lab. One is this polycystic kidney disease. The other one that I'm very excited about is another example of how serendipity has run a lot of the projects in my lab.

I was sitting in my lab one day, and I got a call from a chemist, at Yale, and he had Googled "PC12 cells" and "Yale." PC12s are a neuroblastoma cell line, and my name came up, because we had done experiments with PC12 cells. And he was looking for some living PC12 cells. So his postdoc and my postdoc were going to meet halfway at a coffee shop, and I asked him the important question: "Why does a chemist want living PC12 cells?"

Well, he was doing a phage display of natural product. He said, "Well, we were using taxol, which is taken from the Pacific Yew tree. It's a molecule used to treat cancer."

And I said, "Oh, that's interesting. What did you find?"

He said, "Well, we found a nonstandard binding partner for taxol." Everyone knows how taxol treats cancer—it stabilizes microtubules.

I said, "Well, what is it?"

He said, "Oh, you never *heard* of this."

I said, "Well, try me."

And he said, "Well, it's this protein called neuronal calcium sensor-1 (NCS-1)."

I said, "No way."

He said, "What do you mean, 'no way'?" And our paper looking at NCS-1 regulation of the IP_3 receptor had been accepted, but it was before [the] papers had been online [*Editor's note*: see Schlecker et al., 2006].

EMA: So it had been accepted but not yet published?

BEE: Right. And I told him this, and so, an hour later, the four of us were sitting in the coffee shop, talking about experiments. I'll fast-forward 10 years: Based on that, we think we know why, when people get chemotherapy from microtubule-based chemotherapeutics, they get peripheral neuropathy—this is pain or numbness in their hands and feet.

We've done 11 years' worth of work; we pretty much know the pathway, and based on that, we think we have figured out a way to prevent the negative side effects without affecting the ability to treat the cancer. So we're pretty excited about this.

EMA: Oh, sure.

BEE: And it works in mice. So I'm pretty excited. Because part of why I became a scientist: One is to interpret Truth . . .

EMA: Right.

BEE: And the other is to actually think if we can find a way to help people.

EMA: Uh-huh.

BEE: Going back to Arnold Katz, my first chairman, he always used to say, "It's the National Institutes of Health, not the National Institutes of Interesting Biological Phenomena." So he was happy that we did basic science, but it had to have some goal in the future that would have an effect on health. So that's been sort of, I think, our goal on all our projects, and starting as much as we can from the basic, and moving in that direction.

I'd say that right now it's those two projects, the polycystin-2 in the heart, and seeing that we can figure out from basic ideas what may be happening in people, and the NCS-1 and chemotherapy. These are projects that we're all very excited about.

And of course, there's the irony of all this, which is that they all lead back to some of my initial work that I did from being a graduate student; some of my initial work was looking at lithium as a treatment for bipolar disease, and it turns out that NCS-1 is elevated in bipolar patients.

EMA: Really?

BEE: Well, at least as far as we can tell. And it looks like one of the things that may help in chemotherapy-induced peripheral neuropathy may be treatment with lithium.

EMA: Really? That's amazing.

BEE: It works in mice. We'll have to see if it works in human beings.

EMA: It's really amazing that it circled back to the lithium.

BEE: So it circled back, all the way back to my graduate student days. So you can't predict what's going to come back into your life.

My postdoc was looking at paramecium that are ciliated, and one of the ironies there is that there's this hypothesis that polycystic kidney disease is a disease of *cilia*. Which may or may not be true, but, again, I have this long history of understanding things about cilia, because the voltage-gated calcium channels are—actually, there's this really nice work done by Kathy Dunlap showing that they're all on the cilia [*Editor's note*: see Dunlap, 1977].

EMA: Oh, really?

BE: Yes.

EMA: The voltage-gated calcium channels?

BEE: So if you take a paramecium and you deciliate them, they have no voltage-gated calcium currents any more. And the currents return as the cilia regrow. It's really beautiful work that she did, when she was . . . I think as a student, maybe as a postdoc.

EMA: It must have been a while back.

BEE: Yes, a long time ago; so there's these things that seem to cycle back.

EMA: Do you have anything else you feel like talking about that I haven't thought to ask?

BEE: I guess the only other thing that I could say is that I have a hobby that also is part of being a scientist and making puzzles . . . or putting pieces together, which is I started making ice creams and sorbets. I've started making *many* ice creams and sorbets, and I have a *huge* collection of cookbooks, ice cream

cookbooks, and I put together flavors that are not standard. My two most popular-but-not-standard flavors were jalapeno-pineapple sorbet and an avocado–passion fruit sorbet. So those have been my big successes.

My students get to be the tasters.

EMA: Does that draw people to your lab?

BEE: It has both good news and bad news, because I tried one that was a failure; I tried to make a green apple–wasabi sorbet, because I thought the colors would look nice together, and I like the mixture of sweet and spicy. But I learned that the wasabi you get in a can is not actually wasabi; it's actually horseradish with green food coloring.

EMA: Really?

BEE: And, when you mix it with green apples and sugar, there's something that happens chemically that makes it so that it has a very foul taste.

EMA: [laughs]

BE: So my first students who tasted it were pretty surprised. And, of course, there was an issue with the amount of heat that came out. So I think the first student had both the foul taste and the heat, and you could almost see the steam coming out of his ears.

EMA: Had you tasted it first?

BEE: No, I hadn't. Actually, I think I tried it when I first mixed them together, and it was fine.

EMA: So it was something that developed.

BEE: But it didn't take too long.

And that's, I think, an important part of being a scientist: you can't help but carry it over into other parts of one's life, and I like to experiment with the different flavors and combinations of flavors. Also, it becomes something that can be done for everyone in the lab.

EMA: Sounds like fun. Thank you again for agreeing to talk with me.

BEE: Thank you, Liz. It's great to see you as always.

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