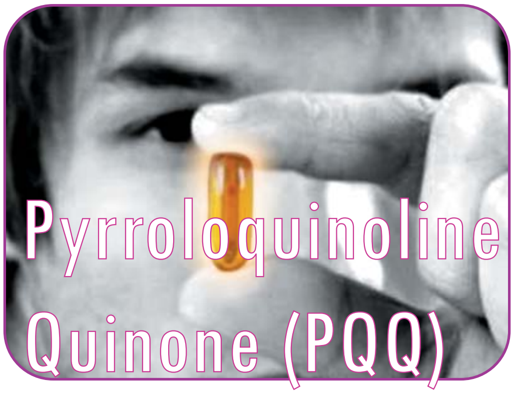

Pyrroloquinoline Quinone (PQQ)

“The First New Vitamin in 55 Years”[1]!

On April 24, 2003, scientists with Japan’s RIKEN Brain Science Institute made a startling announcement in the prestigious science journal *Nature*: they had discovered direct molecular evidence that an enigmatic nutrient was in fact a previously-unidentified B complex vitamin.[2] The “new” vitamin – **Pyrroloquinoline Quinone**, or **PQQ** (see **Figure 1**) – had been discovered decades previously, and had long been known to be essential to the health of *bacteria*. Yet despite decades of research and many tantalizing hints, no one had previously been able to definitively tie **PQQ** into *human* nutritional needs.

A vitamin is, by definition, an organic substance that you don’t make in your body – you *have* to get it directly from your food or supplements – and vitamins are absolutely required to carry out the essential biochemical functions that are indispensable for your survival. Most vitamins do this by forming “**coenzymes**” – molecules that activate the **enzymes** that catalyze these key biochemical reactions – although a few of them, like **vitamin C**, contribute to enzyme function without forming a coenzyme in the body. You can think of coenzymes as being like the PIN number on your debit card: swiping your card (an enzyme) through a card machine (a metabolic substrate) is an essential part of accessing your bank account (completing the biochemical “transaction”), but unless you also punch in your PIN number (the coenzyme) to activate it, the transaction can’t go through.

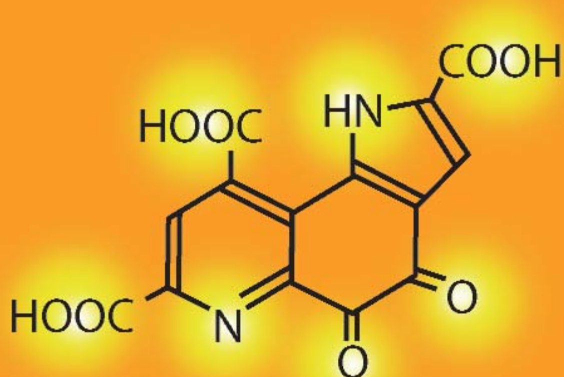

Figure 1: Pyrroloquinoline Quinone (PQQ)

This makes vitamins a very distinct kind of nutrient. While there are relatively many substances that are essential to human life in both health and disease (as a group, these are the **orthomolecules**), only a few of them have *all* of the features that would mark them as vitamins. Some of them (like **R(+)-lipoic acid** or **carnitine**) are undeniably essential to the biochemistry that underlies life, but unlike vitamins they can be made by the body. They are not necessarily made in the amounts required for a lifetime of *optimal* health, but certainly in the minimum quantities needed to keep you struggling on through the twenty or thirty years of hunter-gatherer existence around which evolution tailored your genes through our Paleolithic prehistory.

Other nutrients *are* needed for normal health, and you *do* have to get them from your diet or supplements, but they aren’t *vitamins* because they are not *organic* molecules in the chemical sense: this category includes the essential minerals, which may be *bound to* organic molecules (like citrate, aspartate, or malate) but are not, themselves, chemically organic. So calcium, magnesium and zinc – and, new research suggests, **vanadium**, **silicon**, **lithium**, and **strontium** – are needed for normal biochemistry and have to come from outside the body, but *they aren’t vitamins*.

Then of course, there are the many substances that have been *misidentified* as vitamins. Some of them really are essential, but while they are *orthomolecules,* they aren’t *vitamins,* because they can be made in the body. **Orotic acid**, once called “vitamin B_{13},” is one example of this. Others are **phytochemicals**; substances found in plants that contribute to their health benefits, but whose absence from the diet wouldn’t lead to the total shutdown of an essential biochemical function in the body. (Think of **lycopene**, **indole-3-carbinol**, or **sulforaphane**). Still others are substances claimed to be vitamins that really have no clear biological purpose at all, like **pangamic acid** (so-called “vitamin B_{15},” which not only has no essential biological purpose but may actually be a carcinogen[3,4]) and **laetrile** (which some people claimed to be “vitamin B_{17},” but for which there is no evidence of a role in normal human biochemistry[5]).

So think about what it means to identify **PQQ** as a vitamin for the first time. For *fifty-five years* since the isolation of the last such compound (vitamin B_{12}, isolated in 1948), nutritional science has overlooked an organic molecule whose absence from the diet is as ruinous to your health as scurvy, pellagra, or beriberi!

How do we know that **PQQ** is an essential factor in the human diet, and not just another of the many mistakenly-identified pseudovitamins? The first and most obvious evidence comes from studies in laboratory animals, which have found that **eating a PQQ-free diet leads to a sweeping deficiency syndrome as devastating as scurvy or beriberi**. PQQ-deficient animals suffer impaired reproductive[6-8] and immune function;[7] their growth is impaired;[6,8,9] their skin is thin and breaks easily;[6] and they develop abnormalities in their connective tissues known as **osteolathyrism.**[6] Their offspring are less likely to survive the first few days after birth[8] nor are they likely to make it to weaning.[7] None of these signs and symptoms occur when animals eat the same diets along with supplemental **PQQ.**

Moreover – of particular significance to the life extensionist – animal studies show that **PQQ deficiency leads to abnormalities in their mitochondria**. **PQQ**-deficient mice have 30–40% *fewer* of the cellular "power plants" than do animals given **PQQ** -containing diets[10]. Furthermore, the mitochondria that they *do* have are abnormally small[10] and do not appear to properly control the fuel mix that runs the "fires of life."[9] In fact, these puny mitochondria are so defective that less than half of them are even viable after their extraction in order to study their function, whereas *all* of the mitochondria from animals given **PQQ** survive the process.[10]

The findings were striking – but they were incomplete. What was the molecular explanation for the effects of **PQQ**? Was it actually *essential*, or was it acting in a "druglike" way, making up for a deficiency in some *other*, unknown substance? To definitively prove that **PQQ** is *essential*, scientists would have to map out the exact biochemical need for **PQQ** in normal metabolism. To prove that it was a *vitamin*, scientists would have to go one step further, identifying an enzyme whose function depends on **PQQ**.

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For much of the last two decades, scientists working to solve this puzzle focused their attention on **lysyl oxidase**, the enzyme that completes the last step in the biosynthesis of **collagen** (the basic structural protein in skin, bone, and connective tissue). A connection was suggested by the brittle skin and dysfunctional connective tissue of **PQQ**-deficient animals, which showed that **PQQ** was *somehow* needed for normal collagen biosynthesis. Indeed, investigation showed that **the skin collagen of these animals is abnormally easy to dissolve in strong solutions,**[6] and that their cells underexpress the genes that code for its precursor molecule, **procollagen.**[8]

The case for a lysyl oxidase connection seemed to be all tied up when researchers found that **PQQ**-deficient animals have abnormally low levels of lysyl oxidase protein in key collagen-containing tissues.[6,8] Yet the molecular studies designed to prove the involvement of **PQQ** in the enzyme's activity were difficult to interpret and the results contradictory,[11] leaving some scientists skeptical about **PQQ**'s vitamin status. A 1991 *Science News* article about the **PQQ** puzzle was headlined, "Cryptic molecule leads scientists in circles."[12]

When the RIKEN Brain Science Institute researchers finally proved **PQQ**'s essential coenzyme vitamin role, it was a mixture of good luck and advanced biotechnology. If it weren't for the conservation of genes across huge gulfs of evolutionary divergence in the tree of life, the astonishing power of twenty-first century molecular biology, and unexpected twists in the pursuit of an entirely unrelated line of investigation, science would *still* be ignorant of this fundamental factor in human nutritional biochemistry.

The RIKEN team had originally set out looking for genes that held the blueprints for proteins that control the transport of calcium into the **mitochondria** (the cellular "power plants"). They were working on the theory that **bipolar disorder** ("manic depression") involved abnormalities in this process. Their work at first seemed to be going nowhere, because the only gene that they identified didn't turn out to encode calcium-transport proteins at all. Instead it was a human version (**homolog**) of an enzyme that yeasts use to break down the amino acid

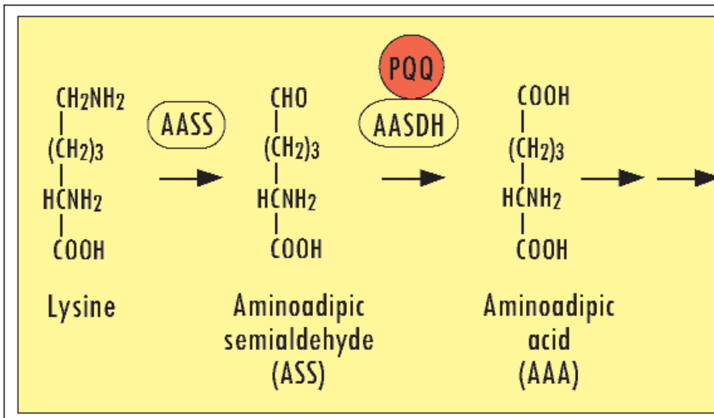

Figure 2. Inital steps of lysine degradation. Redrawn from 2.

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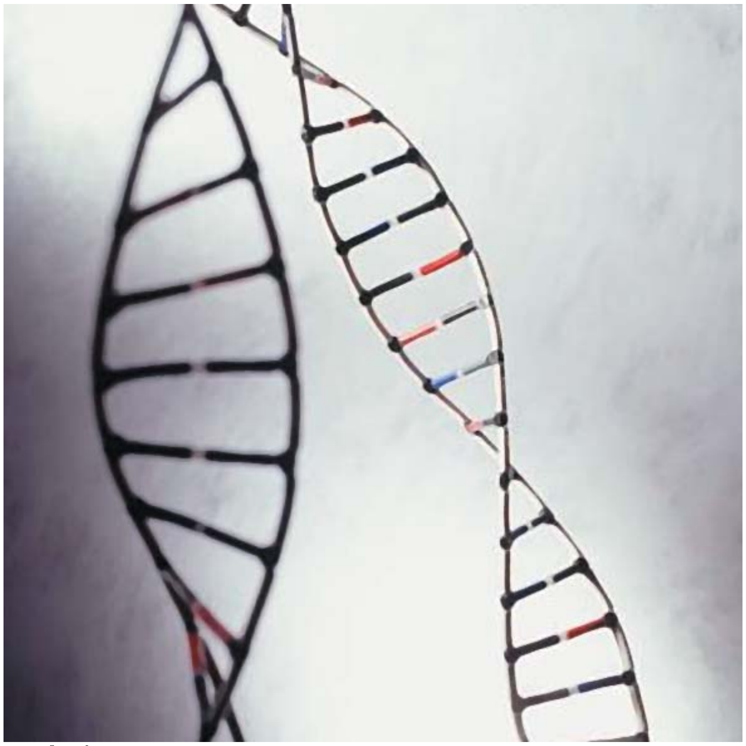

lysine.

While disappointed, the Riken team was still interested in this finding, because the metabolic path for lysine breakdown in animals was not yet understood. It was already known that this process involved two biochemical steps in animals; however, while the *first* enzyme in this process had already been identified, the enzyme responsible for catalyzing the *second* step was still unknown.

Using computer databases, the RIKEN group searched for genes in the common fruit fly that contained instructions for functional sites in their structures that were similar to those encoded in the yeast gene. They got just two "hits," and since one of them was already known to be involved in the metabolism of the brain chemical **dopamine**, they turned their attention to the other gene (**U26**), whose function was still unknown.[2]

The protein coded by U26 was similar to the one in the yeast lysine-metabolizing enzyme, but it had one very clear difference. Both proteins needed to borrow electrons to do their enzymatic work, and in yeast these electrical particles come from the electron carrier **NADPH**, a coenzyme that, like NADH, is built out of **vitamin** $\mathbf{B_3}$ (**niacin** or **niacinamide**). However, where the yeast protein was designed to bind to NADPH, the fruit fly enzyme has a series of sites that was known from bacterial studies to unlock in response to **PQQ**![2]

The enzyme stops working properly if the diet is

At this point, things were becoming very interesting, because the database search showed that a homolog to U26 was already known to exist in mice, which are a big jump closer to humans on the evolutionary tree of life. The RIKEN team looked carefully at the DNA structure of the mouse U26, and found that it, too, contained **PQQ** binding sites in its structure. They then looked for evidence that the gene was active in the mice's cells, and found that the gene was being expressed in every tissue they tested – especially the heart, liver, and kidney.[2]

To test for a role of the mouse U26 gene in lysine metabolism, the researchers' next step was to feed mice diets overly rich in lysine. If U26 was indeed coding for the enzyme that the mice used to complete the two-step breakdown of the amino acid, then you'd expect that the animals' cells would express more of it when they were fed high-lysine diets. As expected, the animals' blood levels of both lysine itself, and of its breakdown product, increased significantly in response to these diets. Yet the important result was the new finding; that the expression of the U26 gene in their liver and heart cells took a huge jump when lysine was overly-abundant – just as they'd predicted.[2]

The last step was to put it all together and answer the ultimate question. The test: feed the mice a **PQQ**-deficient diet, and see the activity of the U26 enzyme is specifically impaired. The result: bang on. Once again, the more lysine the animals were fed, the higher their blood lysine levels climbed. Animals fed diets with **PQQ** produced the normal level of the lysine breakdown product – indicating that the enzyme was fully active – but **animals fed PQQ-deficient diets failed to properly produce normal levels of this lysine metabolite in response.**[2] In other words, the protein for which the U26 gene codes *is* the enzyme that breaks down lysine – and **the enzyme stops working properly if the diet is deficient in PQQ**.

"Here we identify a **PQQ**-dependent dehydrogenase enzyme that is crucial for the degradation of the amino acid lysine in mice," the RIKEN researchers wrote in their *Nature* report. "**PQQ is acting as a mammalian redox cofactor in this reaction, and therefore qualifies as a newcomer to the B group of vitamins.**"[2] In a critical final step, they would shortly discover that humans share the same **PQQ**-dependent gene,[13] bringing the result directly into the realm of human nutrition.

The story doesn't end with this discovery, however. Now that we know that PQQ is a B-complex vitamin, the question will be what other, unexpected roles this particular vitamin plays in human health. For instance, animal studies suggest that **PQQ may have potent antioxidant and anti-inflammatory effects.**[14] Other studies have found that **PQQ is an effective neuroprotectant**, reducing damage to the brain during simulated stroke,[15] reducing the duration of chemically-induced convulsions,[16] and protecting brain cells against the "excitotoxic" overstimulation by the **N-methyl-D-aspartate (NMDA) receptor.**[17,18] An important related finding is that **PQQ enhances the biosynthesis of nerve growth factor (NGF),** a key protein involved in the growth and survival of neurons.[19] (The NGF-enhancing effect has so far only been observed directly in isolated cells: in the whole organism, only its glycine-bound form appeared to be active.[19] Yet the glycine-bound form is synthesized so readily in the presence of glycine[20,21] as to make you optimistic that this benefit, too, may be realized).

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What about the mitochondrial effects of **PQQ**? These appear to be totally unrelated to the lysine-metabolizing role, and instead may be an essential cofactor to a protein in **Complex I** of the mitochondrial energy-generating proton-pumping system.[22] There is even evidence that much of the debility that strikes victims of **lead poisoning** may be due to its interference with this novel PQQ enzyme function.[23] How many other essential biochemical processes will turn out to rely on getting adequate **PQQ**?

Since its role in human nutrition was unknown, **PQQ** has never previously been available in dietary supplements.

Fruits	**Fermented Foods**
Apple 6	Cocoa powder 800
Kiwifruit 27	Natto (Fermented Soybeans) 61
Oranges 7	
Papaya 27	**Animal products**
Tomatoes 9	Human Milk140-180
	Cow's Milk 3.4
Vegetables	Egg yolk 7
Cabbage 16	Egg white 4.1
Carrot 17	
Celery 6	
Green pepper 28	
Parsley 34	
Green Tea 30	
Legumes	
Broad beans 18	
Soybeans 9	
Tofu 24	

Table 1: PQQ in Foods (mcg/kg)[1,10]

Fortunately, many healthy foods contain **PQQ** (see **Table 1**), and a well-balanced diet will contain about one-tenth as much **PQQ** as folic acid on average. For now, with the basic biochemical need for **PQQ** finally established at the gene level, leading-edge supplement companies are taking the initiative to take **PQQ** out of the laboratory and into the hands of the health-conscious and life extension communities. This lets you give yourself the nutritional insurance of a reliable daily dose of **PQQ**, reflective of the amounts found in good diets, and filling the gap in standard B-complex pills to ensure that you get its benefits every day.

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