

## Editorial

# Methodological problems with population cancer studies: The forgotten confounding factors

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## Abstract

Among clinical physicians it is the population study that is considered to be the “gold standard” of medical evidence concerning acceptable treatments. As new information comes to light concerning the many variables and confounding factors that can affect such studies, many older studies lose much of their original impact. While newer population studies take into consideration a far greater number of confounding factors many are still omitted and a number of these omitted factors can have profound effects on interpretation and validity of the study. In this editorial, I will discuss some of the omitted confounding factors and demonstrate how they can alter the interpretation of these papers and their clinical application.

**Key Words:** Cancer, confounding factors, nutraceuticals, population studies

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## EDITORS COMMENT

In this paper Dr. Blaylock explores the scientific world of Natural Supplemental Molecular Agents and their effect on multiple metabolic systems and health and longevity. The term, Supplements, has received a bad reputation, and, thus, is regarded as a Pseudoscience. None of the Supplements that people take are regulated by the Federal Drug Administration (FDA). Thus, the impression is that they are not rigorously investigated. In some cases that is correct, but there are a large number of well conducted scientific publications that indicate the molecular value of these agents in increasing longevity, lowering blood pressure, controlling diabetes, including a value in a wide variety of disease states. Many of these natural agents have been used for centuries with positive results, but the scientific basis for them was not known until recent experimentation.

Natural Supplemental Molecular Agents is a rapidly developing field that will affect all of Medicine. Most

physicians know little about nutrition for their patients. The 21st century will see a focus on the Healing and Repair of tissues. It is not enough to do surgery and “let the body heal by itself”. It is obvious that millions of cells pour into an injured site and produce substances that can be both beneficial and harmful to the organism. To support these cells requires large amounts of basic metabolic components that the patient does not receive after the injury or to prevent the consequences of injury. It is crucial for physicians to understand these processes that accompany the inflammatory response, so that the repair can be supplemented with the appropriate molecular agents and to ensure that the repair proceeds in the best way. It is common sense that supplementation of a deficiency in molecular agents needed for repair are key to recovery. Yet do we do this after surgery? No. Patients are on glucose and or saline after surgery and often do not eat for days. How logical is this treatment for a body with millions of cells needing nutritional supplementation? These agents extend from glucose,

amino acids, transmitting agents, key ligands, signaling agents, molecules in the energy producing Krebs cycle and the mitochondrial energy chain and a knowledge of the complex set of processes that are released upon injury.

Dr. Blaylock has written this paper, which explores the myths that have been propagated about these Natural Supplemental Molecular Agents and quotes many studies that have been done to support his view. There is a bias in the scientific community about Supplements; yet, a high percentage of the public uses them, but without the proper knowledge. Yes, companies profit from these sales. Supplements represent a 60 million dollar business in the USA. In addition, the success of Supplements is a threat to the Pharmaceutical industry and the money

it spends on developing drugs with which Natural Supplemental Molecular Agents can compete on a less costly basis. Dr. Blaylock's paper exposes the complexity of developing treatments for many diseases and the necessity of multimodal therapy to impact the outcome whether using standard therapy or Natural Supplemental Molecular Agents. In the end one learns how complex this process of tissue repair is that we face in the 21<sup>st</sup> century.

I believe that this paper will be a landmark publication in science.

**James I. Ausman**

Editor in Chief

## INTRODUCTION

Much controversy exists concerning the use of various natural products in the treatment of cancer and the effect of diet on cancer prevention. The prevailing view among most clinical oncologists is that natural supplements, which they classify solely as antioxidants, should not be used in cancer patients under active conventional treatment with chemotherapy agents and radiation. The justification for this warning is that both chemotherapy and radiation treatments depend on the production of destructive free radicals to kill the targeted cells and that antioxidants would interfere with this process. While such arguments have been addressed to some degree in this paper, I will consider more the issue of how cancer/diet and supplement studies, especially the larger population studies, have been designed in such a way so as to create more confusion than answers. In this editorial discussion I want to address some of the controversies regarding the reliability of reported conventional research. This paper is not intended to be an extensive discussion of the issues involved but rather to make some brief observations and ask some critical questions regarding how we look at nutritional and plant extract human studies.

## THE PROBLEM WITH RESEARCH STUDIES

Recent papers have dealt with problems of utilizing or mixing older studies with better crafted newer studies when discussing research that most affects clinical treatment protocols. The older studies often suffered from severe design flaws and overlooked confounding factors that were critical for evaluation of the study. Often older studies suffered from the authors making assumptions that proved not to be true in the light of newer information.

## Older studies have flaws of time lapse and new scientific advances

For example, Ioannidis, in a recent editorial on medical research, found that:

An empirical evaluation of the 49 most cited papers on the effectiveness of medical interventions, published in highly visible journals in 1990–2004, showed that a quarter of the randomized trials and five of six nonrandomized studies had already been contradicted or found to have been exaggerated by 2005.<sup>[29]</sup>

## False information, financial bias, expert opinion

Also of great importance, it has been shown that a delay of years often occurs in reporting a negative study to a previously reported and highly cited positive study, especially if it appears in a “prestigious” journal.<sup>[38]</sup> Ioannidis further makes the claim that most published research findings are false and that a major factor in this problem is depending too heavily on statistical significance, that is, an obsession with a *P* value of less than 0.05. Also of importance is financial incentive. As pointed out by Krinsky *et al.* and others, the greater the financial incentive, as well as other prejudices and interest, the less likely the research findings reported are found to be true.<sup>[36,37]</sup> Other studies have also suggested that empirical evidence based on expert opinion shows that such evidence is extremely unreliable.<sup>[4]</sup>

## Double standards of evaluation of studies

I also find it interesting to observe the double standard as regards evidence. Several publications by critics of nutritional treatments of cancers admit there are no scientifically based published studies or hard evidence for harm caused by nutritional supplements during conventional cancer treatments, yet they do not hesitate to cite such poor “evidence” such as single case reports

and even letters-to-the editors as their proof of harm.<sup>[24,52]</sup> In contradistinction, little evidence is accepted from the proponents of supplement benefits on human cancers no matter how careful and abundant the research.

### Failure to understand the biochemistry of the molecular agents (natural substances) and their actions

A great deal of confusion and traveling down the wrong lines of evidence occurred in earlier cancer-nutrition research. For example, most studies before 1970s assumed that the effects on chemoprevention of a selected vegetable or other edible plant was determined by such plants having a high content of a particular component. For example, it was assumed the beneficial anticancer effects of oranges were due to their high vitamin C content. As a result, vitamin C studies were based on using plants high in vitamin C content, such as oranges, tomatoes, strawberries, and leafy green vegetables. We now know that these high C content vegetables and fruits contain a great number of anticancer molecules, such as flavonoids, carotenoids, and other phytochemicals. Likewise, many of these early studies assumed that  $\beta$ -carotene was the active anticancer component of carrots, sweet potatoes, and squash. Again, each contains a number of anticancer components, many of which are much more powerful anticancer compounds than  $\beta$ -carotene itself.

This assumption of high content components as the major anticancer compound then led to testing isolates of various nutrients, such as vitamin C and  $\beta$ -carotene, against various cancers, often with disappointing results. Yet, even here we find that poor study design produced a confusing picture because many papers used forms of the vitamins, mainly synthetic, that were far less effective than natural forms of the vitamins in question.<sup>[8,30]</sup> In addition, the doses used in these studies varied considerably.

## SUPPLEMENTS: SYNTHETIC VERSUS NATURAL

### Failure to understand the pharmacology of the supplemental molecular agents: Synthetic vs natural, dosage, route of administration, bioavailability

In many such studies, especially when large populations were used, usually numbering in the tens or hundreds of thousands, the cost of the supplement being tested is vital. In most cases a manufacturer of the vitamin in question donated the studied vitamins. In reviewing large numbers of these studies I, as well as others, have found that poorly formulated, synthetic or incomplete forms of the vitamin were most often used.<sup>[11,12]</sup> In addition, it has been noted that the dose used and manner of administration often varied considerably between studies, making interpretation very difficult if not impossible.

Finally, little attention was paid to bioavailability of the various products in question, which is essential.

### Natural vs synthetic supplemental (naturally occurring) molecular agents: Is there a difference?

It was assumed in many previous studies (and by many still today) that there is no difference between natural and synthetic vitamins. Synthetic  $\beta$ -carotene was used in both the Carotene and Retinol Efficiency Trial (CARET) and the Alpha-tocopherol,  $\beta$ -carotene Cancer Prevention (ATBC) studies, both of which found increase risk of lung cancer with the use of low dose  $\beta$ -carotene in heavy smokers and drinkers.<sup>[3,48]</sup> No cancer increase was seen in moderate smokers and nonsmokers. The combination of heavy smoking and excessive use of alcohol was proposed to have caused oxidation of the  $\beta$ -carotene, converting it into an oxidant. Interestingly, a postintervention study of the ATBC study found no increase incidence of cancer with either supplement.<sup>[64]</sup>

The ATBC and CARET studies have been heavily criticized mainly based on the fact that a very low dose of the  $\beta$ -carotene was used (15 mg/day), where the anticancer benefits that are reported require a dose of at least 30 mg/day. In addition, the study was of too short a duration to provide any reliable information. A better designed study from the Physicians Health Study reported later, in which the subjects were given  $\beta$ -carotene in a dose of 50 mg every other day for 12 years found no harm or benefit, even in smokers.<sup>[25]</sup> Synthetic  $\beta$ -carotene was used in this study as well.

Demonstration of the difference between synthetic and natural forms of  $\beta$ -carotene was demonstrated in a study, which found that synthetic  $\beta$ -carotene accelerated the death and shortened the life span of rats exposed to 7 or 8 gray (Gy) of radiation, but the natural form decreased the death rate and significantly increased the life span of exposed rats.<sup>[7]</sup>

### The effect of smoking as an uncontrolled variable in studies

Smoking dramatically lowers tissue levels of vitamin C and vitamin C is essential for protecting  $\beta$ -carotene from oxidation in high free radical environments. Vitamin E also can protect  $\beta$ -carotene from oxidation. In one important study conducted in the Linxian province of China, researchers examined the results of using a mixture of antioxidants— $\beta$ -carotene, vitamin E and selenium—in an effort to prevent gastric cancers. What they found, in contradistinction to the ATBC and CARET studies, was that the greatest anticancer effect was seen when the antioxidant mixture was used in smokers.<sup>[13]</sup>

### Different molecular forms of the supplemental molecules: Cis and trans forms of $\beta$ -carotene

Incredibly, very few studies have been done in these population studies using natural forms of  $\beta$ -carotene. Synthetic  $\beta$ -carotene is composed of only

all-trans- $\beta$ -carotene, whereas natural forms contain a mixture of 9-cis- $\beta$ -carotene and all-trans forms. There is evidence that it is the 9-cis form that has the greatest antioxidant effectiveness.<sup>[8]</sup> In humans, ingestion of the algae *Dunaliella* species (which has a very high  $\beta$ -carotene content and also contains an equal portion of 9-cis and all-trans  $\beta$ -carotene), results in elevation only of all-trans  $\beta$ -carotene in the serum and no 9-cis form. Tissue levels of the cis-form were higher than plasma levels, and most of the 9-cis- $\beta$ -carotene is converted in the intestinal wall to retinol. The cited study in humans found that the 9-cis form is better absorbed and is a much more powerful antioxidant, especially for singlet oxygen. In addition, the 9-cis form is transformed in the intestinal cells into 9-cis-retinol which has 10  $\times$  greater absorption than all-trans retinol, which may account for differences in anticancer effectiveness.<sup>[9]</sup> There is also evidence that the 9-cis form of  $\beta$ -carotene is incorporated into the tissues, even though only the all-trans form exist in the plasma.

#### *Natural forms of beta carotene vs synthetic forms and their differential effect on cancer differentiation*

Studies have shown that the synthetic all-trans form of  $\beta$ -carotene actually suppresses several of the anticancer effects of the more polar carotenoids, such as cell differentiation, and can protect cancer cells from growth inhibition by the other forms of carotenes. The power of natural polar forms of carotene to stimulate differentiation of cancers was dramatically demonstrated in a study using human neuroblastoma cells in which the polar  $\beta$ -carotene alone lead to a 20% cell differentiation rate and when PGE1 was also added differentiation increased to 92%.<sup>[18]</sup>

#### *Metabolized products of supplemental molecular agents may have different effects*

Also important is the fact that natural carotenes are metabolized into a number of compounds that can have even stronger anticancer effects than the parent compound. These metabolic compounds include such products as neurosporene, phytofluene, and phytoene in both trans and cis conformations. The conformation can have important implications in antioxidant effectiveness. Studies, for example, have shown that 9-cis-phytoene had stronger antioxidative effects than the all-trans isomer.<sup>[65]</sup>

#### *Tissue specificity of supplemental molecular agents*

Many of the carotenoids and their metabolic products are tissue specific in terms of concentrations.<sup>[16]</sup> For example, phytofluene is found in higher concentrations in breast tissue than other carotenoids and cervical tissue has an affinity for lycopene,  $\beta$ -carotene and phytofluene. Lung tissue has high concentrations of lutein, lycopene,  $\beta$ -carotene + cis-isomers,  $\beta$ -cryptoxanthin, phytofluene, phytoene. What this means is that while phytoene may be effective in lung cancer prevention it may have little effectiveness in breast cancer, as it is not found in breast tissue.

*Variable tissue concentrations of supplemental molecular agents*  
Studies of specific tissues demonstrate that the concentration of  $\beta$ -carotene can vary considerably.<sup>[16]</sup> For example, concentrations of  $\beta$ -carotene were found to vary as much as 10-fold in the prostate gland. Variations as much as 30–85-fold can occur in such tissues as the kidney, liver, and lung. Rarely, are these tissue-specific issues addressed in large population studies.

We find similar differences between synthetic and natural forms of vitamin E. Natural vitamin E is composed of eight subunits,  $\alpha$ -tocopherol,  $\beta$ -tocopherol,  $\gamma$ -tocopherol and  $\delta$ -tocopherol, and four tocotrienols subunits. The most commonly used form of synthetic vitamin E, called all-ras alpha-tocopherol acetate (previously called DL-alpha-tocopherol acetate), has been shown to have little or no anticancer effectiveness. The most effective against cancer has been the natural form (RRR-alpha-tocopherol) or the synthetic form, alpha-tocopherol succinate.<sup>[21]</sup>

## **SYNERGISM AND ADDITIVE EFFECTS OF NUTRIENTS**

### **Interactions of mixtures of supplemental molecular agents not available with single supplemental molecular agents**

The importance of mixtures of nutrient compounds in reducing cancer risk, such as the various carotenoid forms and forms of tocotrienols and tocopherols, have been shown in a number of human studies.<sup>[47]</sup> For example, an extensive review of the highest quality carotenoid studies have shown that the  $\beta$ -carotene studies using supplements (most using the synthetic vitamin) found little or no reduction in lung cancer incidence, but the studies using natural forms of mixed carotenoids in foods found a significant inverse relationship.<sup>[23]</sup> The anticancer effectiveness of eating fruits and vegetables stems not from single compounds, as was once thought, but from an interaction of hundreds of chemical compounds in these plants.<sup>[11,12]</sup>

Rath and Pauling developed a combination anticancer product designed to strengthen the collagen surrounding cancers, so as to reduce invasion and subsequent metastasis.<sup>[50]</sup> Their product contained lysine, proline, green tea extract, ascorbate, and other micronutrients. Each of the components has been shown to have significant anticancer effects when studied alone. When the Rath-Pauling compound was tested alone in rats having a chemically induced mammary carcinoma, researchers demonstrated greater cancer inhibition than when green tea extract was used alone.<sup>[31]</sup> Adding quercetin and other micronutrients further enhanced the cancer-inhibiting effects of the base mixture. Not only were the tumors smaller, but also the number of metastatic nodules was reduced from 24 in the control animals to 6 nodules in the treated animals.



In another study of the Rath-Pauling nutrient mixture researchers found a 68.4% reduction in tumor incidence, a 78% reduction in tumor weight and a 60.5% reduction in tumor burden as compared with the control group of animals. The animals in the control group developed large adenocarcinomas with an increased mitotic index and prominent angiogenesis, while the supplemented animals had small adenomas with a low mitotic index.<sup>[52]</sup> One of the more interesting effects of this nutrient mixture is its ability to powerfully suppress MMP-2 and MMP-9, two of the major gelatinase proteolytic enzymes involved in tumor invasion. Elevations of MMP-2 and MMP-9 have been strongly linked to tumor aggressiveness and invasiveness of tumors in both experimental and clinical studies.<sup>[59]</sup> The antitumor effect of this mixture is strongly dose related, with the highest dose completely preventing metastasis in an animal model using melanoma cells.<sup>[46]</sup>

Since this early effort, a number of effective anticancer plant extract combinations have been designed, such as combinations of green tea extract, quercetin, a number of herbs, curcumin and DHA, extract of *Scutellaria baicalensis*, hesperidin, naringenin, kaempferol, silymarin, and apigenin.<sup>[1,45,67]</sup> The idea of using a single drug to attack a single target is being effectively challenged by the dramatic findings of the effectiveness of combinations of nutrient extracts on cancer pathophysiology.<sup>[51]</sup>

## ANIMAL VERSUS HUMAN STUDIES

### Human studies involving single target therapy not as effective as simultaneous multiple target treatments

As cancer is a very complex disease process involving a great number of cellular and microenvironmental mechanisms, it makes sense that treatments that can attack a number of these mechanisms and cell-signaling pathways simultaneously have a much better chance of success than treatments that are not directed specifically at cancer cells but all cells in general and at only one or two of the cell growth mechanisms.<sup>[40]</sup>

### The fundamental fallacious assumptions in comparing human and animal studies

*Why are the results of animal and human studies different? Differences in models used*

There is a significant difference in human studies and animal studies in terms of the laboratory designs of anticancer studies and in consideration of confounding factors. In many cases the results of studies using animals or even tumor tissues in culture were incredibly impressive and demonstrated dramatic anticancer effects of the studied compounds, while testing in humans led to disappointing results or even, in rare instances, suggestions that the tested agent may be inducing cancers. Understanding the difference between these two

different types of studies is critical. Especially since we see an almost total dependence on human studies being universally dominant among practicing physicians.

### *Differences between a controlled animal model and diverse but statistically similar human cohorts – Internal environmental factors*

If we think about it in terms of actual human behavior in a real world, we see considerable differences between human and animal studies. In most animal studies, especially the more recent studies, as stated, all conditions are carefully controlled including room temperature, lighting, and environmental conditions. The animals all are fed a controlled, identical primary diet of uniform composition. They even have carefully controlled births and genetic histories.

On the other hand, with human studies, depending on how the study was designed, an incredible number of confounding factors are involved, many never considered. For example, the subjects may: (a) Eat widely varied diets, (b) have various genetic influences (including polymorphisms), (c) widely variable birth histories (which can significantly affect epigenetics; controlling later cancer risk), (d) differing levels of inflammation, their general health status varies, (e) varied sleep patterns, (f) exposure to differing levels of environmental toxic substances, and (g) variable immune competence. In addition, the ability to absorb and utilize various nutrients can vary considerably, that is, the bioavailability and metabolism of the nutrients being tested can vary extensively.

For example, studies have shown that low stomach acid impairs the absorption of  $\beta$ -carotene and this includes drug-induced achlorhydria.<sup>[60]</sup> With so many people on stomach acid lowering medications, this factor alone negates many of the studies using food frequency questionnaires alone or who were given  $\beta$ -carotene alone. All of these listed factors can affect the outcome of the study, yet many if not most are never considered.

### *External environmental toxin exposure*

In a real world, for example, we see a great variation in people's exposure to pesticides/herbicides and fungicides. Even with the strong link between agricultural chemical exposure and some cancers, this single factor is never considered in the large population studies no matter how carefully designed otherwise. Likewise, despite the strong link between a number of toxic metals and several cancers, no consideration is given to the dramatic difference in toxic metal exposure among study participants. There exist powerful evidence of synergism between these toxic metals and human disease.

### *Further toxin effects that produce inflammation*

If we examine a typical population in the United States, we would find that virtually everyone has within their tissues a number of toxic metals, such as aluminum, mercury,

lead, cadmium, and arsenic as well as over 100 industrial and agricultural chemicals, many of which are either singly or in combination, carcinogenic. In addition, all of these xenobiotic chemicals and toxic metals induce chronic inflammation, a major driving force for the carcinogenic process. Yet, I have never seen these considered in any of the highly cited population studies or studies of single natural phytochemicals tested against cancers.

#### *Heavy metal exposure in humans*

One of the more commonly ignored factors in cancer studies is the presence of excess iron and ferritin. Free iron is essential for cell reproduction and a great number of studies have shown a very strong link between the levels of ferritin and iron in cancer patients and test animals and the aggressiveness and invasive potential of a number of cancers.<sup>[22,44,49]</sup> For example, it has been shown that leukemia patients with elevated iron levels have a much higher mortality and rapid course than those with levels lower than midrange of normal.<sup>[33,43]</sup> Rarely are iron levels measured and correlated to responses in these studies.

It is obvious, even upon casual consideration, that humans are engaged daily in a great number of harmful practices that could very well alter the results in large population cancer studies. It should also be obvious that they differ considerably in their immune function, metabolism, genetic and epigenetic influences, and degree of frailty.

### WHAT ARE WE NOT BEING TOLD?

Often medical-related stories reported in the general media are rarely accurate. A case in point was the results of the CARET study and the ATBC study. In each case, the journal involved prereleased results of the study and ultimately the story was reported in a completely deceptive way—at least that is how it was reported in the news. What the public read and heard was that  $\beta$ -carotene could cause lung and prostate cancer. Many people, both lay people and medical professionals, approached me at the time declaring this very same statement. It is still repeated both among practicing physicians to their patients and even in medical articles. Similar distorted stories about other supplements have since appeared in the media and medical literature.

To the true scientist, arriving at a scientific truth is done much in the way a detective solves a criminal case, even though the scientist depends heavily on the “scientific method”. There are many misconceptions about science and how it should be done. Many of those on the borderland of science assume that if something cannot be shown by either the “scientific method” or by statistical analysis, it just is not true and we need not bother ourselves with it any further. Few appreciate that statistics can only tell us probability and not actual

linkage. That is, a finding may not meet the magical  $P < 0.05$  and still be an important factor to consider and may be linked to causation. Statistics can never, as most scientists know, determine causation.

One must consider the fact that between being able to demonstrate an absolute truth by the scientific method and during the many years an idea drifts in the area of hypothesis or theory, many lives are at stake. If you are a scientist attempting to discover the Hadron particle, delays in the discovery process may be disappointing but nobody dies. A cancer patient with months to live cannot wait for bickering scientists to decide if their evidence is fully valid and meets all purist scientific criteria, especially when a premature application of the supplement in question has a low possibility of harm and its benefit is supported by a number of other forms of research.

### IGNORING EVIDENCE

#### The importance of understanding the basic pharmacology of supplemental molecular agents

I have spoken to many doctors who told me that they never pay attention to *in vitro* studies or evidence based on *in vitro* studies and many others will not even accept animal studies, even though human tumors were implanted in the animals. The only studies acceptable to a great many such purist physicians are the human studies that involve a great number of people studied under rigid conditions—such as randomization, double blinding, very large number of participants and done with placebo control. While I do not reject this methodology, we cannot ignore the combined results of many types of evidence, especially when this evidence all points in the same direction. Roitberg makes a compelling case against depending on randomized studies as the “gold standard” of research.<sup>[53]</sup>

Scientific investigations should be done in much the same way a criminal investigation is conducted—that is, by examining and carefully evaluating all of the evidence and correlating one’s findings. Excluding 90% of one’s evidence, either in a scientific investigation or a criminal one would be considered by the prudent mind as foolhardy and most likely to lead to a wrong conclusion.

By this I mean that by looking at studies of cells exposed to the natural supplement in culture gives us a greater understanding of the effect of the nutrient in question in terms of how it affects single cells or groups of cells—either homogenous cells or mixed cultures of cells. Such studies demonstrate how the test substance affects membrane function, cell signaling, genetic and epigenetic mechanisms. Understandably, this information tells us little about the effect of the same substance *in vivo*, mainly because in the intact animal many other factors come into play. Yet, this data can demonstrate a plausible series

of mechanisms to explain why a particular supplement or even plant extract would have anticancer activity, for example. We know a considerable amount about curcumin, biacalein, wogonin, berberine, silymarin and many other phytonutrients, especially how they affect cell signaling and membrane function.<sup>[5,26,42,62]</sup> This data should not be ignored, especially when rejection of these studies is based on a poorly done series of population studies.

Fundamentally, what I am saying is that the same careful pharmacological scientific evaluation of these Supplemental Molecular Agents should be carried out as would be required for any drug consumed by humans. Unfortunately, many companies have entered the “Supplement” field for the purpose of making money, regardless of the science, purity or human benefit to the “Supplement”. There are many safeguards one can apply to determine which natural supplement meets these stringent criteria. For example, one can look for pharmaceutical grade manufacturing classification and insist on independent testing of the supplement for purity and content.

Furthermore the use of the term “Supplement” has a negative connotation when compared with drugs that in many physicians’ minds are highly scrutinized. Thus the world of “Supplements” develops a negative connotation. In this paper, I have used the term Supplemental Molecular Agents for that reason. These are biologically very important substances and molecules most of which have been extensively tested by leading experts in the field of the biological effects of natural substances. Most of the literature dealing with natural substances appears in highly specialized journals, which are rarely read by practicing physicians.

### **In vivo studies**

The next stage is to do *in vivo* studies using a variety of animal species. Animal studies have the advantage of being able to eliminate many of the variables that can affect people exposed to a wide range of confounding factors as I will discuss in more detail in the next section. One, as I have stated, can carefully control the animal’s diet content, feeding times, food volume, exposure to light and dark, room temperature, surrounding stressful events, and other variables. By chemically or genetically suppressing the animal’s immune tumor rejection system one can further refine the conditions and be sure that the response is due to the test compound directly and not occurring by immune rejection initiated by the animal’s immune system.

### **Using a standardized tumor model**

Tumors can either be induced in the animals by chemical, viral, or radiological means or one can use spontaneously developing animal tumors for testing these anticancer compounds. More accurate testing would come from using implanted human tumors. To

eliminate species-dependent effects one can use several genetic species of the same animals or even different species altogether. If the test compound were found to be beneficial under all of these animal models, it would be foolhardy to assume that just because it failed in human population studies, it is of no use.

While isolated case studies and small, uncontrolled studies are considered to be of little value, this prevailing view in government-sponsored studies is fundamentally false. Without unique case observations, no new discoveries would be made upon which randomized controlled trials (RCT) could be done. Case studies can be enormously valuable in discovering the many nuances of disease and lead to new discoveries and theories. In today’s scientific environment, RCT are considered the “gold standard” of science. Many such trials are poorly done and have useless information. Well done case reports, or series of case observations, or RCT can be valuable. As Coccia and Ausman have shown, to eliminate one series of scientific observations as “Anecdotal” is a blatantly biased attitude that favors RCT as the only method of contributing to science.<sup>[17]</sup> In the future, RCT will disappear as medical science determines the genetic makeup of the individual and tailors therapeutic approaches to each person based on his/her individual biochemical state. The assumption made in RCT that by having two populations of patients randomly selected will eliminate the variables between the groups is a false assumption for all the reasons stated above.

In dealing with a cancer with a very aggressive nature and a mortality rate close to 100%, a cure or substantial prolongation of survival can be a significant clue to an important finding—even though it is not definitive. For example, several smaller studies have been done on patients with terminal pancreatic cancer, which have shown significant tumor shrinkage and a prolongation of survival using curcumin extracts, despite the researchers using a very small dose in the studies.<sup>[20]</sup> I personally know of a number of women with stage IV metastatic breast cancer who have had very long survivals following treatment with nutritional methods, even after their oncologist sent them home to die. Careful follow-up of these cases with PET scanning and using other reliable biomarkers, prove that the cancer in many such cases has completely disappeared. We ignore such cases only at our peril.

## **UNDERSTANDING WHAT YOU ARE STUDYING**

### **Why supplemental molecular agents are not the same as a single drug and why results in animals and humans can differ**

One of the major reasons for failure to find in humans similar effects as found in the animal studies is a failure

of the researchers to appreciate that nutrients are not necessarily like drugs—that is, usefulness should not be determined by testing a natural compound in isolation. To test a drug one expects it to work when used alone and to function the same under most conditions (which is also an error). Yet a significant difference exists between a single drug and plant extracts, vitamins and minerals in the body. These Supplemental Molecular Agents do not act alone and these same compounds are not found in plants alone, but rather as complexes of hundreds of compounds. Because cancer is a very complex process and often a process that can undergo radical changes during its course, treatment must target a great many cellular systems and pathways to be successful—such as angiogenesis, cell signaling, immune suppression, tumor nutrition, cell communication, gene activation, tumor microenvironmental conditions, cell detoxification systems, multidrug resistance mechanisms, and tumor cell invasion mechanisms.

### Control of diet and other aspects of the environment to which the human is exposed

To gain the full benefit of a nutritional treatment a great many conditions must be controlled, including the diet, exposure to carcinogenic toxic substances, stress, exposure to endocrine disruptive substances in the environment, toxic metal exposure (and removal), abdominal obesity and levels of physical activity. It should also be appreciated that many biochemical systems work as a network and this is especially true of the flavonoids, vitamins, minerals, and other phytochemicals. Unfortunately, in most studies the compounds are used alone. For example, one sees many studies of vitamin C alone or specific carotenoids or selected flavonoids being used in isolation in studies.

### Antioxidation. Is that all that a supplemental natural substance does? The fallacious assumptions about antioxidants

It is accepted that tissue and cellular antioxidant protection is dependent on the antioxidant network—which includes vitamins, minerals, antioxidant enzymes, special molecules, such as, glutathione and thioredoxins and a number of phytochemicals. If one reviews the cancer research literature, at least until quite recently, one will see that often the experimenter did not really understand free radical and lipid peroxidation chemistry. In many such papers we see reference to one or possibly two reactive oxygen or nitrogen species, while ignoring many others—many of which are much more powerful than the ones cited. For example, I see a number of papers referring to superoxide as a powerful radical, when in fact it is rather weak. Hydrogen peroxide is also frequently referred to as a free radical, which is it not—yet, it breaks down into powerful free radicals. Most papers, especially older papers, ignore reactive

nitrogen species, such as peroxyxynitrite, only referring to the reactive oxygen species. The influence of lipid peroxidation products, such as 4-hydroxynonenal and acrolein, are also ignored in many of these studies. These lipid peroxidation products are often not affected by antioxidant vitamins.

Of real importance is that a great number of the human studies are conducted in a manner that demonstrates a complete lack of understanding of antioxidant specificity within cells. For example, vitamin E is an excellent antioxidant for membrane oxidants, but not those in the tissue fluid or cytosol. Vitamin C is an excellent antioxidant for the cytosol and tissue fluids but only protects the membranes by being in apposition to the membrane. The carotenoids can be both water-soluble and fat soluble, depending on the specific carotenoid. The flavonoids, and many other phytonutrients, affect many other antioxidant cell mechanism, but recent studies demonstrate that they are not as powerful as antioxidants *in vivo* as *in vitro*.

Another common mistake I see used in the cancer research literature is attributing the beneficial effects of vitamins and plant extracts to their antioxidant properties alone. While important, often ignored are their more important effects on cell signaling, membrane properties and in reducing inflammation in the tumor microenvironment—often affecting all of these mechanisms.

### The interaction between supplemental molecular agents from natural sources.

It should also be appreciated that the vitamins and many flavonoids depend on each other to maintain a functional biochemical status—that is, there is considerable interaction among these compounds. This is especially true in a high oxidant situation, as we see in smokers and persons exposed to certain pesticides. Vitamin C used alone in a high oxidant environment quickly becomes oxidized and then becomes a free radical itself. Vitamin E, the carotenoids, alpha-lipoic acid and reduced glutathione restore vitamin C to its reduced and antioxidant state. The same is true of vitamin E, the carotenoids and many flavonoids as well—each interacts so as to restore the functional antioxidant state of each other. Alpha-lipoic acid is unusual in that it is an antioxidant in both its oxidized and reduced state.

### Why these natural substances cannot be tested individually

Unfortunately, many studies test these compounds individually and as expected one sees poor results. This is the proposed reason for the inactivity and possible pro-carcinogenic effect of  $\beta$ -carotene in heavy smokers who were also heavy drinkers of alcohol as reported in the CARET and ATBC studies. The lung being a tissue with a high oxygen tension lowers the activity of  $\beta$ -carotene



by oxidizing it and smoking and alcohol greatly increase the free radicals and lipid peroxidation in the lung tissue as well—which increases the carcinogenic risk of the  $\beta$ -carotene by oxidizing it. The flavonoids have shown great promise in preventing and even treating a number of cancers, yet they too can become oxidized if used alone in a high oxidant tissue.

### The fallacious assumption that by varying one component, an understanding of its action can be determined

As an example of this complex interaction, control of inflammation is often difficult because of the complex biochemical reactions involved. Blocking one set of inflammatory chemicals may not only allow other inflammatory pathways to operate, but also these other pathways can upregulate to a higher functional state, thus increasing inflammation. For example, blocking only COX-1 function may increase the activity of COX-2 or COX-3 leading to higher level of inflammation. Curcumin is a potent blocker of COX-1 and COX-2 but not LOX, an alternate inflammatory pathway. Quercetin is a more efficient LOX inhibitor. When used together one attains better overall inflammatory control. There are a number of sites of inflammatory control that are affected by flavonoid supplementation and the effectiveness improves when antiinflammatory vitamins and minerals are also used in conjunction.

Every physician knows from his/her laboratory experiments in medical school that there are many variables that can influence the measurement of blood pressure. So, why do we assume that by controlling one variable that no other factors are involved in the outcome of the experiment?

### LIMITATIONS OF PRESENT HUMAN STUDIES

There are a great many reasons for failure of studies done in the human populations as we have seen. Other factors may also be at play. For example, the test subjects may have powerful synergistic carcinogenic compounds resident in their tissues at the baseline, such as pesticide residues and heavy metals. Cellular immunity varies considerably between individuals, with many gradations of efficiency and this would also affect the outcome. The subjects' diet may also contain compounds that inactivate or reduce the effectiveness of the studied compound. The type of nutrient used, synthetic or natural, can make a considerable difference in outcome as already discussed. Absorption and bioavailability to target tissues is also critical. And most important is the additive or synergistic effects when using whole plants or even plant extracts.

We rarely see well-designed human studies that look at actual human behavior—not in a laboratory—but rather in

everyday conditions under which people normally live. Let us examine a number of these ignored factors. One of the most obvious is that most people do not eat organically grown foods and for a high percentage most of their diet consists of processed foods containing a great number of additive chemicals not found in nature. Americans, for example, consume massive amounts of sugary, high glycemic foods, not only just at mealtime, but also as snacks. These foods have been associated with a higher risk of many cancers.<sup>[11,12]</sup> These foods are also known to promote inflammation, which is strongly associated with cancer development and in all stages of actively growing tumors.

As I have pointed out in my paper, inflammation within the microenvironment plays a major role in tumor aggressiveness, invasion, angiogenesis, and metastasis—the major factors associated with a poor survival. Most Americans, and many others, now that the Western diet has spread across the world, consume far too many omega-6 oils (mainly as linoleic acid) and this also increases inflammation by activation of pro-inflammatory prostanoids. A number of studies have shown that a high intake of omega-6 oils (as linoleic acid) stimulates tumor aggressiveness, invasion, and metastasis.<sup>[41]</sup> In most epidemiological studies I have examined, the intake of omega-6 oils, sugar, and high glycemic foods are not generally considered. Yet, such a diet might strongly counteract the beneficial effects of anticancer phytochemicals and would affect the outcome of such studies.

A potentially more powerful pro-cancer effect is from retained pesticide/herbicide and fungicide residues on fruits and vegetables. While some of these residues are inside the plants most are on the surface and can be washed off. A number of studies have found that several commonly used insect and fungal control agents increase the risk of several cancers, especially hematopoietic cancers. The vast majority of epidemiological diet/cancer studies do not control for washing of fruits and vegetables, even though studies have shown a significant accumulation of these agents in human fat tissue based on consumption of unwashed fruits and vegetables. Differences in the systemic concentration of these pro-carcinogenic agents could explain the studies showing minor effects of high vegetable consumption on cancer risk.

The widespread growing of genetically modified organism (GMO; plants that have genetic alterations), have dramatically increased the concentration of herbicide weed-control agents being used on crops since the widespread use of Round-up.<sup>[10]</sup> While the main ingredient of Round-up is glyphosate, other chemical adjuvants are also used and may be worse carcinogens according to recent studies. One would also need to consider the use of insecticides within and around the home. Rarely are these confounding factors even considered.

Many earlier, and some recent studies, used a rather liberal definition of “vegetables”, which included a number of vegetables of low nutrient density and even such foods as French fries. In most such studies there is no consideration as to how the vegetables are prepared or the source of the vegetables. Preparation can make a lot of difference. Cooking vegetables in water, for example, can leach out a number of water-soluble vitamins, minerals, and phytonutrients.<sup>[56]</sup> Flavonoids vary as to their susceptibility to heat inactivation and this is never considered. Raw vegetables appear to have greater anticancer effects than cooked vegetables.<sup>[55]</sup>

As to source of the vegetables, it matters in terms of nutrient density. Organic vegetables and vegetables grown locally or in a personal garden have higher nutrient density than commercial vegetables. Many people eat most of their vegetables from a can, which can have adulterated nutrients or low nutrient density. Again, no differential is made in these large studies. Studies in which the vegetables were limited to cruciferous vegetables more often demonstrated significantly higher protective and antitumor ability.<sup>[32,35]</sup>

Another often ignored factor in these large population studies is the influence of growing conditions on the content of nutrients in vegetables. For example, the concentration of glucosinolates in Brassica vegetables, such as broccoli, can vary depending on growing conditions, breeding, cultivation, storage, and processing. In addition, these anticancer compounds can vary in different parts of the plant, that is, seed, roots, or leaves.<sup>[63]</sup> Sprouts of broccoli have been found to have as much as  $\times 100$  higher glucosinolate levels than the mature plant.

In essence, one can see that among a heterogeneous group in a population study, should more individuals consuming unwashed, low nutrient-dense vegetables and having the other pro-carcinogenic factors in operation, be included in the group under study, a poorer anticarcinogenic effect would be expected. This would be reflected in a lesser difference in cancer prevention between the typical Western diet and a high-vegetable diet. This is because of the additive and often synergistic carcinogenic effects of pesticides/herbicides and fungicides from unwashed vegetables as well as the combined effects of the other unrecognized factors.

To take another example, if more subjects with high lead or mercury levels were in the test group versus the control group one would also expect a more narrow difference in chemoprevention outcome. For an even greater accuracy one might determine the level and number of industrial and agricultural chemicals and toxic metal residues within the test subjects' fat tissue. Humans are engaged in a number of high-risk activities that would not exist in laboratory raised test animals and these confounding activities can explain much of the difference in the

results of animal tests and human testing of the same compound.

One of the most ignored confounding factors is the test subject's exposure to glutamate in foods and as additives. Compelling evidence exists that links extracellular levels of glutamate to the aggressiveness, invasion, and metastasis of a growing list of human tumors.<sup>[14,15,58,66]</sup> High levels of glutamate have been shown to stimulate tumor growth and invasion.<sup>[54]</sup> A great number of processed foods contain glutamate additives and several foods, such as cheese, mushrooms, red meats, and soy products naturally contain high levels of glutamate. In addition, a number of supplemental nutrition products used in hospitals and included in cancer patient nutritional support, contain significantly high glutamate levels.

Another important difference is that in most human studies the participants were tested after all conventional methods had been completed, such as surgery, chemotherapy, and radiation therapy. It is agreed that these modalities do considerable harm to a number of organs and tissues and can significantly lower a person's resilience.<sup>[57]</sup> Low resilience greatly contributes to morbidity and mortality of the cancer patient. The presence of chronic conditions, such as diabetes and autoimmune disorders, also affect the outcome.<sup>[34]</sup> Chemotherapy and radiation therapy can damage the gastrointestinal (GI) tract, leading to malabsorption of nutrients.

## CONCLUSIONS

A careful analysis and consideration of the past 60 years of cancer research related to nutrition suggests that these studies have been seriously flawed. Many studies were so poorly done as to cause one to question how such huge sums of money could have been approved for projects that were doomed to fail and that resulted in conflicting and often contradictory results. In many of the older population studies, and some animal studies, it is obvious that the researchers were not familiar with free radical and lipid peroxidation pathophysiology and that their knowledge of the antioxidant network was insufficient. As a result, for years we were given a wrong impression concerning the ability of plant compounds and extracts to prevent and even treat cancer and were led down a blind alley.

It is also obvious upon reflection that a great deal of oncology practice is predicated on this poorly done research, much of which continues today. During the Korean war, a number of our pilots would, while chasing enemy aircraft, concentrate with such intensity on the enemy plane that during a dive they would fail to pull up and consequently crashed into the ground. Studies by the air force determined that this was caused by mentally erasing out all surrounding visual cues—such as the ground rushing up toward the pilot. It required special training to overcome this fatal flaw.

This phenomenon of blindly focusing too closely on an object can also happen in the world of research. Often, especially when a certain paradigm is dominant and causing much excitement, researchers focus intently on the accepted paradigm and ignore, what should be, obvious clues to the puzzle under study. It is accepted by historians and philosophers of science that those who paid attention to the anomalies in science, that is, the things that did not fit the paradigm, made most of the great advances. Far too often in today's world, the anomalies are ignored or swept under the rug. We like things to fit in our conceptual world.

It has been said that the cure for cancer already exists hidden in the scientific literature and that we do not need more data. Be that as it may, it is true that the problem is that most of this data has not been analyzed and utilized. In this paper I tried to define some of these blind spots and hopefully stimulate a more objective examination of natural medicine in cancer treatment. Even if it is determined that no single or complex of natural compounds can cure cancer, there is compelling evidence that a great number of such compounds can dramatically improve conventional cancer treatments and make them much less harmful to the patient.

For most such compounds, very few side effects and complications are seen and most such problems are minor or reversible with cessation of use. There is no clinical evidence of harm by using scientifically based nutritional treatments. With the growing evidence that a number of natural compounds can reverse multi-drug resistance, re-establish p53 function, induce cell cycle arrest, retard invasion, reverse tumor immune suppression, and inhibit angiogenesis it would be foolhardy to reject their use.<sup>[19,27,39,61]</sup> A number of studies have shown that many of these compounds can have a differential effect in that they increase cancer cell sensitivity to apoptosis and protect surrounding tissues and cells from damage by conventional modes of treatment, such as radiotherapy and chemotherapy.<sup>[2,6,28]</sup> It is time that we take advantage of these alternative methods of cancer treatment instead of rejecting them based on poorly done research and rumor. The cancer patient, as stated, cannot wait while we engage in scientific bickering.

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