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Narrative Review

Homocysteine and Oxidative Metabolism in Arteriosclerosis, Cancer, and Diseases of Aging

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Discovery of the Homocysteine Theory of Arteriosclerosis

In 1967 creation of a new Human Genetics Unit at Massachusetts General Hospital was announced for the purpose of studying and managing human diseases of genetic origin. This new initiative was to be directed by John Littlefield, Professor of Pediatrics at Harvard Medical School. I became acquainted with Littlefield during my student, fellowship, and residency years at Harvard Medical School and Massachusetts General Hospital and admired his insights in the field of human genetics. Having completed my residency in Pathology and Laboratory Medicine, I became interested in studying the pathology of inherited diseases as an informal member of the Human Genetics Unit. Littlefield and his colleagues invited me to attend Human Genetics clinical rounds and meetings because of my training in pathology and my previous experience in the molecular biology, genetics, and structure of transfer ribonucleic acid (tRNA) [1-3].

At a clinical conference of the Human Genetics Unit a fascinating case of a nine-year old female with accelerated growth in childhood, mental retardation, dislocated ocular lenses and capillary fragility was discussed by the pediatricians who had evaluated this patient in the Human Genetics Clinic [4]. These clinical features resembled abnormalities in reported cases of homocystinuria from Belfast, Philadelphia, and Milwaukee, and chromatography of the urine and plasma disclosed excretion of the amino acid, homocystine. The mother of the child informed the pediatricians that an eight-year-old uncle had died in 1933 of a similar disease, and the case was published as a Clinicopathological report in the New England Journal of Medicine [5]. The autopsy findings of the 1933 case revealed carotid arteriosclerosis with organizing mural thrombus, focal thrombosis of the internal carotid artery with acute cerebral infarct, and congenital coxa vara of the femur. My pathological examination of surviving tissues of this case revealed arteriosclerotic lesions in peripheral arteries, fibrotic arteriosclerotic occlusion of the carotid artery with organizing mural thrombus, and acute cerebral infarct.

Later in 1967 at a subsequent clinical conference of the Human Genetics Unit, another case of homocystinuria was discussed. This two-month-old baby boy with pneumonia, lactic acidosis, and weight loss was found to have cystathionine, methyl malonic acid, homocystine, and homocysteine-cysteine disulfide in the urine [6]. In this index case of cobalamin c disease, the metabolic abnormalities are attributed to deficiency of methyl tetrahydrofolate methyl transferase. Despite vitamin therapy and supportive measures, the patient expired, and autopsy revealed bronchopneumonia, gastritis, and encephalomalacia. Because of my interest in the observation of arteriosclerosis in the 1933 case of homocystinuria, I restudied the tissues from this case and discovered diffuse, advanced arteriosclerotic lesions affecting arteries in multiple organs.

In discussions with John Littlefield and Moses Suzman, a visiting cardiologist from South Africa, we recalled experiments conducted by James Rinehart and Louis Greenberg reporting arteriosclerotic lesions in monkeys subjected to chronic deficiency of pyridoxine (vitamin B6) at the Boston City Hospital in 1949 [7]. Since the most common cause of homocystinuria is enzymatic deficiency of cystathionine synthase, an enzyme that requires the coenzyme, pyridoxal phosphate [8], these experiments support the concept that elevation of blood homocysteine is responsible for initiation of human arteriosclerosis, as observed in homocystinuria. Elevation of blood methyl malonic acid, cystathionine, or methionine was not observed to be associated with arteriosclerosis in other reported cases of human enzyme deficiencies. Because of these observations, the elevation of blood homocysteine levels in cystathionine synthase deficiency and in cobalamin c disease is hypothesized to cause arteriosclerosis in homocystinuria and in subjects in the general population without these rare enzymatic deficiencies [9]. Further support for the association of hyperhomocysteinemia with arteriosclerosis is the observation of fibrous arteriosclerotic plaques in patients with homocystinuria caused by deficiency of methylenetetrahydrofolate reductase [10].

The gene for cystathionine synthase (CBS) is located on Chromosome 21 in the subtelomeric region, 21q22.3 [11]. Alleles of CBS are the most important causes of hyperhomocysteinemia in inherited homocystinuria, and subjects with alleles that are responsive to vitamin B6 therapy exhibit a reduced risk of adverse vascular events. Other genetic determinants of hyperhomocysteinemia are alleles of the gene for methylenetetrahydrofolate reductase (MTHFR) [11]. Subjects with MTHFR deficiency are resistant to therapy and are only partially responsive to 5-formyltetrahydrofolate, vitamin B6, and cobalamin. Subjects with hyperhomocysteinemia caused by inborn errors of cobalamin absorption and transport, such as cobalamin c disease [6], are

resistant to oral cobalamin therapy and are only partially responsive to parenteral hydroxycobalamin [11].

Cellular Conversion of Homocysteine Thiolactone to Sulfate

During my two years' tenure as Associate in Biochemistry in the laboratory of Giulio Cantoni at the National Institute of Mental Health, I became acquainted with Stuart Harvey Mudd, a specialist in the biochemistry of sulfur amino acids. Cantoni was the discoverer of adenosyl methionine [12], and Mudd's team discovered the enzymatic deficiency of cystathionine synthase in cases of homocystinuria [8]. During our weekly conferences, recent developments in the biochemistry of sulfur amino acids were discussed. Through a contact with Mudd, cell cultures of skin fibroblasts from two patients with homocystinuria from two different families with cystathionine synthase deficiency were delivered to my newly established cell culture laboratory at Massachusetts General Hospital.

The cultured cells from both patients with cystathionine synthase deficiency produce moderate quantities of abnormal, metachromatic, aggregated extracellular matrix disposed along normal intracellular fibrillar matrix [13]. When homocysteine or homocysteine thiolactone are added to the culture medium of normal fibroblasts, smaller amounts of the aggregated matrix are observed. The birefringence of fibrillar matrix observed in normal cells is converted to short, irregular, aggregated matrix by added homocysteine in the culture medium. The amino acid composition of the extracellular matrix resembles that of tissue proteoglycans, and a trace fraction of hydroxyproline is demonstrated, excluding the presence of significant numbers of collagen fibrils in these cell cultures. The results with S35 labelled sulfate show that homocysteine produces altered proteoglycan molecules with increased sulfation, which is capable of binding LDL during atherogenesis [14]. S35 labelled homocysteine thiolactone is found to be a precursor of esterified sulfate of extracellular matrix in these cell cultures, demonstrating an alternate pathway for sulfate ester formation, since the cultured cells lack cystathionine synthase, impairing conversion of homocysteine to cysteine and sulfate, the only previously known precursor of sulfate esters [14].

Study of pathologic changes in guinea pigs with experimental scurvy induced by deprivation of dietary ascorbic acid revealed that the sulfated proteoglycan matrix of arterioles and arteries is severely diminished, explaining the pathogenesis of capillary and arteriolar hemorrhages occurring in the human disease of scurvy [15]. To explain this observation, the oxidation of homocysteine thiolactone was studied in microsomes of scorbutic guinea pig liver, demonstrating increased accumulation of homocysteine and inhibition of homocysteine oxidation [16]. In contrast, microsomes from normal guinea pig liver oxidize homocysteine thiolactone to homocysteic acid, homocysteine sulfinic acid and other oxidized derivatives of homocysteine. These results show that failure of formation of sulfated proteoglycan matrix is related to inhibition of oxidation of homocysteine to homocysteic acid and other oxidized homocysteine derivatives in experimental scurvy.

In experiments with S35 labelled homocysteic acid, formation of phosphoadenosine phosphate, the precursor coenzyme of sulfate esters, is demonstrated to occur in liver microsomes from added ATP [16]. These results and the faster growth rate of normal young guinea pigs injected with homocysteic acid [17] suggest that homocysteic acid has the properties of the sulfation factor of liver that mediates the effect of growth hormone [18]. Thus, the accelerated growth in childhood of patients with homocystinuria may be attributed to increased formation of homocysteic acid from increased secretion of growth hormone in untreated homocystinuria [19]. Added homocysteic acid in the cell cultures of human fibroblasts or chick embryo chondrocytes increases the binding of S35 sulfate to the cultured cells, reproducing the effect of somatomedin [17], and homocystine has somatomedin activity in cartilage fragments [20]. Currently accepted terminology for somatomedin C is insulin-like growth factor, IGF-1, a protein synthesized in liver with amino acid sequence similarities to insulin.

In thyroxine-treated hypophysectomized rats, IGF-1 activity is increased by parenteral homocysteic acid, as assayed by the porcine epiphyseal disk assay [21]. In these experiments, the tail growth assay demonstrates a significant growth response to parenteral homocysteic acid, correlating with increased IGF-1 activity. These results suggest that endogenous homocysteic acid stimulates secretion of IGF-1 and promotes skeletal growth in subjects with homocystinuria, since homocysteic acid, homocysteine sulfinic acid and other homocysteine metabolites are demonstrated in the urine of patients with homocystinuria [22].

Homocysteine and Experimental Arteriosclerosis

If plasma homocysteine is a pathogenic factor in production of arteriosclerosis in homocystinuria, caused by different inherited enzyme abnormalities, demonstration of arteriosclerosis in experimental animals with hyperhomocysteinemia provides evidence for the validity of the homocysteine theory of arteriosclerosis [9]. Fibrotic and calcific arteriosclerotic plaques that closely resemble arteriosclerotic changes in homocystinuria are observed in rabbits by subcutaneous injection of homocysteine [23] and by feeding of experimental diets containing methionine, homocysteine thiolactone perchlorate, or homocystine oxidized with H_2O_2 to homocysteic acid, sulfate, homolanthionine sulfoxide, and homolanthionine sulfone [24]. In rabbits injected with high doses of homocysteine thiolactone hydrochloride, methionine or homocysteic acid, venous thrombosis and pulmonary embolism with fatal pulmonary infarct are observed in a few animals. In the aorta, fibrotic and calcified elevated plaques are associated with deposition of sulfated metachromatic matrix and degeneration of elastic lamellae. Thrombosis and embolism are prevented by parenteral administration of pyridoxine, and these results are confirmed by independent observers [25]. Addition of butter to the synthetic diet converts the fibrous arteriosclerotic plaques to fibrolipid plaques produced by parenteral homocysteine in rabbits [26].

The demonstration of arteriosclerosis in monkeys exposed to chronic, intermittent dietary deficiency of vitamin B6 (pyridoxine) supports the production of experimental arteriosclerosis by homocysteine, since dietary deficiency of pyridoxine causes hyperhomocysteinemia [7]. Arteriosclerotic changes occur in the aorta, carotid, and coronary arteries of

rats exposed to a diet that is deficient in choline [27], and choline deficiency produces deficiency of betaine and hyperhomocysteinemia. Betaine is derived from choline, and the enzyme betaine homocysteine methyl transferase converts homocysteine to methionine by transfer of methyl groups from betaine, preventing accumulation of homocysteine [11]. Exposure of monkeys to a diet containing soy protein causes arteriosclerosis, and added dietary methionine prevents atherosclerosis in this animal model [28]. A possible explanation of this result is reduction of plasma homocysteine, because of increased formation of adenosyl methionine from dietary methionine, causing increased allosteric regulation of remethylation and transsulfuration of homocysteine [29]. Intravenous infusion of homocysteine in baboons [30] and rats [31] produces endothelial loss, consumption of platelets, and intra-arterial thrombosis. Feeding a diet containing triglycerides, cholesterol, thiouracil and sodium cholate produces cholesterolosis, arteriosclerosis and myocardial infarction in rats [32, 33]. Thiouracil is a goitrogen that produces hypothyroidism and hyperhomocysteinemia, and sodium cholate is a bile salt that increases absorption of fat and cholesterol, producing cholesterolosis. Injection of the potent carcinogen, nickel subsulfide (Ni_3S_2) in the kidney of rats produces sialyl hyperplasia, glomerulomegaly with mesangial hyperplasia, erythrocytosis, and arteriosclerotic plaques, as visualized by hematoporphyrin fluorescence of the endothelium [34].

Demonstration of arteriosclerosis in these animal models with hyperhomocysteinemia supports the early concepts of human atherogenesis developed by Carl Rokitansky and Rudolph Virchow in the 19th century [35]. Rokitansky demonstrated mural thrombosis in atherosclerosis as an "increased mass of the inner lining of arteries through a pseudomembranous enlargement" by components of blood serum [36]. Virchow demonstrated deposition of sulfated metachromatic matrix as the earliest change in development of arterial plaques, followed by inflammation of arterial media and adventitia, deposition of plasma fats, associated with degeneration of arterial wall, a process he termed "endoarteritis chronica deformans nodosa" [37]. Virchow suggested that increased permeability of arterial intima permitted passage of plasma lipids, producing atheromas containing calcification and deposition of crystals, identified as cholesterol crystals by Ludwig Aschoff [38].

Discovery of Abnormal Metabolism of Homocysteine Thiolactone in Cancer Cells

Because of the interest in the discovery of oncogenes, I discussed studying homocysteine metabolism in cell cultures from malignant tumors with Paul Black, a virologist at Massachusetts General Hospital whom I had met when he had a fellowship at the Glasgow Virology Institute in 1963, at the same time when I was a research fellow in the Genetics Department at the University of Glasgow. Black and his colleague Richard Roblin and my colleague, Urology fellow James Daly, made available several serially cultured malignant cell lines for study [39]. These cell lines included a hamster sarcoma induced by SV40 virus, cells from a human metastatic renal cell carcinoma, a murine mammary carcinoma, cells from a rat bladder carcinoma obtained from a carcinogen-induced tumor, mouse embryo cells transformed by SV40 virus, hamster kidney cells transformed by polyoma virus, and normal fibroblasts cultured from human skin, mouse embryo cells and hamster kidney cells.

Labelling experiments of malignant cells with S35 homocysteine thiolactone, followed by dialysis of cell homogenates, hydrolysis to homocysteine by trypsin and pronase, or by acid or base hydrolysis, but not by mercaptoethanol, demonstrated formation of homocysteine bound to the amino groups of proteins by a peptide bond, a process known as thiolation, since a new thiol group is formed by opening of the homocysteine thiolactone molecule ring [39]. In contrast, fibroblast cultures from normal human skin converted none of the S35 homocysteine thiolactone to peptide-bound homocysteine. Hypothetically therefore, an unknown derivative of homocysteine thiolactone which occurs in normal cells may prevent this thiolation reaction that occurs in malignant cells [39].

Because of my observations on arteriosclerosis in homocystinuria, growth hormone, and thiolation of proteins in cultured malignant cells, I contacted Konrad Bloch, my Professor of Biochemistry at Harvard, for advice and comment on how to investigate the biochemical basis for these discoveries. At Harvard I had previously studied advanced topics in biochemistry in Bloch's course and served as a research assistant in his laboratory, isolating dimethyl lanosterol, an intermediate in cholesterol biosynthesis, from liver homogenates. During a luncheon meeting at the Harvard Faculty Club, Bloch cautioned that other independent scientists must confirm my discoveries in the homocysteine field. He also advised collaboration with a qualified chemist who could assist in investigating the chemical nature of the unknown homocysteine thiolactone derivative that prevents thiolation of proteins in normal cells. Soon thereafter, Pierre Clopath, a post-doctoral fellow from the University of Fribourg in Switzerland with advanced experience with radical complexes, joined our research team at Massachusetts General Hospital.

Thereafter, Clopath and I mounted a campaign of organic synthesis of a variety of about 35 different new derivatives of homocysteine thiolactone and testing them for anti-neoplastic activity in mice with serially transplanted malignant tumors. Pyridoxal homocysteine thiolactone enamine hydrochloride inhibits growth of transplanted spontaneous murine adenocarcinomas when administered before and after transplantation [40]. Oleoyl homocysteine thiolactone increases growth and arachidonoyl homocysteine thiolactone decreases growth of the adenocarcinoma.

Organic Synthesis of Antineoplastic Derivatives of Homocysteine Thiolactone

As Clopath and I were about to continue an expanded program of organic synthesis of antineoplastic derivatives of homocysteine thiolactone, a problem arose in 1978 concerning my appointment and laboratory space in the Pathology Department of Massachusetts General Hospital. The Director of Pathology, who had supported my research program, announced his retirement. Thereafter, the newly appointed Director of Pathology moved my laboratory operations to the Research Building and informed me that my appointment at Harvard Medical School would be terminated. This problem necessitated securing a position at another

institution where my research team could continue our investigation of antineoplastic homocysteine compounds. Because of the prolonged delay in my securing a new position, Clopath and the technician members of my research team decided to leave Massachusetts General Hospital for other opportunities. After a search of two years, I finally secured an appointment as Staff Pathologist at the VA Medical Center in Providence in 1981 [41], where I completed and published a monograph on the homocysteine theory of arteriosclerosis [42].

When Michael Vezeridis, a surgeon who had trained as a research fellow in Surgery at Massachusetts General Hospital, arrived at the Providence VA Medical Center in 1982, we discovered our mutual interest in cancer research and embarked on a collaboration to investigate antineoplastic derivatives of homocysteine thiolactone. We were assigned a laboratory in the Research Building, and a grant was assigned to Vezeridis to support our new initiative. Our experiments with animals were carried out in the newly renovated Animal Research Facility.

In prior experiments with homocysteine thiolactone perchlorate and with the free base of homocysteine thiolactone, stimulation and necrosis of malignant growth of murine adenocarcinoma are observed and attributed to the lipophilic properties of these derivatives [43]. Homocysteine thiolactone perchlorate is prepared by treating homocysteine thiolactone hydrochloride with perchloric acid in chloroform-methanol solvent, yielding crystals of the perchlorate salt. X-ray crystallographic analysis of these crystals demonstrates identity of the 5-membered ring structure with that of homocysteine thiolactone hydrochloride [44].

Because of the solubility of homocysteine thiolactone perchlorate in organic solvents, N-maleyl homocysteine thiolactone amide and N-maleimide homocysteine thiolactone amide are readily synthesized from maleyl anhydride and maleimide, respectively. Both compounds inhibit growth of transplanted murine carcinoma, and the anti-neoplastic activity of N-maleimide homocysteine thiolactone amide is enhanced by delivery within liposomes [45]. Daily intraperitoneal injections of liposomal N-maleimide homocysteine thiolactone amide produces 65% inhibition of growth of transplanted murine mammary adenocarcinoma and 89% inhibition of growth of transplanted murine rhabdomyosarcoma. Two thirds of animals with transplanted rhabdomyosarcoma reveal no evidence of residual tumor after two weeks of intraperitoneal injections of liposomal N-maleimide homocysteine thiolactone amide [45].

The solubility of homocysteine thiolactone perchlorate in organic solvents facilitates synthesis of dimeric homocysteine thiolactone from oxalyl chloride, yielding a dark brown oil without antineoplastic activity. Oxalyl homocysteine thiolactone and rhodium trichloride form a red-brown solid which is soluble in a lipid solvent of triolein, cholesterol, phosphatidyl choline, and cholesteryl palmitate [46]. This rhodium trichloride complex with oxalyl homocysteine thiolactone prominently inhibits growth of transplanted murine rhabdomyosarcoma. Thus, taken together, model N-substituted derivatives of homocysteine thiolactone exhibit antineoplastic activity if they are (a) soluble in lipids, (b) contain a carbonyl group adjacent to the nitrogen atom of homocysteine thiolactone, (c) contain a transition metal ion, and (d) contain a conjugated double bond system. These model compounds exhibit little toxicity at low doses and moderate toxicity at high doses.

Antineoplastic and anticarcinogenic activity of thioretinamide and thioretinaco

Prior to his departure from my laboratory Clopath and I discussed synthesis of a retinoid derivative of homocysteine thiolactone, because of the known antineoplastic and anticarcinogenic activities of retinoids. We found that the free base of homocysteine thiolactone reacts with all-trans retinoic acid to produce N-homocysteine thiolactonyl retinamide, named thioretinamide, which has antineoplastic activity in transplanted murine adenocarcinomas. By repeating these experiments in the Providence VA research laboratory, Vezeridis and I demonstrated the antineoplastic activity of thioretinamide against transplanted murine rhabdomyosarcoma and the anticarcinogenic activity of thioretinamide in preventing pulmonary neoplasms induced by ethyl carbamate in strain A mice [47]. In these experiments the free base of homocysteine thiolactone and homocysteine thiolactone perchlorate both demonstrate augmentation of the carcinogenic effect of ethyl carbamate in induction of pulmonary neoplasms. Thus, addition of the retinoic acid group to the amine group of homocysteine thiolactone abolishes its co-carcinogenic activity and confers anticarcinogenic activity.

Because of the importance of cobalamin in homocysteine and methionine metabolism [11] and because of the enhancement of antineoplastic activity of oxalyl homocysteine thiolactone by complexation with the transition metal, rhodium trichloride [46], a complex was synthesized from adenosyl cobalamin and thioretinamide [48]. This complex, N-homocysteine thiolactonyl retinamido cobalamin, named thioretinaco, contains two molecules of thioretinamide which are bound to the axial ligands of the cobalt octahedral complex of the corrinoid ring of cobalamin. Thioretinaco produces a dark red-brown solution in ethanol or propylene glycol, exhibiting a fruity, aged-wine aroma. In experiments with rats injected with homocysteine thiolactone, both thioretinamide and thioretinaco are demonstrated to have antiatherogenic activity, by preventing formation of arteriosclerotic plaques of the intercostal arteries [49].

The formation of pulmonary neoplasms in strain A mice treated with ethyl carbamate is diminished by thioretinaco, demonstrating anticarcinogenic activity [48]. Adenosyl cobalamin, in contrast, increases the formation of pulmonary neoplasms in these experiments, demonstrating cocarcinogenic activity. Thus, complexation of cobalamin with thioretinamide abolishes the cocarcinogenic activity of adenosyl cobalamin and produces the anticarcinogenic activity of thioretinaco. In the control group of mice not receiving ethyl carbamate, thioretinaco produces hyperactivity, increased food consumption, increased fecal and urinary excretion, and failure of weight gain, effects which are attributed to effects of thioretinaco on metabolic functions.

In experiments with transplanted human pancreatic adenocarcinomas in athymic mice, a single intra-tumor dose of 2.5 mg/kg of thioretinaco produces a 50% reduction in tumor weight, demonstrating antineoplastic activity [50]. None of the precursors of thioretinaco, namely thioretinamide,

adenosyl cobalamin, homocysteine thiolactonyl cobalamin, and homocysteine thiolactone perchlorate, exhibit antineoplastic activity. Thus, thioretinaco is anticarcinogenic, antineoplastic, and antiatherogenic, supporting the hypothesis that the accumulation of homocysteine thiolactone and failure of sulfate synthesis in malignant cells is caused by cellular deficiency of an N-substituted homocysteine thiolactone compound with properties of thioretinaco [39]. In experiments with cultured human pancreatic adenocarcinoma cells, growth and lactate production are inhibited by thioretinamide and thioretinaco [51], explaining inhibition of growth of these malignant cells in athymic mice by thioretinaco [50]. In these experiments, thioco, a complex of cobalamin and homocysteine thiolactone, increases the growth of cultured adenocarcinoma cells and normal diploid fibroblasts cultured from skin.

Thioretinaco, Homocysteine Thiolactone, and Carcinogenesis

The chemical carcinogen diethylnitrosamine alters metabolism of methionine and cobalamin in normal liver and hepatomas of rats [52]. Thioretinaco is anticarcinogenic in strain A mice [48], and depletion of cellular thioretinaco by chemical carcinogens may inhibit methyl transfer reactions, producing accumulation of homocysteine thiolactone [53,54] and the reduced concentrations of methyl cobalamin [55], methionine, and adenosyl methionine that are observed in malignant cells [56]. Inhibition of endogenous biosynthesis of thioretinaco may also occur because of cellular methionine deficiency produced by choline deficiency [57], ethionine [58], dehydroepiandrosterone [59], or a methyl-deficient diet [60]. The proposal that carcinogenesis produces deficiency of cellular thioretinaco [61] is supported by the observation of invasive squamous cell carcinoma of murine skin exposed to the free base of homocysteine thiolactone [62]. Depletion of thioretinaco in carcinogenesis is attributed to increased conversion of thioretinaco to thioco, the complex formed by homocysteine thiolactone and cobalamin [61].

Intraperitoneal injection of the free base of homocysteine thiolactone in mice produces acute toxicity and increased mortality because of necrosis of tissues at the injection site [62]. Congestion and fibrin thrombi in pulmonary arterioles, focal necrosis of liver and kidney, and hepatic fat deposition are also observed. Intramuscular injection of homocysteine thiolactone free base produces fibrosis, acute and chronic inflammation, angiogenesis, proliferation of muscle cells, calcification, hypertrophy of nerves and ducts, and acanthosis with dysplasia of squamous epithelium [62]. Thus, the desmoplastic reaction of connective tissues surrounding malignant neoplasms, and the increased occurrence of thrombosis may be attributed to effects of homocysteine thiolactone on connective tissues and platelets, because of increased platelet aggregation [63]. Thiolation of low-density lipoprotein (LDL) by homocysteine thiolactone causes aggregation of LDL and phagocytosis by cultured macrophages, and consequent release of homocysteine thiolactone may cause intimal injury in atherogenesis [64].

Thioretinaco Ozonide, Oxidative Phosphorylation, and Tocotrienol

Methionine inhibits aerobic glycolysis in malignant or transformed cells, suggesting that increased intracellular methionine may increase oxidative phosphorylation and aerobic respiration [65]. Deficiency of methionine within malignant cells may be related to increased conversion of methionine to homocysteine thiolactone, because of cellular deficiency of thioretinaco [61]. In scorbutic guinea pigs, oxidation of homocysteine thiolactone to homocysteic acid and homocysteine sulfinic acid is diminished because of deficiency of ascorbic acid [16]. The biosynthesis of ATP is increased by retinoic acid in cultured normal fibroblasts, suggesting that thioretinamide may facilitate oxidative phosphorylation [66]. The suggestion of a function of thioretinaco in cellular respiration [48] is supported by the observations that retinoids and ascorbate facilitate cellular oxidation reactions and ATP biosynthesis.

Oxidized thioethers [67] and oxidized N-acetyl homocysteine thiolactone [68] increase ATP synthesis from AMP, ADP, and phosphate in model systems of oxidative phosphorylation. Microsomal electron transport, oxidation and hydroxylation reactions are catalyzed by monodehydroascorbate radical [69,70,71]. Mitochondrial oxygen consumption is increased by atmospheric ozone, suggesting that ozone may oxidize the sulfur atoms of thioretinaco to disulfonium groups which catalyze ATP biosynthesis by oxidative phosphorylation [72,73]. Growth of cultured malignant cells is inhibited by ozone [74], and regression of arteriosclerotic plaques is caused by hydrogen peroxide [75], providing evidence for ozone and peroxide as factors in oxidative metabolism catalyzed by thioretinaco ozonide. Further evidence for ozone in atherogenesis is the demonstration of ozone in arteriosclerotic plaques [76].

According to a model proposal [61], binding of thioretinaco to the lipid bilayers of mitochondria occurs by hydrophobic bonding of the thioretinamide groups of the molecule to the lipid bilayer. Orientation of the hydrophilic dimethylbenzimidazole group occurs at the surface of the lipid bilayer, and the sulfur and carbonyl groups of thioretinaco are oriented to the opposite surface. The corrinoid group of the cobalamin of thioretinaco is perpendicular to the plane of the membrane, and the rings of the homocysteine thiolactone groups are oriented in parallel to that of the corrinoid group. In this model, ozone oxidizes the two sulfur atoms of the two thioretinamide groups (TR_2) to form thioretinaco ozonide (TR_2CoO3), the disulfonium derivative which theoretically catalyzes ATP biosynthesis by oxidative phosphorylation [61].

The active site of oxidative phosphorylation is proposed to consist of thioretinaco ozonide, which is complexed with oxygen, nicotinamide adenine dinucleotide and inorganic phosphate, $TR_2CoO_3O_2NAD^+H_2PO_4^-$ [77]. According to this proposal, reduction of the pyridinium nitrogen of the nicotinamide group by an electron from electron transport complexes initiates polymerization of phosphate with adenosine diphosphate, yielding nicotinamide riboside and ATP bound to thioretinaco ozonide oxygen. Binding of ATP to the active site of oxidative phosphorylation occurs by ionic bonding of the two oxygen anions of ATP with the two sulfonium atoms of thioretinaco ozonide and by interaction of the oxygen ozone cluster of thioretinaco ozonide with the phosphorus atoms of ATP. A second electron is proposed to reduce oxygen to hydroperoxide radical, causing release of

ATP from the active site. This process creates a proton gradient within F1F0 ATPase complexes of mitochondria by reaction of protons with nicotinamide riboside and hydroperoxyl radicals, causing release of reduced nicotinamide riboside and hydroperoxyl radicals, releasing reduced nicotinamide riboside and hydroperoxide from the active site.

In 1922 a fat-soluble vitamin, isolated from leafy vegetables, later named vitamin E, was discovered to be required for fertility in animals [78,79]. Vitamin E is a complex of eight chemical isomers, consisting of alpha (α), beta (β), gamma (γ) and delta (δ) tocopherols (TP) and the corresponding tocotrienols (T3), each differing in placement of methyl groups on the benzenoid heterocyclic chromanol group of the molecule. The chromanol groups of both tocopherols and tocotrienols contain a benzenoid hydroxyl group, which is susceptible to oxidation by free radicals, allowing formation of benzenoid oxygen radical through loss of a proton and generating a proton gradient across the mitochondrial membrane. The tocopherols contain a saturated phytyl poly-isoprenoid 15-carbon side chain, and the tocotrienols contain an unsaturated farnesyl poly-isoprenoid 15-carbon side chain. Tocotrienol (T3) protects against radiation injury and mitochondrial dysfunction by preventing the opening of the mitochondrial permeability transition pore, theoretically inhibiting loss of the active site for oxidative phosphorylation, thioretinaco ozonide oxygen ATP, from mitochondria by complex formation with the active site, $TR_2CoO_3O_2NAD^+H_2PO_4^-$T3 [80].

Melatonin, Thioretinaco Ozonide, Adenosyl methionine, and Hyperhomocysteinemia

Melatonin is the indolamine hormone that is synthesized in the pineal gland, controls circadian rhythm, and causes aggregation of melanin granules within melanocytes, reversing the darkening effect of melanocyte-stimulating hormone [81]. Pineal secretion of melatonin declines markedly with aging and dementia, and the final two enzymatic processes in the biosynthesis of melatonin produce N-acetyl serotonin by acetylation of serotonin and methylation of N-acetyl serotonin by adenosyl methionine to produce melatonin, catalyzed by hydroxyindole-O-methyl transferase [82]. Progressive elevation of homocysteine levels is attributable to the decreased biosynthesis of adenosyl methionine, as observed in aging [83]. Adenosyl methionine biosynthesis is proposed to be catalyzed by ATP and thioretinaco ozonide, the concentration of which declines in aging, theoretically causing the consequent decreased biosynthesis of adenosyl methionine [84].

The melatonin of mitochondria theoretically inhibits opening of the mitochondrial permeability transition pore, explaining its antioxidant and anti-apoptotic effects by reducing oxygen consumption, restoration of membrane potential, and reduction of superoxide production [85]. In aging, thioretinaco ozonide is proposed to be lost from dysfunctional mitochondria by disruption of the outer mitochondrial membrane, leading to its depletion during the aging process. Decreased adenosyl methionine causes dysregulation of methionine metabolism and hyperhomocysteinemia from decreased allosteric inhibition of methylenetetrahydrofolate reductase [86] and decreased allosteric activation of cystathionine synthase [87]. Thus, the hyperhomocysteinemia and suppressed immunity that are observed in atherosclerosis and dementia may be attributable to deficiency of adenosyl methionine [85]. Deficiency of adenosyl methionine is attributed to increased polyamine synthesis and decreased nitric oxide synthesis by vascular and neural cells infected by pathogenic microbes. Increased depletion of adenosyl methionine may occur in these diseases because of its increased utilization in the biosynthesis of polyamines by microbial metabolism [85,88].

Homocysteine, Melatonin, and Mitochondrial Dysfunction

In addition to its biosynthesis and secretion by the pineal gland, melatonin is also present in the mitochondria of bone marrow, gut, and skin, where melatonin may protect against oxidative stress [89]. Melatonin inhibits opening of the mitochondrial permeability transition pore in cultured neurons, explaining its anti-apoptotic effects [90]. In hippocampal neurons of hyperhomocysteinemic rats, melatonin inhibits neural apoptosis induced by homocysteine by inhibiting release of mitochondrial cytochrome c, by reducing activation of caspase-3, by decreasing the levels of the pro-apoptotic Bax protein, and by increasing the levels of the anti-apoptotic Bcl protein [91].

Excitotoxicity, oxidative stress, endothelial dysfunction, and inflammation are related to effects of homocysteine on the function of thoretinaco ozonide within mitochondria and endoplasmic reticulum (ER) [92]. Melatonin protects mitochondria from oxidative damage by reduction of oxygen consumption, reduced membrane potential, and decreased superoxide production in mouse liver cells [93]. Activation of matrix metalloproteinase-9 occurs in hyperhomocysteinemic rats through induction of the mitochondrial permeability transition pore by antagonizing the N-methyl-D-aspartate receptor-1 (NMDA-R1), causing myocyte dysfunction, increased calcium overload, and oxidative stress [94]. The homocysteine-inducible, ubiquitin-like domain member 2 (HERPUD1) protects cultured HELA cells against oxidative stress by down-regulating the inositol 1,4,5-triphosphate receptor and decreasing transfer of calcium ions from the ER to mitochondria [95].

Effects of Melatonin on Mitochondrial Dysfunction in Aging

Myelin and mitochondria contain the enzyme 2',3'-cyclic nucleotide 3'-phophodiesterase (CNP) which hydrolyzes the cyclic nucleotides, 2',3'-cyclic adenosine monophosphate (cAMP) and 2',3'-cyclic nicotinamide adenine dinucleotide phosphate (cNADP) [96]. The cyclic nucleotides, cAMP and cNADP, produce calcium-overload-induced release of calcium ions from mitochondrial matrix in rat brain mitochondria, causing reduced membrane potential and mitochondrial swelling [97]. Cyclosporin A inhibits opening of the permeability transition pore, preventing loss of the CNP enzyme from mitochondria and explaining the participation of cAMP and cNADP in cellular apoptosis [97]. The CNP enzyme is associated with the inner membrane complexes I-IV of mitochondria, and pore opening correlates with release of CNP, cytochrome c, apoptosis-inducing factor (AIF), and endonuclease G from mitochondria [98].

The progressive dysfunction of mitochondria and reduced ATP biosynthesis that occur in aging are attributed to loss of the active site for oxidative phosphorylation, $TR_2CoO_3O_2NAD^+H_2PO_4^-$, from mitochondrial membranes [99]. Increased opening of the permeability transition pore in brain mitochondria of aged animals causes decreased levels of CNP enzyme and increased levels of cAMP [100]. Chronic treatment of rats with melatonin inhibits opening of the permeability transition pore, preventing release of CNP and cytochrome c from both aged and young rats and protecting against mitochondrial dysfunction induced by cAMP in aging [101].

Opening of the Permeability Transition Pore is Proposed to Cause Loss of Thioretinaco Ozonide from Mitochondria

Osmotic swelling of mitochondria, rupture of the outer membrane, and release of inter-membrane components are related to elevated matrix calcium ions, oxidative stress, and depletion of cAMP and cNADP [102]. These effects are associated with membrane depolarization, uncoupling of oxidative phosphorylation, and reversal of the mitochondrial proton-translocating ATPase, increasing hydrolysis of ATP produced by glycolysis. The permeability transition pore permits passage of molecules less than 1500 in molecular weight across the inner membrane. These effects cause the increased colloid osmotic pressure and mitochondrial swelling that are observed in mitochondrial dysfunction. Opening of the inner membrane permeability pore and rupture of the outer membrane lead to release of the high molecular weight proteins, cytochrome c, endonuclease G, cyclic nucleotide 3'-phosphodiesterase, and apoptosis-inducing factor, from mitochondria [102].

During opening of the transition pore, thioretinaco, molecular weight 2045, is proposed to be unable to cross the inner membrane because its size exceeds the 1500 size limitation. However, dissociation of thioretinaco into thioretinamide, molecular weight 399 and cobalamin, molecular weight 1245, allows passage of these components through the pore, causing depletion of the active site of oxidative phosphorylation, $TR_2CoO_3O_2NAD^+H_2PO_4^-$ [77]. When disruption of the outer membrane is associated with opening of the inner membrane permeability pore, thioretinaco ozonide is proposed to be released from mitochondria along with high molecular weight proteins [102]. Hormonally increased mitochondrial calcium and activation of pyruvate dehydrogenase, isocitrate dehydrogenase, and 2-oxoglutarate dehydrogenase, increase matrix NADH/NAD^+, promote proton entry, membrane repolarization, and biosynthesis of ATP [102]. Increased matrix NAD^+ causes increased formation of the active site of oxidative phosphorylation, $TR_2CoO_3O_2NAD^+H_2PO_4^-$ [77]. Thus, opening of the mitochondrial permeability transition pore may explain the depletion of thioretinaco ozonide and mitochondrial dysfunction that are observed in aging, dementia, and cancer [61,77,88].

Thioretinaco, Mitochondrial Dysfunction, and Cellular Senescence in Aging

Progressive vascular disease, including heart disease, stroke, kidney failure, and thrombosis, shortens the lifespan of subjects with hereditary homocystinuria [103]. In addition, reversal of gray to normal hair color, prevention of vascular disease, and prolongation of lifespan occur in cases of hereditary cystathionine synthase deficiency that respond to vitamin B6 (pyridoxine) therapy. Ultraviolet light skin damage, neurodegenerative disease, and premature aging occur in disorders of DNA repair enzymes, including Cockayne syndrome, ataxia telangiectasia and xeroderma pigmentosum. Repair of double stranded DNA breakage by DNA repair enzymes causes decreased concentrations of the coenzyme, NAD^+, because of activation of the enzyme poly-adenosine diphosphate ribose polymerase (PARP), during repair of DNA breakage [104].

Aging is associated with depletion of NAD^+ from cells of yeast, worms, flies, and mammals [105]. Extension of lifespan is caused by increased activity of sirtuins, the lysine deacetylase enzymes, activities of which are dependent upon NAD^+ [105]. Caloric restriction and dietary antioxidants such as resveratrol cause increased activities of sirtuins because of increased intracellular NAD^+ [105]. The activation of sirtuins by nicotinamide riboside, the precursor of intracellular NAD^+, increases oxidative metabolism, biogenesis of mitochondria, and insulin sensitivity because of increased concentration of the active site of oxidative phosphorylation, $TR_2CoO_3O_2NAD^+H_2PO_4^-$, which contains NAD^+, within mitochondria [77].

In cellular senescence, cultured normal human diploid fibroblasts are unable to divide beyond approximately 45 to 50 cycles before mitotic activity ceases [106]. Shortening of chromosomal telomeres, the DNA and protein complexes that form a cap on the ends of chromosomes, occurs in cellular senescence [107]. The enzyme telomerase is a ribonucleoprotein reverse transcriptase enzyme which adds telomeres to the ends of chromosomes by utilizing its ribonucleoprotein component as a template for lengthening of telomeres. Cellular senescence and growth arrest are associated with shortened telomeres. Telomerase counteracts cellular senescence by lengthening of telomeres and alteration of gene expression [108].

Homocysteine and Cellular Senescence in Atherosclerosis

Cellular senescence of vascular cells in atherosclerotic plaques is demonstrated by observation of increased acidic β-galactosidase, a biomarker of aging and senescence [109]. In atherosclerotic plaques, smooth muscle cells and endothelial cells are flattened and enlarged, characteristic abnormalities of cellular senescence found in atherosclerosis [110]. Decreased biosynthesis of nitric oxide (NO) and decreased endothelial nitric oxide synthase (eNOS) activity are demonstrated in senescent aortic endothelial cells, compared with non-senescent cells and cells transfected with human telomerase [111]. Increased binding of monocytes to senescent endothelial cells is observed, compared with non-senescent endothelial cells. Elevated acidic β-galactosidase and reduction of telomere length of cultured senescent human umbilical vein cells are reversed by exposure to the NO-donor, S-nitro-penicillamine, which reduces cellular senescence by activation of telomerase [112]. These results provide evidence that NO prevents age-related downregulation of telomerase and delays senescence of endothelial cells.

In cultured endothelial cells, homocysteine increases the activity of acidic β-galactosidase and shortens telomere length, demonstrating acceleration of cellular senescence [113]. In addition, homocysteine increases cellular expression of intracellular adhesion molecule-1 (ICAM-1) and plasminogen activator inhibitor-1 (PAI-1), molecules that contribute to the pathogenesis of atherosclerosis. Exposure of endothelial cells to catalase, the enzyme that decomposes hydrogen peroxide, inhibits the cellular senescence induced by homocysteine, suggesting that oxidative stress may increase cellular senescence in atherosclerosis. Endothelial dysfunction and oxidative stress are characteristic abnormalities of the atherogenic effect of hyperhomocysteinemia [92].

Thioretinaco and Mitochondrial Dysfunction in Theories of Aging

The proposal that depletion of thioretinaco ozonide from mitochondrial membranes occurs in aging [84] is supported by demonstration of decreased levels of the cobalamin coenzymes, methyl cobalamin and adenosyl cobalamin, in postmortem brain specimens aged 19 weeks to 80 years of age [114]. The free radical theory of aging [115] and the neuroendocrine theory of aging [116] are both supported by the effects of hyperhomocysteinemia and melatonin on mitochondrial function in aging [85]. In aging the elevation of the hypothalamic threshold to feedback control [116] may affect melatonin biosynthesis and secretion by inhibitory effects on the supra-chiasmatic nucleus and regulation of pineal function. The increased free radical production by mitochondrial dysfunction in aging [115] may be caused by deficient melatonin synthesis by the pineal gland and increased opening of the permeability transition pore. The consequent, proposed loss of thioretinaco ozonide and the active site for oxidative phosphorylation, $TR_2CoO_3O_2NAD^+H_2PO_4^-$, from the F1F0 complex of mitochondria, may produce decreased ATP biosynthesis, as observed in aging, atherosclerosis, and dementia [61,77, 117].

Mitochondrial Dysfunction, Thioretinaco Ozonide, and Carcinogenesis

Retinol is transported to cells by plasma transthyretin, and retinol is oxidized to retinoic acid by superoxide that is produced by the heme oxygenase function of cystathionine synthase. Biosynthesis of thioretinamide (TR) is produced by reaction of retinoic acid with homocysteine thiolactone to form thioretinamide, catalyzed by cystathionine synthase [118]. Thioretinaco (TR_2Co) is formed by complexation of two molecules of thioretinamide with cobalamin. Complex formation of thioretinaco occurs by interaction of the corrin group of cobalamin with the heme oxygenase of cystathionine synthase. In liver of thyroidectomized rats, retinoic acid enhances the stimulation by thyroid hormone of heme oxygenase activity [119]. The thyroxine that is transported by transthyretin increases the biosynthesis of thioretinaco from thioretinamide, catalyzed by cystathionine synthase, explaining how thyroxine stimulates oxidative metabolism.

The deficient activity of cystathionine synthase in embryonic [120] and malignant cells [121] explains why malignant cells are deficient in oxidation of homocysteine thiolactone to sulfate by superoxide [39]. Decreased formation of superoxide is caused by deficient heme oxygenase function of cystathionine synthase [122]. Thus, malignant cells are unable to synthesize thioretinamide and thioretinaco because of deficient production of superoxide by cystathionine synthase, deficient activity of heme oxygenase function, and failure of oxidation of retinol to retinoic acid [118,122].

Opening of the permeability transition pore and dissociation of thioretinamide from cobalamin, ozone, oxygen, NAD^+, and $H_2PO_4^-$, are proposed to cause loss of thioretinaco ozonide from mitochondria by escape of components of the active site of oxidative phosphorylation through the 1500 molecular weight limit of the inner mitochondrial membrane [85]. Consequently, opening of the permeability transition pore may explain the origin of oxidative stress and mitochondrial dysfunction that are observed in aging, dementia, and cancer because of loss of $TR_2CoO_3O_2H_2PO_4^-$ [61,77,84].

Comprehensive analysis of the chemical properties of chemical carcinogens concluded that carcinogenesis is caused by electrophilic molecules [123]. The carcinogens which are electrophilic in their reactive form are nitrogen mustards, alkyl methane sulfonates, beta propiolactone and dimethyl carbamyl chloride. The carcinogens which are converted to electrophilic molecules by cellular metabolism are benzo(a)pyrene, dimethyl nitrosamine, 2-acetylaminofluorene, carbon tetrachloride, 4-nitroquinoline-oxide, and ethyl carbamate. Electrophilic carcinogens derived from plants are pyrrolizidine alkaloids, safrole, aflatoxin B1, cycasin, azaserine, and mitomycin C. Although these early investigations were concerned with potential reactions of ultimate carcinogens with DNA, RNA or proteins, the proposed loss of thioretinaco ozonide from mitochondria in carcinogenesis is related to discovery of the anticarcinogenic and antineoplastic effects of thioretinamide and thioretinaco [124]. Thus, the interaction of electrophilic carcinogens with the nucleophilic properties of thioretinaco ozonide of mitochondria may be essential to the process of chemical carcinogenesis [124].

2-acetylaminofluorene is a potent carcinogen that is metabolized to the proximate carcinogen, N-hydroxy-2-acetylaminofluorene, and converted to its sulfuric acid ester, which is the principal electrophilic carcinogen [123]. Susceptibility of rodents to carcinogenesis by N-hydroxy-2-acetylaminofluorene is related to activity of the enzyme, 3'-phophoadenosine-5'-phosphosulfate transferase, which catalyzes formation of the sulfuric acid ester of N-hydroxy-2-acetylaminofluorene. Synthesis of adenosine phosphosulfate (APS) from NAD^+ and HSO_4^- is proposed to be catalyzed by the active site of oxidative phosphorylation, $TR_2CoO_3O_2NAD^+H_2PO_4^-$, and by reduction of the complex by electrons from electron transport complexes of mitochondria [125]. Reaction of APS with guanosine triphosphate (GTP) produces 3'-phospho-5'-phosphosulfate (PAPS), which catalyzes formation of the sulfate ester of N-hydroxy-2-acetylaminofluorene, the ultimate carcinogen of 2-acetylaminofluorene [123].

Electron transfer between the K and L regions of polycyclic hydrocarbons produces the ultimate carcinogen and carcinogenesis by reactivity with cellular constituents [126]. Conversion of benzo(a)pyrene to the epoxide by cytochrome P450 monoxygenase produces the electrophilic ultimate

carcinogen. The electrophilic properties of metal ions are responsible for their carcinogenic activity [123]. Nickel subsulfide, Ni_3S_2, is a potent carcinogen which causes intimal atherosclerotic plaques, polycythemia, and renal malignancies in animals [34,127]. Thus, this analysis supports the view that the electrophilic properties of diverse carcinogens cause depletion of thioretinaco ozonide oxygen from mitochondria of malignant and vascular cells during carcinogenesis and atherogenesis [61].

The ozone oxygen cluster of thioretinaco ozonide, which is the active site of oxidative phosphorylation, $TR_2CoO_3O_2NAD^+H_2PO_4^-$, is nucleophilic, explaining its stabilization of the electrophilic disulfonium centers of thioretinaco [61]. Therefore, interaction between electrophilic carcinogens with the nucleophilic ozone oxygen complex of thioretinaco ozonide causes decomposition of the active site for oxidative phosphorylation by dissociation of the ozone and oxygen atoms from thioretinaco ozonide. Dissociation of two thioretinamide molecules from the cobalamin of thioretinaco permits passage through opening of the permeability transition pore of the inner mitochondrial membrane because these constituents do not exceed the 1500 molecular weight size limitations [85]. This analysis supports the concept that electrophilic carcinogens irreversibly inactivate the active site of oxidative phosphorylation, producing aerobic glycolysis of malignant cells [128].

Mitochondrial Dysfunction, Infections, Cholesterol, Immunity and Atherogenesis

Although for decades elevation of blood cholesterol has been considered by many observers to be a causal factor in atherosclerosis, multiple epidemiological studies demonstrate an increased risk of cancer, infections, and all-cause mortality in subjects with low blood cholesterol levels [129,130]. A review of published studies demonstrates an inverse association of low-density lipoprotein (LDL) with mortality [131], and a review of published studies concludes that LDL does not cause cardiovascular disease [132]. Subjects with low blood cholesterol are associated with increased mortality rates from cancer and infectious diseases, as determined 10-30 years earlier in multiple cohort studies [133].

Many published studies implicate infections by a variety of pathogenic microbes in the pathogenesis of atherosclerosis [134]. Pathogenic microbes and microbial toxins are inactivated by complex formation with LDL, producing microbial-lipoprotein aggregates that obstruct the vasa vasorum of arterial walls, leading to ischemia, necrosis, capillary rupture, and escape of microbes into the intima [135]. Complex formation and aggregation of LDL with microbes are enhanced by hyperhomocysteinemia, which causes homocysteinylation of LDL [64], endothelial dysfunction [9,92], and autoantibodies to homocysteinylated LDL [136]. These pathogenic processes produce inflammation, oxidation of LDL, and creation of intimal micro-abscesses, which are the vulnerable plaques predisposing to thrombosis of arteries in advanced atherosclerosis. In addition, low blood levels of LDL cholesterol impair the innate immune system by decreasing formation of lipoprotein-microbial aggregates and increasing susceptibility to infection of arterial plaques by pathogenic microbes.

Atherosclerotic plaques exhibit evidence of pathogenic microbes, including *Chlamydia pneumoniae*, *cytomegalovirus*, and diverse bacterial signatures of common bacteria [135,137,138]. In a study of cultured human stem cells infected by *Chlamydia trachomatis*, the infectious agent of trachoma and venereal disease, upregulation of ornithine decarboxylase, the rate-limiting enzyme in polyamine biosynthesis, is demonstrated to inhibit nitric oxide (NO) biosynthesis by inducible nitric oxide synthase (iNOS) [139]. In this example, an infectious microbe inhibits the synthesis of NO, an essential component of the immune system.

Infected cells synthesize increased quantities of NO, and the anti-microbial activity of NO is enhanced by formation of peroxynitrite ($OONOO^-$) from NO and superoxide (O_2^-) [140]. Peroxynitrite, oxygen radicals and nitrogen radicals are utilized by phago-lysosomes of neutrophils and macrophages to mediate destruction of pathogenic microbes that are implicated in atherosclerosis and dementia [117,134,141]. The reduction of nitrite to NO is catalyzed by the heme co-factor of cystathionine synthase, providing evidence for cystathionine synthase as a source of peroxynitrite and NO [142,143]. The decreased concentration of adenosyl methionine in infected cells and in senescent cells impairs the function of NO and peroxynitrite, therefore, because of decreased allosteric activation of cystathionine synthase by adenosyl methionine [87] and decreased biosynthesis of thioretinamide and thioretinaco [118].

Although the outer mitochondrial membrane of normal cells contains 10% cholesterol, the inner membrane contains almost none [144]. The concentration of cholesterol within the inner membrane is increased in multiple tumors, and the increased membrane potential of many tumors is attributed to up-regulation of the ATPase inhibitory factor (IF-1) and alteration of the ATP synthase protein, decreasing proton conductance through the F0 subunit, decreasing ATP synthesis and contributing to aerobic glycolysis [145]. The increased cholesterol concentration within tumor cells decreases mitochondrial membrane permeabilization and impairs BAX-driven release of cytochrome c to the cytosol [146]. Increased cholesterol concentration within tumor cells is attributable to increased biosynthesis from acetyl-Coenzyme A [147]. Decreased ATP biosynthesis from oxidative phosphorylation is secondary to loss of the active site, $TR_2CoO_3O_2NAD^+H_2PO_4^-$, from mitochondria during carcinogenesis, aging, brain injury and dementia, decreasing consumption of acetyl-Coenzyme A and ATP biosynthesis by oxidative phosphorylation, and increasing ATP synthesis by aerobic glycolysis [124].

The increased cancer mortality secondary to low blood cholesterol levels [129] may be attributed to increased loss of thioretinaco ozonide by opening of the mitochondrial permeability pore by decreased membrane cholesterol, increasing susceptibility to carcinogenesis by electrophilic carcinogens and oncogenic viruses [124]. In support of this concept, increased membrane cholesterol concentration inhibits the ability of oligomers of BAX to transition from a membrane-associated protein to a membrane-integral protein [148]. Therefore, increased membrane cholesterol, associated with increased blood cholesterol, may inhibit permeability transition pore opening, thereby inhibiting loss of the active site for oxidative phosphorylation from mitochondria and preventing mitochondrial dysfunction [149].

Environmental Pollution, Thioretinaco Ozonide and Mitochondrial Dysfunction

Environmental pollutants, including pesticides, herbicides, food additives, and electromagnetic fields are proposed to cause mitochondrial dysfunction and oxidative stress through loss of the active site complex for oxidative phosphorylation, $TR_2CoO_3O_2NAD^+H_2PO_4^-$, from opening of the mitochondrial permeability transition pore [150]. Glyphosate, fluoride, and electromagnetic fields are examples of the vast number of technological pollutants that cause multiple adverse health effects, including carcinogenesis, cytotoxicity, cataracts, infertility, congenital malformations, cancer, lymphocytosis, leukemia, hearing loss, blindness, retinal hemorrhages, cardiac arrythmias, dermatitis, hair loss, depression, memory loss, premature aging, heart disease and weaponized mind control.

The herbicide glyphosate (n-phosphonomethylglycine) is carcinogenic in animals and in human exposure, increasing the risk of malignant tumors of skin and breast in rats and leukemia and multiple myeloma in herbicide applicators [151,152]. Glyphosate suppresses the shikimate pathway in bacteria and down-regulates genes encoding ATP synthase and cytochrome P450 oxidative enzymes [151]. The electrophilic and nucleophilic zwitterion of glyphosate is proposed to interact with the nucleophilic O_3O_2 cluster and electrophilic disulfonium centers of thioretinaco ozonide, causing decomposition of the active site of oxidative phosphorylation, $TR_2CoO_3O_2NAD^+H_2PO_4^-$, and its loss through opening of the mitochondrial permeability transition pore [61,124,150].

Fluoride anion stimulates oxygen consumption and superoxide production of polymorphonuclear leukocytes [153,154] and inhibits ATP biosynthesis and oxidative metabolism [155]. Fluoride disrupts enzymatic function by interfering with hydrogen bonding, as shown by strong bonding in amide-fluoride systems [156] and by analysis of the crystal structure of fluoride-inhibited yeast cytochrome c peroxidase [157]. Thus, fluoride inhibits enzymatic function by altering the conformation of polypeptides through interactions with peptidyl amide groups [155]. The interaction of fluoride with the amide groups of thioretinaco ozonide is proposed to cause a conformational change in binding of thioretinamide to the cobalt atom of cobalamin, inhibiting binding of superoxide and other oxygen radicals to thioretinaco ozonide, inhibiting ATP synthesis and causing oxidative stress [92]. The extreme toxicity of oxygen difluoride gas is attributed to displacement of ozone from thioretinaco ozonide because of its molecular similarity to the structure of ozone [92].

Ionizing radiation causes oxidative stress by increased production of oxygen radicals and reactive nitrogen radicals within cultured cells, associated with transient, reversible opening of the mitochondrial permeability transition pore and loss of membrane potential [158]. Ionizing radiation is carcinogenic, and the proposed loss of the active site for oxidative phosphorylation from mitochondria is attributed to radiation-induced depletion of thioretinaco ozonide from malignant cells [61,124]. Non-ionizing radiation from electromagnetic fields (EMF) from microwaves, radar, telecommunication technology, electrical transmission lines, cell phones, television and computer screens has long been suspected of carcinogenesis and adverse effects on capillaries, nerve cells, blood brain barrier, embryonic cells, germ cells, lenses, and cardiac function [159].

Prolonged exposure of animals to EMF produces malignant schwannomas of lung, malignant gliomas of brain, and cardiomyopathy [160]. Cardiac arrhythmias are produced in anesthetized dogs by exposure of the sympathetic and parasympathetic nervous system to externally administered low-level EMF [161]. The cardiac arrythmias induced by EMF include bradycardia, atrial premature depolarizations, atrial tachycardia, and atrial fibrillation. Exposure of human cultured breast cancer, melanoma, gastric and colon cancer cells to EMF inhibits proliferation and produces increased mitochondrial membrane potential, but no changes in ATP levels are observed [162]. Deleterious effects of EMF on fertility are related to inhibition of oogenesis and spermatogenesis, which are attributable to oxidative stress and decreased scavenging of reactive oxygen species (ROS) by mitochondria [163].

Hyperhomocysteinemia and coagulation dysfunction in COVID-19 Disease

In patients with coronavirus disease (COVID-19), logistical models reveal that age, monocyte/lymphocyte ratio, and plasma homocysteine predict progression of disease, as determined by chest computerized tomography (CT) [164]. Coagulation dysfunction is a characteristic abnormality associated with COVID-19 disease, and fulminant thrombotic complications are observed with severe, progressive disease [165]. Elevated levels of D-dimer are associated with COVID-19 disease progression and decreased survival, and a broad range of abnormal coagulation parameters, including alterations of prothrombin time, fibrinogen, platelet count, antithrombin (AT), factor VIII (FVIII) and von Willebrand factor (VWF) is also associated with disease progression. Hyperhomocysteinemia is associated with a procoagulant state because of multiple effects on platelet function and the coagulation cascade [11,63], and plasma homocysteine is a potent predictor of cardiovascular risk in COVID-19 disease [166]. Both cobalamin and folate are molecular factors that interact with key COVID-19 viral proteins, suggesting a potential therapeutic benefit of these vitamins in preventing progression of COVID-19 disease [166].

A lowered level of plasma prealbumin predicts mortality in COVID-19 disease, in comparison with most routine laboratory indicators [167]. Prealbumin contains transthyretin, an important carrier of plasma retinol, and transthyretin is a sensitive indicator of protein energy malnutrition, which is associated with hyperhomocysteinemia and atherosclerosis [168]. The biosynthesis of thioretinamide is catalyzed by cystathionine synthase from retinoic acid, the product of oxidation of retinol, and homocysteine thiolactone [118]. Thus, subjects with protein energy malnutrition and hyperhomocysteinemia are potentially susceptible to an adverse outcome of COVID-19 disease because of decreased biosynthesis of thioretinamide and thioretinaco, a key component of the active site of oxidative phosphorylation [77]. In addition, altered innate immunity from statin therapy and suppression of the mevalonate pathway is suggested to affect COVID-19 disease adversely by decreased formation of the isopentenyl-adenosine group of the transfer ribonucleic

acid (tRNA) that is necessary for biosynthesis of selenoproteins containing selenocysteine [169].

Mitochondrial Dysfunction and Atrial Fibrillation

The packing of nuclear chromatin is dependent upon acetylation of histones, as controlled by acetyl transferases and histone deacetylases, and genetically modified mice with increased histone deacetylase activity develop atrial arrythmias, including atrial fibrillation [170]. Atrial fibrillation produces remodeling and loss of contractility of cardiomyocytes by activation of histone deacetylase-6 and subsequent deacetylation of α-tubulin and disruption of microtubule structure, as demonstrated in animal models and human cardiomyocytes [170]. Furthermore, both human and experimental atrial fibrillation are associated with mitochondrial dysfunction, altered Ca^{2+} transport, and decreased ATP production, respiration, and membrane potential, associated with depletion of NAD^+ and decreased oxidative metabolism [171]. These findings suggest that loss of the active site of oxidative metabolism, $TR_2CoO_3O_2NAD^+H_2PO_4^-$, may explain the origin of dysfunctional mitochondria in atrial fibrillation [77].

In experimental cardiomyocyte dysfunction induced by tachypacing of cultured cardiomyocytes and *Drosophila* pupae hearts, DNA damage and activation of poly-(ADP)-ribose polymerase (PARP-1) and depletion of NAD^+ are observed [172]. Accordingly, inhibition of PARP-1 or replenishment of NAD^+ may be effective in preventing DNA damage and contractile dysfunction of cardiomyocytes in atrial fibrillation because of increased formation of the active site of oxidative phosphorylation [77].

Autism, Mitochondrial Dysfunction, Vaccines, and Gulf War Illness

A cohort analysis of patients with autism spectrum disorder disclosed mitochondrial dysfunction, characterized by enzyme- or mutation-defined abnormalities of mitochondrial electron transport [173]. The most common disorders of electron transport in autism involve deficiencies of complex I (64%) and complex III (20%), suggesting a disturbance of mitochondrial energy production in individuals with autism. These findings confirm case reports of developmental regression and mitochondrial dysfunction, which are associated with deficiencies of complexes I and III in children with autism [174].

There is increasing concern that the adjuvant components of vaccines may be causal in autism because of a temporal relation of onset of autism with vaccination in some cases [175]. Gulf War Illness (GWI) is a neurological abnormality characterized by fatigue, muscle and joint pain, mood disorders, post-traumatic stress disorder, headaches, memory loss and increased risk of amyotrophic lateral sclerosis. Since troops that were vaccinated against anthrax but not deployed are at increased risk for GWI, behavioral and neuropathological studies of the toxic effects of the anthrax vaccine adjuvants, aluminum hydroxide and squalene, were carried out in mice [175]. Industry and regulatory authorities have long approved of the safety of aluminum hydroxide as a vaccine adjuvant, but aluminum is implicated in neurotoxicity of cultured neurons and in neurological disorders, including Alzheimer dementia. In addition, many cases of GWI exhibit antibodies to squalene, and the experiments with mice exposed to aluminum hydroxide and squalene demonstrate cognitive defects and apoptosis of neurons in the central nervous system and lumbar spinal cord [175].

In studies of cultured human hepatocytes, aluminum trichloride exposure produces mitochondrial dysfunction and lipid accumulation, characterized by decreased ATP synthesis, diminution of enzyme activities mediating oxidative phosphorylation, and increased production of very low-density lipoprotein (VLDL) [176]. The results of the experimental studies in mice and cultured human hepatocytes [175,176] support the possible etiological participation of aluminum and squalene adjuvants of the anthrax vaccine in the pathogenesis of GWI.

Thimerosal is used as a preservative in many vaccines for multiuse formulations, and ethyl mercury is the component which is believed to be responsible for the neurodevelopmental toxicity of thimerosal. Approval of use of thimerosal as a preservative in pediatric vaccines was withdrawn by the Centers for Disease Control and Prevention (CDC) in 1999, but thimerosal is currently used in influenza, diphtheria toxoid, acellular pertussis, and tetanus toxoid vaccines. Because of the critical function of dendritic cells in antigen presentation in immune responses, the effect of thimerosal on regulation of interleukin-6 (IL-6) secretion was studied in dendritic cells [177]. The results demonstrate that nanomolar concentrations of thimerosal uncouple ATP-mediated calcium signaling by effects on inositol triphosphate receptors and ryanodine receptors of immature dendritic cells. In a study of cultured human astrocytes, the ethyl mercury of thimerosal is demonstrated to cause mitochondrial dysfunction, reduced membrane potential, increased reactive oxygen species, and increased opening of the mitochondrial permeability transition pore [178]. These effects on mitochondrial function and oxidative stress are associated with oxidation and strand breakage of mitochondrial DNA.

Strategies for Prevention of Diseases Associated with Mitochondrial Dysfunction and Hyperhomocysteinemia

In diseases of aging, mitochondrial dysfunction is associated with loss of the active site for oxidative phosphorylation by opening of the mitochondrial permeability transition pore [124]. The consequent deficiency of adenosyl methionine formation produces hyperhomocysteinemia by dysregulation of methionine metabolism [29,83,86,87]. Therefore, strategies for prevention of hyperhomocysteinemia and mitochondrial dysfunction depend upon prevention of loss and replacement of $TR_2CoO_3O_2NAD^+H_2PO_4^-$ within mitochondria by correction of nutritional deficiencies of vitamin precursors or by utilizing cellular vectors for introducing the synthetic active site of oxidative phosphorylation within dysfunctional mitochondria [84,99].

The leading cause of death in the United States is coronary heart disease, the incidence of which reached a peak of 560 deaths per 100,000 population in 1958. This extraordinary incidence of cardiovascular disease in the population is related to dietary deficiencies of vitamins B6 (pyridoxal) and B9 (folate) and consumption of highly processed nutritionally depleted foods [179]. The dramatic decreases of mortality from coronary heart disease and

cerebrovascular disease in the past half century reached declines of 68% and 79%, respectively by 2010 [42]. These declines in atherosclerotic disease are attributed to increased consumption of folate and pyridoxine from voluntary supplementation, increased absorption of cobalamin, and decreased homocysteine levels [42], as demonstrated by the Framingham Heart Study of elderly participants [180].

In 1998 synthetic folic acid (vitamin B9) was mandated for fortification of processed grain foods in the United States and Canada. Subsequently, the plasma folate in the population was doubled, and the plasma homocysteine decreased 15%, compared with values prior to folic acid fortification, contributing to the decline in cardiovascular disease and neural tube birth defects [181]. Thus, the dramatic decline in coronary heart disease and cerebrovascular disease mortality may be attributed in part to voluntary fortification with pyridoxine and folic acid and to mandated fortification with folic acid since 1998 [182]. In support of this conclusion, an accelerated decline in stroke mortality occurred in the United States and Canada beginning in 1998, when folic acid fortification was mandated in these countries, whereas no such change in stroke mortality was observed in the United Kingdom, where folic acid fortification was not mandated [183]. Among patients who were given pyridoxine (vitamin B6) for carpal tunnel syndrome, a 73% decrease in risk of myocardial infarction and an 8-year delay in death from coronary heart disease were attributed to lowering of plasma homocysteine [184].

Multiple studies of participants with low blood cholesterol levels demonstrate an increased risk of cancer, infection, and all-cause mortality [129,130], and a review of published studies of mortality and LDL in the elderly demonstrates a lack of or an inverse association between LDL cholesterol and mortality [131]. Accordingly, in these studies subjects with high blood cholesterol live the longest [132], and subjects with low blood cholesterol are associated with increased mortality from cancer and infectious diseases, as determined 10-30 years earlier in multiple cohort studies [133]. The decreased mortality associated with elevated LDL may be attributed to prevention of mitochondrial dysfunction by increased membrane cholesterol, which is proposed to decrease loss of the active site for oxidative phosphorylation by preventing mitochondrial permeability transition pore opening [149].

A detailed interventional protocol for a novel therapeutic strategy is designed for treatment of subjects with cancer, hyperhomocysteinemia, cardiovascular disease, and myelodysplasia [185]. Myelodysplasia is a pre-leukemic clonal hematological stem cell disorder, which is associated with hyperhomocysteinemia, oxidative cell damage, adverse cardiovascular events, anemia, and autoimmune disease, including rheumatoid arthritis, Behcet's disease, anti-platelet antibodies, and anti-mitochondrial antibodies. In myelodysplasia, the hypercellular bone marrow displays megaloblastic maturation, dysplastic megakaryocytes, erythroid hyperplasia, impaired myeloid maturation, increased blast transformation, and ringed sideroblasts. In peripheral blood, monocytosis, macrocytosis, immature myeloid and erythroid forms, and pseudo Pelger-Huet cells are identified. The International Prognostic Scoring System (IPSS) classifies patients as low, intermediate, and high risk, according to presence of cytopenia, cytogenetic profile, and percentage of bone marrow blasts. Low risk patients survive a median of 5.7 years, intermediate risk, 3.5 years, and high risk, 0.4 years.

Existing supportive therapy of myelodysplasia includes red blood cell and platelet transfusions to treat life-threatening anemias and pan-cytopenias, and antibiotics for infections, but remission or cure are seldom observed. Additional therapeutic options include erythropoietin, darbepoetin, granulocyte colony-stimulating factor, immunomodulators such as lenalidomide, anti-thymocyte globulin, cyclosporin A, or thalidomide, chemotherapy with cytarabine, and hypomethylating agents, azacytidine or decitabine. Many myelodysplasia patients experience little benefit from current therapeutics, and except for stem cell transplantation, these modalities are not curative. Most myelodysplasia patients are not candidates for stem cell transplantation therapy because of age and comorbidities.

The benefit of the proposed thioretinamide protocol is expected to result from increased endogenous biosynthesis of thioretinamide ozonide from synthetic thioretinamide [49,118] in dysplastic and regenerative cells of bone marrow, conversion of aerobic glycolysis to oxidative phosphorylation, and inhibition of premalignant stem cells of bone marrow through down-regulation of Stat3 signaling by diallyl trisulfide and napabucasin. This thioretinamide protocol is a patented method for treatment and prevention of diseases of aging [186]. Addition of allyl sulfides, napthoquinones, and pancreatic enzymes to the thioretinamide protocol provides a patented composition which promotes apoptosis of malignant cells while preventing apoptosis of normal cells [187].

The proposed thioretinamide protocol [185] comprises administration of synthetic thioretinamide, diallyl trisulfide, and napabucasin with pancreatic enzymes for enzymatic degradation of homocysteinylated proteins, nucleic acids, and glycosaminoglycans, together with vitamins, amino acids, and nitrilosides, in order to enhance metabolic conversion of endogenous homocysteine thiolactone and retinol to thioretinamide and thioretinaco by cystathionine synthase [118]. By promoting endogenous biosynthesis of thioretinaco ozonide, this protocol may prevent mitochondrial dysfunction, enhance immune function, and prevent progression of the myelodysplasia syndrome, including adverse vascular events, hyperhomocysteinemia and development of leukemia.

Dementia from Alzheimer disease and other causes is an increasing threat to public health, as cited by the World Health Organization. Increasing efforts to understand the origin of dementia have centered upon an infectious etiology, as originally proposed by Alzheimer and Fischer [188]. A study of 1,092 subjects of the Framingham Heart Study without dementia demonstrated elevated plasma homocysteine as a risk factor for development of dementia and Alzheimer's disease after 8 years of observation [189]. Low blood levels of folate and cobalamin and elevated homocysteine are associated with dementia, and a 2-year interventional study to lower homocysteine levels with folate, pyridoxine, and cobalamin in elderly subjects with mild cognitive impairment demonstrated reduced cerebral atrophy of the temporal lobe [190].

The reduction of cerebral glucose utilization and blood flow in Alzheimer's disease is accompanied by down-regulation of glucose transport, Na, K-ATPase, oxidative phosphorylation, and energy consumption. These metabolic abnormalities of oxidative metabolism are attributed to loss of

the active site of oxidative phosphorylation, $TR_2CoO_3O_2NAD^+H_2PO_4^-$, from the F1F0 complex of mitochondria in dementia [88]. Pathogenic microbes cause increased synthesis of polyamines in neurons by increasing the transfer of aminopropyl groups of adenosyl methionine to putrescine, resulting in depletion of intracellular adenosyl methionine, dysregulation of methionine metabolism, and hyperhomocysteinemia [88].

Decreased immune function in aging [191] is attributed to declining mitochondrial concentrations of thioretinaco ozonide and adenosyl methionine within lymphocytes, macrophages, polymorphonuclear leukocytes, and dendritic cells [84]. Loss of intracellular thioretinaco ozonide may explain the exponential increase in susceptibility to microbial infections in atherosclerosis and dementia with aging [117], because of suppressed immunity. During immune reactions, antibodies release singlet oxygen, $^1O^2$*, from production of hydrogen peroxide by oxidation of H_2O, and singlet oxygen destroys antigens that are bound to antibodies [192]. The production of singlet oxygen involves hydrogen trioxide, H_2O_3, as a key intermediate, and ozone originates from antibodies during killing of bacteria [193]. In the peroxone process for killing microbes in purification of water, ozone destroys hydrogen peroxide. Other oxidants, including peroxynitrite, hydrogen peroxide, superoxide, hydrogen trioxide, and hypochlorite, which are produced by antibody catalysis by macrophages or neutrophils, kill pathogens by the immune system, but ozone is the most reactive of these oxidants [193].

Early detection of dementia is accomplished by assessment of cognitive impairment by abnormal Mini-Mental State Examination (MMSE) scores, computed tomography or magnetic resonance imaging of medial temporal lobe thickness, cerebrospinal fluid $A\beta_{40}$, $A\beta_{42}$, or tau protein, plasma homocysteine, C-reactive protein, and ocular biomarkers [190,194]. Identification of pathogenic microbes by culture, sero-positivity, or other methods directs the antibiotic, vaccination, or other anti-microbial strategy [188].

A treatment strategy for correction of the metabolic abnormalities produced by pathogenic microbes in dementia, including hyperhomocysteinemia, increased polyamine biosynthesis, impaired biosynthesis of NO, and impaired oxidative metabolism from depletion of thioretinaco ozonide, may be accomplished by a proposed homocysteine-lowering protocol [117]. The protocol consists of synthetic thioretinamide, methyl-cobalamin, methyl-folate, pyridoxine, nicotinamide riboside, ascorbate, coenzyme Q10, adenosyl methionine, menoquinone, cod liver oil, vitamin D3, pancreatic enzymes, and dietary improvement to minimize processed foods and to prevent protein energy malnutrition [168]. Pancreatic enzymes may disperse biofilms of cerebral plaques, increasing susceptibility of microbial pathogens to antibiotic therapy [195].

The observations of hyperhomocysteinemia as a factor determining progression of COVID-19 disease [164,166] and coagulopathy secondary to hyperhomocysteinemia [165] suggest that a homocysteine-lowering protocol may be useful in treatment of this disease. The observations of mitochondrial dysfunction in human and experimental atrial fibrillation [171,172] suggest that replenishment of the active site of oxidative phosphorylation, $TR_2CoO_3O_2NAD^+H_2PO_4^-$, may reverse mitochondrial dysfunction and provide an effective therapeutic strategy for atrial fibrillation. The demonstration of mitochondrial dysfunction in cases of autism and in GWI suggests that treatment of neurodegeneration and amyotrophic lateral sclerosis to increase mitochondrial oxidative phosphorylation may also be effective in these diseases.

The protective effect of tocotrienol against opening of the mitochondrial permeability transition pore may have a synergistic effect in replenishment of the active site complex, $TR_2CoO_3O_2NAD^+H_2PO_4^-T_3$, within mitochondria in neurodegenerative diseases [80]. Thus, a homocysteine-lowering protocol for prevention of Alzheimer dementia with added tocotrienol may prove to be effective in treatment of autism and amyotrophic lateral sclerosis [117].

Cellular Vectors for Treatment of Hyperhomocysteinemia and Mitochondrial Dysfunction

The strategy for correction of vitamin deficiencies utilizes oral synthetic thioretinamide, vitamins, pancreatic enzymes, and dietary improvements as a method for counteracting diseases associated with hyperhomocysteinemia [117,188]. In contrast, the proposed use of cellular vectors for reversing mitochondrial dysfunction and hyperhomocysteinemia comprises exposure of human mesenchymal stem cells to a culture medium containing synthetic thioretinaco (TR_2Co) and nicotinamide adenine diphosphate ($NAD^+H_2PO_4^-$) and an ozone (O_3) atmosphere [196]. When the cultured stem cells reach confluency, the harvested cells contain an increased concentration of the active site of oxidative phosphorylation, $TR_2CoO_3O_2NAD^+H_2PO_4^-$, for administration to target tissues by direct injection or intravenous administration.

Mesenchymal stem cells, which are derived from human adipose tissue, bone marrow, or induced pluripotent cells, migrate into malignant tissues and tissues with deficiency of the active site of oxidative phosphorylation within mitochondria [197,198]. Thus, delivery of increased concentrations of the active site by cultured stem cells containing increased $TR_2CoO_3O_2NAD^+H_2PO_4^-$ may cause repletion of the active site, which is depleted from mitochondria during the pathogenesis of atherosclerosis, cancer, dementia, autoimmunity and diseases of aging, providing a therapeutic transformation of a malignant cellular phenotype to a benign cellular phenotype, prevention of hyperhomocysteinemia and mortality, enhancement of immunity, and a decrease of cellular senescence, promoting an increased lifespan and beneficial therapeutic effects [196].

In recent years, development of engineered chimeric antigen receptor T-lymphocytes (CAR-T) provides a novel method of immunotherapy of cancer against hematological malignancies [199]. However, because of risk of toxicity from graft versus host disease and cytokine release syndrome by CAR-T immunotherapy, the use of chimeric antigen receptor natural killer cells (CAR-NK) has been proposed for immunotherapy of solid malignant tumors [200]. Induced pluripotent stem cell-natural killer cells (iPSC-NK) are engineered to express CAR-NK derivatives and provide ability to kill mesothelin-expressing ovarian tumors without inducing toxicity from the cytokine release syndrome [201]. To implement immunotherapy with CAR-NK cells, a source of readily manufactured immune cells for cancer therapy from iPSC cells is required [202]. Because of their improved safety

profile, CAR-NK cells have been the subject of clinical trials [203]. Efforts are in progress to reprogram NK cells to enhance their anti-neoplastic activity [199], and incubation with precursors of the active site of oxidative phosphorylation, $TR_2CoO_3O_2NAD^+H_2PO_4^-$, may provide a targeted therapeutic strategy for immunotherapy [196].

Conclusions

As originally proposed a century ago in 1923 by Sir Archibald E. Garrod, study of "inborn errors of metabolism" may be productive in understanding the pathogenesis of inherited metabolic diseases [204]. In the case of homocystinuria, study of rare inherited enzymatic abnormalities that produce hyperhomocysteinemia, arteriosclerosis and thrombotic vascular disease led to the conclusion that elevation of plasma homocysteine may play an etiological role in the pathogenesis of diseases occurring in the general population [9]. Indeed, more than 100 diseases or conditions are associated with hyperhomocysteinemia, as described in more than 26,000 reports referenced in PubMed until 2021. In the recent comprehensive study of conditions associated with hyperhomocysteinemia, published reports demonstrate that five diseases, including neural tube defects, childhood cognition impairment, macular degeneration, primary stroke, and cognitive impairment of the elderly, can be prevented at least in part by lowering total plasma homocysteine [205]. As discussed in the present review, the importance of thioretinaco ozonide and its critical function in oxidative phosphorylation increase understanding of the pathogenesis of diseases of aging and conditions associated with hyperhomocysteinemia and mitochondrial dysfunction.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. McCully KS, Cantoni GL (1961) On the specificity of takadiastase T1 ribonuclease. Biochim Biophys Acta 51: 190-192.
2. McCully KS, Cantoni GL (1962) Studies on soluble ribonucleic acid (S-RNA) of rabbit liver. VII. A base sequence model of S-RNA. J Molec Biol 5: 497-505.
3. McCully KS, Forbes E (1966) The use of p-fluorophenylalanine with "master strains" of Aspergillus nidulans for assigning genes to linkage groups. Genet Res Cambridge 6(3): 352-359.
4. Shih V, Efron ML (1970) Pyridoxine-unresponsive homocystinuria. Final diagnosis of MGH Case 19471, 1933. New Engl J Med 283: 1206-1208.
5. Case Records of the Massachusetts General Hospital (1933) Marked cerebral symptoms following a limp of three months' duration. New Engl J Med 209: 1063-1066.
6. Mudd SH, Levy HL, Abeles RH (1969) A derangement in the metabolism of vitamin B12 leading to homocystinuria, cystathionuria, and methyl malonic aciduria. Biochem Biophys Res Commun 35(1): 121-126.
7. Rinehart JF, Greenberg LD (1949) Arteriosclerotic lesions in pyridoxine-deficient monkeys. Amer J Pathol 25(3): 481-491.
8. Mudd SH, Finklestein JD, Irreverre F, et al. (1964) Homocystinuria: An enzymatic defect. Science 143: 1443-1445.
9. McCully KS (1969) Vascular pathology of homocysteinemia: Implications for the pathogenesis of arteriosclerosis. Amer J Pathol 56(1): 111-128.
10. Kanwar YS, Manaligod JR, Wong WK (1976) Morphologic studies in a patient with homocystinuria due to 5,10-methylenetetrahydrofolate reductase deficiency. Pediatr Res 10(6): 598-609.
11. Carmel R, Jacobsen DW (2001) Homocysteine in Health and Disease. Cambridge University Press, UK, pp: 357-465.
12. Cantoni, GL (1953) The nature of the active methyl donor formed enzymatically from L-methionine and adenosinetriphosphate. J Amer Chem Soc 74(11): 2942-2943.
13. McCully KS (1970) Importance of homocysteine-induced abnormalities of proteoglycan structure in arteriosclerosis. Amer J Pathol 59(1): 181-193.
14. McCully KS (1972) Macromolecular basis for homocysteine-induced changes in proteoglycan structure in growth and arteriosclerosis. Amer J Pathol 66(1): 83-96.
15. Wolbach SB, Howe PR (1926) Intercellular substances in experimental scorbutus (scurvy, a vitamin C deficiency state). Arch Pathol Lab Med 1(1): 1-24.
16. McCully KS (1971) Homocysteine metabolism in scurvy, growth, and arteriosclerosis. Nature 231: 391–392.
17. McCully KS (1975) Growth disorders and homocysteine metabolism. Ann Clin Lab Sci 5(3): 147-152.
18. Salmon WD, Daughaday WH (1957) A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. J Lab Clin Med 49(6): 825-836.
19. Schedewe HK, Lipinski C, Schmidt E (1977) Elevated growth hormone levels in untreated homocystinuria: Mechanism of tall stature? Clin Res 25: 67A.
20. Dehnel JM, Francis MJO (1972) Somatomedin (sulfation factor)-like activity of homocystine. Clin Sci 43(6): 903-906.
21. Clopath P, Smith VC, McCully KS (1976) Growth promotion by homocysteic acid. Science 192(4237): 372-374.
22. Ohmori S, Kodama H, Ikedami T, et al. (1972) Unusual sulfur-containing amino acids in the urine of homocystinuric patients. III. Homocysteic acid, homocysteine sulfinic acid, S-(carboxymethylthio)homocysteine, and S-(3-hydroxy-3-carboxy-n-propyl)homocysteine. Physiol Chem Phys 4(3): 286-294.
23. McCully KS, Ragsdale BD (1970) Production of arteriosclerosis by homocysteinemia. Amer J Pathol 61: 1-11.
24. McCully KS, Wilson RB (1975) Homocysteine theory of arteriosclerosis. Atherosclerosis 22: 215-227.
25. Kuzuya F, Yoshimine N (1978) Homocysteine theory of arteriosclerosis. J Japan Atheroscler Soc 6: 135-139.
26. McCully KS (1996) Atherogenesis and the chemical pathology of homocysteine. Eur J Lab Med 4: 121-128.
27. Hartroft WS, Ridout JH, Sellers EA, et al. (1952) Atheromatous changes in aorta, carotid, and coronary arteries of choline-deficient rats. Proc Soc Exp Biol Med 81(2): 384-393.
28. Mann GV, Andrus SB, McNally A, et al. (1953) Experimental atherosclerosis in cebus monkeys. J Exp Med 98: 195-218.

29. Selhub J, Miller JW (1992) The pathogenesis of homocysteinemia: Interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. Amer J Clin Nutr 55(1): 131-138.
30. Harker LA, Ross R, Slichter CS, et al. (1976) Homocystine-induced arteriosclerosis. The role of endothelial cell injury and platelet response in its genesis. J Clin Invest 58(3): 731-741.
31. Hladovec J (1979) Experimental homocystinemia, endothelial lesions, and thrombosis. Blood Vessels 16: 202-205.
32. Mann GV, Andrus SB, McNally A, et al. (1953) Experimental atherosclerosis in cebus monkeys. J Exp Med 98(3): 195-218.
33. Thomas WA, Hartroft WE (1959) Myocardial infarction in rats fed diets containing high fat, cholesterol, thiouracil and sodium cholate. Circulation 19(1): 65-72.
34. McCully KS, Rineheimer LA, Gillies CG, et al. (1982) Erythrocytosis, glomerulomegaly, mesangial hyperplasia, sialyl hyperplasia, and arteriosclerosis induced in rats by nickel subsulfide. Virchows Arch A Pathol Anat Histol 394(3): 207-220.
35. McCully KS (2005) Hyperhomocysteinemia and arteriosclerosis: Historical perspectives. Clin Chem Lab Med 43(10): 980-986.
36. Rokitansky C (1856) Lehrbuch der Pathologischen Anatomie. Specialle Pathologische Anatomie. Wein: W Braumueller, 2, pp. 305-315.
37. Virchow R (1856) Gasammelte Abhandlung zur Wissenschaftlichen Medicin. Meidinger, Frankfurt, pp: 499-513.
38. Aschoff L (1924) Lectures in pathology. Hoeber, New York, pp: 131-153.
39. McCully KS (1976) Homocysteine thiolactone metabolism in malignant cells. Cancer Res 36: 3198-3202.
40. McCully KS, Clopath P (1977) Homocysteine compounds which influence the growth of a malignant neoplasm. Chemotherapy 23(1): 44-49.
41. McCully KS (2013) Pioneer of the Homocysteine Theory. Nova Science Publishers, New York, pp: 61-76.
42. McCully KS (1983) Homocysteine theory of arteriosclerosis: development and current status. In Gotto MGJr and Paoletti R, eds, Atherosclerosis Reviews, Raven Press, New York, 11; pp. 157-246.
43. McCully KS (1981) Homocysteine thiolactone perchlorate as a tumor promotor. US Patent 4,255,443.
44. McCully KS, Boyko ER, Carpenter GB (1985) Homocysteine thiolactone perchlorate: X-ray crystallography of a lipophilic salt. Chem-Biol Interact 56: 121-124.
45. McCully KS, Vezeridis MP (1985) Anti-neoplastic activity of N-maleimide homocysteine thiolactone imide encapsulated within liposomes. Proc Soc Exp Biol Med 180: 57-61.
46. McCully KS, Vezeridis MP (1987) Anti-neoplastic activity of a rhodium trichloride complex of oxalyl homocysteine thiolactone. Cancer Invest 5(1): 25-30.
47. McCully KS, Vezeridis MP (1987) Chemopreventive and antineoplastic activity of N-homocysteine thiolactonyl retinamide. Carcinogenesis 8(10): 1559-1562.
48. McCully KS, Vezeridis MP (1989) Chemopreventive effect of N-homocysteine thiolactonyl retinamido cobalamin on carcinogenesis by ethyl carbamate in mice. Proc Soc Exp Biol Med 191(4): 346-351.
49. Kazimir M, Wilson FR (2002) Prevention of homocysteine thiolactone induced atherogenesis in rats. Res Commun Molec Pathol Pharmacol 111(5-6): 179-198.
50. McCully KS, Tzanakakis G, Vezeridis MP (1989) Inhibition of neoplastic growth by N-homocysteine thiolactonyl retinamido cobalamin. Res Commun Chem Pathol Pharmacol 66(1): 117-122.
51. McCully KS, Tzanakakis GN, Vezeridis MP (1992) Effect of the synthetic N-homocysteine thiolactonyl derivatives, thioretinaco, thioretinamide, and thioco on growth and lactate production by malignant cells. Res Commun Chem Pathol Pharmacol 77(1): 125-128.
52. Linnell JC, Quadros EV, Matthews DM, et al. (1977) Altered cobalamin distribution in rat hepatomas and in the liver of rats treated with diethylnitrosamine. Cancer Res 37(9): 2975-2978.
53. Jakubowski H, Goldman E (1993) Synthesis of homocysteine thiolactone by methionyl-tRNA synthetase in cultured mammalian cells. FEBS Lett 317(3): 237-240.
54. McCully KS, Vezeridis MP (1988) Homocysteine thiolactone in arteriosclerosis and cancer. Res Commun Chem Pathol Pharmacol 59(1): 107-119.
55. Liteplo RG, Hipwell SE, Rosenblatt DS, et al. (1991) Changes in cobalamin metabolism are associated with methionine auxotrophy of highly growth autonomous melanoma cells. J Cell Physiol 149(2): 332-338.
56. Hoffman RM (1984) Altered methionine metabolism DNA methylation and oncogene expression in carcinogenesis. A review and synthesis. Biochim Biophys Acta 738(1-2): 49-87.
57. Copeland DA, Salmon WD (1946) Neoplasms in the liver, lungs, and other tissues of rats as the result of prolonged choline deficiency. Amer J Pathol 22: 1059-1079.
58. Popper H, DeLaHuerga J, Yesnick C (1953) Hepatic tumors due to prolonged ethionine feeding. Science 118(3055) : 80-82.
59. Gordon GB, Shantz LM, Talalay P (1987) Modulation of growth, differentiation, and carcinogenesis by dehydroepiandrosterone. Adv Enzyme Regul 26: 355-382.
60. Mikol YB, Hoover KL, Creasia D, et al. (1983) Hepatocarcinogenesis in rats fed methyl-deficient, amino acid defined diets. Carcinogenesis 4(12): 1619-1629.
61. McCully KS (1994) Chemical pathology of homocysteine. II. Carcinogenesis and homocysteine thiolactone metabolism. Ann Clin Lab Sci 24(1): 27-59.
62. McCully KS, Vezeridis MP (1989) Histopathological effects of homocysteine thiolactone on epithelial and stromal tissues. Exp Molec Pathol 51(2): 159-170.
63. McCully KS, Carvalho ACA (1987) Homocysteine thiolactone, N-thiolactonyl retinamide and platelet aggregation. Res Commun Chem Pathol Pharmacol 56(3): 349-360.
64. Naruszewicz M, Mirkiewicz E, Olszewski AJ, et al. (1994) Thiolation of low density lipoprotein by homocysteine thiolactone causes increased aggregation and interaction with cultured macrophages. Nutr Metab Cardiovas Dis 4: 70-79.
65. Boerner P, Racker E (1985) Methionine-sensitive glycolysis in transformed cells. Proc Natl Acad Sci USA 82(20): 6750-6754.

66. Rapoport E, Schroder EW, Black PW (1982) Retinoic acid-promoted expansion of total cellular ATP pools in 3T3 cells can mediate its stimulatory and growth inhibitory effects. J Cell Physiol 110(3): 318-322.
67. Lambeth DO, Lardy HA (1969) The oxidation of thioethers by bromine: A model system for oxidative phosphorylation. Biochemistry 8(8): 3395-3402.
68. Wieland T, Bauerlein E (1964) N-acetyl homocysteine thiolactone als Vermitter einer oxidativen Synthese von Adenosindiphosphat und Adenosintriphosphate aus Adenosinmonophosphat und Orthophosphat. Chem Ber 100(12): 3869-3876.
69. Kern M, Racker E (1954) Activation of a DPN oxidase by an oxidation product of ascorbic acid. Arch Biochem Biophys 48(1): 235-236.
70. Nason A, Wosilait WD, Terell AJ (1954) The enzymatic oxidation of reduced pyridine nucleotides by an oxidation product of ascorbic acid. Arch Biochem Biophys 48(1): 233-235.
71. Staudinger H, Krisch K, Leonhauser S (1961) Role for ascorbic acid in microsomal electron transport and the possible relation to hydroxylation reactions. Ann New York Acad Sci 92(1): 195-207.
72. Mustafa MG, DeLuca AJ, York GK, et al. (1973) Ozone interaction with rodent lung. II. Effects on oxygen consumption of mitochondria. J Lab Clin Med 82(3): 357-365.
73. Mustafa MG (1990) Biochemical basis of ozone toxicity. Free Rad Biol Med 9(3): 245-265.
74. Sweet F, Kao MS, Lee SC, et al. (1980) Ozone selectively inhibits growth of human cancer cells. Science 209(4459): 931-933.
75. Urschel HC Jr (1967) Cardiovascular effects of hydrogen peroxide: Current status. Dis Chest 51(2): 180-192.
76. Wentworth PJr, Nieva J, Taceuchi C, et al. (2003) Evidence for ozone formation in human atherosclerotic arteries. Science 302(5647): 1053-1056.
77. McCully KS (2015) The active site of oxidative phosphorylation and the origin of hyperhomocysteinemia in aging and dementia. Ann Clin Lab Sci 45(2): 222-225.
78. Tan B, Watson RR, Preedy VR, (2013) Tocotrienols Vitamin E Beyond Tocopherols, (2nd Edn), CRC Press, Boca Raton, pp: 1-78.
79. Aggarwal BB, Sundaram C, Prasad S, et al. (2010) Tocotrienols, the vitamin E of the 21st century. Biochem Pharmacol 80(11): 1613-1631.
80. McCully KS (2020) Chemical pathology of homocysteine. VIII. Effects of tocotrienol, geranylgeraniol, and squalene on thioretinaco ozonide, mitochondrial permeability, and oxidative phosphorylation in arteriosclerosis, cancer, neurodegeneration, and aging. Ann Clin Lab Sci 50(5): 567-577.
81. Lerner AB, Case JD, Takahashi Y, et al. (1958) Isolation of melatonin, the pineal factor that lightens melanocytes. J Amer Chem Soc 890(10): 2587.
82. Ebels I, Balemans MGM (1986) Physiological aspects of pineal function in mammals. Physiol Rev 66(3): 581-605.
83. Bohuon C, Caillard L (1971) S-adenosyl methionine in blood. Clin Chim Acta 33(1): 256.
84. McCully KS (1994) Chemical pathology of homocysteine. III. Cellular function and aging. Ann Clin Lab Sci 24(2): 134-152.
85. McCully KS (2018) Melatonin, hyperhomocysteinemia, thioretinaco ozonide, adenosyl methionine and mitochondrial dysfunction in aging and dementia. Ann Clin Lab Sci 48(1): 126-131.
86. Jencks DA, Matthews RG (1987) Allosteric inhibition of methylenetetrahydrofolate reductase by adenosylmethionine. J Biol Chem 262(6): 2485-2493.
87. Finklestein JC, Kyle WE, Martin JJ, et al. (1975) Activation of cystathionine synthase by adenosylmethionine. Biochem Biophys Res Commun 66(1): 81-87.
88. McCully KS (2016) Homocysteine, infections, polyamines, oxidative metabolism, and the pathogenesis of dementia and atherosclerosis. J Alzheimer's Dis 54(4): 1283-1290.
89. Paul R, Borah A (2015) The potential physiological crosstalk and interrelationship between the two sovereign endogenous amines, melatonin, and homocysteine. Life Sci 139: 97-107.
90. Andrabi SA, Sayeed I, Siemen D, et al. (2004) Direct inhibition of the mitochondrial permeability transition pore: A possible mechanism for anti-apoptotic effects of melatonin. FASEB J 81(7): 869-871.
91. Baydas G, Reiter RJ, Akbulut M, et al. (2005) Melatonin inhibits neural apoptosis induced by homocysteine in rats via inhibition of cytochrome c translocation and caspase-3 activation and by regulating pro- and anti-apoptotic protein levels. Neurosci 135(3): 879-886.
92. McCully KS (2009) Chemical pathology of homocysteine. IV. Excitotoxicity, oxidative stress, endothelial dysfunction, and inflammation. Ann Clin Lab Sci 39(3): 219-232.
93. Lopez A, Garcia JA, Escames G, et al. (2009) Melatonin protects the mitochondria from oxidative damage by reducing oxygen consumption, membrane potential, and superoxide anion production. J Pineal Res 469(2): 188-198.
94. Moshal KS, Tipparaju SM, Vacek TP, et al. (2008) Mitochondrial matrix metalloproteinase activation decreases myocyte contractility in hyperhomocysteinemia. Amer J Physiol Heart Circ Physiol 295(2): H890-H897.
95. Paredes F, Parra V, Torrealba N, et al. (1981) HERPUD1 protects against oxidative stress-induced apoptosis through down regulation of the inositol 1,4,5-triphosphate receptor. Free Rad Biol Med 90: 206-218.
96. Dreiling CE, Schilling RJ, Reitz RC (1981) 2'-3'-cyclic nucleotide phosphodiesterase in rat liver mitochondrial membranes. Biochim Biophys Acta 640(1): 114-120.
97. Azarashvili T, Krestinina O, Galvita A, et al. (2009) Ca2+-dependent permeability transition regulation in rat brain mitochondria by 2',3'-cyclic nucleotides and 2',3'-cyclic nucleotide 3'-phosphodiesterase. Amer J Physiol Cell Physiol 296(6): C1428-C1439.
98. Baburina Y, Azarashvili T, Grachev D, et al. (2015) Mitochondrial 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) interacts with mPTP modulators and functional complexes (I-IV) coupled with release of apoptotic factors. Neurochem Int 90: 46-55.
99. McCully KS (2018) Chemical pathology of homocysteine. VI. Aging, cellular senescence, and mitochondrial dysfunction. Ann Clin Lab Sci 48(5): 677-687.
100. Krestinina O, Azarashvili T, Baburina Y, et al. (2015) In aging the vulnerability of rat brain mitochondria is enhanced due to reduced level of 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) and subsequently increased

permeability transition in brain mitochondria in old animals. Neurochem Int 80: 41-50.
101. Baburina Y, Odinokova I, Azarashvili T, et al. (2017) 2',3'-cyclic nucleotide 3'-phosphodiesterase as a messenger of protection of mitochondrial function during melatonin treatment in aging. Biochim Biophs Acta 1859(1): 94-103.
102. Halestrap AP, McStay GP, Clarke SJ (2002) The permeability transition pore complex: another view. Biochimie 84(2-3): 153-166.
103. Mudd SH, Skovby F, Levy H, et al. (1985) The natural history of homocystinuria due to cystathionine beta synthase deficiency. Amer J Hum Genet 37(1): 1-31.
104. Verdin E (2015) NAD+ in aging, metabolism, and neurodegeneration. Science 350(6265): 1208-1213.
105. Rajmin L, Chwalek K, Sinclair DA (2018) Therapeutic potential of NAD-boosting molecules: the in vivo evidence. Cell Metab 27(3): 529-547.
106. Hayflick L, Moorhead PS (1961) The serial cultivation of human diploid cell strains. Exp Cell Res 25: 585-621.
107. Campisi J, Kim SH, Lim CS, et al. (2001) Cellular senescence, cancer, and aging: The telomere connection. Exp Gerontol 36(10): 1619-1637.
108. Hornsby PJ (2007) Telomerase and the aging process. Exp Gerontol 42: 575-581.
109. Minamino T, Komuro I (2008) Role of telomeres in vascular senescence. Front Biosci 13: 2972-2979.
110. Minamino T, Hiyauchi H, Yoshida T, et al. (2004) Vascular cell senescence and vascular aging. J Molec Cell Cardiol 36(2): 175-183.
111. Matsushita H, Chang E, Glassford AJ, et al. (2001) eNOS activity is reduced in senescent human endothelial cells. Circ Res 89(9): 793-798.
112. Vasa M, Breitschopf K, Zeiher AM, et al. (2000) Nitric oxide activates telomerase and delays endothelial cell senescence. Circ Res 87(7): 540-542.
113. Xu D, Neville R, Finkel T (2000) Homocysteine accelerates endothelial cell senescence. FEBS Lett 470(1): 20-24.
114. Zhang Y, Hodgson NW, Trivedi MS, et al. (2016) Decreased brain levels of vitamin B12 in aging, autism and schizophrenia. PLoSOne 11(1): e146797.
115. Harman D (1956) Aging: A theory based on free radical and radiation chemistry. J Gerontol 11(3): 298-300.
116. Dilman VM (1971) Age-associated elevation of hypothalamic threshold to feedback control, and its role in development, aging and disease. Lancet 297(7711): 1211-1219.
117. McCully KS (2017) Hyperhomocysteinemia, suppressed immunity and altered oxidative metabolism caused by pathogenic microbes in atherosclerosis and dementia. Front Aging Neurosci 9: 324.
118. McCully KS (2011) Chemical pathology of homocysteine. V. Thioretinamide, thioretinaco and cystathionine synthase function in degenerative disease. Ann Clin Lab Sci 41(4): 301-314.
119. Smith TJ, Drummond GS (1991) Retinoic acid can enhance the stimulation of heme oxygenase activity in the liver of thyroidectomized rats. Biochim Biophys Acta 1075(2): 119-122.
120. Gaull G, Sturman JA, Raiha NCR (1972) Development of mammalian sulfur metabolism: Absence of cystathionase in human fetal tissues. Pediat Res 6(6): 538-547.
121. Kim J, Hong SJ, Park SY, et al. (2009) Expression of cystathionine β-synthase is downregulated in hepatocellular carcinoma and associated with poor prognosis. Oncol Reports 21(6): 1449-1454.
122. Carballal S, Madzelan P, Zinola CF, et al. (2008) Dioxygen reactivity and heme redox potential of truncated human cystathionine β-synthase. Biochemistry 47(10): 3194-3202.
123. Miller MC, Miller JA (1981) Searches for ultimate chemical carcinogens and their reaction with cellular macromolecules. Cancer 47(10): 2327-2345.
124. McCully KS (2018) Loss of the thioretinaco ozonide oxygen adenosine triphosphate complex from mitochondria produces mitochondrial dysfunction and carcinogenesis. Ann Clin Lab Sci 48(3): 386-393.
125. McCully KS (2016) Homocysteine, thioretinaco ozonide, oxidative phosphorylation, biosynthesis of phosphoadenosine phosphosulfate, and the pathogenesis of atherosclerosis. Ann Clin Lab Sci 46(6): 701-704.
126. Pullman A, Pullman B (1962) Electron transfer and carcinogenesis. Nature 196: 228-229.
127. Hopfer SM, Sunderman FWJr, McCully KS, et al. (1984) Studies of the pathogenesis of arteriosclerosis induced in rats by intrarenal injection of a carcinogen, nickel subsulfide. Ann Clin Lab Sci 14(5): 355-365.
128. Warburg O (1956) On the origin of cancer cells. Science 123(3191): 309-314.
129. Ravnskov U (2003) High cholesterol may protect against infections and atherosclerosis. Quar J Med 96(12): 927-934.
130. Ravnskov U, Rosch PJ, McCully, KS (2012) The statin-low density cholesterol-cancer conundrum. Quar J Med 105(4): 383-388.
131. Ravnskov U, Diamond DM, Hama R, et al. (2016) Lack of an association or an inverse association between low-density-lipoprotein cholesterol and mortality in the elderly: A systematic review. Brit Med J Open 6(6): e010401.
132. Ravnskov U, DeLorgeril M, Diamond DM, et al. (2018) LDL-C does not cause cardiovascular disease: A comprehensive review of the current literature. Exp Rev Clin Pharmacol 11(10): 959-970.
133. Ravnskov U, McCully KS, Rosch PJ (2018) Increased mortality associated with low cholesterol does not reflect reverse causation but causes it. Brit Med J Heart Open 5: e000789.
134. Ravnskov U, McCully KS (2012) Infections may be causal in the pathogenesis of atherosclerosis. Amer J Med Sci 344(5): 391-394.
135. Ravnskov U, McCully KS (2009) Vulnerable plaque formation from obstruction of vasa vasorum by homocysteinylated and oxidized lipoprotein aggregates complexed with microbial remnants and LDL autoantibodies. Ann Clin Lab Sci 39(1): 3-16.
136. Ferguson E, Parthasarathy S, Joseph J, et al. (1998) Generation and initial characterization of a novel polyclonal antibody directed against homocysteine thiolactone-modified low density lipoprotein. J Lipid Res 39(4): 925-933.
137. Ott SJ, Mokhtari NE, Musfeldt M, et al. (2006) Detection of diverse bacterial signatures in atherosclerotic lesions of patients with coronary artery disease. Circulation 113(7): 929-937.

138. Melnick JL, Petrie BL, Dreesman GR, et al. (1983) Cytomegalovirus antigen within human arterial smooth muscle cells. Lancet 2(8351): 644-647.
139. Abu-Lubad M, Meyer TF, AlZeer MA (2014) Chlamydia trachomatis inhibits inducible NO synthesis in human mesenchymal stem cells by stimulating polyamine synthesis. J Immunol 193(6): 2941-2951.
140. MacMicking J, Xie QW, Nathan C (1997) Nitric oxide and macrophage function. Annu Rev Immunol 15: 323-350.
141. Zaki MH, Akuta T, Akaike T (2005) Nitric oxide-induced stress involved in microbial pathogenesis. J Pharmacol Sci 98(2): 117-129.
142. Gherasim C, Yadav PK, Kabil O, et al. (2014) Nitrite reductase activity and inhibition of H2S biogenesis by human cystathionine-β-synthase. PLoS One 9(1): e85544.
143. Carballal S, Cuevasanta E, Yadav PK, et al. (2016) Kinetics of nitrite reduction and peroxynitrite formation by ferrous heme in cystathionine-β-synthase. J Biol Chem 291(15): 8004-8014.
144. Monteiro JP, Oliveira PJ, Jurado AS (2013) Mitochondrial membrane lipid remodeling in pathophysiology: A new target for diet and therapeutic intervention. Prog Lipid Res 52(4): 513-528.
145. Sanchez-Cenizo L, Formentini L, Aldea M, et al. (2010) Up-regulation of the ATPase inhibitory factor (IF-1) of the mitochondrial H+-ATP synthase in human tumors mediates the metabolic shift of cancer cells to a Warburg phenotype. J Biol Chem 285(33): 25308-25313.
146. Grimm S, Brdiczka D (2007) The permeability transition pore in cell death. Apoptosis 12(5): 841-855.
147. Bloch KE (1965) The biological synthesis of cholesterol. Science 150(3692): 19-28.
148. Christenson E, Merlin S, Saito M, et al. (2008) Cholesterol effects on BAX pore activation. J Mol Biol 381(5): 1166-1183.
149. McCully KS (2019) Chemical pathology of homocysteine. VII. Cholesterol, thioretinaco ozonide, mitochondrial dysfunction, and prevention of mortality. Ann Clin Lab Sci 49(4): 55-68.
150. McCully KS (2020) Environmental pollution, oxidative stress and thioretinaco ozonide: Effects of glyphosate, fluoride, and electromagnetic fields on mitochondrial dysfunction in carcinogenesis, atherogenesis and aging. Ann Clin Lab Sci 50(3): 408-411.
151. Samsel A, Seneff S (2013) Glyphosate's suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: pathways to modern disease. Entropy 15(4): 1416-1463.
152. Andreotti G, Freeman LEB (2018) Glyphosate use and cancer incidence in the Agricultural Health Study. J Natl Cancer Inst 110 (5): 509-516.
153. Sbarra AJ, Karnovsky ML (1959) The biochemical basis of phagocytosis. I. Metabolic changes during the ingestion of particles by polymorphonuclear leukocytes. J Biol Chem 234(6): 1355-1362.
154. Gabler WL, Leong PA (1979) Fluoride inhibition of polymorphonuclear leukocytes. J Dent Res 58(9): 1933-1939.
155. Yiamouyiannis J (1993) Fluoride: The Aging Factor. Health Action Press: Delaware, Ohio, USA, pp: 26-33, 94-99.
156. Emsly J, Jones DJ, Miller JM, et al. (1981) An unexpectedly strong hydrogen bond. Ab initio calculations and spectroscopic studies of amide-fluoride systems. J Amer Chem Soc 103(1): 24-28.
157. Edwards SL, Poulos TL, Kraut J (1984) The crystal structure of fluoride-inhibited cytochrome c peroxidase. J Biol Chem 259(21): 12984-12988.
158. Leach JK, Van Tuyle G, Lin PS, et al. (2001) Ionizing radiation-induced, mitochondria-dependent generation of reactive oxygen/nitrogen. Cancer Res 61(10): 3894-3901.
159. Brodeur P (1977) The Zapping of America. Microwaves, Their Deadly Risk, and the Cover-up. W.W.Norton & Company, New York.
160. Wyde M, Cesta M, Blystone C, et al. (2018) Report of the partial findings from the National Toxicology Program Carcinogenesis Studies of cell phone radiofrequency radiation in Hsd: Sprague Dawley rats (whole body exposure). Cold Spring Harbor Laboratory bioRχiv.
161. Scherling BJ, Yamanashi WS, Hou Y, et al. (2004) Magnetism and cardiac arrythmias. Cardiol Rev 12(2): 85-96.
162. Destafanis M, Viano M, Leo C, Gervino G, et al. (2015) Extremely low frequency electromagnetic fields affect proliferation and mitochondrial activity of human cancer cell lines. Int J Radiat Biol 91(12): 964-972.
163. Santini SJ, Cordone V, Falone S, et al. (2018) Role of mitochondria in the oxidative stress induced by electromagnetic fields: Focus on reproductive systems. Oxid Med Cell Longev pp: 1-18.
164. Yang Z, Shi J, Lu Y, et al. (2020) Predictors for imaging progression on chest CT from coronavirus disease 2019 (COVD-19) patients. Aging 12(7): 6037-6048.
165. Fei Y, Tang N, Li H, et al. (2020) Coagulation dysfunction: a hallmark in COVID-19. Arch Pathol Lab Med 144(10): 1223-1229.
166. Ponti G, Ruini A, Tomasia A (2020) Homocysteine as a potential predictor of prognosis in patients with coronavirus disease 2019. Med Hypoth 143: 109859.
167. Luo Y, Xue Y, Mao L, et al. (2020) Prealbumin as a predictor of prognosis in patients with coronavirus disease 2019. Front Med 7: 374.
168. Ingenbleek Y (2009) Plasma transthyretin reflects the fluctuations of lean body mass in health and disease. In Recent Advances in Transthyretin Evolution, Structure and Biological Functions; Richardson SJ, Cody V, Eds; Berlin Springer-Verlag, Heidelberg, pp: 329-357.
169. Minetti G (2020) Mevalonate pathway, selenoproteins, redox balance, immune system, COVID-19: reasoning about connections. Med Hypoth 144: 110128.
170. Zhang D, Wu CT, Qi XY, et al. (2014) Activation of histone deacetylase-6 induces contractile dysfunction through derailment of α-tubulin proteostasis in experimental and human atrial fibrillation. Circulation 129(3): 346-358.
171. Wiersma M, Van Marion DMS, Wust RCI, et al. (2019) Mitochondrial dysfunction underlies cardiomyocyte remodeling in experimental and clinical atrial fibrillation. Cells 8(10): 1202-1223.
172. Zhang D, Hu X, Li J, et al. (2019) DNA damage-induced PARP-1 activation confers cardiomyocyte dysfunction through NAD+ depletion in experimental atrial fibrillation. Nature Commun 10(1): 1307.
173. Weissman JR, Kelle, RI, Bauman ML, et al. (2008) Mitochondrial disease autism spectrum disorder: A cohort analysis. PLoS One 3(11): e3815.

174. Poling JS, Frye RE, Schoffner J, et al. (2006) Developmental regression and mitochondrial dysfunction in a child with autism. J Child Neurol 21(2): 170-172.
175. Petrik MS, Wong MC, Rabata RC, et al. (2007) Aluminum adjuvant linked to Gulf War Illness induces motor neuron death in mice. Neuromolec Med 9(1): 83-100.
176. Mailloux R, Lemire J, Appanna V (2007) Aluminum-induced mitochondrial dysfunction leads to lipid accumulation in human hepatocytes: a link to obesity. Cell Physiol Biochem 20(5): 6217-638.
177. Goth SR, Chu RA, Gregg JP, et al. (2006) Uncoupling of ATP-mediated calcium signaling and dysregulated interleukin-6 secretion in dendritic cells by nanomolar thimerosal. Environ Health Perspect 114(7): 1083-1091.
178. Sharpe MA, Livingston AD, Baskin DS (2012) Thimerosal-derived ethylmercury is a mitochondrial toxin in human astrocytes: possible role of Fenton chemistry in the oxidation and breakage of mtDNA. J Toxicol 373678.
179. Schroeder HA (1971) Losses of vitamins and trace minerals resulting from processing and preservation of foods. Amer J Clin Nutr 24(5): 562-573.
180. Selhub J, Jacques PF, Wilson PWF, et al. (1993) Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. J Amer Med Assoc 270(22): 2693-2698.
181. Jacques PF, Selhub J, Bostom AG, et al. (1999) The effect of folic acid fortification on plasma folate and total homocysteine concentrations. New Engl J Med 340(19): 1449-1454.
182. McCully KS (2007) Homocysteine, vitamins, and vascular disease prevention. Amer J Clin Nutr 86(5): 1563S-1568S.
183. Yang T, Friedman JM, Botto LD (2006) Improvement in stroke mortality in Canada and the United States, 1990 to 2002. Circulation 113(10): 1335-1343.
184. Ellis JM, McCully KS (1995) Prevention of myocardial infarction by vitamin B6. Res Commun Molec Pathol Pharmacol 89(2): 208-220.
185. McCully KS (2019) Homocysteine, thioretinaco ozonide, and oxidative phosphorylation in cancer and aging: a proposed clinical trial protocol. In Methionine Dependence of Cancer and Aging, Methods and Protocols; Hofmann RM, Ed; Humana Press, New York, pp: 285-310.
186. McCully KS (2015) Compositions and method for utilization of thioretinamide in therapy of diseases of aging. US Patent No. 9,216,209.
187. McCully KS (2017) Thioretinamide compositions for the apoptosis of malignant cells while preventing the apoptosis of normal cells and related methods. US Continuing Patent Application S.N. 15/475,103.
188. Miklossy J (2017) Ed. Handbook of Infection and Alzheimer's Disease; IOS Press, Amsterdam, pp: 41-294.
189. Seshadri S, Beiser A, Selhub J, et al. (2002) Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. New Engl J Med 346(7): 476-483.
190. Douad G, Refsum H, de Jager CA, et al. (2013) Preventing Alzheimer's disease-related gray matter atrophy by B-vitamin treatment. Proc Natl Acad Sci USA 110(23): 9523-9528.
191. Siskind GW (1981) Immunological aspects of aging: an overview. In Biological Mechanisms of Aging, Schimke, RT, Ed.; NIH Publication, Bethesda, pp: 455-466.
192. Wentworth AD, Jones LH, Wentworth PJr, et al. (2000) Antibodies have the intrinsic capacity to destroy antigens. Proc Natl Acad Sci USA 97(20): 10931-10935.
193. Lerner RA, Eschenmoser A (2003) Ozone in biology. Proc Natl Acad Sci USA 100(6): 3013-3015.
194. Frost S, Martins RN, Kanagasingam Y (2010) Ocular biomarkers for early detection of Alzheimer's disease. J Alzheimer's Dis 22(1): 1-16.
195. Allen HB (2016) Alzheimer's disease: Assessing the role of spirochetes, biofilms, the immune system and amyloid-β with regard to potential treatment and prevention. J Alzheimer's Dis53: 1271-1276.
196. McCully KS (2018) Novel therapeutic stem cell compositions with an active oxidative phosphorylation site and their preparation. US Patent Application S.N 16/138,898.
197. Grisendi G, Bussolari R, Cafarelli L, et al. (2010) Adipose-derived mesenchymal stem cells as stable source of tumor necrosis factor-related apoptosis-inducing ligand delivery for cancer therapy. Cancer Res 70(9): 3718-3729.
198. Choi SA, Lee JY, Kwon SE, et al. (2015) Human adipose tissue-derived mesenchymal stem cells target brain tumor-initiating cells. PLoS One 10: e0129292.
199. Rezvani K, Rouce R, Shpall E (2017) Engineering natural killer cells for cancer immunotherapy. Molec Therapy 25(8): 1769-1781.
200. Wang W, Jiang J, Wu C (2020) CAR-NK for tumor immunotherapy: Clinical transformation and future prospects. Cancer Lett 472: 175-180.
201. Li Y, Hermanson DL, Moriarity BS, et al. (2018) Human iPSC-derived natural killer cells engineered with chimeric antigen receptor enhance anti-tumor activity. Cell Stem Cell 23(2): 181-192.
202. Siegler EL, Zhu Y, Wang P, et al. (2018) Off the shelf CAR-NK cells for cancer immunotherapy. Cell Stem Cell 23(2): 160-161.
203. Kloess S, Kretschmer A, Stahl L, et al. (2019) CAR-expressing natural killer cells for cancer targeting. Transfus Med Hemother 46(1): 4-13.
204. Garrod AE (1923) Inborn Errors of Metabolism. (2nd Edn), Oxford University Press, London.
205. Smith AD, Refsum H (2021) Homocysteine – from disease biomarker to disease prevention. J Int Med 290(4): 826-854.

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Biography

Kilmer Serjus McCully currently serves as Consultant in Pathology, Veterans Affairs Boston Healthcare System, and Associate Professor of Pathology, Harvard Medical School, Boston, Massachusetts USA. He was born in 1933 in Daykin, Nebraska. His mother, Lulu Viola (Litwinenco) McCully, was a teacher, musician, and artist. His father, Cyrus Harold McCully EdD, was a teacher, Dean of Students at Denver University, and Educational Psychologist serving as Director of Counselling for the US Veterans Administration. Kilmer attended public schools in Denver, Chicago, Memphis, and Alexandria, Virginia, where he was valedictorian of the Class of 1951, George Washington High School. Kilmer was awarded the AB degree magna cum laude (chemistry) by Harvard College in 1955 and the MD degree cum laude by Harvard Medical School in 1959. His internship in Medicine and his residency in Anatomic and Clinical Pathology were served at Massachusetts General Hospital. His research training fellowships in Medicine, Molecular Biology, Genetics and Pathology were served at Harvard University, Massachusetts General Hospital, and Glasgow University, Scotland. His work experience was served as Associate in Biochemistry, National Institutes of Health, Visiting Professor of Laboratory Medicine, University of Connecticut, and Pathologist, Massachusetts General Hospital and the Veterans Affairs Medical Centers in Providence, Rhode Island and West Roxbury, Massachusetts. His current research interest is the homocysteine theory of aging and disease, and previous research interests included structure of transfer RNA, genetic recombination in Aspergillus, carcinogenesis, polyribosome structure, and protein biosynthesis. He serves as a member of American Society for Investigative Pathology, American Society for the Advancement of Science, American Chemical Society, and Association of Clinical Scientists, past President. Among other awards, he received the Linus Pauling Functional Medicine Award by the Institute for Functional Medicine, and the F.William Sunderman Jr. Diploma of Honor by the Association of Clinical Scientists. An amateur violinist, Kilmer played with the Harvard Radcliffe Orchestra and was a founding member of the Harvard Bach Society Orchestra, while a student at Harvard College. After college, Kilmer joined the Harvard Musical Association of Boston, serving on the Board of Directors and as past President. In 1955 Kilmer married Annina Elena Jacobs, a graduate of Tufts and UCLA. His immediate family includes daughter, son, son's wife, two grandchildren, grandson's wife, and two great grandchildren. His sister, Marilyn Jane McCully, her husband Michael Raeburn, and family reside in London, England. Kilmer and Annina currently reside in Winchester, Massachusetts, USA.