Journal of Scientific Exploration, Vol. 15, No. 1, pp. 5–20, 2001 0892-3310/01

The Biomedical Significance of Homocysteine

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Abstract—In recent years the amino acid homocysteine has achieved the status of an important factor in vascular disease, diseases of aging, and other fundamental processes in biology and medicine. After its discovery in 1932, homocysteine was characterized as an important intermediate in methionine metabolism. Little was known about its biomedical significance until 1962, when children with mental retardation, accelerated growth, and propensity to thrombosis of arteries and veins were found to excrete homocysteine in the urine. My analysis of an archival case of homocystinuria from 1933 disclosed widespread arteriosclerotic plaques and thrombosis of carotid artery with death from stroke in an 8-year-old boy. The cause of homocystinuria in most of these cases is deficiency of the enzyme cystathionine synthase, a pyridoxal phosphate-dependent enzyme. In 1968, my analysis of a second case of homocystinuria caused by deficiency of a different enzyme, methionine synthase, a folate and vitamin B12–dependent enzyme, was critical in the discovery of the atherogenic potential of homocysteine. This 2-month-old baby boy was demonstrated to have advanced arteriosclerotic plaques in arteries of organs throughout the body. Because of the difference in enzyme abnormality in these two cases, it was possible for me to conclude that homocysteine causes arteriosclerosis by a direct effect of the amino acid on the cells and tissues of the arteries. Several years later, arteriosclerotic plaques were demonstrated in a third major type of homocystinuria caused by deficiency of the enzyme methylenetetrahydrofolate reductase, independently corroborating this conclusion.

The conclusion that homocysteine is atherogenic is supported by demonstration of arteriosclerotic plaques in experimental animals with hyperhomocysteinemia produced by injection or feeding of the amino acid. The homocysteine theory of arteriosclerosis attributes the underlying cause of the disease to dietary deficiencies of vitamin B6 and folic acid, which lead to hyperhomocysteinemia in the general population. Dietary deficiencies of these B vitamins are caused by losses of these sensitive vitamins through important methods of food processing, including milling of grains, canning, extraction of sugar and oils, radiation, and chemical additives. This revolutionary new view of the underlying cause of arteriosclerosis appeared to contradict the conventional wisdom concerning the role of dietary cholesterol and saturated

fats in atherogenesis. For this reason the homocysteine theory was actively suppressed in the 1970s by adherents of the cholesterol approach and by academic and administrative officials at Harvard Medical School and Massachusetts General Hospital.

Within the past decade, hundreds of major prospective and retrospective clinical and epidemiological studies have proven the underlying validity of the homocysteine theory by showing that hyperhomocysteinemia is a potent independent major risk factor for vascular disease. At present at least 18 major interventional trials are in progress worldwide to prove that lowering blood homocysteine by dietary improvement and supplemental B vitamins will prevent vascular disease.

Recent research on homocysteine has also implicated abnormal homocysteine metabolism in a wide range of other important disease processes, including developmental birth defects, neurodegenerative diseases like Alzheimer's disease, autoimmune diseases like rheumatoid arthritis, hormonal imbalances, renal failure, cancer, and degenerative diseases of aging. In the future the biomedical significance of homocysteine will become extraordinarily important for prevention and therapy of a wide range of major diseases.

Keywords: homocysteine — heart disease — cholesterol — Dinsdale Award

Homocysteine and Homocystinuria

In recent years the amino acid homocysteine has achieved the status of a "hot topic" in vascular disease. Besides arteriosclerosis and heart disease, the significance of this amino acid has also rapidly expanded to areas of biology, physiology, and medicine ranging from endothelial function to aging, oxidative stress, oxidative metabolism, embryology, reproductive physiology, cancer, growth and cell division, endocrinology, neural transmission, and neurodegenerative disease. This essay examines the discovery of the extraordinary importance of this previously obscure amino acid in fundamental processes in biology and medicine.

In 1932 the American biochemist Vincent DuVigneaud (1952) discovered a new amino acid resulting from removal of the methyl group of methionine. Since this amino acid has the same functional groups as the amino acid cysteine, namely, sulfhydryl, amino and carboxyl groups, and one additional carbon atom, he named it homocysteine. The four-carbon backbone of homocysteine enables formation of an internal cyclic anhydride called homocysteine thiolactone. This compound with a five-member ring forms stable salts with strong acids, but when neutralized with weak bases, the ring opens, forming two peptide bonds between two molecules of the thiolactone and producing homocysteine diketopiperazine. Homocysteine is produced by hydrolysis of homocysteine thiolactone with strong bases. The chemical synthesis, hydrolysis, oxidation, and polymerization reactions of homocysteine thiolactone are shown in Figure 1.

In the 1930s when DuVigneaud and others investigated the properties of homocysteine, the amino acid was not found in the polypeptide backbone of pro-

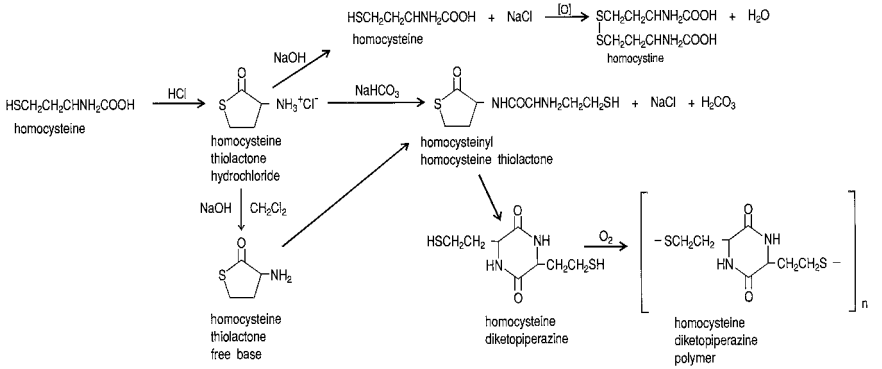

Fig. 1. Chemical synthesis, hydrolysis, oxidation, and polymerization reactions of homocysteine thiolactone are indicated.

teins. The precursor of homocysteine is methionine, which is found in all proteins as the initial amino acid of all polypeptides. Cysteine is present in the polypeptide chain of almost all proteins.

Homocysteine is converted to methionine by transfer of methyl groups from compounds such as choline and betaine, a process called transmethylation. DuVigneaud also discovered that homocysteine was converted to cysteine in metabolism through the intermediate formation of cystathionine.

Mueller had previously discovered the amino acid methionine in 1922, and studies with animals in the 1930s showed that it is essential for growth. Because methionine can be converted in metabolism to homocysteine, cystathionine, and cysteine, as discovered by DuVigneaud, the amino acid cysteine is not essential for growth if methionine is available in the diet.

In the 1940s and 1950s little more was learned about homocysteine. Nothing was known about its significance in clinical medicine, and the compound was relegated to an obscure status as an intermediate in sulfur-amino-acid metabolism. Apparently members of DuVigneaud's department of biochemistry had identified several children with homocysteine in their urine, because these cases were discussed in teaching conferences at New York Hospital in the 1950s. However, nothing was known about the clinical findings in these children, and the cases were never published.

In 1962 a group of investigators in Belfast, Northern Ireland, began to screen the urine of children with mental retardation for the presence of amino acids, using newly developed techniques of paper and column chromatography (McCully, 1983). Several of the children were found to have homocysteine in their urine, and the disease was called homocystinuria. These children had other abnormalities in addition to mental retardation, including accelerated growth, dislocated ocular lenses, osteoporosis and other skeletal problems, and a tendency to develop thrombi in arteries and veins. Almost simultane-

ously, cases of homocystinuria were found in Wisconsin and at the Wills Eye Hospital in Philadelphia. Mudd and coworkers at the National Institutes of Health discovered that cystathionine synthase, a vitamin B6–dependent enzyme, is deficient in many of these cases. Spaeth and coworkers in Philadelphia discovered that vitamin B6 therapy was effective for many cases of homocystinuria.

Homocysteine and Arteriosclerosis

In the autopsy studies of children dying with homocystinuria, as first described in 1964, the cause of death was often related to thrombosis of arteries and veins to the major organs. The changes that were found in the arteries were attributed to a lathyrogenic effect of homocysteine on connective tissues because of the molecular similarity with penicillamine. (In experimental lathyrism, compounds like penicillamine and beta propionitrile interfere with collagen cross-linking.) In one or two cases of homocystinuria, the arterial lesions were described as atheromas, but in most reports there was no mention of a relation to arteriosclerosis. The tendency to arterial and venous thrombosis was attributed to increased adhesiveness of platelets.

Because of my fellowship training in biochemistry, molecular biology, genetics, and pathology, I decided to study pathological aspects of inherited human diseases. For several months I attended the newly instituted Human Genetics Conferences at Massachusetts General Hospital in 1968. In one of these conferences I heard the remarkable story of medical sleuth work by pediatricians investigating a case of homocystinuria in 1965. The case was a 9-year-old girl with mental retardation, dislocated ocular lenses, and accelerated growth. The mother of the girl told the pediatricians that an uncle of the girl had died in the 1930s of a similar disease, and the case was published in the medical literature. They found the case reported as a Clinical Pathological Conference in the *New England Journal of Medicine* in 1933. The uncle was an 8-year-old boy with mental retardation and dislocated ocular lenses who had died of a stroke.

In reviewing the original protocol, several original slides, and new slides made from several fragments of tissue that had survived from 1933, I was interested to find that the pathologist, Tracey Mallory, had diagnosed carotid arteriosclerosis, thrombosis, and cerebral infarct as the cause of death. He commented that the carotid arteries were narrowed by a fibrotic thickening that "one might expect to find in an elderly man." In restudying this case I found that in addition to carotid arteriosclerosis, fibrous arteriosclerotic plaques were scattered through the arteries throughout the body.

Although the case from 1933 was not definitively diagnosed until 1970, it remains the first published case of homocystinuria and cystathionine synthase deficiency in the medical literature. The diagnosis of arteriosclerosis was puzzling because there was no evidence of lipid deposition in arterial plaques, and other cases of homocystinuria had no evidence of dyslipidemia. In 1968 the

leading hypothesis concerning the origin of arteriosclerosis was that dietary cholesterol caused elevated low-density lipoprotein (LDL), predisposing to atherogenesis.

Although I had never worked directly with arteriosclerosis during medical school or fellowship training, I had studied cholesterol chemistry, metabolism, and biosynthesis with Louis Fieser and Konrad Bloch during college and medical school at Harvard. During my 2 years as a biochemist at the National Institutes of Health, I had worked with Giulio Cantoni, the discoverer of adenosyl methionine, and Harvey Mudd, who later pioneered investigations of the biochemical basis of homocystinuria. I had studied protein biosynthesis with Paul Zamecnik at Harvard and genetic analysis of protein synthesis with Guido Pontecorvo in Glasgow and James Watson at Harvard. These experiences enabled me to take a fresh look at an old problem, the pathogenesis of arteriosclerosis.

Discovery of the Atherogenic Effect of Homocysteine

Although the presence of arteriosclerosis in the newly described disease homocystinuria was intriguing, there was no way to be sure that homocysteine was the cause of the plaques that I had observed in the arteries. Other investigators of homocystinuria had suggested that elevated levels of methionine in blood and tissues might be related to the various abnormalities found in these patients. In 1955 Frederick Stare had suggested in a lecture in medical school that methionine might be beneficial, because it lowered cholesterol levels in monkeys fed an atherogenic diet. In the 1940s James Rinehart had discovered arteriosclerotic plaques in monkeys fed a diet partially deficient in pyridoxine, the precursor of pyridoxal phosphate, the coenzyme of many reactions in protein metabolism.

My opportunity to clarify these questions came several months later, when a second unique case of homocystinuria was discussed at the Human Genetics Conference. This 2-month-old boy had expired after 2 weeks of study at Massachusetts General Hospital. The baby was found to have both homocysteine and cystathionine in the urine, indicating that a different enzyme deficiency caused his metabolic abnormalities. The enzyme analyses revealed that a deficiency of methionine synthase was responsible for homocysteine and cystathionine excretion. This enzyme is dependent upon methyltetrahydrofolate and methylcobalamin for its action in converting homocysteine to methionine. An autopsy had been completed, and the protocol and case materials were filed in the Pathology Department. The enzyme deficiencies leading to homocystinuria are indicated in Figure 2.

Immediately I realized that examination of this new case with a different enzyme abnormality would answer the question concerning the atherogenic effect of homocysteine. If the baby had normal arteries, it would mean that high levels of homocysteine could be present without causing arterial plaques. On the other hand, if the baby had arterial plaques, it might mean that elevated ho-

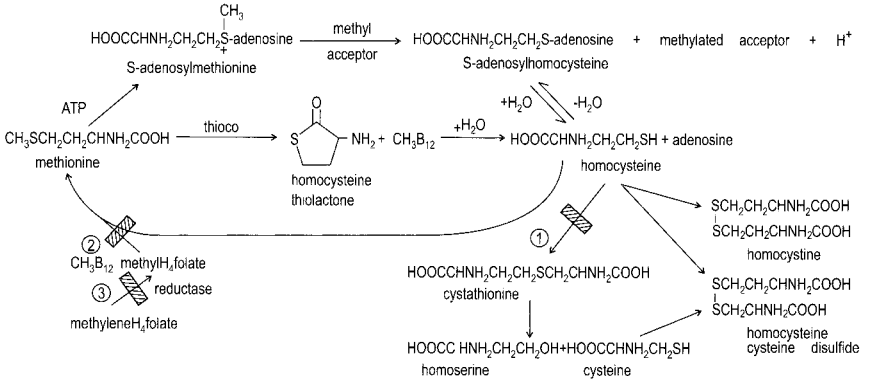

Fig. 2. Biochemical pathways of synthesis of homocysteine thiolactone, adenosyl methionine, and homocysteine are indicated. Inherited deficiencies of cystathionine synthase, enzyme **1**, methionine synthase, enzyme **2**, and methylenetetrahydrofolate reductase, enzyme **3**, cause accumulation of homocystine and homocysteine cysteine disulfide in tissues and body fluids.

mocysteine levels cause arteriosclerotic plaques, regardless of which enzyme abnormality was responsible for hyperhomocysteinemia.

In looking at the completed autopsy protocol, I was discouraged to find that there was no mention of the arteries in the microscopic description. In order to settle the question about the possible atherogenic effect of homocysteine, I decided to restudy the case, using stored tissues of all the organs. I was astounded to find that the baby had both early and astonishingly advanced arteriosclerotic plaques, involving arteries throughout the body! Because of this observation, I immediately suspected that homocysteine caused plaques by a direct effect of the amino acid on the cells and tissues of the arteries.

In my report of the analysis of these two cases of homocystinuria, I suggested that elevation of plasma levels of homocysteine is likely to be of great importance in the pathogenesis of arteriosclerosis in the population (McCully, 1969). Whether the underlying cause of hyperhomocysteinemia is genetic, dietary, toxic, or hormonal, the atherogenic effect of homocysteine on the cells and tissues of the arteries would be expected to cause arteriosclerotic plaques. The consequence of this disease process is ultimately coronary heart disease, cerebrovascular disease, and peripheral vascular disease.

Furthermore, the analysis of these cases provided an explanation for the cause of arteriosclerotic plaques in two important animal models, deficiency of vitamin B6 in monkeys and deficiency of choline, folic acid, and vitamin B12 in rats. Dietary deficiency of vitamin B6 produces hyperhomocysteinemia because of inhibition of cystathionine synthase, the enzyme that is deficient in most cases of homocystinuria. Dietary choline deficiency produces hyperhomocysteinemia because of inhibition of transmethylation of homo-

cysteine to methionine, the abnormality present in cases of homocystinuria caused by methionine synthase deficiency.

Several years later, in 1972, a third major type of homocystinuria was found to be caused by deficiency of the enzyme methylenetetrahydrofolate reductase (Figure 2). In 1976 an autopsy of a child with this new disease was published in the medical literature, showing arteriosclerotic plaques identical to those found in the more common type of homocystinuria. This observation independently supported my hypothesis that homocysteine causes plaques by a direct effect on the cells and tissues of the arteries, regardless of which enzyme deficiency causes hyperhomocysteinemia.

Homocysteine Theory of Arteriosclerosis

A careful review of current research on arteriosclerosis in the late 1960s failed to reveal any suggestion of altered protein metabolism as the cause of the disease. However, Moses Suzman, a cardiologist from South Africa, became interested in my work on homocysteine and suggested further readings in the history of arteriosclerosis research. Suzman was a colleague of James Rinehart in the 1940s; both were Rockefeller fellows in nutrition at Harvard. Rinehart had discovered the atherogenic effect of pyridoxine deficiency in monkeys in the late 1940s, and Suzman found that pyridoxine benefited his patients with coronary heart disease.

Earlier in the 20th century, Harry Newburgh in Michigan had repeated Ignatowsky's 1908 experimental induction of arteriosclerosis by feeding milk, eggs, and meat to rabbits. Newburgh used meat protein that had been extracted with organic solvents to remove all cholesterol and lipids, showing atherogenesis without lipid feeding. Subsequently, Newburgh infused amino acids intravenously in dogs, but he failed to find arteriosclerotic plaques in the arteries—because methionine and homocysteine had not been discovered in the early 1920s when these experiments were completed. Nevertheless, these early experiments with protein feeding and with vitamin B6 deficiency seemed to suggest an important, as yet poorly understood, role of altered protein metabolism in atherogenesis.

Since feeding homocysteine in a synthetic diet investigated by Klavins had failed to produce arterial plaques in rats, my initial experiments to demonstrate atherogenesis involved injection of homocysteine into rabbits. Within 3 weeks of these injections, early fibrous arteriosclerotic plaques were demonstrated in coronary arteries. Feeding cholesterol in the diet resulted in lipid deposition in these plaques, and feeding a diet deficient in vitamin B6 increased their severity. Increased doses of homocysteine or methionine given for periods of 2 months resulted in more advanced plaques and death of several rabbits from pulmonary embolism, a complication found in children with homocystinuria. The thrombosis and embolism were prevented by simultaneously injecting vitamin B6 in some animals. Feeding of homocysteine or methionine in a synthetic diet also produced arteriosclerotic plaques in rabbits, and addi-

tion of lipids in the form of butter converted the fibrous plaques to fibrolipid plaques. An elegant experiment by other investigators, involving intravenous administration of homocysteine to baboons (Harker et al., 1976), confirmed my results with rabbits, showing endothelial damage, thrombosis of peripheral arteries, and fibrous arteriosclerotic plaques.

These animal experiments directly demonstrated the atherogenic potential of homocysteine. In relating these results to arteriosclerosis in human populations, a number of earlier reports suggested that deficiency of vitamin B6 was observed in persons with heart disease. An important study by Schroeder in 1971 demonstrated that processing of a wide variety of foods caused significant losses of vitamin B6, folic acid, minerals, and fiber (Schroeder, 1971). For example, milling of wheat into white flour is associated with losses of these micronutrients on the order of 85% compared with the content of whole-wheat kernels. This study indicated that food processing, including milling of grains, canning, chemical additives, extraction of sugar and oils from plant foods, and other common practices were responsible for a widespread deficiency of vitamin B6 and folic acid in populations susceptible to vascular disease (McCully & McCully, 1999). The homocysteine theory of arteriosclerosis that I formulated in 1972 explains the underlying cause of vascular disease as a deficiency disease of these B vitamins rather than the supposed effects of dietary cholesterol and fats envisioned by the traditional diet-heart hypothesis.

Homocysteine Metabolism, Growth, and Cancer

In order to understand molecular aspects of the arteriosclerotic plaques observed in homocystinuria and in experimental atherogenesis by homocysteine, my associates and I cultured cells from the skin of patients with homocystinuria. These cells are deficient in cystathionine synthase, the enzyme that is deficient in the most common form of homocystinuria. The cultured cells produce an aggregated proteoglycan extracellular matrix that binds increased numbers of sulfate groups because of conversion of helical to random coil configuration (McCully, 1998). Experiments with radioactively labeled homocysteine thiolactone demonstrated a new pathway for conversion of the sulfur of homocysteine to sulfate without the formation of cystathionine, cysteine, or sulfate in these cells. Experiments with guinea pig liver demonstrated conversion of methionine to homocysteine thiolactone, followed by conversion of homocysteic acid to phosphoadenosine phosphosulfate, the sulfating coenzyme required for sulfation of proteoglycans (McCully, 1971).

Because of the accelerated growth observed in many children with homocystinuria and because of the abnormal growth pattern of cultured skin cells from these children, we decided to study the function of homocysteine in normal growth in animal models. Children with homocystinuria secrete increased growth hormone, and homocysteine increases the binding of sulfate to cultured cartilage fragments, an effect of insulin-like growth factor. Our experiments showed that homocysteic acid increases the binding of sulfate both to

normal skin cells and to cultured chick cartilage cells. In addition, homocysteic acid increases the growth of normal guinea pigs and rabbits. Our experiments with hypophysectomized rats treated with thyroxin demonstrated a growth response to homocysteic acid and increased levels of insulin-like growth factor in plasma (Clopath et al., 1976). These results help to explain the accelerated skeletal growth observed in homocystinuria and the increased growth of smooth muscle cells in arteriosclerotic plaques produced by hyperhomocysteinemia.

The cultured skin cells from children with homocystinuria grow in an abnormal pattern, in which the cells become layered, similar to the growth of malignant cells in culture. In our experiments with guinea pigs with scurvy, we discovered that ascorbic acid (vitamin C) is required for the oxidation of homocysteine to homocystine and homocysteic acid (McCully, 1971). In the 1950s Albert Szent-Gyorgi had demonstrated that normal tissues contain low-molecular-weight factors of unknown composition that stimulate or retard the growth of malignant tumors in mice. Because of these observations, I studied the metabolism of homocysteine thiolactone in several examples of malignant cells in culture. These experiments showed that malignant cells are unable to convert the sulfur atom of homocysteine thiolactone to sulfate (McCully, 1976). Normal cells or homocystinuric cells rapidly complete this conversion.

These experiments also showed that excess homocysteine thiolactone reacts with amino groups of proteins and other macromolecules, forming peptide-bound homocysteine groups, similar to the homocysteinylation reaction discovered by DuVigneaud in the 1930s. In recent years Jakubowski has confirmed the production of homocysteine thiolactone in cultured malignant cells and shown that methionine aminoacyl transfer-RNA synthetase is the enzyme responsible for homocysteine thiolactone synthesis from methionine (Jakubowski & Goldman, 1993).

In order to explain the abnormal metabolism of homocysteine thiolactone in malignant cells, I hypothesized that normal cells contain a derivative of homocysteine thiolactone that facilitates the conversion of homocysteine thiolactone to sulfate and that this derivative is lost during malignant transformation (McCully, 1976). My associates and I investigated the nature of this substance by organic synthesis of homocysteine thiolactone derivatives and testing for inhibition of growth of malignant tumors in mice.

We demonstrated that the homocysteine thiolactonyl amide of retinoic acid, called thioretinamide, is anticarcinogenic and antineoplastic in mice (McCully, 1994a). Thioretinamide is presumed to be an intermediate in normal sulfate-ester synthesis, since experiments in the 1960s demonstrated participation of vitamin A in sulfation reactions. I also discovered that thioretinamide forms a complex with vitamin B12, called thioretinaco, in which two molecules of thioretinamide are bound to the cobalt atom of cobalamin. This substance is also anticarcinogenic and antineoplastic in mice. In addition, recent experiments by my colleague Kazimir (1999) have demonstrated potent an-

tiatherogenic activity of thioretinamide and thioretinaco in rats injected with homocysteine.

Linus Pauling attended a symposium dedicated to Albert Szent-Gyorgi in 1974 at Boston University. During his lecture Szent-Gyorgi showed photographs of dark brown solutions of his growth-promoting or growth-inhibiting extracts of normal tissues; he stated that malignant tissues contain little of these substances. Pauling related these findings to the oxidation reactions in tissues catalyzed by ascorbic acid, which was discovered by Szent-Gyorgi in 1928. Solutions of my synthetic homocysteine compound thioretinaco in alcohol are dark red-brown, resembling Szent-Gyorgi's tissue extracts.

In the 1920s and 1930s Otto Warburg had demonstrated an abnormal Pasteur reaction, the production of lactic acid in the presence of oxygen by aerobic glycolysis, in malignant tissues. Later work by others showed this abnormal reaction in embryonic and regenerating tissues. In the excitement of the scientific revolution following the discovery of the DNA double helix by Watson and Crick, the importance of abnormalities of oxidative metabolism in malignant tissues was neglected by young scientists. The experiments with thioretinamide and thioretinaco suggested their participation in normal oxidative metabolism and their loss in malignant cells.

In my review of the chemical pathology of homocysteine in 1994, I suggested that thioretinaco ozonide is the active site of oxidative phosphorylation in mitochondria (McCully, 1994a). Ozone stimulates oxidative phosphorylation at moderate concentrations, and molecular modeling suggests that thioretinaco forms a disulfonium-ozone-oxygen ion cluster that binds the alpha and gamma phosphate groups of adenosine triphosphate (ATP). During oxidative phosphorylation this binding reaction facilitates electron transport to reduce the bound oxygen, and ATP is released by proton flow through the F1F0 complex of the inner mitochondrial membrane. Synthetic corrin analogues inhibit oxidative phosphorylation, and ozonization catalyzes the formation of ATP from adenosine diphosphate (ADP) and phosphate in model reactions. In the 1960s, experiments conducted in Germany and Wisconsin showed that oxidation of sulfur amino acids, especially homocysteine thiolactone, catalyzes ATP synthesis in chemical models of oxidative phosphorylation (McCully, 1994a).

It is fascinating that Pasteur was interested in the biological properties of ozone, since an early ozone generator is prominently displayed in the museum of the Pasteur Institute in Paris. While the thioretinaco-ozonide theory needs further exploration and proof, it explains the inhibition of oxidative metabolism in malignant tissues and the abnormal accumulation of oxygen radicals in aging and degenerative diseases (McCully, 1994b). Furthermore, the reaction of homocysteine thiolactone with proteins, nucleic acids, and glycosaminoglycans to form homocysteinylated macromolecules explains the alterations in cellular membranes, protein structure and function, and genetic expression observed in malignant cells.

Reaction and Resistance

Stimulated by discussions with Moses Suzman, two young neurophysiologists, Stephen Raymond and Edward Gruberg at Massachusetts Institute of Technology, became interested in the homocysteine theory of arteriosclerosis in 1976. As a result of their interest and inquiry into the new theory, they wrote a book for the general reader, *Beyond Cholesterol,* first published as an article in *Atlantic Monthly* in 1979 (Gruberg & Raymond, 1981). These publications called attention to the shortcomings of the lipid/cholesterol hypothesis concerning heart disease and proposed that elevated blood homocysteine and dietary deficiency of vitamin B6 are the underlying cause of arteriosclerosis.

Because of the publicity surrounding the publication of *Beyond Cholesterol* and because of other press attention to homocysteine in the late 1970s, a reaction against this new theory came from prominent members of the lipid/cholesterol camp. In an article in the *Boston Globe* (April 25, 1979) the homocysteine approach was dismissed by a prominent heart disease researcher at Massachusetts Institute of Technology as "errant nonsense" and a "hoax that is being perpetrated on the public." The approach of giving B vitamins to prevent arteriosclerosis, while avoiding cholesterol-lowering drugs, was considered malpractice or worse. In reaction to an article in *Time Magazine* (August 6, 1979), a proponent of the lipid/cholesterol hypothesis affiliated with the Framingham Heart Study labeled the homocysteine approach as "crazy."

Earlier reaction to the controversy concerning the homocysteine theory had already jeopardized my own position as Associate Pathologist at Massachusetts General Hospital and Assistant Professor of Pathology at Harvard Medical School. My laboratory was removed from the department and relocated to the basement, key staff were lost, and it became impossible to renew my research grants under these conditions. The chairman of my department had retired, and the new chairman presented an ultimatum amounting to "renew your grants or leave." The director of the hospital relayed the information that the elders at Harvard felt that I "had not proven my theory."

After leaving in 1979, I was told by the director of public relations at the hospital that they did not want the name of Harvard or Massachusetts General Hospital associated with homocysteine or my name. It became impossible to secure another position suitable for continuing my homocysteine research for 27 months, until after legal advice I was able to work again as a pathologist at the Veterans Affairs Medical Center in Providence.

The intensity of the reaction against the homocysteine theory is difficult to fathom, even in the perspective of 21 elapsed years. The ostracism from Harvard medicine seems to be a disproportionately severe response for introducing a new theory that relegates conventional wisdom about dietary fats and cholesterol to a secondary role. It is true that clinical and epidemiological studies of human populations did not become available until after 1976, when the first human study of homocysteine metabolism in coronary heart disease was published (Wilcken & Wilcken, 1976). However, the hypothetical role of

dietary cholesterol in causing elevation of blood cholesterol and LDL levels has never been proven, and the Framingham study over the years has consistently failed to support this hypothesis.

Another major problem with the cholesterol/fat hypothesis is that a majority of victims of arteriosclerotic vascular disease have no evidence of elevation of blood cholesterol levels or other traditional risk factors. A further difficulty with this hypothesis is that the major decline in mortality from coronary heart disease since the 1960s in the United States is not correlated with changes in dietary fats or blood cholesterol levels. Major interventional trials in the 1970s and 1980s aimed at lowering blood cholesterol levels and controlling other risk factors for vascular disease were largely failures. Only in the 1990s has the introduction of statin drugs to lower blood-cholesterol levels resulted in modest reduction of risk in selected high-risk populations.

Recently my colleagues and I have demonstrated that homocysteine thiolactone converts LDLs to the small dense form of these particles that is associated with increased risk of vascular disease (Naruszewicz et al., 1994). Homocysteine becomes linked to the apoB protein of LDL by peptide-bound homocysteinyl groups, causing aggregation and precipitation of the LDL particles. The homocysteine-LDL aggregates are taken up by macrophages to form foam cells. In the artery wall, foam cells lead to deposition of cholesterol and fats within the arteriosclerotic plaques induced by the effect of homocysteine on arterial cells. These observations show that LDL is a carrier of homocysteine in the pathogenesis of arteriosclerotic plaques.

Ironically, recent research on dietary fats has increasingly incriminated the abnormal *trans* form of partially saturated fatty acids, the so-called transfats, in increased risk of vascular disease. These transfats arise through food processing when hydrogen atoms are introduced into unsaturated fatty acids to stabilize oils that are susceptible to rancidity. These partially saturated transfats are the likely explanation of the correlation of dietary saturated fat with increased risk of vascular disease.

In addition to the effect of transfats, the oxidized forms of cholesterol that are produced by food processing, the so-called oxycholesterols, rapidly produce plaques in the arteries of experimental animals. Oxycholesterols are produced by spray drying of eggs, dehydration of milk, and cooking of animal foods in heated vegetable oils. In contrast, highly purified cholesterol, containing no oxycholesterol contaminants, produces no plaques in animals and is a potent protective antioxidant. These oxidized forms of cholesterol are formed within plaques by the modification of LDL, catalyzed by reactive oxygen radicals, homocysteine, and ferric ions. These results with transfats and oxycholesterols explain how dietary fats and cholesterol are involved in the atherogenic process.

For many years the treatment of animals with large doses of homocysteine has been known to increase the production of cholesterol, triglycerides, and LDL. In addition, the thioretinaco ozonide theory of participation of homo-

cysteine in oxidative metabolism offers a biochemical explanation for this effect (McCully, 1994a). According to this theory, inhibition of oxidative metabolism and increased oxidant stress by hyperhomocysteinemia is caused by depletion of thioretinaco ozonide. Theoretically, hyperhomocysteinemia causes depletion of thioretinaco ozonide because increased homocysteine thiolactone displaces thioretinamide from thioretinaco and forms thioco, the complex formed from homocysteine thiolactone and cobalamin. Because of decreased oxidative metabolism resulting from depletion of thioretinaco ozonide, excess acetyl coenzyme-A accumulates within mitochondria and is converted to fatty acids and cholesterol.

With the benefit of a 21-year retrospective view, the intensity of the reaction against the homocysteine theory of arteriosclerosis is now judged to be clearly misplaced. Because of the entrenched opinion about the importance of dietary fats and cholesterol in 1979, it was difficult for adherents of the cholesterol/lipid hypothesis to accept a revolutionary new perspective in understanding the underlying cause of vascular disease. Instead, the homocysteine approach of the past 2 decades has explained many otherwise inexplicable observations and placed abnormal cholesterol and lipoprotein metabolism in a clearer light. Rather than displacing cholesterol as an important factor in atherogenesis, the homocysteine theory has placed elevated blood cholesterol in the proper context as a reactive change to an underlying deficiency of the B vitamins, vitamin B6, folic acid, and vitamin B12.

Confirmation and Redemption

Within the past decade a veritable avalanche of data from thousands of research studies from laboratories, clinics, and epidemiologists worldwide has now established the importance of homocysteine in mainstream medicine. Prospective and retrospective studies have proven elevated blood homocysteine to be a potent independent risk factor for arteriosclerotic vascular disease. Major studies, such as the Physicians' Health Study, the Framingham Heart Study, the Nurses' Health Study, the Nutrition Canada Study, the European Concerted Action Project on Homocysteine, the Hordaland Homocysteine Study from Norway, the British United Provident Association Study, and many more, have proven the significance of homocysteine in the pathogenesis of arteriosclerosis. At the present time at least 18 major prospective intervention trials are in progress worldwide to prove that lowering blood homocysteine by dietary improvement and supplemental B vitamins will prevent vascular disease.

The acceptance of the homocysteine theory of arteriosclerosis by mainstream medicine has increased within the past 5 years, as evidenced by publication of review articles, national and international symposia on homocysteine, scientific monographs, and multi-authored books. A comprehensive review entitled *Homocysteine Metabolism: From Basic Science to Clinical Medicine* was published in 1997 (Graham et al., 1997). A review dedicated to

vascular disease entitled *Homocysteine and Vascular Disease* was published in 2000 (Robinson, 2000). Two books for the general reader, *The Homocysteine Revolution* (McCully, 1997) and *The Heart Revolution* (McCully & McCully, 1999), explain the significance of the homocysteine approach to prevention of vascular disease. Publicity surrounding the publication of these books, the major clinical and epidemiological studies, and marketing of B vitamin supplements has begun to introduce homocysteine into the vocabulary of the general public.

Finally and significantly, research on homocysteine has recently implicated abnormal homocysteine metabolism in a wide range of disease processes. Homocysteine reacts with nitric oxide to prevent the relaxing effect of this chemical mediator on smooth muscle tone. Hyperhomocysteinemia causes endothelial dysfunction both in experimental animals and human subjects, and folate therapy effectively prevents endothelial dysfunction by preventing hyperhomocysteinemia.

The effect of folate deficiency in causing birth defects of the neural tube is mediated by overproduction of homocysteine with increased levels both in maternal plasma and in amniotic fluid. Growth and development in childhood are correlated with increasing homocysteine levels, and adult levels are achieved during puberty and adolescence. Aging is correlated with increased levels of homocysteine, as explained by a detailed theory of aging involving loss of thioretinaco ozonide from cellular membranes, increased oxidant stress, and decreased formation of adenosyl methionine (McCully, 1994b). Abnormalities of gestation, including toxemia and abruptio placentae, are correlated with increased homocysteine levels.

Hypothyroidism, a hormonal abnormality long known to be associated with increased vascular disease risk, is correlated with increased homocysteine levels, and therapy with thyroid hormone reduces the homocysteine levels to normal. Increased levels of homocysteine and decreased folate and vitamin B12 levels have been documented in a variety of neurodegenerative diseases, including Alzheimer's disease, schizophrenia, and cognitive decline of the elderly. Autoimmune diseases such as rheumatoid arthritis and lupus erythematosus are associated with increased homocysteine levels. Many types of cancer are associated with increased homocysteine production, and homocysteine metabolism is characteristically altered in malignant cells (McCully, 1976, 1994b). Renal failure is associated with high levels of blood homocysteine and greatly increased risk of morbidity and mortality from arteriosclerotic vascular disease.

From a preventive and therapeutic point of view, the biomedical significance of homocysteine is extraordinarily important. The decline in cardiovascular-disease mortality in the United States in the past 35 years is attributable to addition of vitamin B6 and folic acid to the food supply in the form of voluntary fortification of cereals and vitamin supplements (McCully, 1983). Millions of deaths have been prevented because of control of elevated homo-

cysteine levels by these B vitamins. With the anticipated success of prospective interventional trials in the next few years, the protective effect of these vitamins and dietary improvement against vascular disease will be extended, promising to increase life expectancy of the population.

Since the implications of the homocysteine theory are primarily in the field of improved nutrition and preventive therapy, the benefits of the approach will follow improvements in food preservation, fortification of processed foods, and widespread use of vitamin B6, folic acid, and vitamin B12 to prevent vascular disease. These benefits will be increased by improved understanding of the genetic basis of abnormal homocysteine metabolism, such as thermolabile methylenetetrahydrofolate reductase, and the interaction of folic acid and other nutrients to prevent hyperhomocysteinemia. Finally, successful application of the thioretinaco ozonide theory of oxidative metabolism to degenerative diseases of aging will open a new chapter in treatment of cancer, autoimmune disease, and neurodegenerative disease.

Ackowledgments

A lecture based on this essay was presented at the 19th Annual Meeting of the Society for Scientific Exploration, London, Ontario, 8–10 June 2000.

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