Reflections
A PAPER IN A SERIES COMMISSIONED TO CELEBRATE THE CENTENARY OF THE JBC IN 2005

JBC Centennial
1905–2005
100 Years of Biochemistry and Molecular Biology

An Enthusiasm for Metabolism

Published, JBC Papers in Press, December 20, 2002, DOI 10.1074/jbc.X200010200

Bruce N. Ames
From the Children’s Hospital of Oakland Research Institute, Oakland, California 94609-1673

Becoming a Scientist

I was born on December 16, 1928 in New York City. My father, Maurice U. Ames, who was born in 1900 in New York City, had a J.D. in law and an M.A. in chemistry. He had originally planned to practice law, but when the depression hit, he became a high school chemistry teacher, which he viewed as a less risky occupation. He later became a high school principal, then supervisor of science for the New York City public school system, and eventually Assistant Superintendent of Schools. My father was very smart, not eager to try new things, and quite placid by nature. My mother, Dorothy Andres Ames, had come to New York as a young child from Poland. My mother had a tremendous joie de vivre, and I think I inherited my ebullience and creativity from her. Despite their very different personalities my parents got on marvelously well. Someone once remarked to my mother that my father “has such a wonderful disposition.” My mother replied, “Oh he doesn’t have a wonderful disposition. He has no disposition.”

I grew up in the Washington Heights area of Manhattan. Every summer during my childhood I went with my family to Warrensburg in the Adirondack Mountains, where my father and a group of other New York City school teachers rented houses on Echo Lake. Those were wonderful summers. I would collect and study most of the creatures that existed in the woods around the lake, though my mother never was too enthusiastic about the mice and the snakes. Every week I would go and get another stack of books to read from the town library. I was always curious about the world and loved digging into still another subject, and my reading interests have remained quite eclectic to this day. I attended the Bronx High School of Science where I became immersed in biology and chemistry. I did my first scientific experiments there; I grew tomato root tips in culture to determine the effect of plant hormones on their growth. The picture of those white roots growing on their own when stimulated by hormones stays in my mind. The pleasures of doing those experiments set me on the path to becoming a scientist.

Cornell

I attended Cornell University from 1946 to 1950 and received my B.A. degree with a major in chemistry/biochemistry. I never was a top student, either in high school or college. I had only a so-so memory and was easily distracted by some new enthusiasm (reading all of Tolstoy or mastering some new folk dance) when I should have been studying for an exam. Taking required courses was not a thing I could get very excited about (I am too undisciplined and driven by my own enthusiasms) though I did well in those few that sparked my interest. Two such courses from my Cornell days stay in my memory. One was a history class taught by Professor Marcham in which we investigated historical incidents by reading all of the original documents, which of course were quite contradictory, and then tried to determine what was the most likely reality. The other was a course in biochemical genetics taught by Adrian Srb. I had already taken several genetics courses, as I was interested in the subject, and Srb’s course got me all excited because of my background in biochemistry. I applied to various graduate schools as I was finishing up but was somewhat apprehensive because of my less
than stellar grades. I was, in fact, turned down by Wisconsin, but luckily I was accepted by Cal Tech, perhaps because Srb, or one of my other references, saw potential in me.

Cal Tech

I arrived at Cal Tech in August 1950. I chose Professor Herschel K. Mitchell, a former postdoctoral fellow of George Beadle, as a mentor and was doing experiments within a few weeks of arriving. Finally I was in my element, lots of research and relatively few courses. Beadle was chairman of the biology department; he and Tatum had previously pioneered the use of biochemical genetic techniques in the mold Neurospora, which led to their Nobel prize.

I studied the biosynthesis of histidine in Neurospora, using mutant histidine-requiring strains involving at least 4 different genes that Mitchell had isolated. A few months after I got to Cal Tech, I adapted a sensitive reagent for the imidazole ring, which is the heterocyclic ring in histidine, to be used as a spray reagent for paper chromatograms (1). This was the key to elucidating the biosynthetic pathway. I grew a culture of each mutant strain in low histidine medium and chromatographed the supernatant. There were about six different imidazoles in the collection of mutants, though each defective gene had a unique imidazole set, and one had none (2). My next task was to identify these imidazole intermediates. I had one very lucky break. While searching the literature on imidazoles I came across an old German paper that reported cooking up glucose, ammonia, and formaldehyde to form an imidazole with a side chain of four carbons, each containing a hydroxyl group. My intuition told me this was the solution to my problem. I then cooked up ribose, ammonia, and formaldehyde, chromatographed the mixture, and found an imidazole-containing compound with the same mobility and properties as one of the compounds accumulated by one of the histidine-requiring mutants. I guessed, and soon showed, that it was imidazole glycerol (2).

The rest of my thesis went very quickly. While I was doing all of this, Bernie Davis' laboratory published a paper showing that histidinol was a precursor of histidine. That turned out to be another of our imidazoles. A pathway of imidazole glycerol, imidazole acetal, histidinol, and finally histidine seemed to make biochemical sense. We also made the double mutants from all of the genes that were involved, and I determined which imidazole intermediate accumulated. Using this trick I was able to order the steps in the pathway and found that this fit with the biochemistry (3). I made the same compounds from the various pentoses and showed that the substance from ribose, the D-erythro isomer, was the right one as it was the same as the accumulated compound from the mutant (4). I soon found that some minor, slow moving, imidazole-containing spots on the chromatograms were the true intermediates; these were the phosphate esters of the compounds. I synthesized imidazole glycerol phosphate by cooking up ammonia, formaldehyde, and ribose 5-phosphate. I suggested that the first step of the pathway involved ribose phosphate. Later at NIH Bob Martin and I showed that phosphoribosyl pyrophosphate was in fact the precursor of imidazole glycerol phosphate and that it condensed with ATP, which donated the nitrogen and carbon of the imidazole ring (5). I worked out a good part of the pathway of histidine biosynthesis and finished most of the work for my doctoral thesis during my first year at Cal Tech.

Cal Tech was an exciting place for a budding scientist. I became part of the group revolving around Max and Manny Delbruck, who had a salon of sorts, which included play readings, dinners, musicals (I played the alto recorder, though not very well), and camping trips into the desert. While at Cal Tech I took both summers off; one summer I took C. B. van Niel's bacterial physiology course at Stanford University's Hopkins Marine Station in Pacific Grove, CA and the next the physiology course at Woods Hole, MA. Both laboratory courses were extraordinary and expanded my interests. I completed my Ph.D. degree in June 1953 at the age of 24. I had arrived at Cal Tech as a very green, very young researcher who was very uncertain that my memory and focus were good enough to make it in the competitive world of science. I left with at least some conviction that my curiosity and creativity might carry me through and that with a little luck I might make it.

NIH

I knew I needed to learn enzymology, so after completing my Ph.D. degree, I took a postdoctoral position in September 1953 in Bernard Horecker's laboratory at the National Institutes of Health (NIH). At NIH I fished out the enzymes of the histidine pathway using the intermediates I had isolated and synthesized at Cal Tech. I had switched from Neurospora to Salmonella typhimurium as a result of a collaboration with Phil Hartman at Johns Hopkins,
who was studying the genetics of the histidine mutants of Salmonella. In 1954, as an independent investigator at NIH, I began work on gene regulation in histidine biosynthesis using Salmonella. We showed that the histidine genes, which were in a cluster in Salmonella, could be overexpressed if histidine availability limited the growth rate; we also showed that the enzymes were controlled as a group, “coordinate repression” (6). We became interested in a mutant found by Hartman, which had a short region at one end of the cluster deleted, but turned off the function of all of the intact histidine genes. We concluded that the cluster of genes was controlled together as a unit by a regulatory sequence.

NIH was a wonderful place to do science. There was enough money for research and no teaching or committee duties. I interacted with outstanding scientists and formed many lasting friendships: Gordon Tomkins, Earl and Terry Stadtman, Maxine Singer, Ira Pastan, Herb and Celia Tabor, Leon Heppel, David Davies, Marty Gellert, Gary Felsenfeld, and many more.

I married Giovanna Ferro-Luzzi in 1960. She had come from Rome to do postdoctoral work at Johns Hopkins University in 1958, and I met her at the Baltimore-Washington Enzyme Club. We are still remarkably happily married some 40 years later. When she finished at Hopkins, Gordon Tomkins gave her a position in his laboratory.

Cambridge/Paris

In 1961, I took a year of sabbatical leave from NIH. Giovanna and I divided our time between Francis Crick’s laboratory in Cambridge and Francois Jacob’s laboratory at the Pasteur Institute in Paris. It was a honeymoon year, both personally and intellectually. This was an exciting time in Cambridge with Crick, Brenner, Perutz, Kendrew, and innumerable distinguished visitors in the incubator for what would become molecular biology. The Institut Pasteur was also an exciting place where Jacob, Monod, Lwoff, Francois Gros, Jean-Pierre Changeux, Giuseppe Attardi, and numerous bright young people worked in a ferment of activity.

NIH Again

I returned to Bethesda in 1962 to become a section chief at NIH in a group Gordon Tomkins had formed, the Laboratory of Molecular Biology. My research focused on the regulation of the histidine operon (the cluster of genes involved with the biosynthesis of histidine in Salmonella) and the role of transfer ribonucleic acid (tRNA) in this regulation. I was very lucky in that the first three postdoctoral fellows who came to my laboratory were Gerry Fink, John Roth, and Robert G. Martin, a tremendously talented and enthusiastic group. Among the significant contributions during this period was a paper Bob Martin and I wrote on using sucrose gradient centrifugation to determine the molecular weight of enzymes (7). Bob was a medical student at Harvard who had come to my laboratory for a semester as part of an NIH program for medical students interested in biomedical research. I was enamored of the idea that the histidine biosynthetic enzymes were in a complex in the cell, and I encouraged Bob to see if this was true, using sucrose gradient centrifugation, a method that had been developed for analyzing ribosomes and larger molecules. He worked out a method for doing this during his semester at NIH. Though there was not much to the idea of a complex of histidine biosynthetic enzymes, our paper on the method became one of the most cited papers in biochemistry. Bob enjoyed his time at NIH so much that he came back after he finished medical school and stayed on with me as a postdoctoral fellow. He later showed that the histidine biosynthetic genes were turned on and off as a unit and that a single mRNA was produced from the cluster of genes. His wife, Judith Martin, got a job as a reporter at the Washington Post, and she eventually became “Miss Manners,” the columnist.

The Test for Mutagens

Sometime in 1964, I read the list of ingredients on a box of potato chips and began to wonder whether preservatives and other chemicals could cause genetic damage to humans. I had been working on some aspects of mutagenesis with Harvey Whitfield (8, 9). I thought it would be useful to have a test for chemical mutagens and so I decided to develop one. Because we had thousands of mutants of Salmonella that required histidine for growth, mainly isolated by Phil Hartman over the years, I made use of them in my experiments. The experiments involve placing a few hundred million bacteria onto a Petri dish containing agar medium with a trace amount of histidine. This small amount of histidine allows all of the plated histidine-depend-
ent bacteria to undergo a few cell divisions. After the histidine is depleted from the medium only those bacteria that have mutated back to wild type continue to grow and form visible colonies on a light lawn of the mutant. The spontaneous mutation rate for each strain is relatively constant. However, when a mutagen is added to the assay mixture, there is an increase in the number of histidine-independent colonies and a dose-response curve can be obtained. During the next few years I developed a set of the most sensitive tester strains using all of the known mutagens I could get my hands on; I further improved the sensitivity of the test by eliminating some DNA repair systems in the strains (10).

Leaving NIH

I was happy at NIH; Giovanna and I had two children, and we liked the area. NIH was a great place to work, and we had a wide circle of friends. What prompted my moving was that Gordon Tomkins, the director of the Laboratory of Molecular Biology in which I worked, started looking at job offers. Tomkins, who had both an M.D. and a Ph.D., was an immensely bright polymath with extraordinary charisma, charm, breadth, and intelligence. He was making a mark in science and was known to everyone at NIH. Among the section chiefs in his laboratory were David Davies, Marty Gellert, Gary Felsenfeld, Todd Miles, and myself. Tomkins was at the center of our little universe, but he was getting offers to become department chair at one university or medical school after another and was seriously thinking about moving. I became convinced that he was likely to accept one of these offers and that our tight and compatible group would likely break up. I mentioned to my friend, Jesse Rabinowitz at the University of California, Berkeley that I might be on the market. Berkeley offered me a job soon after, and as I always had a soft spot for California since graduate school, Giovanna and I decided to move. I had been at NIH for 15 years. Tomkins moved to the University of California, San Francisco soon afterward and we were close friends with Gordon and his wife Millicent until his tragic early death. I felt privileged to have known such an extraordinary fellow, as did almost everyone who knew him. I have discussed his life in a commemorative essay (11).

Berkeley

I arrived at the University of California at Berkeley in December 1967 as a Professor of Biochemistry. I continued to work on regulation of the histidine operon with a series of graduate students and postdoctoral fellows. I was particularly interested in the role of histidine transfer RNA and its modified bases, which we had shown were important in the regulation. In later years I also worked on the regulatory system for defense against oxidants. I also continued my work on mutagen detection, though for many years I considered it more of a hobby until it became a major research focus. In trying to get funding for the mutagen project I was turned down by the National Cancer Institute (they did not think bacteria could teach us much about cancer), but I finally got funded by the Atomic Energy Commission, as they were interested in mutation.

My 30 some years at Berkeley were remarkably happy ones, and despite some shiny job offers I never was tempted to leave. I enjoyed and respected my colleagues, who were an amazingly responsible and competent crew. I was fortunate in attracting excellent graduate students and a series of first rate postdoctoral fellows from all over the world, aided no doubt by the allure of the San Francisco Bay Area.

The Biochemistry Department had a rotating chairmanship, and though I tried to avoid the job for as long as I could, I served as the department chairman from 1983 to 1989 out of a sense of duty. I am not particularly good at administration (I am incorrigibly distractable and find myself drifting off in committee meetings and thinking about experiments) so I try to avoid administration whenever I can. I did, however, form the National Institute of Environmental Health Science Center at University of California, Berkeley in 1979 and served as the director until 2002. There are now 22 such centers. I heard we were known as the Center with poor administration but great science.

Mutagens Again

Because the mutagen project was viewed as more applied research and was not basic enough for the graduate students and postdoctoral fellows who came to my laboratory, I utilized mostly a succession of wonderful undergraduate students who did honors work in my laboratory. I have always had the policy of having about six or so undergraduate students in the
laboratory at any one time; I particularly enjoy their youthful enthusiasm. The word must have gotten around that my laboratory was a good place to work so I think I attracted some of the best undergraduates. Each undergraduate student normally chooses a postdoctoral fellow to work with based on their interest in the project. A high percentage of my papers from Berkeley have undergraduate students as coauthors.

In the early 1970s we continued to improve the sensitivity of the tester strains (12) and added to the mixture a liver homogenate fraction from rodents, which contains various metabolic enzymes (13). Some chemicals are not mutagens themselves but become mutagens in the presence of the liver homogenate, which can metabolize the chemicals to an active form, which then mutates the bacteria.

I also became more and more interested in the relation of mutagens to carcinogens. We showed that cigarette smoke was highly mutagenic (14) and that we could detect most common chemical carcinogens with the test, particularly after we added the liver homogenate (15). Having had a background in genetics as well as biochemistry, all of my intuition told me that mutagens ought to be carcinogens, though this was not the prevailing view at the time or for many years. I became a proselytizer for this view, though in retrospect I should have also emphasized the role of cell division rates in mutation and carcinogenesis; I tried to make up for this later (16–19). It now seems obvious that increasing either DNA damage or cell division rates, e.g. by hormones, increases cancer rates.

In contrast to the expensive and time-consuming rodent cancer test, our method of assaying the mutagenicity of chemicals was simple, rapid, and inexpensive. As a result, it was quickly adopted by thousands of laboratories worldwide, particularly by drug and chemical companies, for the detection of mutagens and potential carcinogens. Our method made it possible to weed out mutagenic chemicals inexpensively early in their development before they were introduced into commerce. I never patented the test, in part because I thought it might detract from my effectiveness in promoting mutagen testing, though I did have a brief pang of regret when it seemed that almost every industry in the world was asking for the strains. I started with the notion that industry would be reluctant to use the test and that regulators would force them to. I soon realized that industry was eager to adopt the test, in part, I concluded, because they had a huge incentive to weed out nasty chemicals. Regulatory agencies only took notice of the test years afterward, perhaps because a lack of competition created no incentive to change their routine way of doing things. This experience, together with subsequent interactions with bureaucracies such as the Environmental Protection Agency and my readings in economics, reinforced a growing conviction that to accomplish anything of importance incentives matter.

Two major conclusions from our work were that mutation is one aspect of the mechanism of cancer causation and that a high percentage of carcinogens are detectable as mutagens. A series of wonderful students did the work, especially Frank Lee and Bill Durston, particularly brilliant undergraduates; David Levin, a graduate student; and Joyce McCann, a postdoctoral fellow, who made a major contribution. An extraordinarily competent and devoted laboratory technician, Edie Yamasaki, also was a major contributor. I gradually drifted out of bacterial work and into rats and humans. In recent years when people ask me “Are you the Ames of the Ames test?” our work on the test seems so long ago that I reply “Oh no, it was my father.”

In the early 1990s we made one more improvement in mutagen testing. A postdoctoral fellow, Pauline Gee, some students, and Dorothy Maron, a laboratory technician, developed a new set of six strains that were at least as sensitive as the old tester strains and also diagnosed the 6 possible base pair mutations (20). These strains not only showed whether the test chemicals were mutagenic, they also indicated the type of mutation. The University of California did take out a patent on this improved test. In the beginning I thought that the mutagenicity test would be outdated in a few years, and I still find it surprising that people are using it 30 years later, despite all of the new genetic tools that have come along.

Carcinogens

My interests in cancer prevention and toxicology stemmed from the mutagen test, and I soon became deeply immersed in both fields. I enjoy learning new fields and I always seem to be in the midst of learning a new one. I can often bring a fresh perspective to a new area because of my broad interests in science. My laboratory at the time was researching gene regulation and later regulation of antioxidant defenses in Salmonella. I was helped enormously in entering these new fields by finding a few extraordinarily intelligent and competent associates who could help with the scholarship. A major find was Lois Gold, who walked into my office after
she saw a story in the newspaper about a paper we had published in *Science* (21) that Tris-BP was a mutagen. Tris-BP was one of the major flame retardants, which a government agency had decreed were to be in all children’s pajamas. She was a mother with a young daughter and wanted to know everything about flame retardants, burn statistics, risk, evidence, etc. She was clearly unusually smart and thorough. After an exhausting hour answering questions, I asked her what her background was. She had a Ph.D. from Stanford and had a background in statistical methods; she had taught at Berkeley and Stanford and was taking a few years off to be with her child. She also had an interest in public health policy. In my excitement about innovative ideas in new fields there is a danger in getting carried away by enthusiasm, so I am always looking for smart, tough minded associates who are willing to challenge my assumptions and data. I hired Lois on the spot to work whatever hours she could put in on various projects, including the Carcinogenic Potency Database I had started.

I had started the Carcinogenic Potency Database after I realized, in trying to compare mutagenic potency with carcinogenic potency, that no one had ever systematized the literature on the quantitative aspects of animal cancer tests. We also found that carcinogens could vary by a million-fold in potency. I applied for a grant to set up the database, but it was turned down as I did not have any experience in animal cancer tests, statistics, or pathology. This was all true, but we thought it was important to do, wanted to do it, and knew we would do it as well as it could be done if we consulted the best people in the various fields, so we decided to go ahead anyway. Lois Gold stayed on to develop the database, which is now the definitive quantitative database in the world on animal cancer tests. Together Gold and I have written over 100 papers based on our analyses of the database. We have challenged most of the assumptions in the field, so we have engendered reams of controversy.

One important finding to come out of our analysis was that over half of all the chemicals tested, whether natural or synthetic, were carcinogenic when tested chronically in rodents at the maximum tolerated dose (MTD), the standard procedure in the rodent cancer bioassay (22). Our analysis suggested that carcinogenesis in the high dose rodent tests was due to the use of a high dose and that the high dose could cause chronic cell killing, inflammation, and cell proliferation, which could account for the carcinogenic effects. We concluded, therefore, that the tests did not provide information to calculate low dose risks (18). These conclusions did not endear us to scientists who have spent their lives testing synthetic chemicals at the MTD, or environmental activists who have tried to purge the world of tiny traces of synthetic chemicals, or regulators whose jobs depend on eliminating traces of “toxic chemicals.” I have become inured to *ad hominem* attacks on Gold and myself that allege we are a tool of industry, despite the fact Gold and I have always had a policy not to accept money from industry, or to testify in lawsuits, or to consult. It is clear that our critics do not like our conclusions, but we have seen no convincing rebuttal of our science.

### Natural Chemicals, the Forgotten Control

One observation that struck me fairly soon after our mutagenicity test became widely used was the high rate at which we and others were finding mutagens in the natural world of plant chemicals. That got me thinking about the natural chemicals that humans ingest, such as the natural pesticides plants produce to kill off insects and other predators (23) and the burnt material in cups of coffee (24). Because almost all of the chemicals that humans are ingesting are natural (23, 25) it seemed very improbable that synthetic chemicals were likely to be more than a tiny fraction of our total exposure to mutagens/carcinogens, other than from high dose occupational exposures or medicinal drugs.

To put synthetic carcinogens in perspective, Gold and I thought it necessary to examine the carcinogenicity of the natural background of chemicals as an appropriate control for synthetic chemicals. We estimated that 99.9% of all chemical exposure is from ingesting natural chemicals in food, *e.g.* 99.99% of exposure to pesticides is from ingesting natural pesticides produced by plants (23). We published a paper entitled “Ranking Carcinogenic Hazards” in *Science* in 1987 (17). To compare the average daily dose of chemicals which humans might receive with the dose that induced cancer in rodents, we created an index called HERP (human exposure dose/rodent potency dose). The results of HERP showed that the possible cancer hazard of traces of synthetic chemicals such as pesticide residues are tiny compared with natural chemicals in the diet. Even the possible hazards from “rodent carcinogens” in natural chemicals should be viewed with skepticism because of the problem of high dose testing.
We also pointed out that diversion of resources and attention from programs that focus on major risks to those that focus on minor hypothetical risks might hurt public health. As I have become more and more concerned with cancer prevention, I have concluded that we must concentrate on major risks if we are to make any progress and that concern with hundreds of minor, hypothetical risks is a distraction from major risks, such as unbalanced diets and cigarette smoking. Epidemiology is fraught with difficulties. “In Miami, study finds everyone born Hispanic, dies Jewish.” Epidemiology is useful when there are large risks, but lacks the power to provide convincing evidence that traces of synthetic chemicals cause small amounts of human cancer. Though I am passionate about cancer prevention, I remain skeptical of the purported dangers from traces of synthetic chemicals, such as pesticides, and do not see much plausibility from either toxicological or epidemiological analysis. Spending time debunking the dubious assumptions (26) behind the environmentalist fervor against traces of industrial chemicals does not prevent any cancer. Having demonstrated the implausibility of such assumptions, I turned to finding more effective ways to prevent cancer.

**Diet and Health**

In reading what was known about cancer prevention I was attracted to the idea that unbalanced diets are a major contributor to cancer because all the leading epidemiologists thought dietary factors were likely to be in the same league as smoking. My intuition told me there was a lot of interesting science in the diet-cancer area and that the field was murky enough so there were not many people exploring it (27). When I enter a new field, which I seem to do fairly often, I always spend a fair amount of time getting an overview to see where the least amount of effort will bring the maximum return, *i.e.* exploring several different approaches with potentially high payoffs. I am reluctant to enter, or stay in, a field that is very active, as I find it too difficult to focus on just one thing. I think that my talents lie in my finding new ways of looking at a problem and opening up new fields, which is a result of my broad scientific reading and interests. This, of course, makes it much harder to get grants. I was fortunate to have received a large NCI Outstanding Investigator Grant for 15 years, at which point NCI stopped that type of grant. The grant was made for me as it permitted me to do work on whatever interested me, and that saved my neck. I am enormously grateful someone had the vision to fund such a program, at least for a while.

I was intrigued by the reviews of Potter and Block on 200 or so epidemiological studies, which showed that the quarter of the population eating the fewest fruits and vegetables had about double the cancer rate for most types of cancer, compared with the quarter eating the most. Our work on endogenous oxidants as a major source of DNA damage had interested me in antioxidants, and I began to view vitamin C, vitamin E, selenium, and other vitamins and minerals, many of which came from the fruits and vegetables in the diet, as anti-mutagens and anti-carcinogens (27). I began to think that much cancer in certain human populations might be because of the less than optimal amounts of anti-carcinogens and protective vitamins and minerals consumed in the diet.

The work that finally got me seriously involved in this area was the research of Jim MacGregor on folic acid. Jim was a cytogeneticist who spent a year in my laboratory (28). While assaying chromosome breaks in humans and in mice, he stumbled on the fact that folic acid deficiency breaks chromosomes in mice, just as radiation does. He then showed that a person with a very high level of chromosome breaks was folate-deficient and that a folate intervention lowered the level of breaks. Folic acid comes from the Latin word *folia* (*i.e.* leaf); one gets it from green leafy vegetables. (My graduate mentor, H. K. Mitchell, first discovered folic acid and isolated it from 4 tons of spinach.)

Because low folate levels were very common in the population of the United States, I talked a graduate student, Ben Blount, and then a postdoctoral fellow, Matt Mack, into investigating the mechanism. They showed that folate deficiency causes a block in the methylation of dUMP to dTMP, which results in the misincorporation of millions of uracils into the DNA of each rat cell, which causes chromosome breaks (29). Removal of the uracil by uracil glycosylase causes a transient single strand break (nick) in the DNA. Two opposing nicks, *e.g.* from repair of a uracil across from an oxidative lesion, cause a double strand break, the most serious DNA lesion. The chromosome breaks from radiation are made by an analogous mechanism, the repair of opposing oxidative lesions.

This prompted me to look into the whole array of vitamins and essential minerals, as I think it likely that when one input in the metabolic network is inadequate, repercussions will be felt...
on a large number of systems and lead to degenerative disease. For example, deficiencies of folate, B12, or B6 lead to an increase in DNA damage and cancer (30); iron deficiency leads to neuron decay and cognitive dysfunction (31) and mitochondrial decay and premature aging (32). We have shown that inadequate levels of many vitamins or minerals, such as iron, zinc, folate, B12, and B6, result in DNA, mitochondrial, and other types of damage (30–35). Emily Ho, a postdoctoral fellow in my laboratory has shown, for example, that zinc deficiency in human cells in culture not only fills the cell up with oxidants that damage DNA but disables p53, a zinc enzyme, and also various other components of the DNA defense network (33). Low intake of each of these vitamins and minerals is found in 10% or more of the population, particularly in the poor (30).

I am convinced that by tuning up metabolism by ensuring vitamin and mineral adequacy we can effect a major improvement in public health (35), particularly for the poor. Numerous efforts and programs to convince people to change their diets have not been particularly successful. Fortification, e.g. the folic acid fortification of flour, has a role to play. Vitamins and minerals are amazingly inexpensive; a multivitamin/mineral pill containing the recommended daily allowance (RDA) for the essential vitamins and minerals costs less than a penny to make. In fact, I think that everyone should take one every day as “insurance” (35), though of course efforts to encourage eating a balanced diet should continue. Vitamin and mineral adequacy is important but is not the only part of our dietary needs, which also include fiber, essential fatty acids, and other components not found in a multivitamin/mineral pill (36).

Oxidants

I became interested in the early 1980s in oxidants from metabolism, smoking, and chronic inflammation as a major source of mutagens; I started working in this area both in Salmonella and in higher organisms (37–41). With some wonderful graduate students, particularly Gigi Storz, Lou Tartaglia, and Mike Christman, and postdoctoral fellows Robin Morgan and Fred Jacobson, we clarified for the first time the strategies employed by bacteria in their response to oxidants such as hydrogen peroxide (42, 43). The discovery of the oxyR regulatory protein, which involved isolating it and determining its sequence and DNA-binding site, provided general insights into which cell constituents are damaged by oxidants and how cells sense and respond to oxidative stress. It also showed that an oxyR thiol operated directly as a sensor of oxidative stress (44). A series of studies showed that oxyR controls a variety of genes, including those that code for catalase and a new enzyme, alkyl hydroperoxide reductase (42, 43, 45, 46), which was later cloned and sequenced. Studies on the oxyR regulon led to the elucidation of the mechanisms by which exposure of bacterial cells to low doses of oxidants allows these cells to adapt to subsequent challenges of higher doses of oxidants. These studies also provided insights in understanding how higher organisms such as mammals adapt to oxidant exposure. Work by Rhee and Stadtman identified the mammalian counterpart to the alkyl hydroperoxide reductase. Other laboratories have since identified similar oxidant-responsive elements in mammals.

We documented that endogenous oxidants from normal metabolism are important in damaging DNA in both bacteria (47) and mammals (41, 48). At one point, I had the vision that the lesions that were excised from DNA by repair enzymes should be excreted in the urine and thus could be analyzed. With the help of several sensational postdoctoral fellows, including Mark Shigenaga, Robert Saul, and Rick Cathcart, we looked for known oxidized DNA bases (the radiation biologists had worked out the chemistry) in rat and human urine as a measure of oxidative DNA damage (41, 48–50). This work suggests that there is a large rate of endogenous oxidative damage to DNA (about 100,000 hits/cell/day in the rat) (51). Moreover, though repair is very effective, some oxidative lesions escape repair, the steady state level of oxidative lesions increases with age, and an old rat has accumulated about 66,000 oxidative DNA lesions per cell (51).

Two extraordinarily good Swiss postdoctoral fellows, Roland Stocker and Balz Frei, came to my laboratory to work on oxidation in the late 1980s. They and other students clarified the role of various antioxidants in human plasma (52, 53) and discovered some major antioxidants that were previously not fully appreciated, including uric acid (37), bilirubin (54–56), and ubiquinol (57, 58). We showed that ascorbate serves as a first line defense in blood plasma against lipid oxidation (53) and as a key protective agent against oxidative damage to sperm DNA (59). In the course of this work we developed many new methods for measuring oxidative damage and defenses in tissues, as well as in biological fluids such as urine and plasma. Throughout my
career I have always felt that developing new analytical methods helps to open up a field and
is well worth the effort. I take some satisfaction in knowing that many of the methods we have
developed are among the most highly cited papers. Because mitochondria are the main source
of endogenous oxidants and mitochondria are the main targets of oxidants, this led directly to
our work on the mitochondrial decay of aging.

**Delaying the Mitochondrial Decay of Aging**

Aging has been a major interest of mine for some time, in particular the role of mitochondrial
decay as a major contributor to aging and age-related degenerative diseases. Mark Shigenaga
and Tory Hagen, two brilliant senior postdoctoral fellows, and I wrote a review in 1994 (60) on
why mitochondrial decay due to oxidant leakage from the electron transport chain was likely
to be a major factor in aging. This idea was not original with us; Denham Harman and then
Jaime Miquel had discussed the free radical theory of aging, but we feel we contributed some
insights in the review. Writing the review got us all fired up to work on the subject, and Hagen
figured out an experimental approach in rats that worked; we showed that there is a large
amount of oxidative damage to the mitochondria and mitochondrial decay during aging (61).
Kenny Beckman, another unusually creative postdoctoral fellow, and I also reviewed the free
radical theory of aging (62). These radicals can cause oxidative damage, which in turn
contributes to mitochondrial decay and degenerative diseases such as cancer, aging, heart
disease, cataract, and brain dysfunction.

In a series of experiments by Hagen, and later after he left to go to Oregon State, by another
excellent postdoctoral fellow, Jiankang Liu, we made progress in reversing some of this
mitochondrial decay in old rats by feeding them the normal mitochondrial metabolites,
acetylcarnitine (ALCAR) and lipoic acid (LA), at high levels (63–67). The principle behind this
effect appears to be that with age, increased oxidative damage to mitochondrial protein causes
a deformation of structure of key enzymes, with a consequent lessening of affinity ($K_m$) for the
enzyme substrate (67). The effect of age on the ALCAR binding affinity of carnitine acetyl-
transferase can be mimicked by reacting it with malondialdehyde (a lipid peroxidation product
that increases with age). Feeding the substrate ALCAR with LA, a mitochondrial antioxidant,
restores the velocity of the reaction, $K_m$ for ALCAR-CoA transferase, and mitochondrial
function. In old rats (versus young rats) mitochondrial membrane potential, cardiolipin level,
respiratory control ratio, and cellular $O_2$ uptake are lower; oxidants/$O_2$, neuron RNA oxid-
adion, and mutagenic aldehydes from lipid peroxidation are higher. Ambulatory activity and
cognition decline with age. Feeding old rats ALCAR plus LA for a few weeks improves
mitochondrial function; lowers oxidants, neuron RNA oxidation, and mutagenic aldehydes;
and increases rat ambulatory activity and cognition (as assayed with the Skinner box and
Morris water maze). I have been so excited about this work that I am sure it has added (or
perhaps subtracted) a year or two to my own life.

Two more outstanding postdoctoral fellows, Hani Atamna and Patrick Walter, have shown
that common micronutrient deficiencies accelerate mitochondrial decay. Heme biosynthesis
takes place predominantly in the mitochondria. Interfering with heme synthesis causes a
specific loss of Complex IV with a consequent release of oxidants (68, 69). Iron deficiency (25%'
of menstruating women in the United States ingest <50% of the RDA) also causes release of
oxidants and mitochondrial decay (32) presumably through lack of heme (69). Vitamin B$_6$
deficiency (10% of Americans ingest <50% of the RDA) also causes heme deficiency (69). In a
beautiful new paper Atamna shows that the consequences are likely to be accelerated aging,
nervous decay, and Alzheimer's disease (31).

**The $K_m$ in Genetic Disease, Polymorphisms, and Aging**

One of my great pleasures in the last few years has been working on an innovative,
integrative review (70) with an undergraduate student, Ilan Elson-Schwab. We think this
review will change thinking in both human genetics and nutrition. As many as one-third of
mutations in a gene result in the corresponding enzyme having an increased Michaelis
constant/$K_m$ (decreased binding affinity) for a coenzyme, resulting in a lower rate of reaction.
We review 50 human genetic diseases due to defective enzymes that can be remedied or
ameliorated by the administration of high doses of the B vitamin component of the correspond-
ing coenzyme, which we show raises levels of the coenzyme and at least partially restores
enzymatic activity (70).
Reflections: An Enthusiasm for Metabolism

We also review five single-nucleotide polymorphisms in which the variant amino acid reduces coenzyme binding and thus enzymatic activity; the reduced levels of activity are likely to be remediable by raising cellular concentrations of the cofactor through high dose vitamin therapy (70). Some examples of polymorphisms include the (C677T; Ala-222 → Val) methyltetrahydrofolate reductase (NADPH) and the cofactor FAD (in relation to cardiovascular disease, migraines, and rages), the (C609T; Pro-187 → Ser) mutation in NAD(P):quinone oxidoreductase 1 (NQO1) and FAD (in relation to cancer), the (C131G; Ala-44 → Gly) mutation in glucose-6-phosphate 1-dehydrogenase and NADP (in relation to favism and hemolytic anemia), and the (Glu-487 → Lys) mutation (present in about half of Asians) in aldehyde dehydrogenase and NAD (in relation to alcohol intolerance, Alzheimer's disease, and cancer). As all of the polymorphisms are sorted out in humans, this Km concept may be relevant for tuning up the metabolism of much of the population. I suspect this might be one of the first major contributions of genomics to public health. We also are actively working on whether high doses of some of the B vitamins might help delay the mitochondrial decay of aging. To encourage further discussion and new information on this topic we have set up a web site (www.KmMutants.org).

Children's Hospital of Oakland Research Institute (CHORI)

At the end of 1999 our building on campus, Barker Hall, reached the top of the list of buildings that needed to be renovated for earthquake reinforcements. We were asked to vacate the building. I was told to cut my laboratory in half and that the University would figure out where to squeeze me in for 2–3 years until the renovations were completed. Instead, I said goodbye to campus and moved to CHORI, with the encouragement of its director Bert Lubin. CHORI is a nearby research facility that has recently renovated a beautiful old high school and turned it into laboratories. We recently occupied a newly renovated wing, which will be a Nutrition-Genomics Center within CHORI. I have been exceptionally happy here these last few years in the company of first rate colleagues, and I do not plan to ever move again.

I recently told a colleague that I thought I was doing the best work of my scientific career. He replied, “Bruce, you’ve been telling me that for 30 years.” Thus, aging has not damaged my enthusiasm genes, though I am not as certain about my neurons. My current passion, as can be seen from the above sections, is tuning up metabolism in humans, both in the young and the old, by vitamins, minerals, and biochemicals. I think this will lead to a marked improvement in health and an increase in longevity. With so much work to do, I have no plans to retire from science.

Address correspondence to: bames@chori.org.

REFERENCES
1. Ames, B. N., and Mitchell, H. K. (1952) The paper chromatography of imidazoles. J. Am. Chem. Soc. 74, 252–253
2. Ames, B. N., Mitchell, H. K., and Mitchell, M. B. (1955) Some new naturally occurring imidazoles related to the biosynthesis of histidine. J. Am. Chem. Soc. 77, 1015–1018
3. Haas, F., Mitchell, M. B., and Ames, B. N. (1952) A series of histidineless mutants of Neurospora crassa. Genetics 37, 217–226
4. Ames, B. N., and Mitchell, H. K. (1955) The biosynthesis of histidine: imidazoleglycerol phosphate, imidazoleacetol phosphate, and histidinol phosphate. J. Biol. Chem. 212, 687–696
5. Ames, B. N., Martin, R. G., and Garry, B. J. (1961) The first step of histidine biosynthesis. J. Biol. Chem. 236, 2019–2026
6. Ames, B. N., and Garry, B. (1969) Coordinate repression of the synthesis of four histidine biosynthetic enzymes by histidine. Proc. Natl. Acad. Sci. U. S. A. 45, 1453–1463
7. Martin, R. G., and Ames, B. N. (1961) A method for determining the sedimentation behavior of enzymes: application to protein mixtures. J. Biol. Chem. 236, 1372–1379
8. Ames, B. N., and Whitfield, H. J., Jr. (1966) Frameshift mutagenesis in Salmonella. Cold Spring Harb. Symp. Quant. Biol. 31, 221–229
9. Whitfield, H. J., Jr., Martin, R. G., and Ames, B. N. (1966) Classification of aminotransferase (C gene) mutants in the histidine operon. J. Mol. Biol. 21, 335–355
10. Ames, B. N. (1971) The detection of chemical mutagens with enteric bacteria. In Chemical Mutagens: Principles and Methods for Their Detection (Hollaender, A., ed) Vol. 1, pp. 267–282, Plenum Press, New York
11. Ames, B. N. (1977) Gordon M. Tomkins (1926–1975). In Biochemical Actions of Hormones (Litwack, G., ed) Vol. IV, pp. xvi–xxxvi, Academic Press, New York
12. Ames, B. N., Lee, F. D., and Durston, W. E. (1973) An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc. Natl. Acad. Sci. U. S. A. 70, 782–786
13. Ames, B. N., Durston, W. E., Yamashita, E., and Lee, F. D. (1973) Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proc. Natl. Acad. Sci. U. S. A. 70, 2281–2285
14. Kier, L. D., Yamashita, E., and Ames, B. N. (1974) Detection of mutagenic activity in cigarette smoke condensates.
DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. *Proc. Natl. Acad. Sci. U. S. A.* **95**, 288–293

52. Frei, B., Stocker, R., and Ames, B. N. (1988) Antioxidant defenses and lipid peroxidation in human blood plasma. *Proc. Natl. Acad. Sci. U. S. A.* **85**, 9748–9752

53. Frei, B., England, L., and Ames, B. N. (1989) Ascorbate is an outstanding antioxidant in human blood plasma. *Proc. Natl. Acad. Sci. U. S. A.* **86**, 6377–6381

54. Stocker, R., Yamamoto, Y., McDonagh, A. F., Glazer, A. N., and Ames, B. N. (1987) Bilirubin is an antioxidant of possible physiological importance. *Science* **235**, 1043–1046

55. Stocker, R., Glazer, A. N., and Ames, B. N. (1987) Antioxidant activity of albumin-bound bilirubin. *Proc. Natl. Acad. Sci. U. S. A.* **84**, 5918–5922

56. Stocker, R., and Ames, B. N. (1987) Potential role of conjugated bilirubin and copper in the metabolism of lipid peroxides in bile. *Proc. Natl. Acad. Sci. U. S. A.* **84**, 5918–5922

57. Frei, B., Kim, M. C., and Ames, B. N. (1990) Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. *Proc. Natl. Acad. Sci. U. S. A.* **87**, 4879–4883

58. Tribble, D. L., van den Berg, J. J., Motchnik, P. A., Ames, B. N., Lewis, D. M., Chait, A., and Krauss, R. M. (1994) Oxidative susceptibility of low density lipoprotein subfractions is related to their ubiquinol-10 and alphatocopherol content. *Proc. Natl. Acad. Sci. U. S. A.* **91**, 10771–10778

59. Fraga, C. G., Motchnik, P. A., Shigenaga, M. K., Helbock, H. J., Jacob, R. A., and Ames, B. N. (1991) Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. *Proc. Natl. Acad. Sci. U. S. A.* **88**, 11003–11006

60. Shigenaga, M. K., Hagen, T. M., and Ames, B. N. (1994) Oxidative damage and mitochondrial decay in aging. *Proc. Natl. Acad. Sci. U. S. A.* **91**, 10771–10778

61. Hagen, T. M., Yowe, D. L., Bartholomew, J. C., Wehr, C. M., Do, K. L., Park, J. Y., and Ames, B. N. (1997) Mitochondrial decay in hepatocytes from old rats: membrane potential declines, heterogeneity and oxidants increase. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 3064–3069

62. Beckman, K. B., and Ames, B. N. (1995) The free radical theory of aging matures. *Physiol. Rev.* **75**, 547–581

63. Hagen, T. M., Ingersoll, R. T., Wehr, C. M., Lykkesfeldt, J., Vinarsky, V., Bartholomew, J. C., Song, M. H., and Ames, B. N. (1998) Acetyl-l-carnitine fed to old rats partially restores mitochondrial function and ambulatory activity. *Proc. Natl. Acad. Sci. U. S. A.* **95**, 9562–9566

64. Hagen, T. M., Ingersoll, R. T., Lykkesfeldt, J., Liu, J., Wehr, C. M., Vinarsky, V., Bartholomew, J. C., and Ames, B. N. (1999) (R)-α-Lipoic acid-supplemented old rats have improved mitochondrial function, decreased oxidative damage, and increased metabolic rate. *FASEB J.* **13**, 411–418

65. Liu, J., Heed, E., Gharib, A. M., Yuan, W., Ingersoll, R. T., Hagen, T. M., Cotman, C. W., and Ames, B. N. (2002) Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: partial reversal by feeding acetyl-l-carnitine and/or R-alpha-lipoic acid. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 2356–2361

66. Hagen, T. M., Liu, J., Lykkesfeldt, J., Wehr, C. M., Ingersoll, R. T., Vinarsky, V., Bartholomew, J. C., and Ames, B. N. (2002) Feeding acetyl-l-carnitine and lipoic acid to old rats significantly improves metabolic function while decreasing oxidative stress. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 1870–1875

67. Liu, J., Killilea, D. W., and Ames, B. N. (2002) Age-associated mitochondrial oxidative decay: improvement of carnitine acetyltransferase substrate-binding affinity and activity in brain by feeding old rats acetyl-l-carnitine and/or R-alpha-lipoic acid. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 1876–1881

68. Atamna, H., Liu, J., and Ames, B. N. (2001) Heme deficiency selectively interrupts assembly of mitochondrial complex IV in human fibroblasts: relevance to aging. *J. Biol. Chem.* **276**, 48410–48416

69. Atamna, H., Walter, P. B., and Ames, B. N. (2002) The role of heme and iron-sulfur clusters in mitochondrial biogenesis, maintenance, and decay with age. *Arch. Biochem. Biophys.* **397**, 345–353

70. Ames, B. N., Elson-Schwab, I., and Silver, E. A. (2002) High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased K(m)): relevance to genetic disease and polymorphisms. *Am. J. Clin. Nutr.* **75**, 616–658