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Solving Biochemistry's Biggest Mystery: How We Produce Energy

Part 1: The discovery of coenzyme Q-10.

An Interview with Dr. Fred L Crane

by Richard A. Passwater, Ph.D.

More than half of the people in the United States take a daily vitamin supplement. Most of these individuals don't even realize that this was not possible not too awfully long ago. Thanks to a small number of scientists, we can improve our health and reduce our risk of disease.

Have you ever wondered about the scientists behind the supplements that make and keep you healthy and how difficult it was for them to make their discoveries? How did it happen? This story is about one of these scientists and the "detective" work that went into the discovery of coenzyme Q-10 (CoQ).

One of the neat things about being a scientist is that I have had the pleasure of knowing and exchanging ideas with many other scientists, including those who have discovered most of the vitamins and other nutrients that we take in our daily supplements. Consequently, it takes me longer to take my vitamins than most people. Usually, while I hold the pills in my hand I often think about how many people have been helped by vitamin supplements.

Often, I think about my friends who discovered the vitamins and made it possible to benefit from the supplements. I fondly remember sharing the podium with the discoverers and/or synthesizers of several vitamins, among them Dr. Albert Szent-Gyorgyi (vitamin C), Dr. Roger J. Williams (pantothenic acid and folic acid) and Dr. Karl Folkers, (vitamin B-6, vitamin B-12, and biotin). Maybe after reading this interview with Dr. Fred Crane, you, too, will think of him occasionally when you take your CoQ supplement and are reminded about how important his discovery of CoQ is to our health.

This story is not just about the discovery of a compound but the discovery of an important biochemical pathway. When I was studying biochemistry in the 1950s at the University of Delaware, I considered the two greatest unsolved mysteries to be how the body produced energy and what was the structure of DNA. The latter problem often is described as one of the more difficult mysteries. But, in my opinion, it was nowhere near as difficult as the former. Most scientifically oriented people are aware that Drs. James Watson and Francis Crick elucidated the structure of the double helix of DNA in 1953, and that after everyone accepted this over the next few years, understanding this

structure had led to many important understandings and advancements in biochemistry.

The harder problem, however, has been to elucidate the chemical process of how we convert food into energy. Even today, the eyes of many biochemists glaze over when the topic turns to the movement of electrons along the respiratory chain to produce energy. A good way to impress other biochemists is to bring up the topic, and, like dropping names of important people to impress a friend, spit out the details of the transfer of electrons and proteins along one of the complexes in the respiratory chain.

Unfortunately, few people, including most scientists, are aware that Dr. Peter Mitchell was awarded the Nobel Prize in Chemistry in 1978 for unraveling the mystery of how energy is derived from reactions in the mitochondria in cells to produce energy. Dr. Mitchell developed his hypothesis by integrating the experimental results from a handful of laboratories working in the field of bioenergetics. Dr. Mitchell's "chemiosmotic hypothesis" was developed in 1961, but it was rejected by most of the mainstream scientists at the time.

Dr. Crane is best known as the discoverer of CoQ. However, this discovery was much more important than discovering an important compound that is a conditional nutrient. This discovery was the missing link in understanding how our bodies produce energy.

The biochemical detective work of Dr. Crane is a great story and this is what we will chat about shortly. In this column, Dr. Crane will share with us some basic information about CoQ and the thrill of the chase in making this all-important discovery. We will not bog the story down with biochemistry, but for those interested in the biochemistry behind the story, we will present that more technical information in a series of separate "text boxes."

The work of Dr. Crane leads us to ponder many deep and difficult questions, including the following:

- What are the compounds necessary to produce the energy of life?
- What compounds had to be thrown together to sustain the production of energy?
- What is "life"? (We can leave the discussion of "What is the meaning of life" for another time.)

Let us begin with the premise that live organisms produce their own energy that carries out all of the life processes, including growth and repair. Live organisms also can gather and assimilate stored energy sources—such as food—and convert it into chemical energy that drives the life processes of the body.

Energy and repair are critical to life. If we can't make energy, we can't repair ourselves. Therefore, making energy is the single most important requirement for life. How do we make this essence of life called energy? Dr. Emile Bliznakov, whom we will chat with in a future interview, sums it up with the following formula: **CoQ = Energy = Life**.

Of course, we had long known that food was first broken down from large polymeric compounds into their component monomers and dimers by digestion in the gastrointestinal tract. Then these smaller compounds are taken into cells where they are further broken up into very basic compounds and two-carbon fragments of compounds. These small fragments are then converted to water and carbon dioxide by combination with oxygen.

Normally, this process of combining these compounds and fragments in open air produces visible burning accompanied with the release of considerable heat. How does the body combine oxygen with food without burning itself up and how can it store the energy that is produced? This process in the body is called the "respiratory chain" or "oxidative respiration," and it involves moving electrons along a path (sometimes called an electron transfer chain).

In science it is often helpful to bring in fresh ideas and techniques from other fields of expertise. Thinking "outside the box" is an overused cliché today, but believe it or not, many people have done that throughout history without the benefit of the cliché. This interview illustrates how fortuitous this can be. Dr. Fred Crane was able to use his experience in one field to solve a major mystery in another field. He was the right scientist in the right place at the right time—even though it may not have appeared that way.

Passwater: In the 1950's, we knew only small portions of the mystery of how the body turned food into energy. We had to look at the gaps in our knowledge as mysterious "little black boxes" in which unknown chemical reactions take place. At that time, we knew that groups of chemicals called "complexes" serve like little buckets to carry electrons along the membranes of mitochondria where energy is produced, but not much more than that.

At the time you discovered CoQ, it was not known how energy was produced in the body. Was it understood how the body stored energy?

Crane: Well, it was known that the body made adenine triphosphate (ATP) and creatine phosphate, and that other compounds were made through reactions that utilized ATP.

Passwater: But we didn't know how ATP was created?

Crane: The crucial thing that we were looking to learn was how ATP is produced in the mitochondria of the cells. Mitochondria are the powerhouses of the cells. They can be

considered little chemical energy factories in cells that provide the energy or power to make other reactions happen.

Passwater: So there you were, involved in solving one of the most important discoveries in animal physiology, yet you trained in plant physiology. Why did you decide to become a plant physiologist, and why did you switch to animal physiology?

Crane: I found the chemistry of plants very interesting. But then, in 1952, I was invited to join the Enzyme Institute at the University of Wisconsin under Dr. David Green's group there. Their main project was to determine the function of heart mitochondria.

Passwater: How did your chain of research take you from your original quest to the point where you isolated this yellow substance and determined that it was what you were looking for?

Crane: It took time because I didn't know that much about enzymes even when I went to work for Dr. Green. As we discussed, I got my degree in plant physiology and studied niacin synthesis in plants, but they took me as a postdoctoral student and started teaching me how to do enzymology. I worked there for some years and actually at first we were studying fatty acid oxidation by mitochondria. Eventually, we found some enzymes which were involved in fatty acid oxidation and in the process I found a new enzyme, the electron transferring flavoprotein, which helped carry electrons from the dehydrogenase enzyme to the mitochondria system.

Passwater: For our non-biochemist readers, enzymes are proteins that facilitate chemical reactions in the body. Without enzymes, chemical reactions would not occur or would occur at speeds too slow to do any good. If you are interested in how the enzymes function in the energy process, please see Box 1.

Box 1

How Enzymes are Classified

There are six major classifications of enzymes. An important class of enzymes is the oxidoreductase class. Oxidoreductases catalyze oxidation and reduction reactions. This class can be subdivided into reductases and oxidases.

Enzymes called "dehydrogenases" are reductases. Their function is to catalyze the removal of a pair of electrons (and usually one or two protons) from another molecule. The name arises from the fact that protons are actually hydrogen ions (H^+) and that two electrons plus two protons is actually one hydrogen molecule (H_2).

The compound that gains an electron is said to be "reduced." (Its oxidation number or positive valence is lowered because of the addition of a negative charge.) A compound that adds an electron to another compound is called a reducing agent. The same term "reducing agent" is used for a compound that adds hydrogen to another substance. Thus, a reaction in which either hydrogen or an electron is added to another substance is called a "reduction."

An "oxidizing" reaction is the opposite from a "reducing" reaction. There are also enzymes called "oxidases" which help transfer two electrons from a donor compound to an oxygen, resulting in either water or hydrogen peroxide being produced. (Oxidases are not to be confused with "Oxygenases,"

which catalyze the incorporation of oxygen into compounds.) An atom or molecule that loses an electron is said to be “oxidized.”

Another major class of enzymes called “transferases” help transfer functional groups between donors and acceptors.

While we are discussing biochemical nomenclature, it is useful to know that “coenzymes” are organic compounds that associate with enzymes and affect their activity. Many vitamins serve as direct coenzymes or as portions of coenzymes.

So, Dr. Crane, your first important discovery in this process was not CoQ, but the enzyme, the electron transferring flavoprotein. At the time you isolated this enzyme, did you understand what its function was, or did that come later?

Crane: We knew these enzymes were necessary for getting the electrons out of fatty acids to go to the synthesis of ATP. But what we were working was just the front end of the story—in other words, the dehydrogenase that did the first step.

Passwater: Is this the little black box we now call fatty acid dehydrogenase system?

Crane: Yes, it is this fatty acid dehydrogenase system that actually feeds in separately through the electron transferring flavoprotein which reduces CoQ.

Passwater: For our non-biochemist readers, this system in “reducing” CoQ means either that CoQ has gained an electron (thus its valence state has been reduced) or that hydrogen has been added. This is the opposite of oxidation. See the figure below to see how electrons and hydrogen ions (protons) can change CoQ back and forth stepwise between its oxidized state and its reduced state, which is written as CoQH₂.

(Figure)

In order for CoQ to act as an antioxidant in the body, it must be in its reduced state. However, in the energy-making process, CoQ works by changing back and forth between CoQH₂(reduced) and oxidized CoQ. This process wherein a compound moves back and forth between its oxidized and reduced states is called a “redox” system.

Crane: I don’t know where in the current biochemistry texts you might find an effective representation of this system in which fatty acid dehydrogenase feeds in separately through the electron transferring flavoprotein to reduce CoQ. Nevertheless, this was a fatty acid oxidase sideline.

As a result of finding this new enzyme, Dr. green got some confidence in me and put me on a major study of breaking up the mitochondria into pieces to try to find the

individual parts that are involved in the overall electron transport. That is what eventually led to the discovery of the complexes I, II and III.

Passwater: So now, instead of having little unknown “boxes”, we now know the structures of the chemical compounds that group together as “complexes” to function. In order to isolate enough of these compounds, how much beef heart would you have to start with?

Crane: We had huge amounts of beef heart. We got our beef from Oscar Meyer in large tubs and they had a big 13-liter centrifuge downstairs where they centrifuged the ground-up beef heart. We would take a liter of concentrated mitochondria at a time for our experiments so we had a huge mitochondria factory. I guess it took about ten pounds of beef heart to give us a liter or so of concentrated mitochondria.

Passwater: Why did you choose the heart and not the liver?

Crane: Dr. Green chose the heart. I think he got grants from the National Heart Institute, and that is probably the most important reason.

The year was 1956. We were fractionating the mitochondria membrane with detergents so we would separate out various fragments. Some of the fragments would have dehydrogenase activity, some of them would have cytochrome reductase activity, and of course, there was the well-known fraction which had cytochrome oxidase activity. That had already been done years ago. So, we were getting pieces, but the idea was that in order to prove the structure you had to put the pieces back together again.

Passwater: A cytochrome is a red or brown protein that contains iron in the form of a “heme” group. There are three types of cytochromes, a, b and c. Subgroups within the main classes are designated with a numerical subscript. Cytochromes serve to transfer electrons by means of oxidoreduction cycling of the iron atoms in the heme groups. An enzyme that helps oxidize cytochromes is called a cytochrome oxidase, whereas an enzyme that reduces a cytochrome is called a cytochrome reductase.

So, if a part of the system were missing, then putting all of the known ingredients back together would not produce energy. Only when the system would function normally, would you prove you had isolated all of the components.

Crane: We could put cytochrome c reductase back with cytochrome oxidase and that part would work, but when we took the dehydrogenases off from what was eventually the cytochrome bc_1 complex, then we would lose cytochrome c reduction, we would lose oxidase activity, we lost everything. We knew there was something missing.

Passwater: Other researchers had seen pieces of this puzzle but they weren't able to figure out what was missing, and this was what you zeroed in on?

Crane: Yes, exactly. As I remember, there were only three or four labs that were really studying this, and I don't know that any of them took the approach that Dr. Green took of breaking the mitochondrial membrane up with detergent and then trying to put the pieces together. At the time, Dr. Britton Chance of the University of Pennsylvania was studying the spectrum of cytochrome. He was studying cytochrome b, cytochrome c₁, cytochrome a, and cytochrome a₃ in the mitochondria.

Passwater: He had invented a dual-wavelength spectrophotometer to obtain the spectra of individual compounds through these murky solutions.

Crane: This is how he made a terrific advance for all of us. He managed to get us to a point where you could see the cytochromes through this milky mess.

Passwater: That's how I met Dr. Chance. I helped refine his instrument into the Amino-Chance Dual Wavelength Spectrophotometer. You say there are only three or four groups that were looking into it. I think this is one of the greatest mysteries of all time and I am surprised that there weren't several dozens of groups looking into it. I guess it was too difficult a problem for many to tackle.

Crane: There were a lot of groups working with isolated mitochondria but there weren't very many taking them apart like that. They didn't have enough mitochondria. We had the unique advantage that we had so much mitochondria and actually Dr. Green was a bit of an iconoclast. The belief at that time was that beef heart mitochondria were not good mitochondria and that the only true mitochondria were liver mitochondria.

Dr. Albert Lehninger and Dr. E. Slater and other researchers such as Dr. E Racker, working with liver mitochondria. They would kill a few rats every day and make a little bit of concentrated mitochondria. They couldn't do much fractionation with them because they didn't have enough mitochondria to fractionate.

Passwater: You would think that maybe they would have considered beef liver instead of rat liver.

Crane: Eventually they did.

Passwater: So you were setting about to isolate one or more unknown compounds from this mess.

Crane: At first we were isolating the different parts of the system. In other words, we were isolating the flavoprotein dehydrogenases, which we could identify. Then we would divide the mitochondria extract into a red fraction and a green fraction. The red fraction had the cytochrome b and c and the green fraction had the cytochrome a.

We hoped that if we put the red and green fractions back together, that the system would work—but nothing happened. I got to thinking that we were missing something.

So we got a big batch of mitochondria and sent it over to Wisconsin Alumni Foundation for a vitamin analysis on the assumption that maybe some coenzyme was missing.

They came back with an analysis that the mitochondria have almost all the vitamins in them you could think of, except they didn't analyze for vitamin A. Not knowing very much about Vitamin A, I had to go and try to find out how much vitamin A was in the mitochondria. I learned how to assay for vitamin A, but I didn't find any vitamin A. All I found was a little bit of carotene.

This was partly because I used to go in on Saturdays and Sundays while no one was in there and work on cauliflower mitochondria. Being an old plant physiologist, I thought this was lovely opportunity to study cauliflower mitochondria.

Passwater: Why cauliflowers?

Crane: Cauliflower buds are a rich source of mitochondria.

Passwater: Did studying the cauliflower mitochondria help?

Crane: Well, I got some cauliflower mitochondria and the funny thing was they were yellow, whereas beef heart mitochondria were brown because of all the cytochromes. The cauliflower mitochondria were yellow, so I thought, "gosh, they might have carotene in them. I looked into the beef heart mitochondria and, sure enough, they had some carotene, but there was something else there, too, and in much greater quantity. That's where we found this CoQ.

Passwater: That's a great story in itself. Because of your experience in plant physiology, you wanted to look at plant mitochondria, which do the same energy conversion as animal mitochondria, but have less cytochrome to obscure the yellow color, which you then set out to identify. It wasn't a carotenoid, but it was another yellow substance. Did you then analyze it?

Crane: Yes. It turned out it was very easy because there was so much of it. I used a technique called column chromatography that separates the various components from complex mixtures on the extract with the carotenes and all. The chromatography process resulted in the carotenes being separated into a little tiny band up at the top of the column, and then there was this great big yellow band down below.

So we collected the big yellow band and stuck it in the fridge. About a week later, we looked in and my goodness, there were great big yellow crystals in this preparation.

Passwater: What is the significance of the crystals? Purity?

Crane: Crystallization is the last step in purification of a compound.

Passwater: You were probably getting pretty excited by this time.

Crane: We thought, “Gee, this must be something.” I actually kept thinking of vitamin A. So we analyzed the stuff in the spectrophotometer hoping to obtain qualitative information from its spectrum.

Passwater: The wavelengths at which absorption peaks occur in the spectra often supply some information as to what compounds or class of compounds may or may not be present. What did you find?

Crane: We found the spectrum had a peak having a maximum at 275 nanometers, whereas vitamin A has its maximum at 290 nonometers. My first thought was that this could be a modified form of vitamin A. I then got to looking at it. In my plant biochemistry training, I had been indoctrinated in the idea that a class of compounds called “quinones” could be involved in respiration because they can easily be oxidized and reduced, and plants have a lot of quinones.

I thought maybe there is a quinone in here. So we went to the library and got a book by Dr. R. Morton, in which he described the spectrum of a strange compound he had found that happened to have the same spectrum as the compound that we found. He claimed it could not be a quinone because the absorption maximum was not in the right place. A quinone is supposed to have its peak absorption at 265 nonometers.

Dr. Morton did analyze how one could determine a quinone formation. We tested various things and found out our substance was acting like a quinone. We could reduce it with hydrides and other reducing agents. Going by my old plant physiology experience, I kept thinking this must be a quinone. And, when we actually determined the structure, it turned out that is what it was.

Passwater: We have a very fortuitous event that a plant physiologist was looking at mitochondria from animals with certain knowledge in hand and then made several deductions.

Crane: It was well known at the time that animals don’t have any quinones.

Passwater: Oh, I see, that was “well known.” Ha!

Crane: This is where you run into the little beauties of life.

Passwater: I can imagine what happened when you announced to the world that you had isolated this quinone-like substance and all of the establishment voices insisted, “There is no quinone in animals.” Your discovery, presumably, was not received with ready belief or great joy.

Crane: Actually they didn’t dispute that some kind of compound was in animals, but they argued that what we’d found was just an artifact of a tocopherol oxidation. They were

trying to say this was just tocopherol quinone, which it wasn't. The spectrum wasn't anywhere near that of tocopherol.

Passwater: What did you call this quinone that you had isolated?

Crane: We started out calling it Q-275 because it was a quinone and it had an absorption maximum of 275 nonometers.

Passwater: Did you describe this in the scientific literature at this point?

Crane: Yes, we submitted our findings to *Biochemica et Biophysica Acta* and it was published in 1957. [Isolation of a quinone from beef heart mitochondria. Crane, FL; Hatefi, J.; Lester, RL and Widmer, C. *Biochemic et Biophysica Acta* 25:220-221, 1957].

Passwater: And so history was made. Let's stop with the publication of your discovery and continue in our next installment with an account of how CoQ actually helps produce energy. **WF**