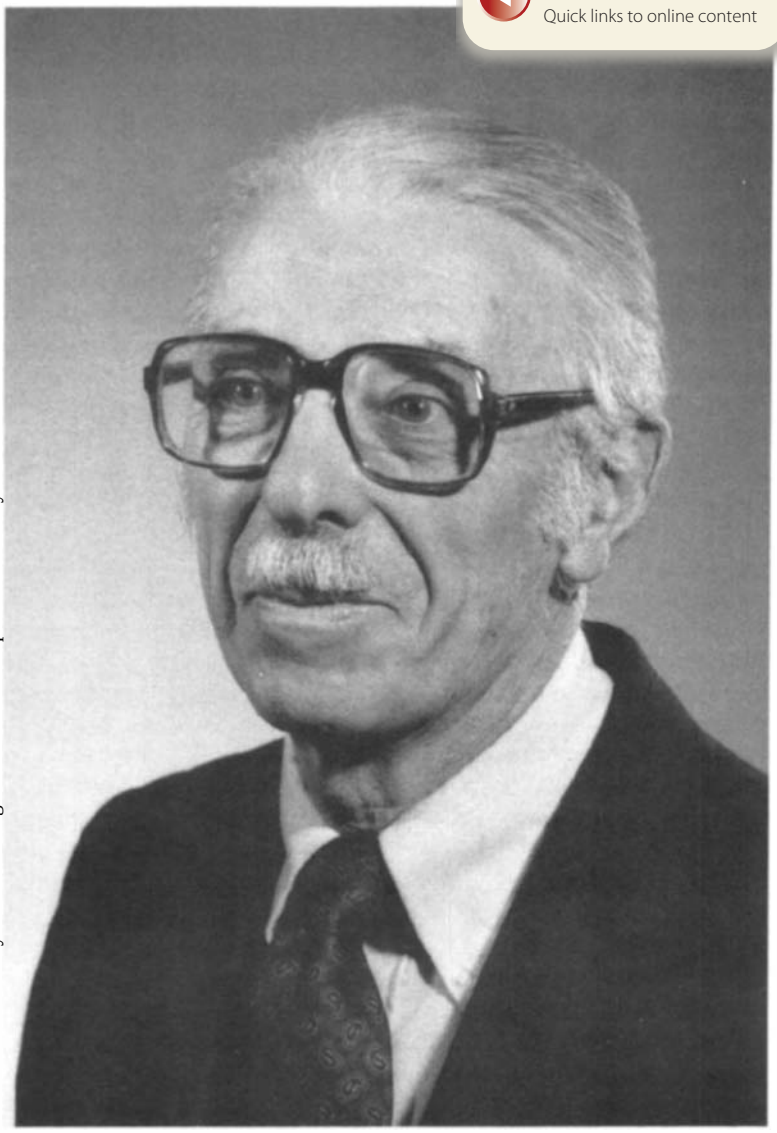



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# NUTRITION SCIENCE FROM VITAMINS TO MOLECULAR BIOLOGY[1]

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## CONTENTS



Much have I travell'd in the realms of gold
And many goodly states and kingdoms seen

John Keats, "On First Looking Into Chapman's *Homer*"

[1]I dedicate this essay to my wife, Marguerite, our three children, and our seven grandchildren.



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# INTRODUCTION—EARLY YEARS

Nutrition is an unusual science. Most Americans, and probably most other people, believe they know something about it. They like talking about food, food preferences, and how food affects them. Food fads are common, and they vary from year to year. At present, oat bran, broccoli, and low dietary cholesterol are in the news, supported by segments of the "nutrition establishment." Nutrition, more than any other science, is exploited and used for money-making schemes based on superstition. A walk through a health food store is something like a visit to an intellectual Dante's inferno. Many newspapers have full sections on food but would never think of publishing a section on enzymes. Yet professors who work in departments of nutrition quite often list themselves as biochemists rather than as nutritionists.

The editors invited me to write a review on "Nutrition Science from Vitamins to Molecular Biology." I worked in the vitamin field from 1934 to 1957, during the "discovery period." In 1962, the emergence of the genetic code led me to transfer my main scientific interest to molecular evolution and molecular biology. Also that year, I devoted much time to defending science against environmental activists and later against creationists. During the same period, I was also active in exposing various forms of nutritional quackery.

In such an essay, I could describe how much harder life was in the old days, how science was just great 50 years ago, tell my readers what they should do, or describe what went on behind the scenes in research. I shall touch on all these matters, and I shall try to be entertaining.

I divide my scientific experiences into two periods. The first was the expansive era of 1930 to 1962, when science was the endless frontier and we strove to prevent hunger and disease. The second was the contentious years following 1962, during the rise of the ideas that human beings are intruders in the biosphere, that their growth must be held in check, and that science is harmful because, even at its best, it enables more people to live.

My memories and experiences of the past 60 years started in the golden age of nutrition: the period when the vitamins, the essential trace minerals, and the essential amino acids were identified and the vitamins were synthesized. These discoveries ended much suffering from nutritional deficiency diseases and led to further improvements in the health of people all over the world.

I came to Canada as an immigrant in 1924, at the age of 17, by myself. I hoped to make my way, on my own, in the New World. I started by working on a farm in southwestern Ontario, where the farmer and his family "lived off the land" in the traditional manner. Fuel was obtained by cutting trees in a wood lot; the crops, livestock, and home garden supplied most of the food; there was no electricity, but there was a party-line telephone and a crystal radio set with earphones. Relaxation was rare. Most of the time was spent in

hard work: hoeing weeds, pitching hay, sawing wood, husking corn, harvesting grain, and picking tomatoes.

Across the border from Canada beckoned the glamorous city of Detroit, where wages were high for unskilled labor. Night shifts ran for 12 hours but for only five nights a week. It was exhausting work, but easier than farming, and I soon saved enough for my first year in college.

In the fall of 1926, I entered the Ontario Agricultural College, then part of the University of Toronto. After toiling on the farm and in the factories, life as a student was like a rest-cure, punctuated by my return to Detroit each summer to earn money for the next college year. At the end of my junior year, I took a job in a biochemical laboratory on campus, to study the effect of nutrition on the hatchability of hens' eggs. Various crude protein supplements gave different effects on hatchability, and Pollard & Carr (cited in Ref. 43) proposed that this was related to an effect of diet on the amino acid content of eggs. If we had confirmed this hypothesis, we would have contradicted the basis of the then-unknown science of biochemical genetics. Fortunately for science (and us), my boss, W. D. McFarlane, and I found no such effect, but we did record our observation that cod liver meal in the diet increased a yellowish tinge in egg whites, which, of course, was due to the then-unknown riboflavin. These eggs hatched well (43).

I spent the next three years as a graduate student in the Department of Biochemistry at the University of Toronto, studying proteins. With my PhD in hand, and with National Research Council support, I then headed for Berkeley as a postdoctoral fellow in biochemistry. The Depression caught up with me in 1934 in California: my postdoctoral fellowship was suddenly terminated because of lack of funds. I went back into nutrition as an instructor in poultry husbandry at the University of California, Davis, then known as the "University Farm."

I felt that I had been "sent to Siberia," except that Davis was noted for being too hot rather than too cold. I had no budget and no laboratory. I was told that my assignment was to study the calcium and phosphorus requirements of turkeys. This was at a time when most of the nutritional requirements of turkeys, or anything else, were unknown. Of course, turkeys had to have calcium and phosphorus to make bones; in retrospect, I should have written a memorandum saying that 2% of bone meal and 2% of ground limestone should be included in turkey diets, because this was what was actually being used—project completed and now let's get on with something else. Probably I would have been fired. Instead, I used patience and cunning. I put the turkey problem "on the back burner" and wrote a proposal for constructing a purified diet for chicks that would supply all the requirements for growth and reproduction. No one had been able to keep baby chicks alive for more than about 6 weeks on a purified diet, even one that contained up to

40% yeast. I was told that my proposal was unpromising, but somehow I was allowed to work on it, probably because I wasn't given any funds. A long list of vitamins and trace minerals had to be discovered before the objective was finally reached in 1948.

## SORTING OUT THE B-COMPLEX VITAMINS: 1935 TO 1948

My first purified diet consisted mainly of starch, casein, and rice-bran extract. The chicks did fairly well for a while, but as a result of an article by Lee Kline and his coworkers at the University of Wisconsin (37) I decided to go in a different direction (41). Kline et al had produced in chicks a distinctive nutritional deficiency, characterized by dermatitis, by feeding a diet of corn, wheat middlings, and casein that had been subjected to prolonged dry heat. Their chicks did not develop polyneuritis, and this showed that the diet was not deficient in vitamin $B_1$. Instead they grew slowly and had a severe dermatitis. Thiamine had not yet been identified or named, and "vitamin B" was considered to consist of $B_1$, destroyed by autoclaving, and $B_2$ or G, a thermostable growth-promoting component, which was thought to be riboflavin. By good luck, I used a supply of acid-washed casein in the diet that was practically free from riboflavin. My chicks on the heated diet developed a double deficiency of riboflavin and of the antidermatitis factor that had been destroyed by dry heat treatment. In 1935, I started to collaborate with Sam Lepkovsky at Berkeley. We found that riboflavin could be removed from a water extract of beef liver by treating it with fuller's earth, and the filtrate contained the chick antidermatitis factor (41). I named the antidermatitis component "filtrate factor." Elvehjem and Koehn, in similar experiments, used the name "chick pellagra" for the dermatitis (7). We announced (42) that so-called "vitamin G" contained two vitamins, and that our experiments showed that a third one existed that was needed for prevention of dermatitis in rats. We called this "factor 1". Paul György named it "vitamin $B_6$" (12).

By 1936 there were four known members in the "vitamin B complex." A big prize loomed on the horizon—the anti-pellagra vitamin, or "PP-factor." After years of work, Joseph Goldberger and his colleagues had shown that pellagra was indeed a nutritional-deficiency disease (11). Their studies also showed that dogs in the households of families with pellagra developed an analogous nutritional deficiency disease, "black tongue." So dogs could be used in the hunt for the PP factor.

We were not allowed to keep dogs for nutritional research in a poultry department. The nearest approach was watchdogs to guard turkeys. However, Sam Lepkovsky at Berkeley had working relationships with the Lilly Research Laboratories at Indianapolis City Hospital, where pellagra was being studied in human patients.

The end of the search came at Madison, Wisconsin, where, as I have told elsewhere (23), a group of young researchers decided to test nicotinic acid on dogs with black tongue. This took place in the biochemistry department at the University of Wisconsin, where the use of dogs was permitted. Doug Frost has related how in 1936 he bought a small bottle of nicotinic acid from Eastman Kodak Company, with a skull and crossbones and "POISON" in capitals on the label. I purchased some nicotinic acid from Eastman Kodak at about the same time, but my bottle did not have a poison label. Interest in nicotinic acid had come from the discovery by B. C. J. G. Knight in England (38) that it was a growth factor for *Staphylococcus*. Now the Wisconsin group showed that nicotinic acid and nicotinic acid amide cured black tongue in dogs. My bottle of nicotinic acid went to Indianapolis, where Paul Fouts and Oscar Helmer showed that this new vitamin promptly cured pellagra in human patients, 1937 (10). Thus in 1937 we could list five vitamins in the B-complex.

In 1938, crystallization of vitamin $B_6$ was reported by Lepkovsky (40) at Berkeley and by four other groups elsewhere (12, 14, 33, 39). Vitamin $B_6$ had been previously crystallized, isolated, and its empirical formula determined by Ohdake in 1932 (45), but he did not recognize it as a vitamin. This suggests the precept that a vitamin is not a vitamin until it has been identified as such. Several substances, including carotene, lactoflavin or lactochrome (riboflavin), nicotinic acid, pyridoxine, hexuronic acid (ascorbic acid), and phthiocol (vitamin K) were known chemically before their nutritional role was discovered. Lepkovsky's isolation of $B_6$ (factor 1) was aided by using a "filtrate factor" supplement, tested for potency with chicks (41), from which factor 1 had been removed by fullers earth. The filtrate factor preparation probably also contained nicotinic acid amide. I was unable to get any crystalline vitamin $B_6$ from Lepkovsky, but soon it was synthesized at the Merck Laboratories, and John Keresztesy sent me a small sample. I put together a simplified diet that enabled me to produce uncomplicated vitamin $B_6$ deficiency in chicks. The deficient birds developed convulsions (16). Later, it was shown that infants on "formula diets" had convulsions as a result of vitamin $B_6$ deficiency.

In 1939, I found that a substance in yeast prevented turkeys from developing a bone deformity termed "perosis." In 1940, in two short experiments, I identified the substance as choline (17).

By 1939, the race was on for identification of the filtrate factor, or "chick antidermatitis factor." Early in 1939, R. J. Williams, H. Weinstock, and their collaborators at Oregon State College described the concentration of an unidentified substance present in liver and elsewhere that yeast cells needed for growth. They named the substance "pantothenic acid" in reference to its allegedly universal occurrence (65). The properties they recorded seemed like those of the filtrate factor. I wrote to Dr. Williams, and asked him for a

sample of pantothenic acid concentrate. It did not have to be pure, as long as its potency was expressed in terms of equivalence to a known weight of whole liver. He kindly sent me a sample of a concentrate prepared from liver. By feeding it to chicks, I found within 43 hours that the filtrate factor was indeed pantothenic acid (15). Simultaneously the same conclusion was reached at the University of Wisconsin (67), and the two announcements appeared in the same issue of the *Journal of the American Chemical Society*. William's group showed that beta-alanine was a component of pantothenic acid. The other half of the molecule was identified by Jake Finkelstein at the Merck Laboratories, who found a description of it in Beilstein's compilation of organic chemicals. It was a lactone, 2,4-dihydroxy-3,3-dimethylbutyric acid-gamma-lactone (60). The Merck group synthesized pantothenic acid in 1940. I collaborated with Sidney Babcock, an organic chemist at the University of California, Davis, who synthesized the lactone and coupled it with beta-alanine in alkaline solution. The chick test showed that the product was the filtrate factor, synthetic pantothenic acid (2).

In the meantime, Esmond Snell and Frank Strong, in a ground-breaking paper, showed that *Lactobacillus casei* could be used as an assay organism for riboflavin (59). Animal tests were much slower and more cumbersome than overnight assays with bacterial cultures, and their procedure revolutionized the hunt for new B-complex vitamins. Esmond Snell described this at length in the 1989 *Annual Review of Nutrition* (58). Snell's work with lactic acid bacteria as assay organisms for growth factors pointed to a new member of the B-complex group—the "yeast norite eluate factor," later named folic acid. Its existence was first shown, in 1931, by Lucy Wills (66) in studies with pregnant women in India. Bob Stokstad pioneered in studies with chicks and called it "factor U" (62). He then switched from the slow chick tests to the use of *Lactobacillus casei* as an assay procedure, and eventually he isolated folic acid from liver (61). Folic acid was identified and synthesized by a large group of organic chemists and biochemists at Lederle Laboratories in 1945 (1). The same feat was performed simultaneously by scientists at Parke Davis & Co. (51).

World War II intervened during these years, but when it ended, I was put in charge of nutrition and physiology research at Lederle Laboratories, Pearl River, NY, a division of American Cyanamid Company. Robert Stokstad and Alfred Franklin joined my group in 1945, and Harry Broquist in 1949. The search started in earnest for the most elusive of the B-vitamins, the anti-pernicious anemia (APA) factor of liver. By this time, it was obvious that vitamins extended their domain over many living species, including bacteria that had become too finicky to live on simple media, rats and chicks on purified diets, and human beings who did not eat the right food. Rats needed a substance called "factor X," or "zoopherin." Chicks needed the "animal

protein factor," sometimes called the "cow manure factor." Cattle and sheep developed a wasting disease caused by lack of cobalt in their diet or by living on crops grown on soils deficient in cobalt. An unknown dietary factor was needed by humans; its lack caused pernicious anemia. Patients with this disease needed both "extrinsic factor" and "intrinsic factor." The intrinsic factor was present in normal human gastric juice, but absent from the gastric juice of patients with pernicious anemia. Intrinsic factor enabled extrinsic factor to be absorbed from the intestine. Injection of purified liver extract bypassed the gastric juice-intestinal absorption route.

The big prize to be won was discovering the extrinsic (APA) factor. Patients with pernicious anemia died from the disease unless regularly treated. For hereditary reasons the disease was most common among people in the north temperate countries. It had no cure, and the only reliable treatment was injection with concentrated liver extract, which was not well tolerated by some patients. We produced a bacterial extract that was tolerated.

Eventually, a lactic acid bacterium was found that responded to the factor, but only one or two research groups knew how to do the test. Finally, APA factor was found to be bright red, and chromatography could be used visually to concentrate it.

In 1948, the APA factor was isolated by the Merck Laboratories in New Jersey (52) and the Glaxo Laboratories in England (57). It was named vitamin $B_{12}$. Everything fell into place; the red color was caused by cobalt. In our lab we found that we could pour commercial liver extract down a silicic acid column, and the red band of the active factor came immediately into view. The disease of ruminants, which resulted from cobalt deficiency, occurred because bacteria in their digestive tracts could not make the vitamin unless cobalt was supplied. Vitamin $B_{12}$ was shown to be the only one of the B-complex vitamins that was not made by green plants. Except for pernicious anemia patients, vitamin $B_{12}$ deficiency in humans is comparatively rare, occurring mainly in vegetarians who do not consume milk or eggs.

Thus ends the first part of my narrative. During the 1930s and 1940s, the fat-soluble vitamins A, D, E, and K, and all the nine water-soluble vitamins were chemically identified, and most of the vitamins were synthesized (24, 53). The golden age of discovery of vitamins had come to an end.

## OTHER GROWTH FACTORS

Our group at Lederle continued the search for unidentified growth factors. Kidder & Dewey (34) found that the protozoan *Tetrahymena geleii* needed a substance in liver for growth. Later, members of our group, including John Brockman, Harry Broquist, Milon Bullock, Jack Pierce and Robert Stokstad, among others, showed that the substance was identical with the "acetate

factor" and "pyruvate oxidation factor" needed by *Streptococcus faecalis* bacteria, and that its chemical structure, obtained by synthesis, was 6,8-thioctic acid (1,2-dithiolane-3-valeric acid) (4, 48). Seymour Hutner urged us to go after other growth factors needed by protozoa. Ernie Patterson et al identified and synthesized biopterin: 2-amino-4-hydroxy-6(1,2-hydroxy propyl)pteridine, needed for growth of *Crithidia fasciculata* (46).

We thought that a new vitamin might be needed for prevention of alimentary exudative diathesis in chicks fed a diet high in Torula yeast. The factor was present in pork liver. However, the ash of the pork liver was biologically active, and Patterson et al identified it as selenium (47). Almost immediately, selenium deficiency in livestock was then reported in many parts of the world. Prior to this, selenium was universally regarded as a toxic element without nutritional value.



## ANTIBIOTICS AS FEED ADDITIVES

Introducing the antibiotic aureomycin (chlortetracycline) into animal feeds was very exciting and a great deal of fun. An article by Moore, Luckey, Elvehjem & Hart of University of Wisconsin in 1946 (44) described growth promotion in chicks by streptomycin and growth depression by streptothricin. This article was largely ignored; indeed, Elvehjem wrote a review a year later (6) on effects of intestinal bacteria on growth without mentioning the 1946 publication, of which he was a coauthor! Our finding, in 1949, was that addition of a crude aureomycin culture to the diet promoted growth of chicks in excess of that obtained with a complete diet. We distributed samples of aureomycin fermentation residues to many research groups that were working with farm animals; large growth responses were obtained, especially in animals with diarrhea. A strong commercial demand developed for the product. The year 1949 was a truly remarkable adventure for us, because of the severity of bloody diarrhea in pigs in the middle west of the United States, including the herds of the agricultural experiment stations of Illinois, Ohio, Florida, and Hormel Institute, Minnesota. The disease was rapidly cured by adding small amounts of aureomycin fermentation residues in the feed. An entrepreneur bought runt pigs in Iowa and brought them to normal size. A druggist in Minnesota prospered greatly when he purchased aureomycin residues from Lederle and repackaged them for sale to pig growers. Senator Wherry of Nebraska complained that an undue proportion of the available supply of the aureomycin supplement ("Aurofac") was going to Iowa (25). The next step was the finding by Bob White-Stevens that higher levels of aureomycin, up to 200 grams per ton of feed, prevented the common ailment air-sac disease, which was usually attributed to *Mycoplasma* infections in broiler chickens (64). Soon antibiotics, especially aureomycin (chlortetracy-

cline), oxytetracycline, and penicillin became standard additives in animal feeds. These antibiotics are still in use for this purpose, despite numerous objections based on claims that low-level feeding of antibiotics to animals produces a spread of transferable resistance, passing from nonpathogenic to pathogenic bacteria. This issue has been debated for about 20 years without resolution. The most recent report on it was in 1989 by a committee appointed by the National Institute of Medicine (5). This voluminous report stated that "the committee was unable to find a substantial body of direct evidence that established the existence of a definite health hazard in the use of subtherapeutic concentrations of penicillin and the tetracyclines in animal feeds."

The antibiotic growth effect is produced by action on intestinal bacteria. This fact has been established in various ways, the most definite of which is that "germ-free animals" grow faster than "contaminated" (i.e. normal) controls, and that these controls show a growth response to antibiotics (9) but the germ-free animals do not. The growth effect occurs simultaneously with an increase in the count of both total and resistant intestinal bacteria. A sparing effect on the requirement for vitamins occurs on diets that are deficient in the vitamin being studied. There is also a sparing effect on the mineral requirement for some minerals (25). Therefore, antibiotic feeding has a definite relationship to nutrition. These two effects are not seen under practical conditions, for under these circumstances, diets deficient in vitamins and minerals are not fed. Instead, the level of "disease" is lowered by antibiotics, and animals can be reared more successfully in confinement. The proportion of total antibiotic production used in animal feeds was about 31% (9.9 million pounds) in 1983 (5).

## METHOTREXATE, LEUCOVORIN AND CANCER

A biochemical adventure started for us in 1946, because of the synthesis of folic acid. The first truly successful chemotherapeutic drug was sulfanilamide, and its effect was due to blocking the synthesis of folic acid in bacteria. Unlike bacteria, animals need preformed, dietary folic acid, so sulfanilamide is without effect on animals. The synthesis of folic acid does not take place in animal cells and therefore cannot be blocked. The possibility arose that folic acid antagonists would block the utilization of folic acid rather than its synthesis. Perhaps, therefore, a chemical antagonist of folic acid could be used to stop the formation of DNA and hence the unwanted proliferation of cells, such as occurs in neoplastic diseases. Our colleagues in organic chemistry made a crude folic acid antagonist by adding a methyl group to one of the compounds used in its chemical preparation. We fed this crude compound, "X-methyl folic acid", to rats, and we found that it reduced their white blood cell count to very low levels; indeed, the granulocyte count went to zero.

Addition of large amounts of folic acid to the diet prevented all the changes. We thought we might have opened a door to the chemotherapy of leukemia, by the administration of a compound whose inhibitory effects on the proliferation of white blood cells could be reversed, if necessary.

Our hopes were short-lived. X-methyl folic acid was ineffective in patients with leukemia. This disappointment soon vanished when another folic acid antagonist, 4-amino folic acid, termed *aminopterin,* was synthesized in 1947 by Seeger, Smith & Hultquist (56). We found that this compound was intensely toxic to mice and killed them within a week or two when fed at only one part per million of the diet. In 1948, Dr. Sidney Farber at Children's Hospital in Boston found that aminopterin produced temporary remission in leukemia in young children. However, its high toxicity, which results from blocking dihydrofolic reductase, made it difficult to use in chemotherapy on a sustained basis. To make aminopterin safe for clinical use, we needed a compound that would block its action, just as folic acid blocked the action of X-methyl folic acid. A lead came from publications by Sauberlich & Baumann (54, 55), who reported that liver contained a substance, needed by the microorganism *Leuconostoc citrovorum.* The substance was evidently a chemical relative of folic acid because slow growth could be obtained by adding large amounts of folic acid to the culture. Sauberlich showed that the "citrovorum factor," but not folic acid, reversed the toxic effects of aminopterin on the test microorganism. Our research group, led by John Brockman and Harry Broquist, synthesized citrovorum factor as 5-formyltetrahydrofolic acid (3). We named it *leucovorin,* and it is used clinically in the procedure termed *leucovorin rescue,* in which methotrexate (a close relative of aminopterin, but somewhat less toxic) is used to inhibit the growth of cancer cells, and the danger to the patient caused by the toxicity of methotrexate is avoided by administering leucovorin at appropriate times.

Just as sulfanilamide opened the door to chemotherapy against pathogenic bacteria, so did the folic acid antagonists make a limited beginning in the chemotherapy of certain forms of cancer; notably childhood leukemia and choriocarcinoma in women. I have described the participation of J. M. Smith, D. Seeger, D. Cosulich, and M. E. Hultquist, organic chemists, and A. L. Franklin, M. Belt, and E. L. R. Stokstad in these studies (30). Methotrexate is now used also in the treatment of noncancerous diseases such as psoriasis and rheumatoid arthritis.

## MOLECULAR EVOLUTION: THE GENETIC CODE: THE NEUTRAL THEORY

In 1962, I heard two lectures that described the beginnings of solving the genetic code. The science of biology was about to be transformed by molecular biology, and, for the same reason, evolution would be transformed into

molecular evolution. I decided to enter a new field, so I resigned my job and returned in 1963 to the University of California, at Berkeley, to become Professor of Biophysics and principal investigator on a grant from NASA, "The Chemistry of Living Systems." NASA was interested in the origin of life and hence was willing to support fundamental studies of molecular biology and molecular evolution. This was in the early years after Sputnik, when NASA was well funded to support research. I hired several young molecular biologists, I put laboratories in an old building that the university owned, and I proceeded to write a book called *Molecules and Evolution* (21). In this book, I discussed the fact that cytochrome *c* in different species has different amino acid sequences, although its function is always the same. I asked if the differences in the amino acid sequences were needed by the individual species, or whether the molecule had diverged because the species, such as dogs and horses, had separated during evolution, and the changes had been "carried along" during the separation because they were the result of random mutations. The changes produced in proteins by mutations will in some cases destroy their essential functions, but in other cases the change allows the protein molecule to continue to serve its purpose. This was my first statement of the "neutral theory" of molecular evolution, 1966.

Earlier, N. Sueoka (63) in 1961 had shown, before the genetic code was known, that the nucleotide composition of DNA in bacteria varied over a wide range in different species. High A+T content of DNA in bacteria was correlated with increases in isoleucine, lysine, phenylalanine, and tyrosine in total proteins, and high G+T with increases in alanine, arginine, and glycine. When the code was solved, it showed that these increases corresponded to the composition of the codons; for example, UAU and UAC both are codons for tyrosine. A codon is the sequence of three nucleotides in RNA (where U replaces T in DNA) that specifies an amino acid in a protein molecule. Evidently, the amino acid composition of bacterial proteins could change without loss of viability. In 1965 (20), I pointed out that changing all the silent positions in codons, from A and U to G and C, for example, changing UAU to UAC, would change the base composition of the genes in DNA from 40% (high AT) to 73% (high GC) without any change in the amino acid content of the organism. This was even more striking evidence for neutral changes than Sueoka (63) had been able to obtain before the code was known.

In 1968, I collaborated with Jack King, a population geneticist, in further exploration of this possibility, and we published a long article in *Science*, "Non-Darwinian Evolution" (36). The idea behind the title was that Darwinian evolution was based on natural selection, implying that all evolutionary changes were adaptive. We pointed out that this was not necessarily always the case: The same proteins, such as cytochrome or hemoglobin, in different species were different according to the length of time the species had been separated, because molecular changes, without changing function, had be-

come fixed by random drift. Our manuscript was rejected by *Science;* one referee said that the idea was so obvious that it did not need to be published, and the other referee said that the idea was completely wrong. We appealed this decision, a step that is not possible to take with *Science* today, and we won the appeal so that our article was published in 1969. In 1977, we received a "citation award" because so many references had appeared to our article. (A few months later Jack King tragically and suddenly died of leukemia.) The same idea of neutral changes was published by Motoo Kimura of Mishima, Japan in a short note in *Nature* in 1968 (35). Our 1969 publication was vigorously attacked by classical evolutionists, many of whom insisted that every change in proteins had an adaptive value. Kimura proceeded to support the neutral theory by means of mathematical studies based on population genetics, and in 1983 he wrote a textbook, *The Neutral Theory of Molecular Evolution*.

Determination of the sequences of codons in DNA molecules provided additional support for the neutral theory as I had predicted in 1965. From 1966 to 1989 I published various articles on evolution of the genetic code. It is now evident that the code itself is evolving, and this is shown by differences between the code in cells and the code in their mitochondria, also by departures from the usual code in certain bacteria and ciliated protozoa.

## EVOLUTION AND VITAMIN REQUIREMENT

Jack King and I used the neutral theory in explaining a nutritional phenomenon. The ability to synthesize ascorbic acid exists in most terrestrial vertebrates. However, scurvy occurs because human beings have lost this ability. So have other members of Anthropoidea, also guinea-pigs, fruit-eating bats, and certain passerine birds. Obviously the loss of ASA (ascorbic acid synthesizing ability) could not be tolerated in a species unless its diet was adequately supplied with ascorbic acid. The guinea-pig, for example, eats green leaves and does not need to synthesize ascorbic acid. But goats also eat green leaves, and they are able to synthesize ascorbic acid. Among the birds, why did the red-vented bulbul, but not the omnivorous mynah bird, stop making its own vitamin C?

Our proposal was that species without ASA lost this ability by a neutral evolutionary change that occurred sporadically by mutation. The change was adopted by genetic drift in the DNA of a few groups of birds and mammals that are widely scattered in phylogeny (31).

In addition to the fairly recent loss of ascorbic-acid synthesizing ability by some species, there is the fact that many animals, including mammals, depend on food or microbial sources for other vitamins (except vitamin D)

and for eight to ten amino acids. This dietary dependence probably means that during evolution, animals lost the metabolic pathways for synthesizing these substances. Here is a field for consideration by nutritionists. Were these losses neutral evolutionary changes? Why are some amino acids made by animals while others must be supplied in the diet? Are there some cases in which animals never had the necessary anabolic pathway, such as that of vitamin $B_{12}$ which is not made by green plants? Vitamin A, retinal, is derived from carotene, which probably originated in cyanobacteria as an antioxidant to protect against oxygen produced in photosynthesis. Carotene is now recommended by some in nutrition for the same purpose: to detoxify free radicals produced by oxidation. We seem to be mimicking our remote ancestors who appeared 3.5 billion years ago.



## DEFENSE OF PESTICIDES

In June 1962, *The New Yorker* published "Silent Spring" by Rachel Carson. This article was mainly an attack on DDT, which had saved more lives and prevented more illnesses than any single chemical in history. This fact was not mentioned by Rachel Carson. I saw that I had to take the side of the people of the Third World against American environmentalists and bird-watchers. I promptly wrote a short manuscript: "A Town in Harmony," which described the privations and dangers of life in the days before the control of pests. *The New Yorker* didn't even acknowledge receipt of it, but it was published in *Chemical Week* (18). I then wrote one of my favorite articles "People and Pesticides," an account of the effects of DDT, for *American Scientist,* the Sigma Xi journal (19). One member of Sigma Xi cancelled her subscription immediately in protest, and officials of the Audubon Society and the American Museum of Natural History retorted with a letter containing long, blistering attacks. These attacks were based on allegations of the effects of insecticides on birds and ignored the information I had presented on control of malaria and other vector-borne diseases. I also received many letters praising the article and asking for bulk supplies of reprints. My defense of pesticides and the attacks on me because of it were to be repeated for many years, but "People and Pesticides" pretty much summed up the whole story. In it, I drew on some of the Audubon bird counts that showed birds counted per observer had increased after DDT was introduced. The defense of DDT occupied me for 13 years, and I was joined in it by Bob White-Stevens, Gordon Edwards, and Norman Borlaug.

In 1972, *The New York Times* (NYT) published an article saying that five people—Gordon Edwards, Bob White-Stevens, myself, Norman Borlaug, and Donald Spencer—were paid liars acting on behalf of the pesticide industry. The first three of us filed a libel suit against Audubon and NYT. We

won a jury verdict and an award of damages in a New York District Court. NYT appealed, and Judge Irving Kaufmann, who was a long-standing friend of *The New York Times,* presided over the appeal at his own request. He wrote an opinion reversing the lower court, saying, in effect, that although we had been unjustifiably slandered, we would have to put up with it so as to protect the freedom of the press. Ironically, NYT had refused to print my immediate rebuttal of the original "paid liars" article. Evidently "freedom of the press" is intended for publishers rather than those who are slandered. The US Supreme Court would not hear our appeal. The episode was a fascinating insight into the workings of the judiciary.

Despite repeated pleas of the World Health Organization (WHO), DDT was banned in 1972 by William Ruckelshaus, head of the Environmental Protection Agency, following long hearings. He gave no indication that he read the proceedings of the hearings, and his ban overturned the recommendations of his own Hearing Examiner. At the request of the editor of *Die Naturwissenschaften* (22) I wrote a long article describing the campaigns against pesticides and rebutting the anecdotal claims that pesticides were injurious to wildlife. Pesticides are essential to the production of enough food.



## THE RISE OF ENVIRONMENTALISM

Starting in the 1960s, the public perception of science largely changed from acceptance to rejection. According to H. Fairlie, "the origins of the widespread refusal to accept . . . risk as a normal and necessary hazard of life began in the early 1970's . . . beyond this has been the growth of the larger belief that science . . . promises evil and not beneficence" (8). This change, influenced by the environmental movement, was so great that most younger people take it for granted, and only a dwindling group of their elders can recall a day when science was the "endless frontier," pushing back what we regarded as the bad old days. The phrase "contains no additives" is used on food labels as a sales aid. The media treat us to regular scares, and quite often pesticide residues are the target, even though their effects are trivial (22).

Nevertheless, in the United States, life expectancy steadily increases, year after year.

## DIETHYLSTILBESTROL IN BEEF PRODUCTION

I had a long involvement with the ban on diethylstibestrol (DES) in beef production. Low levels of supplementary estrogens (rather unexpectedly) increase the production of lean meat by beef cattle. DES is a cheap synthetic estrogen that has this effect on an animal when a small pellet is implanted in its ear, which is discarded at slaughter, or when low levels are fed and then withdrawn.

This use of DES was approved on the basis of absence of residues in meat, but the FDA soon found itself "chasing a receding zero" (28) as methods of assay became more sensitive. DES became strongly politicized in 1971 when A. L. Herbst and co-workers in Boston reported (13) that a small number of young women developed cancer attributed to large doses of DES given to their mothers during pregnancy as an attempted but ineffective medication to prevent miscarriages. The wrath of Senator Edward Kennedy fell, not upon the clinical use of DES, but upon the luckless cattle growers, and he was helped by several prominent scientists including Roy Hertz, who said that one molecule of DES consumed in a portion of beef liver might be enough to start the cancerous process. Hertz did not point out that the daily endogenous production of estrogens in women is about 6 times $10^{17}$ molecules. The average amount of DES given to women during pregnancy was the same as the amount present in 122,500 tons of beef liver containing 0.1 ppb of DES, more than the total annual production of beef liver in the United States. I estimated the average daily intake of DES, resulting from its use in beef production, as between 4 and 5 nanograms. The total for a lifetime of 90 years would be about 0.16 mg. DES is metabolized, is not cumulative, and the clinical dose is several milligrams *daily*. These facts and figures led me to participate in the defense of the use of DES in beef production. The US Food and Drug Administration (FDA) Commissioner, A. M. Schmidt, MD, was against a ban, but he was succeeded by Donald Kennedy, PhD, who placed high priority on the ban. While the hearings were still in progress, Kennedy announced that the ban would take place (sentence first, verdict afterwards), and it went into effect in November 1979. In 1980, Mr. and Mrs. Jud Lackey were brought into court in Wichita, Kansas, accused by the FDA of implanting their cattle with DES a few days after the ban. During the trial, in which I was a witness, FDA witnesses alleged that 1 part per trillion of DES in beef was a finite carcinogenic hazard, but Judge Patrick Kelly was deaf to their blandishments (29). The FDA withdrew its appeal; the disputed beef was liberated from custody, and a barbecue party featuring the said beef was given by the Lackeys in Kansas for its defenders. Of course, the FDA did not rescind the ban.

This experience was my only court appearance against the FDA. I was witness for the FDA and other governmental prosecutors in several antiquackery trials, starting with laetrile.

## QUACK REMEDIES FOR CANCER

My involvement with laetrile, a name under which the cyanogenic glycoside, amygdalin, was illicitly peddled for treating cancer, started when I wrote a short note for the *Journal of the American Medical Association* (26), rebutting the therapeutic claims for laetrile. Its use was promoted by Ernst Krebs, Jr.,

on the mythical basis that amygdalin would be broken down by an enzyme in cancerous tissue to liberate cyanide which would "kill" the cancer. This nonsense was exposed by David Greenberg, who showed there were only traces of beta-glucosidase in animal tissues and even less in experimental tumors. Even if cyanide were liberated from amygdalin, it would diffuse rapidly and poison surrounding normal tissues.

Krebs then alleged that laetrile was "vitamin $B_{17}$," although it had not the slightest resemblance to a vitamin; the crucial property of a vitamin is that its absence from the diet produces a specific deficiency disease in vertebrate animals. I testified to this effect in several court trials, all of which ended in victories for the prosecution. One was in San Francisco, where Krebs, the originator of the name laetrile, received a 6-months jail sentence, which he eventually served. Preparation for these trials involved much work in which the lawyers prepared me for the courtroom, and in turn, I lectured them about nutrition. I found that I was "on the list" as a witness for topics other than laetrile. Another fable concocted by Ernst Krebs with the support of Dean Burk was the claim that a hypothetical "pangamic acid" was "vitamin $B_{15}$". This claim was gladly accepted by many "health food" purveyors. Even the name "pangamic acid" was spurious; the actual product was usually a mixture of betaine and glucuronic acid.

In general, legal action against quack remedies is a protracted process. By the time a court decision is reached, the defendants have moved on to other, greener pastures.

Vitamin C came into prominence as a universal remedy. Bogus claims for vitamin C were made as a result of its hyperenthusiastic promotion by Dr. Linus Pauling (49), and I appeared as a rebuttal witness against him in San Francisco and Santa Rosa, California.

Linus Pauling published the following account of a discussion with me (50):

> I am reminded of an experience I had in 1984 on a radio medical program (on station KQED) in San Francisco. There was another guest on the program, a retired professor of nutrition from the University of California in Berkeley. I made a statement about the value of a high intake of vitamin C . . . The retired professor of nutrition said simply, "No one needs more than 60 mg of vitamin C per day," without giving any evidence to support his flat statement. I then presented some more evidence for my large intake, and he responded by saying, "Sixty mg of vitamin C per day is adequate for any person." After I had presented some more evidence, this retired professor said, "For fifty years I and other leading authorities in nutrition have been saying that 60 mg of vitamin C per day is all that any person needs!" There was just time enough left on the live radio program for me to say "Yes—that's just the trouble: you are fifty years behind the times."

What actually happened is described on the official audiotape of the broadcast, May 7, 1984. (LP = Linus Pauling, DW = moderator, TJ = T. Jukes).

LP: Well, I failed to mention that if I am traveling and people are sneezing in my face and [I] get pretty tired and think that I may be coming down with a cold I go up to as much as 50 grams of Vitamin C in a day and that stops it.
DW: Do you think that is recommended for all people, Dr. Jukes, to take that much Vitamin C?
TJ: Well, I certainly don't.
DW: Based upon your previous observations.
TJ: Based upon my previous observations and my studies of literature and my conversations with vitamin experts for about the last 50 years.
DW: All right.
LP: You know it's too bad that these vitamin experts have been making the same statements for the last 50 years and haven't caught up with the times yet.
TJ: Yes, but their statements are based on controlled observations.

This shows a lack of agreement between what Pauling reported and what actually happened.

## MEGAVITAMINS

The Food and Drug Administration proposed that an upper limit should be set on over-the-counter vitamins of 150% of Recommended Daily Allowances (RDAs) per tablet. This modest proposal was seen as a challenge by the megavitamin industry. The National Health Federation organized a letter-writing campaign to Congress for the support of the "Proxmire Bill" that specifically prevented FDA from making such a regulation. The Proxmire Bill was opposed by the American Institute of Nutrition and other scientific groups. It was supported by *Prevention* magazine, the National Health Federation, Linus Pauling, and Roger Williams. The Proxmire Bill passed the Senate by a vote of 81 to 10 in September 1974.

Another successful nutritional deception is the "organic food" industry. The term *organic* to describe food and a particular farming style was originated by the late Jerome Rodale, an electrical contractor who moved from New York City to Pennsylvania and decided to become an expert on farming and health. His ideas feature the promotion of garlic, dolomite, fertilized eggs, sunflower seeds, honey, and dried seaweed. His son, Robert Rodale, offered the following definition at a public hearing on "organic foods" in New York City in 1972: "Organically grown food is food grown without pesticides, grown without artificial fertilizers, grown in soil whose humus content is increased by the additions of organic matter, grown in soil whose mineral content is increased with applications of natural mineral fertilizers, has not been treated with preservatives, hormones, antibiotics, etc."

By incessant repetition, and conspicuous publicity in the media, the "organic" concept of farming has gained headway. It has been helped by unfounded fears of pesticide residues in food.

## CREATIONISM AS AN ENEMY OF SCIENTIFIC EDUCATION

The creationists are a religious sect of biblical fundamentalists. They assert that any scientific conclusions that differ from the literal reading of the Book of Genesis are in error. Creationists distort and pervert science by fabricating "creation science," in which the age of the Earth (and the Universe) is placed at 10,000 years, all species of life were created instantaneously, the Great Flood of Noah was an actual event, fossils of animals are the remains of creatures drowned in the Great Flood, and nations and languages originated at the Tower of Babel. They lobby incessantly for the teaching of "creation science" on an equal-time basis with science in schools. Creationists harass schoolteachers who present subject matter on evolution in public school science classes. They intimidate publishers into deleting mention of evolution from school science textbooks.

Their main objective is to deny and discredit evolution as evil, but they extend this objective to include condemnation of geology, physics, and astronomy. By incessant Bible-quoting and appeals to "fair play," and aided by the mass media, creationists have succeeded in getting support from a substantial proportion of the US population. I have spent a lot of time during the past 20 years exposing the creationist attacks on science. Currently, creationists have been successful in obtaining revisions in the new Science Framework for California Schools. Creationism is an intolerable burden on the scientific community.

In 1982, I organized and chaired a symposium, "The Creationist Attack on Science" for the American Society of Biological Chemists at the Federation Meetings in New Orleans. One of the speakers was Julian Bartlett, D. D., Dean of Grace Cathedral, San Francisco. It was the first and only time that a clergyman addressed the Society on a religious topic (27).



## SUMMARY

Nutrition is a science of great importance. Indeed, unless one studies it, one might be afraid to eat anything, in view of all the scare stories about food. Today is the age of molecular biology and above all of DNA. The human genome project will bring new understanding of genetic diseases, and many of these will be "inborn errors of metabolism," in which nutrition has an important role. The challenge of nutrition is to help provide a healthy diet for all the world.

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