

# Everything Is Connected to Everything Else

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## Bill Lands

When I applied to graduate school at the University of Illinois in Urbana in 1951, my naive plan was to get a masters in biochemistry and a Ph.D. in biophysics to build a career in what I named at the time “biochemocytology.” The biochemistry faculty gently suggested that I would probably find enough to do in biochemistry without a need to move on to other departments. They were correct. The narrative below tells you some of what happened after that.

**T**he more that I reflect on what I learned in science, the more it seems that I have been using over and over two basic concepts learned during my graduate studies at the University of Illinois. They both are embedded in all that I did and do.

### Concept 1: Connect the Dots

The first text I remember about metabolic processes is *Dynamic Aspects of Biochemistry* by Ernest Baldwin. This early book showed fascinating interconnected cycles of substrates, cofactors, and products that had an almost artistic appeal to me. In those days, the Krebs tricarboxylic acid cycle had just been through the controversial removal and replacement of citric acid as an intermediate. My mentor, Herb Carter, was actively arguing that the two sides of a “meso”-carbon were identifiably equal and opposite. This idea was important in reconciling citrate’s plane of symmetry in a metabolic cycle that releases CO<sub>2</sub> from only one carboxyl group to form  $\alpha$ -ketoglutaric acid.

Over the next sixty years, I sensed again and again a need to connect cause to consequence by identifying the intermediate mediators that cause the consequences. I believe that “connecting the dots” remains a major biochemical discipline in interpreting associated biomedical events. Training in biochemistry at the University of Illinois in those days included attending all of the courses and seminars required for organic chemists. Memorable presentations by R. B. Woodward and E. J. Corey filled blackboards with complicated paths of intermediates in an elegant total synthesis that led to some complex natural product. We had no doubt that connecting the dots was an important intellectual activity.

When studying for preliminary examinations in 1953, Bob Fisher and I laid large sheets of shelf paper on the floor to sketch out and integrate all the metabolic interrelationships for carbohydrates, amino acids, and lipids known at the time. It was fun and a unique experience. Bob used “silkscreen” technology to print copies, which we sold for \$1 each to anyone interested. Some years later, more professional versions with similar arrays of increasingly complex details were provided by companies that supply materials to biochemists. The charts increased in size and complexity over the next forty years. While visiting a laboratory recently, I saw an intimidating four-sheet display covered with hundreds of details in small print. It made me glad that I passed my exams in the “good old days,” when things were a bit simpler.

### Concept 2: Quantitative Models of Reality

Studies of enzyme catalysis at the beginning of the twentieth century created a controversial paradox, with some reaction rates dependent on substrate abundance (first-order kinetics) and others independent of substrate abundance (zero-order kinetics). Moving from a logically

expected outcome to an apparently miraculous outcome was finally reconciled by introducing the concept of a saturable catalytic site. For biochemists, the Michaelis-Menten relationship,  $V = V_{\max}/(1 + K_m/S)$ , remains a profoundly important concept when interpreting reality. I was fascinated by the simplicity with which it reconciles paradoxical saturable hyperbolic dynamic catalytic events.

As soon as I obtained my Ph.D. degree, I received one of the first dozen National Science Foundation (NSF) post-doctoral awards in chemistry and spent a year at California Institute of Technology with Carl Niemann to study chymotrypsin kinetics. Our spectrophotometric assay results were laboriously punch card-coded and entered into Linus Pauling's state-of-the-art computer for analysis. We quickly found that sophisticated polynomial representations of the data had more variance and less interpretability than using  $V = V_{\max}/(1 + K_m/S)$ . Even today, I encounter controversies in which others have neglected using that simple relationship and its accompanying model with competing ligands,  $V = V_{\max}/(1 + K_m(1 + I/K_i)/S)$ .

### Starting Research with Glycerolipids

To begin academic research as an instructor in biochemistry at the University of Michigan Medical School (Ann Arbor, Michigan) in 1955, I picked plasmalogens, a minor phospholipid about which I (and everyone else) knew nearly nothing. It was abundant in brain and heart, but it had no known biological role. Phospholipid metabolism was still in its infancy, and we spent much time developing methods for isolating, handling, and analyzing the diverse mixed lipid components that occur in Nature. A fateful distraction occurred during some of our first experiments incorporating radioactive acetate and glycerol into tissue glycerolipids. The proportions of isotope in triglycerides and phospholipids of lung tissue were opposite those expected for the recently discovered "*de novo* pathway." This finding led to the discovery of enzymes remodeling the acyl chains of phospholipids independent of the *de novo* pathway. Fifty years later, the *Journal of Biological Chemistry* named the report a "Classic" (1).

In the 1950s, we knew that natural phospholipids had saturated and unsaturated<sup>1</sup> fatty acids esterified at different positions. Snake venom hydrolyzed the unsaturated acids, but their esterified location was not yet certain. To

<sup>1</sup> Unsaturated acids are indicated with number notations that give chain length (16, 18, 20, etc.) plus the number of ethylenic bonds and the metabolic type of methyl terminus (*n*-3, *n*-6, *n*-9, etc.). The *n*-3 (omega-3) and *n*-6 (omega-6) types are vitamin-like essential nutrients, of which 18:2*n*-6 and 18:3*n*-3 are the most abundant in typical foods. The *n*-3 and *n*-6 20- and 22-carbon HUFAs are important as precursors and modulators of potent hormone-mediated events.

determine the phospholipase selectivity, we used mild cleavage of the 1-alkenyl group from plasmalogen to prepare 1-hydroxy-2-acylglycerophosphorylcholine. We then used the remodeling system of liver microsomes to acylate it with radioactive oleic acid. The radiolabeled lipid did not release isotope when hydrolyzed with venom phospholipase. Thus, this phospholipase is specific for hydrolyzing the 2-acyl group. This meant that natural phospholipids tend to have saturated and unsaturated fatty acids at the 1- and 2-positions, respectively. This was heady stuff, letting us dream about biological consequences of acyltransferases, such as membrane fluidity and adaptation to environmental temperatures.

In the laboratory, we asked, "Why does Nature put saturated acids at position 1 and unsaturated at position 2?" However, I soon realized that "why" is teleological and not experimentally testable. Rather, good science designs controlled experiments on "how" and "what consequence." The acyltransferases gave us a tool to investigate "How does Nature put saturated acids at position 1 and unsaturated at position 2?" We prepared 1-hydroxyl-2-acyl- and 1-acyl-2-hydroxyphospholipids and showed selective transfer of common saturated fatty acids from their CoA esters to the 1-position and common unsaturated acids from their CoA esters to the 2-position.

In the early 1960s, the Michigan biochemistry group had frequent evening faculty research seminars. At one, Minor Coon advised me to develop a spectrophotometric assay to collect data that was easier and faster than the tedious isotope-monitored reactions. Many (but not all) acyltransferase events are active in the presence of sulfhydryl reagents, such as *N*-ethylmaleimide or *p*-chloromercuribenzoate. Using 5,5'-dithiobis(2-nitrobenzoic acid), I was thrilled to see continuous recording of selective acyl transfer rates from CoA esters to both the 1- and 2-positions of phospholipids. With this new tool, the question of "how" rapidly changed to "What does the acyltransferase 'see' when it selects a saturated fatty acyl chain for transfer?"

We were fascinated that transfers to each position seemed to be independent of the acyl group in the adjacent position even though transfers differed with an ether link at the 1-position. The results gave no evidence for an anticipated preference to combine acyl chain pairs and form distinct phospholipid molecular species common in natural phospholipids (*e.g.* the 04 species, 1-stearoyl-2-arachidonoylglycerophosphocholine). Also, selectivity was not affected by the ambient temperature (confounding my friends, who thought lipid formation would adapt to environmental temperatures). We saw that the unsatu-

rated acids accumulated at the 2-position of erythrocyte lecithins had a rational relationship to the relative abundance of non-esterified acids in plasma combined with transferase activity selectivities.

Liver lecithins also responded to dietary acids in a way that combined substrate abundance with acyltransferase selectivity. Although I focused on selective transfers, many of our studies confirmed a great plasticity of acyl chain composition that responded promiscuously to the abundance of whatever substrate was available. For example, the pattern of molecular species tediously measured in rat liver lecithins (11% 01, 2% 11, 28% 02, 6% 12, and 35% 04) became dramatically different when fasting, and feeding a fat-free diet greatly increased the oleate available for lipid synthesis (28% 01, 18% 11, 11% 02, 4% 12, and 20% 04). The available supply of substrate seems to be a major determinant of overall acyl chain composition.

Frank Gunstone offered us a unique collection of 18-carbon fatty acid positional isomers with *cis*- and *trans*-ethylenic, acetylenic, and cyclopropyl groups synthesized by his young colleagues at the University of St. Andrews (Fife, Scotland). We converted them to CoA esters and quickly found that the defining features used by organic chemists are probably not used by acyltransferases when they form natural products. As expected, the 9-*cis*-isomer transferred more rapidly than other *cis*-isomers to the 2-hydroxyl group. However, transfers to the 1-position (expected for high-melting saturated acids) were rapid with many low-melting *cis*-ethylenic isomers (8-, 10-, 12-, and 13-) and slower with the naturally occurring 9-*cis*- and 11-*cis*-octadecenoyl-CoA esters. A similar "sawtooth" pattern of selectivity was seen when a *cis*-methylene group replaced the *cis*-ethylenic bond. This reassured us that the enzyme's active site can "sense" a *cis*-configuration as well as its position along the acyl chain.

In contrast, transfer rates to the 2-position by *cis*-ethylenic and methylene analogs were very different, making us wonder if the 2-acyltransferase had a  $\pi$ -bond detector (and also what would comprise such a detector). More surprisingly, transfer rates for *trans*-ethylenic and acetylenic analogs showed a "frameshift" of one carbon relative to the *cis*-acids. We made space-filling models that clearly showed the conformation of a 9-*cis*-isomer superimposed neatly on the 10-*yne* (or 10-*trans*)-analog. The acyltransferase activity was teaching us basic organic chemistry!

The many unexpected transferase selectivities no longer fit convenient terms like "saturated" or "unsaturated." Clearly, the acyltransferase active site interacted with soluble substrate acyl-CoA esters using acyl chain properties different from usual bulk phase aspects of fatty acids that

biologists often discuss. Stories that biologists tell about high- and low-melting fatty acids may have no relation to the chemistry with which Nature selects acids to be in our membrane phospholipids. The human weakness for a Panglossian hope that our metabolism will always provide us with the best possible outcome needs careful reassessment. Much discussion in the laboratory was spent attempting to "think like the enzyme thinks." Readers interested in the acyl chain chemistry "seen" by microsomal transferases can find our many detailed reports cited in two reflective reviews (2, 3). The topic now waits for new methods of cloning and isolating the many individual acyltransferases to avoid wasting clean thoughts on a dirty system.

The mirrored sides of the *meso*-carbon in glycerol led us to design a way to see the very different acyl chain contents at the *sn*-1- and *sn*-3-positions in triglycerides of rat liver. In this tissue, the 1-ester was mostly palmitate (from acylating glycerol *sn*-3-phosphate), whereas the 2-ester was mostly oleate and linoleate (from acylating 1-acylglycerol 3-phosphate), and the 3-esters were a mixture of all acids available. The triglyceride composition reflected *in vivo* selectivities of the *de novo* pathway not seen in cell-free systems. The unusual loss of selectivity going from *in vivo* studies to increasingly purified microsomal studies made us wonder what sort of "stupidity factor" was being generated. We need more reports to explain how esterification of 1-acylglycerol 3-phosphate becomes increasingly indiscriminant under conditions that provide greater substrate amounts than usually occur *in vivo* (4). New ideas in lipid metabolism were appearing every month, and I gave numerous talks about "shoot first and ask questions later" when describing the different selectivities of the *de novo* and retailoring processes. Sometime around my thirty-fifth birthday, I wrote an invited review of lipid metabolism for *Annual Reviews in Biochemistry* (5).

### Quantitative Nutrition in Microorganisms

Knowing so many "how" and "what" details about acyl chain incorporation prompted me to turn my attention to "what consequence" the structural details might have on the life of a cell. We addressed that with fatty acid auxotrophs of *Escherichia coli* and *Saccharomyces cerevisiae*, which made palmitic acid but could not synthesize unsaturated fatty acids and required them for growth and replication. Would living cells "care" about small details in acyl chain structures? The answer was a surprising "Yes." With an initial amount of needed fatty acid, we saw growth continue as generated daughter cells diluted the nutrient with their synthesized palmitate until growth stopped.

Effectiveness of an acyl chain in supporting “membrane function” was expressed as the nanomoles/cell needed to maintain cell division. As expected, palmitoleate and oleate (normally found in wild-type yeast) were less effective than the lower melting highly unsaturated fatty acids (HUFAs; 20:4, 20:5, and 22:6) not normally seen in yeast. The final content (nanomoles/cell) of the essential nutrient in membrane phospholipids was lower with more “fluid” acids, fitting a concept of growth being limited by fluidity (6). Isomers of *cis*-octadecenoate with the double bond near the middle of the chain have lower melting points and supported more growth with *E. coli* and *S. cerevisiae*. Growth effectiveness matched the inverted bell-shaped transition temperatures for a set of synthetic lecithins made with those isomers. The finding that higher melting *trans*-ethylenic acids did not support appreciable growth of either cell type seemed to add support for fluidity-limited growth. However, we had much more to learn about lipids.

A few positional isomers of octadecynoate (8-, 9-, 10-, and 11-) supported growth of *S. cerevisiae*, fitting expected outcomes. However, only three isomers (7-, 8-, and 10-) supported growth of *E. coli*, and we saw little growth for the 9-isomer, which we expected to be the most effective. We had discovered that *E. coli* has a not yet recognized enzyme that has dramatic acyl chain selectivities similar to those seen with rat liver acyltransferases. Where is the future researcher who will identify the protein in *E. coli* capable of responding so acutely to adjacent positional isomers of fatty acids?

The one-carbon frameshift seen earlier with rat liver acyltransferases was again evident when only three *cis*-methylene isomers (8-, 9-, and 11-) supported growth of *E. coli* with little growth for the 10-isomer (7). More mysterious was the discovery that those sharp differences disappeared when glycerol was the carbon (energy) source to give a bell-shaped pattern of growth with the 10 middle isomers like that predicted from fluidity. A similar “fluidity-limited” bell-shaped pattern occurred when cyclic AMP was added to the glucose medium. A new area of metabolic regulation linked to specific acyl chain chemistry may yet be developed if we can connect the dots.

The inability of *trans*-fatty acids to support growth occurred with cultures grown with glucose as the energy source. However, when we shifted to glycerol or added cyclic AMP, we saw the 9- and 11-*trans*-isomers can provide about half the effectiveness of the *cis*-isomers for *E. coli* division. This agrees better with their melting properties and opens the issue of what metabolic process is impaired by *trans*-isomers. With today's concerns for

harmful effects of *trans*-acids in human health, there is good reason to explore further this non-fluid impairment of metabolism. There is much more than fluidity involved in acyl chain actions.

An important role for phospholipids in DNA replication seemed to be involved when *S. cerevisiae* division was inhibited as *trans*-fatty acids shifted lipid metabolism from phospholipid to triglyceride synthesis. Also, a selective loss of mitochondrial DNA was caused by certain unsaturated fatty acids that support only nuclear DNA replication, leading to rapid accumulation of petite mutants (8). The links between acyl chain structure, metabolic regulation, and DNA replication remain a fascinating area to explore. I wish I had more time to connect the dots.

### **Essential Fatty Acids, Hormones, and Peroxide Tone**

When the Karolinska group reported that essential fatty acids form potent hormones, I bet Bengt Samuelsson that a major tissue source for hormone precursors would be the 2-acyl esters of membrane phospholipids. Supported by a NSF grant, I took my first sabbatical leave in the form of an exciting family expedition by Icelandic Airline to Stockholm. Arriving with four exhausted children, we slept right through our first very short Scandinavian New Year's day. The goal of my grant was to learn whether the hormone was formed as an ester and then released “on demand,” or if phospholipase released the precursor acid, which was then converted to active hormone. The latter quickly proved to be the case, and we found time to travel all over Europe in a VW minibus. While back in Stockholm between trips, an unidentified spot on thin-layer chromatograms gave me the chance to discover yet another new hormone, prostaglandin D. Over the years, I have greatly enjoyed reading reports from others about its interesting actions in immune-inflammatory events and slow wave sleep.

A few years after returning to glycerolipid research at the University of Michigan Medical School, I decided to look at the enzyme kinetics of the fatty acid oxygenases, cyclooxygenase and lipoxygenase. Knowing the immediate product of the reaction was a lipid hydroperoxide, I added glutathione peroxidase to accumulate the product in an alcohol form. That never happened. Rather, I found that the substrate recovered intact with no reaction at all. We quickly learned that fatty acid oxygenases require continual hydroperoxide activation and show positive feedback by product activation. The oxygenase reaction also has a self-limiting aspect of a “suicide” reaction inactiva-

tion that limits the explosive reaction without a need for accumulated product to initiate negative feedback.

A consequence of such bizarre kinetic features is that elevated hydrogen peroxide and lipid hydroperoxides present in inflammatory conditions enhance the synthesis of active eicosanoids (reviewed later in Ref. 9). Another consequence is that self-catalyzed inactivation ensures a negative feedback control of synthesis even when the water-soluble product rapidly leaves the site. As with acyltransferases, we developed a method for continual monitoring of oxygenase reaction rates using a highly sensitive oxygen electrode assembly. The 1970s brought a unique set of Ph.D. candidates (Bill Smith, Lenny Rome, and Martin Hemler) to the University of Michigan Medical School to work on oxygenase kinetics and its inhibition. We described reversible competitive inhibition with substrate by non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen; irreversible time-dependent inhibition by aspirin, indomethacin, and certain other NSAIDs; and a peroxide-sensitive action of acetamidophenol and radical chain transfer NSAIDs.

At my first large international conference in 1972, I discovered that pharmacologists did not share my excitement in finding that dietary *n*-3 fatty acids stored in membrane phospholipids could slow the formation of inflammatory *n*-6 prostaglandins that were targets for drug development. I liked the concept of a nutrient providing a “resident antagonist” of chronic inflammatory disorders, but patents and profits were more desired by corporate pharmacologists. Nevertheless, our tools for monitoring cyclooxygenase reactions and their inhibition were of interest to pharmaceutical companies. This led to many interesting years of serving on Science Advisory Board reviews of research on a wide range of corporate drug development topics. The decade was filled with invited scientific discussions with colleagues in industry plus annual prostaglandin meetings at pleasant ski resorts while more and more active hormone drug targets became recognized to be derived from arachidonic acid (20:4*n*-6).

In 1975, the Karolinska group described thromboxane generation during platelet aggregation, linking the release of arachidonate from membrane phospholipids to heart attacks. We now had vitamin-like nutrients (18:2*n*-6 and 20:4*n*-6) being converted into a powerful hormone that mediated a major cause of death worldwide. This led us to show that feeding menhaden oil (with its *n*-3 HUFAs) could decrease the proportion of *n*-6 in tissue HUFAs and decrease the severity of heart attack and stroke in animal models. As I approached the age of 50, I was invited to write a second review for *Annual Reviews*, this time in

physiology rather than biochemistry (10). I remember also a stimulating short course for cardiovascular surgeons in Davos, Switzerland (with afternoons out in fresh powder snow). It was easy to teach something the group did not know because most had not yet heard of either prostaglandins or omega-6 acids in inflammatory and thrombotic events. It led to a return European visit for an honorary Verhagen Lecture in Rotterdam, The Netherlands. Later, leukotrienes were added to the mediators of chronic immune-inflammatory disorders, and the 1982 Nobel Prize in physiology and medicine recognized the importance of the growing family of lipid hormones made from arachidonate (20:4*n*-6).

### Quantitative Nutrition in Humans

In 1980, the University of Illinois College of Medicine asked me to head the biochemistry department in Chicago, and I moved to that urban campus with its very different environment from the collegial Ann Arbor campus. I found an apartment a few blocks from the laboratory and, without owning a car for ten years, immersed myself in research, theater, opera, and good restaurants. The research turned to quantitatively measuring very low levels of hydroperoxides (*i.e.* peroxide tone) that occur in normal and inflamed conditions. The best method used the sensitive requirement of cyclooxygenase to amplify hydroperoxides to detectable signals not possible with other reagents. After a few years, we measured elevated levels of hydroperoxides in blood-draining septic regions and regions of non-septic trauma (11). Our work on “peroxide tone” was summarized in a later review chapter (9).

I began writing a small monograph about how the HUFAs maintained at the 2-position of membrane phospholipids mediate many chronic disorders for aging humans (12). Then, Pfizer gave me an unrestricted biomedical research award of \$500,000 to study whatever I regarded most important. Inevitably, I chose to study the dynamics of how daily intakes of *n*-3 and *n*-6 essential fatty acids maintain a balance in the 20- and 22-carbon HUFAs accumulated in human tissues.

First, we confirmed that the quantitative diet-tissue relationship reported for laboratory rats twenty years earlier by Mohrhauer and Holman fit the general pattern of  $V = V_{\max}/(1 + K_m(1 + I/K_i)/S)$ . When we applied this quantitative hyperbolic diet-tissue relationship to volunteers in Chicago, I was amazed to see how closely it fit data for both rats and humans (13). It was almost as if the Chicagoans were eating rat chow with very little dietary *n*-3 HUFAs. To see the effect of dietary *n*-3 HUFAs, we analyzed blood from patients taking supplemental fish

oil capsules and developed an overall empirical relationship that fit observed diet-tissue data for rats, mice, and humans (13).

Clearly, our laboratory animals were suitable models for lipid metabolism in humans, and we could predict fairly well the impact of dietary intakes on the resulting proportions of *n*-3 and *n*-6 maintained in tissue HUFAs. To monitor dietary interventions, we developed a streamlined micromethod for monitoring fatty acid composition in the lipids of 50  $\mu$ l of blood. Knowing one's tissue HUFA status and knowing the nutrient contents of daily foods that maintain healthy HUFA proportions should allow people to decrease the severity of many chronic disorders shown to be mediated by excessive actions of *n*-6 HUFAs.

While doing research on dietary impacts, I met annually with Japanese colleagues on the Lipid Subpanel of the U.S.-Japan Malnutrition Panel. We were concerned with the likely consequences of a changing dietary pattern in Japan (14). All of my experience warned that accumulation of 2-acyl esters was indifferent to *n*-3 or *n*-6 structures. If we intend for our tissues to maintain equal amounts of *n*-3 and *n*-6 HUFAs, we need to make careful choices of the dietary supplies. As I approached my sixtieth birthday, a third invitation to write for *Annual Reviews* was for nutrition (15).

My last two academic research reports focused on how lower proportions of *n*-6 eicosanoid precursors accumulate in tissues in response to dietary *n*-3 fatty acids (13) and how detailed kinetic constants of prostaglandin-endoperoxide synthase allow low peroxide tone to give a selective preference for 20:4*n*-6 over 20:5*n*-3 (16). I felt then that I had published information that others would use to diminish the severity of the many chronic diseases mediated by excessive *n*-6 HUFA actions.

I then retired from academic research and accepted a job in Bethesda, Maryland, as Director of Extramural Basic Research at the National Institute on Alcohol Abuse and Alcoholism. The new duties brought an opportunity to assemble information on research for which the Institute provided over \$70 million in grants annually. I saw fascinating research opportunities on the kinetics of ethanol metabolism (17), the molecular basis of ethanol-induced liver injury (18), ethanol's impact on slow wave sleep (19), and the history and politics of alcohol abuse (20). Then, at 72, I retired to what I thought might be a quiet life of reflection on all the biochemocytology that I had learned in the previous fifty years.

### **Paradox of Having Continual Preventable Disease**

When directing young researchers, I repeatedly assured them that a productive approach is to find a

natural paradox in which two known facts seem contradictory. Often, a missing fact can reconcile the paradox. Paraphrasing the two conflicting facts can steer you toward discovering the missing fact. With lots of uncommitted time, I soon found my "quiet retirement" disturbed by an unresolved paradox that continually tugs at my curiosity. How (not why) do we have a nation with continually rising healthcare costs for treatment of preventable diseases? The Centers for Disease Control and Prevention regard cardiovascular disease as preventable while noting that it accounted for 1 of every 2.9 deaths in 2007 in the United States. I believe that a major cause of excessive medical losses in the United States is excessive diversion of attention to treating signs and symptoms while neglecting to prevent the factors that cause them.

I had hoped that my research reports on how to maintain lower proportions of *n*-6 in tissue HUFAs (13) showed a way to "connect the dots" to identify and remove a primary cause and prevent preventable disease (21). This could resolve the "health cost paradox." Somehow, the people who can make the needed changes have not been informed or motivated to participate in making effective preventive interventions. To approach this paradox, I paraphrased the quantitative empirical diet-tissue relationship ([efaeducation.nih.gov/sig/hufacalc.html](http://efaeducation.nih.gov/sig/hufacalc.html)) by placing it into a small Excel spreadsheet ([efaeducation.nih.gov/sig/dietbalance.html](http://efaeducation.nih.gov/sig/dietbalance.html)) to assist effective design of food-based prevention clinical trials. Few people used it because they did not know the amounts of nutrients in foods being eaten.

To correct this deficit, the quantitative relationship was built into an interactive personalized daily menu-planning software program ([efaeducation.nih.gov/sig/kim.html](http://efaeducation.nih.gov/sig/kim.html)) that included *n*-3 and *n*-6 nutrient contents for nearly 12,000 servings in the United States Department of Agriculture Nutrient Database. The program helped people choose foods that fit their own personal tastes and their degree of risk aversion. Also, I revised my twenty-year-old monograph with a second edition that paraphrased the situation using more recent quantitative tools and insights (22). The book describes ways to lower treatment needs with informed food choices, but it did little good because few people read it. Also, my friends told me that the computer software was "too clunky" and involved too many numbers and too many keystrokes. They wanted a single number to use in making food choice priorities. This led me to paraphrase the balance of eleven *n*-3 and *n*-6 nutrients in each food item into a single "Omega-3 Balance Score" and place

thousands of individual food scores on a website (retrieved as “Omega-3 Balance Scores” by web browsers). The weighted average score for daily foods eaten predicts the likely proportions of *n*-6 measured in the HUFAs of a fingertip blood spot, and the % *n*-6 in HUFAs directly relates to the risk of heart attacks (23). We had built a quantitative model connecting daily foods to clinical realities.

Slowly, I began to sense that I was not communicating with the people most motivated to *prevent the need* for treatments. Treatments are important in the daily business of doctors, nurses, hospitals, insurers, actuaries, drug companies, and biomedical researchers. All of them have little to gain from preventing the flow of funds for treatments. In contrast, the employees and employers who share the costs for treatment preventable diseases will gain the freedom of a better quality of life and the use of their resources for important things other than treatments. For many reasons, biomedical experts have not rigorously employed the important concept of connecting the dots to identify valid causal surrogates when planning clinical interventions (21, 23). This failure may be partly due to another “missing fact” in medical statistics. Predicting future disease costs is improved by including all possible associated factors in making risk estimates, whereas preventing disease is better done when “connected dots” identify explicit causal factors to remove. Prediction has different connections than prevention.

After my eightieth birthday, I was invited to talk at the annual prostaglandin conference (which no longer meets at Vail and Keystone in Colorado). I, too, had changed, and I apologized to the audience for not providing a typical academic research report or review. Rather, my data showed how Omega-3 Balance Scores can guide employees to voluntary food choices that quantitatively connect to lower % *n*-6 in HUFAs and lower annual healthcare claim costs (24). The existing fragments of data suggest that a self-insured corporation that arranges to make Omega-3 Balance Scores available plus informative feedback to employees about their individual HUFA status might see average daily balance scores decrease from the current  $-7$  toward  $-3$  with a lowering of the average % *n*-6 in HUFAs from 78 to 60%. These voluntary results can be expected to be accompanied by significantly lower health-related corporate financial losses (24). The return on investment for Pfizer’s one time \$500,000 gift to me in 1985 might now lead Pfizer and its 100,000 employees to make an

estimated lowering of health-related financial losses by \$500 million every year. I hope it does.

*Acknowledgments*—I greatly appreciate the invitation from the JBC editors to reflect once again on my amazing good luck in life. To keep these reflections uncharacteristically brief, I did not cite the many alert young scientists who joined me in trying to make sense of what we need to know about Nature. Their enthusiasm and effort taught me how much fun a life of research can be. I am deeply grateful for their contributions. All are cited in the reviews noted in these reflections. In particular, I benefited much from repeated interactions with Bill Smith, Harumi Okuyama, and Rich Kulmacz over the past several decades. They have steadily helped me better interpret the puzzling and paradoxical reality that surrounds us all.

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Address correspondence to: [wemlands@att.net](mailto:wemlands@att.net).

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