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Musings in the twilight of my career

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ABSTRACT

I present a summary of my research during the last few decades of research which focused on understanding the biochemical basis for maintaining an optimum metabolism to support long-term health. I realized that adequate levels of ~40 vitamins and minerals needed as cofactors in thousands of metabolic reactions were critical for maintaining a healthy metabolism, and thus for longevity and prevention of chronic disease. Inadequate dietary intake of vitamins and minerals accelerates the risk of aging-associated diseases, leading to insidious damage. The Triage Theory provides a mechanistic rationale for such damage: shortage of a nutrient triggers a built-in rationing mechanism that allocates the scarce nutrient to proteins needed for immediate survival (survival proteins), at the expense of those needed for long-term survival (longevity proteins). Many as-yet-unknown longevity vitamins and proteins likely remain to be discovered. The fiber and nutrient-rich CHORI-bar was developed to fill gaps in inadequate diets; it yielded broadscale metabolic improvements. The health-related damages resulting from vitamin D deficiency and the positive effects of vitamin D supplementation were connected to numerous health-related problems, including the higher level of deficiency in people of color residing at northern latitudes. In general, prevention of degenerative diseases of aging requires expertise in metabolism, nutrition, biochemistry and regulatory functions.

1. Introduction

In my 92 nd year, during the twilight of my career, I am using this occasion to summarize how I came to some new ideas. In the 1990s I was happily researching the connection between DNA damage and cancer, e.g., Refs. [1,2], inflammation, and oxidative damage to DNA from naturally-generated oxygen radicals. Around 1997 I was intrigued by the work of a colleague, James MacGregor, who discovered that deficiency of the vitamin folic acid led to chromosome breaks and the formation of micronuclei (i.e., fragments of chromosomes) in developing red blood cells in otherwise normal humans [3,4]. My lab collaborated with MacGregor to understand the molecular basis of the chromosome damage [5] and our findings were consistent with the necessity of the vitamin folic acid for the methylation of dUMP to dTMP. Accumulation of dUMP leads to a misincorporation of uracil into DNA and whenever two uracil molecules are incorporated in both DNA matching strands in close proximity and are simultaneously removed by uracil-repair enzymes a double-strand break would occur. This would be expected to lead to chromosome breaks and/or rearrangements, and to micronuclei formation. Consistent with this interpretation, folate supplementation reduced both uracil misincorporation and micronuclei formation [5,6]. I was particularly struck by the fact that chromosome breaks were similarly created by irradiation (which causes oxidation of DNA) and that, comparing the two, folate deficiency could be as damaging as relatively high doses of ionizing radiation [7]. The finding of this strong connection between folic acid status and DNA damage reinforced my interest in the relationship between nutritional status and the maintenance of genetic integrity [4].

In addition, I was surprised when I realized that, although there were several publications describing these and related findings, there were minimal citations to this work despite the important relevance of these findings to human nutrition and health. This was particularly surprising considering the high level of folic acid and B12 deficiencies in the US at that time (presently improved by several supplementations). I think that my awareness of the magnitude of the problems arising from poor nutrition was the main push leading to my changing the focus of my research. This work also led to my development of the Triage Theory (see below).

2. Vitamin and mineral inadequacy accelerates aging-associated disease

There is much evidence that the nutrition status is quite poor in the USA (and in most of the world's population, even in developed countries) as determined by the inadequate intake of ~40 essential vitamins

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Abbreviations

V/M	vitamins and minerals
KO	knock-out
VDD	vitamin D deficiency

and essential minerals (V/M) [8]. While severe deficiency is now uncommon, modest V/M deficiency is very common. Astoundingly, more than half of the population is deficient in one or more of the V/M (referenced in Ref. [8]), with low intakes being especially true for children, adolescents, elders, and the obese. I proposed [9] that such an incomplete and insufficient nutrition increases many health risks typical of old age, such as immune dysfunction, cancer, osteoporosis, cardiovascular disease, stroke, and cognitive decline, as shown by numerous studies (as extensively referenced in Ref. [8]).

3. Experimental evidence of a connection between V/M deficiency and disease

While pursuing my interest into the causes of cancer, I had proposed long ago that the risk of cancer was increased by a chronic, suboptimal consumption of V/M, and I also suggested at the time that oxidants produced by mitochondria as by-products of normal metabolism, would also contribute to the aging damage [10,11].

Over the years I realized that it should be possible to explore the consequences of nutrient inadequacies using *in vitro* assays (and supporting evidence by analyzing the literature). Several researchers in my lab, and others, lowered the concentration of a number of essential growth components in cell culture. This approach provided evidence of the cellular aging resulting from such shortages, as shown for the following cases: zinc: DNA damage and increased level of P53 [12,13]; choline: an analysis of the literature showed that it has effects on development and cognition [14]; vitamin B6: slows the cell cycle by causing uracil incorporation in DNA [10,11]; magnesium: accelerated senescence in cultured cells and telomere shortening [15]; biotin: heme deficiency and loss of mitochondrial complex IV [16] (biotin is known to be a potent teratogen [17]); folic acid: besides the DNA breaks mentioned above [6], a decrease in the proliferation of $CD8^+$ cells, thus affecting immune cell proliferation and responsiveness [7]. In addition, we discovered that supplementation with the important metabolites acetyl-L-carnitine and lipoic acid improved metabolic mitochondrial functions in old rats [18–21]. Thus, it had become clear to me at this point that there is ample evidence that V/M deficiency is linked to increased risk of diseases of aging. I had also suggested early on, as my interest in diet and health increased, that it would be advisable to "tune-up" metabolism in general by consuming higher levels of V/M [22–26].

4. The CHORI-bar

An obvious approach for improving nutrition would be to change bad dietary habits in general. However, efforts at instituting wholesale changes in diet and behavior have not been successful. I and my colleague Mark Shigenaga came up with the idea that a non-traditional approach would be to directly improve the specific metabolic dysregulation that is the primary cause of increased risk of many diseases, such as diabetes and cardiovascular disease, by consumption of a specially formulated nutrient bar designed to fill gaps in poor diets. The bar would supply all of the necessary vitamins and minerals, fiber, unsaturated oils, etc., in a single edible product. It would be an economical, further improvable, and efficient way to restore micronutrient adequacy. The "CHORI-bar "was developed as such a tool.

The CHORI-bar is a high-fiber, low calorie, nutrient-rich, fruit-based supplement bar. It was developed by a team of biochemists, nutrition scientists, and clinicians, operating out of my group at the Children's Hospital of Oakland Research Institute ("CHORI").[1] Scientists at the USDA Western Regional Research Center in Albany CA provided essential collaboration on refining palatability and also produced all of the CHORI-bars used in the more than 15 small clinical trials we conducted during development of the bar over a period of more than 10 years.

The selection of the ingredients for the CHORI-bar emphasized food components known to be beneficial for healthy metabolism that are deficient in typical Western diets. These included in addition to V/M, polyphenolics, omega-3 fatty acids, several types of fiber and other small molecules important for maintaining a healthy gut [27–29].

The clinical trials (which did not require a change of diet) demonstrated that consumption of two CHORI-bars each day for as short a period as 2-weeks by predominantly lean adults significantly increased HDL-c, particularly large HDL [30]. Longer trials (2-months) in overweight/obese adults resulted in additional significant changes in the direction of decreased risk of future disease in both HDL and LDL lipid particle profiles, triglycerides, insulin resistance and, in addition, statistically significant decreases in weight, waist circumference, diastolic blood pressure and heart rate [31]. These results provided strong support for the concept that the key to reducing disease risk in all people is to restore a healthy metabolism.

In particular, poor diets are one of the causes of obesity, a major health problem plaguing the US, and other countries. Most of the obese have a higher frequency of every age-associated disease that has been examined. The obese eat very poor diets, as judged by the calorie to V/M ratio, thus they are starving for V/M, which may overrule satiety signals and/or activate triage mechanisms [8,9,32,33]. In fact, one of the motivating factors that guided the CHORI-bar research had been the conviction that the main culprit in obesity is the metabolic dysregulation that accompanies it in about 70% of the obese (and about 30% of the lean), rather than the obesity *per se*.

A particular problem associated with obesity in adolescents was recently recognized as a unique type of asthma. It is often severe, difficult to treat by standard procedures, and characterized by less eosinophilic inflammation than in the type of asthma in the non-obese (i.e., it is not predominantly an allergic response). We conducted a clinical trial, in collaboration with the Children's Hospital of Oakland Pulmonary Department, in overweight/obese teen-agers with poorly controlled asthma: consumption of 2 CHORI-bars each day resulted in significant improvements in lung function and positive changes in metabolism [34].

In conclusion, the CHORI-bar is a unique, tasty, fruit-based, high fiber, low-calorie, micronutrient-complete nutrition bar, which offers good hopes of improving many health-related problems. The broadscale improvements in virtually all aspects of the dysregulation of poor nutrition and obesity by CHORI-bar consumption supports the concept that impaired critical underlying functions, such as poor energy production by mitochondria and an unhealthy gut wall, can be restored by supplying dietary components deficient in Western diets. It also suggests that improvements in the diet would be expected to have multiple favorable consequences similar to those observed with the CHORI-bar.

5. High-dose vitamin supplementation therapy to cure genetic disease

I entered this area by chance in the early nineteen seventies and it had important repercussions on my research. One of my responsibilities as a Professor at the University of California at Berkeley was to teach a laboratory course to undergraduate students. The students had to

[1] Presently renamed as: UCSF Benioff Children's Hospital Oakland Research Institute.

perform real experiments, which most of them (and I) particularly enjoyed because they were required to solve actual problems instead of just repeating information they had memorized from a textbook; one of the experiments required them to characterize new bacterial mutants that, unlike the parent "wild type", would not grow on minimal medium, but would grow on a complex nutrient-rich medium. Most of the mutants analyzed turned out to be simple, requiring an amino acid or a purine/pyrimidine, or some other biochemical.

However, one interesting class of mutants emerged that could grow either on the final pathway product (such as an amino acid) or on one of the vitamins. Upon further analysis of several of such mutants, we realized that the mutation had occurred in one of the genes coding for an enzyme responsible for an intermediate step in that particular biochemical pathway and that such gene coded for a vitamin/coenzyme-dependent enzyme. We hypothesized that if such a mutation had not destroyed the enzyme completely, it would result in a partially defective enzyme that had a damaged binding site for its coenzyme, and consequently a poorer affinity for it, i.e., it would have a higher Km (Michaelis constant). Such a defect would explain why it would be corrected upon supplementation with either the actual intermediate or the respective coenzyme, or with the final essential product. This hypothesis was proven correct in a subsequent research project [35].

This finding raised important questions: how common are these mutations? Do humans carry mutations of this type? And if so, can they be ameliorated by treatment with the final product or some intermediate product or a coenzyme? With the collaboration of two undergraduate students who had participated in the course we researched the medical literature looking for examples of remediable diseases in humans. We wrote an integrative review on the effective use of high-dose vitamins supplements for ameliorating human genetic disease [36].

Generally, genetic diseases have been thought of as being irremediable, other than by gene modification. A similar thought is applied to some of the characteristic decay of aging. However, in many cases it was known that some diseases could be ameliorated by supplementation with V/M, once the relevant mechanisms were understood, as summarized in Ref. [36]. Many human diseases were known to be due to a defect in enzymes requiring specific cofactors, as for example: a pyridoxal phosphate-binding (vitamin B6) cofactor (11 cases); FAD or FMN (8 cases); thiamine pyrophosphate (5 cases); NAD(P) (5 cases); folate (5 cases); and a few cases involving minerals, such as zinc and potassium. Surely many more have been identified since. Many of the mutations (as many as one-third) were shown to result in a poorly functional enzyme, with the enzyme having an increased Km (= poorer affinity) for their respective coenzyme, thus resulting in a lower rate of reaction. About 50 human genetic diseases due to defective enzymes were shown (at the time of our review) to be remedied or ameliorated by the administration of high doses of the vitamin component of the corresponding coenzyme, which was shown to restore enzymatic activity, at least partially.

The analysis also provided the important evidence that the strategy of high vitamin administration was effective in some but not all of the cases, and with various levels of remediation. This finding supports the contention that each partial defect in any specific gene is caused by a different protein modification, thus leading to a variety of modified structural changes depending on the specific nature of each mutation. Example of genetic diseases dependent on a specific coenzyme are: methylenetetrahydrofolate reductase that uses FAD (riboflavin) (in relation to cardiovascular disease, migraines, and rages); NAD(P) (niacin):quinone oxidoreductase 1 and the cofactor FAD (in relation to cancer); glucose-6-phosphate 1-dehydrogenase using the cofactor NADP (in relation to favism and hemolytic anemia); aldehyde dehydrogenase (ALDH2) using the cofactor NAD (niacin) (in relation to alcohol intolerance, Alzheimer disease, and cancer) in one-half of Asians.

Two examples of remediation by supplementation are: 1) cystathionine beta-synthase (CBS) which catalyzes the pyridoxal phosphate-dependent condensation of homocysteine and serine to form cystathionine; individuals carrying a defective form of CBS (with either an increased Km or a decreased Vmax), accumulate the very toxic compound homocysteine in their blood and urine, causing mental retardation, vascular and skeletal problems, and optic lens dislocation. Pyridoxine supplementation was shown to have dramatic results towards remediation, with the patient returning in some cases to complete normality [37–39]; 2) ornithine aminotransferase (OAT), a pyridoxal phosphate-dependent mitochondrial matrix protein involved in the conversion of ornithine to proline: defects in OAT result in blindness due to ornithine accumulation [40]; in the pyridoxine-responsive forms of the disease, ornithine accumulation was shown to be decreased upon administration of high doses of pyridoxine.

Interestingly, and in conclusion, it was at this point that it dawned on me that the interest I developed earlier, the damages resulting from poor nutrition, overlapped this earlier work and that it was possible to identify enzymatic problems generated by poor nutrition and remedy them.

6. Insidious metabolic damage

The concepts of inborn or newly-acquired mutations causing sub-optimal functionality of parts of the metabolism and the consequences of common nutritional deficiencies led me to question how commonly would abnormalities exists that do not show up as an obvious disease. Thus, the possibility was considered that some individuals, although being outwardly apparently normal, might harbor a partially defective metabolic protein as the consequence of an inherited dominant somatic mutation (or of some inherited polymorphism), resulting in a deformed protein with poor binding for a substrate/cofactor. If the defect had caused a relatively small change, its consequent damage would not show up as causing a full-blown disease, or even a mild disease, and thus it would affect life in small and undefinable ways. Therefore, such an individual would not necessarily be aware early on of harboring any significant problem, although such a defect might have serious consequences in the later stages of life.

Such a sub-optimal long-term functional defect would not have been detectable by the classical discovery pathway by which all the known vitamins were discovered: i.e., by removing a particular vitamin or mineral from the diet of an experimental animal (or under a known nutritional shortage in a human population) and identifying clear signs of a disease within a relatively short period of time (such as, for example, scurvy, beriberi, pernicious anemia, etc). A long-term effect of a shortage would not have been apparent with such an approach. Thus, such defects are not easily identifiable and are dangerously insidious [9].

7. Mitochondria are particularly sensitive to damage with age

Mitochondria present a unique case because of their particular sensitivity to oxidant damage. Fourteen genetic diseases due to defective mitochondrial proteins have been discussed [36]. The damage was shown to be particularly acute in mitochondrial membranes [18,41,42], presumably due to the presence of high levels of oxidants over time. The latter involves the production of reactive aldehydes arising from lipid peroxidation and the direct oxidation and/or adduction of aldehydes to lysine residues in mitochondrial proteins. Increased oxidant generation is known to cause damage to membrane enzymes and, in particular, to membrane fluidity [43–48], which would lead to deformation of membrane proteins in general. Presumably such damage would also occur in mitochondrial membranes despite their different origin. Poor functionality, as seen by a decrease in the affinity of mitochondrial enzymes for their substrates/coenzymes, would be the consequence [21]. Thus, oxidative damage to mitochondria may be a major contributor to aging and various degenerative diseases (as reviewed, for example, in Refs. [20,21,42,49]).

A postdoctoral fellow in my lab, Tory Hagen, obtained evidence that mitochondrial damage could be remedied by supplementation with

mitochondrial nutrients. In an *in vitro* study we found that mitochondria from old rats, when compared to young rats, showed increased amounts of oxidants by-products and a decreased membrane potential, respiratory control ratio, cellular oxygen consumption, and cardiolipin. Supplementation *in vivo* with two mitochondrial nutrients, acetyl-L-carnitine and alpha-lipoic acid, restored various damaged activities in old compared to young rats [18,20] and reviewed [18,21,49–51].

It is relevant that because of the high rate of mitochondrial damage due to stress conditions, the cell has the ability to constantly turn mitochondria over by replacing dysfunctional ones with newly-synthesized ones, depending in part on nutrient abundance [52].

8. The triage theory

Awareness of the menacing presence of much insidious disease stimulated further ruminations about the importance of the variety of mutations existing in the genetic background in "normal" life conditions. These ruminations led to my proposing the Triage Theory in 2006 [9].

Basically, the knowledge that there are mutations causing suboptimal functionality led me to a more specific investigation and a critical new question: what are the long-term consequences of nutrient shortages on a variety of metabolic functions? How did natural selection deal with the natural variation in the availability of nutrients throughout evolution? The concept of the Triage Theory [9] emerged as a consequence of this questioning. A variation in availability would be due to the natural distribution of metals and minerals throughout the earth (e.g. the 15 essential minerals are not distributed evenly on the earth). Similarly, shortages would occur for other essential micronutrients, when dietary sources are gathered from the environment, because their availability would fluctuate markedly with the seasons and unusual weather conditions. However, such dietary shortages are typical of the present American diet, independent of natural fluctuations.

The Triage Theory posits that, as a result of recurrent moderate shortages of V/M, natural selection would activate and ensure a rebalancing mechanism by a strategic rationing response: a scarce micronutrient would be preferentially allocated to those parts of metabolism that are critical and necessary (from an evolutionary perspective) for short-term survival and/or reproduction, thus protecting them; in the process, those parts needed for long-term survival would be starved for the V/M and become less functional, but without causing acute short-term consequences. This would result in an accumulation of insidious effects that are not readily apparent clinically, with important consequences. I later referred to the latter class of proteins as "longevity proteins" because they normally defend against the diseases associated with aging, while proteins needed for short term survival would be properly defined as "survival proteins" [8]. V/M are needed for both short and long-term proteins.

In summary, natural selection favors short-term survival for reproduction over long-term health. The Triage Theory provides a unifying rationale which includes a causal link between modest micronutrient deficiencies and chronic disease in general, and it also provides a rationale for why a particular class of V/M-dependent proteins (i.e., those that are non-essential), even at modest levels of V/M deficiency, may not be fully functional and may not be accompanied by obvious clinical signs. The Triage Theory does not imply that any particular V/M is the only cause of an age-related disease, but rather that it is a contributing factor along with the sum of all other contributing insidious causal factors, as suggested by the various genetic, and epidemiological evidence. If the Triage Theory is generally predictive of how the body manages modest V/M shortages for the thousands of V/M-dependent proteins, it has important implications for public health. It predicts that optimizing intake of the ~40 essential V/M will remedy deficiencies and reduce the risk of many chronic diseases associated with aging, thus increasing lifespan. Triage rationing is not free; it is a zero-sum situation because it favors survival instead of longevity.

These insidious problems might be delayed or remedied by an inexpensive intervention based on supplementation.

9. Supporting evidence for the triage theory: vitamin K and Selenium

The importance of validating the triage theory was energetically expressed to me by a long-time collaborator, Joyce McCann. However, proving or disproving experimentally that diseases of long-term aging are a consequence of the triage mechanism would be virtually impossible to accomplish. Nevertheless, proving or disproving the theory is expected of any scientific theory; thus, I asked McCann to figure out how to do it.

The approach she took was to categorize the essentiality of V/M-dependent proteins according to the viability of mouse knock-out (KO) mutants.

V/M-dependent proteins lacking in KO offspring that were non-viable or did not survive until weaning were classified as required for short-term survival and reproduction (later named "essential proteins"); V/M-dependent proteins lacking in KO offspring that survived beyond weaning were classified as being needed primarily for long-term health (later named as "non-essential" proteins).

Using data in the published literature the effects of V/M deficiencies on activities or concentrations of the two categories of proteins were compared, as were health consequences of genetic dysfunction (e.g., KO, heterozygotes, polymorphisms, knock-down mutants; human mutants). Population studies examining prospective associations of modest V/M deficiencies with age-related diseases were also analyzed and compared with respect to biological effects of V/M deficiency and genetic dysfunction. On this basis 11 vitamin K-dependent proteins [53] and 12 selenium-dependent proteins [54] were identified. In both cases, there was a clear dichotomy between "essential" and "nonessential" proteins.

In both cases, mechanisms were also identified that suggested a built-in hierarchy to preserve "essential" proteins over "non-essential" proteins upon V/M deficiency. Essential vitamin K-dependent proteins are activated primarily in the liver, while the non-essential vitamin K-dependent proteins are activated primarily in non-hepatic tissues. This dichotomy takes advantage of the fact that dietary vitamin K is preferentially distributed to the liver, and only subsequently to extra-hepatic tissues, thus preserving essential functions when vitamin K is limiting.

Selenium is present in selenium-dependent proteins as selenocysteine, which is incorporated during translation. Interestingly, in the case of selenium, the biosynthesis of the modified tRNA that incorporates selenium into non-essential selenoproteins is itself impaired by selenium deficiency, unlike its tRNA counterpart that incorporates it into essential selenoprotein.

It is particularly noteworthy that in both of these V/M cases, the proteins affected negatively by vitamin K or selenium shortages amount to almost half of the total, suggesting that a large price could be paid in terms of increased disease risk from modest V/M deficiencies. It is also of note that vitamin K and selenium adopted entirely different mechanisms to accomplish the triaging goal, suggesting that nature reached the same purpose by independently-developed selective pressures, thus indicating the importance of this process: for vitamin K the solution is to place the proteins in physically unconnected tissues, while for selenium the rationing is based on the use of two different tRNAs controlled by a modified base in the tRNA. These findings together strengthen the argument that triage is a basic and important mechanism responding to evolutionary pressures in a variety of ways.

In summary, the predictions of the Triage Theory were largely borne out by these two analyses, both of which support the concept that a triage-like mechanism is operative: upon V/M deficiency V/M-dependent proteins required for short-term survival and reproduction are preserved in preference to those needed for long-term health.

10. Longevity proteins and longevity vitamins

Classical vitamins were discovered as necessary for the survival occurring within a relatively short time after depletion from the diet (in that sense they can be defined as *survival* vitamins). However, the problem of cumulative and insidious long-term damage was not considered and the prolongation of healthy aging has not been generally understood as being related to vitamin levels. This problem was addressed by developing the concept of the Triage Theory and of longevity proteins: i. e., the proteins we analyzed to prove the Triage Theory and were determined to be essential for healthy aging. This hypothesis implied that some still-unrecognized longevity proteins are likely to exist, and that they would be dependent on a V/M that has not yet been recognized as specifically important for longevity. Such nutrients would constitute a new class of vitamins that I proposed be named *longevity vitamins* [8].

Inherent in the Triage Theory is the concept that most V/M necessary for the function of longevity proteins/enzymes are *also* survival V/M, therefore, such V/M have *two* effects: on short-term survival and on long-term health. For example, the two nutrients analyzed in detail (vitamin K and selenium) were shown to be both essential and longevity V/M. Three additional examples of V/M that similarly have both effects are: vitamin D, marine omega-3 fatty acids (DHA/EPA) and magnesium. The levels of each of these nutrients are inadequate in a large percentage of the American population, thus these nutritional deficiencies are a major contributor to unhealthy aging.

A search of the literature was performed for the association or causality between various diseases of aging and a number of V/M deficiencies, looking for a class of essential nutrients the function of which has not been realized as being necessary specifically for long-term health. The criteria used included clinical trials, epidemiology, Mendelian Randomization studies, and biochemical and medical literature. This search revealed that, indeed, there are many known essential nutrients that already fit in the category of *longevity vitamins,* such as vitamin D, marine omega-3 fatty acids (DHA/EPA), and magnesium (as mentioned earlier), plus vitamin K and selenium [8].

I also proposed the existence of *putative longevity* vitamins, i.e., dietary biochemicals not officially recognized as having age-delaying functions. Evidence for 11 such compounds having a positive age-delaying effect was presented: the fungal antioxidant ergothioneine; the bacterial metabolites pyrroloquinone and queuine; the plant antioxidant carotenoids lutein, zeaxanthin, lycopene, alpha- and beta-carotene, beta-cryptoxanthin, and the marine astaxanthin [8].

In addition, a particular class is that of *conditional vitamins*, i.e., vitamins that are synthesized by the body, but not at a level that is sufficient to optimize metabolism. Choline is the first known example of a conditional vitamin; the average intakes for the population are half to two thirds of this recommendation, with only 11% of adult Americans achieving the recommended level of choline intake [55]. Severe choline deficiency results in DNA strands breaks in rodents, alterations to epigenetic markers and histones, and it affects brain development. Taurine, which was known to be important for preventing numerous health problems, also appears to be a conditional and longevity vitamin that is particularly important in mitochondrial metabolism [8].

11. Vitamin D and latitudinal mismatch

My most recent interest was an effort to emphasize the important consequences of vitamin D deficiency (VDD) [56]. Vitamin D is synthesized in the skin and the final product, a steroid hormone, interacts with about 2700 binding sites present in the human genome in vitamin D receptor-dependent genes [57], that respond in either a positive or negative fashion [58,59]. The hormone synthesis depends on magnesium (which is present in the chlorophyll, i.e., in vegetables [26]) and adequate amounts of two omega-3 fatty acids EPA and DHA (which are found in marine fish such as salmon and tuna).

Vitamin D levels are inadequate in 70% of the U.S. population (over 90% in African-Americans), with a prevalence of a serious deficiency (10 ng/mL or less), in a large percentage of people of color living at northern latitudes. Few foods, mainly fish and fish liver, have substantial amounts of vitamin D. Therefore, VDD is largely determined by melanin levels and contemporary lifestyles (e.g., little sun exposure by staying indoors, covering the body, and using sunscreen extensively) and excess body fat (which traps vitamin D).

Extensive evidence is available that VDD causes, or has been associated with, wide-spread health problems, a large number of which affect healthy aging (extensively referenced in Ref. [56]. Although many factors adversely affect the health of African-Americans, such as high rates of poverty, poor housing and residential environments, limited educational opportunities, and lack of access to affordable health care, VDD is one factor that can be corrected rapidly and with inexpensive vitamin D supplementation.

One of the purposes of investigating the effects of VDD was to improve health in general, but our analysis addressed more specifically the health parameters of people of color, especially those who found themselves living at higher latitudes than the equatorial environment at which their dark skin color had originally evolved because of ancestral habitats [60]. VDD is associated with higher incidence or poorer outcomes for many diseases regardless of skin color. Evidence is particularly strong for several complications of pregnancy, multiple sclerosis, dementia, type 2 diabetes, colorectal cancer, total cancer mortality, rickets, and acute respiratory tract infections, and now COVID-19. Among various malignancies, VDD has been most consistently associated with colorectal cancer [56]. VDD was also shown to be involved in a number of neurobiological functions: the 3 major human social hormones: serotonin, oxytocin, and vasopressin are under control of the vitamin D steroid hormone. Low levels of serotonin are associated with autism and ADHD in children and with the psychiatric diseases bipolar and schizophrenia [58,59].

Considering the high level of VDD and the important implications of vitamin D interactions, I had suggested long ago that it is particularly important to tune up metabolism with respect to vitamin D [33]. This recent research has strengthened this concept.

12. It was a long trip

I thought it would be interesting to go over the "zig-zagging" process by which many of my ideas came about, as also detailed in Ref. [22]. I started my research career as a very distractable undergraduate student at Cornell University in Ithaca NY with a passion for biochemistry and genetics, getting a good background in chemistry, plus a love for folk dancing, history and classical music. At Caltech, I worked on histidine-requiring mutants of Neurospora mold to sort out the various biosynthetic steps, which used my knowledge of genetics, biochemistry and enzymology. This combination of skills proved fundamental in the course of all the following many years of my research.

This multidimensional expertise then allowed me to study the large and complicated histidine biosynthetic pathway in *Salmonella typhimurium* including its regulation, which led to the concept of a single regulatory unit controlling ten sequentially-positioned genes. My understanding of the chemistry of mutations in the histidine biosynthetic genes, together with my intuition that mutagens ought to be carcinogens, led to the Ames Test (which began as a hobby). Later I developed an interest in oxidative damage and the regulation of antioxidant defenses which made use of my chemical and biochemical expertise [61].

The Ames Test for mutagens, and thus for possible carcinogens, initiated a long research period analyzing the information pouring out of the cancer research community, thus entering the perilous field of comparing mutagenic potency with carcinogenic potency and to the Carcinogenic Potency Database and the Human Exposure Dose/Rodent Potency database [62]. This project put in perspective the possible

cancer hazard of traces of synthetic chemicals (such as pesticide residues) versus that due to the natural mutagenic/carcinogenic chemicals in the diet [63].

The interest in cancer prevention in turn led to thoughts about how unbalanced diets are a major contributor to cancer and the connection with micronutrients deficiency. My familiarity with metabolism [22] was essential for understanding the possible connections between health problems and the underlying metabolism being damaged.

Mulling over the natural mechanisms that would have been devised by evolution, I developed the Triage Theory [9], with the particularly important message that the long term pathology is difficult to detect clinically because it is insidious. My expertise in biochemistry and metabolism led me to understand that such pathology is both measurable and remediable by appropriate supplementation and/or an improved diet.

My interest in metabolism led to the concepts of longevity vitamins and longevity enzymes [8], which further led to the suggestion that 11 already known V/M are actually good candidates for being longevity vitamins and that many such nutrients play a dual role for both survival and longevity.

The development of the CHORI-bar [30,31,34] would have been unthinkable without all the understanding we accumulated about the essentiality of a healthy diet. It was derived from my interest in the effect of nutritional shortages relative to cancer and other medical problems. It became a tool for restoring micronutrient adequacy by supplying as many important nutrients as possible in a single edible and palatable product.

In conclusion, my varied and extensive expertise has been an essential characteristic throughout my entire scientific career. I was blessed with the help of innumerable collaborators: undergraduate and graduate students, postdoctoral fellows, colleagues, and just the entire scientific community. Thank you to you all.

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