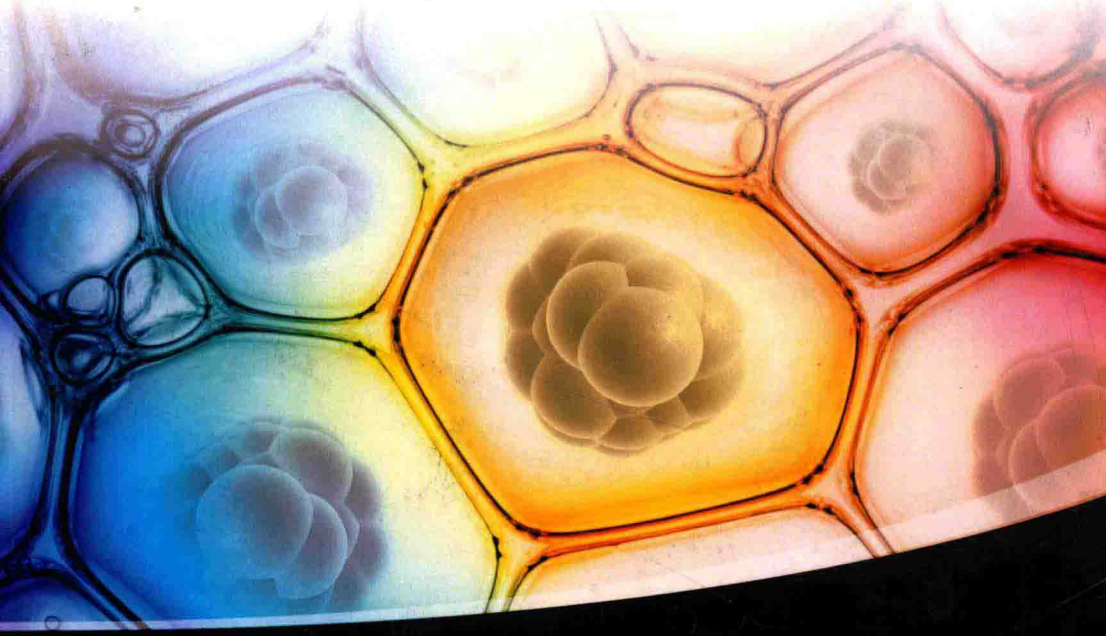
Philip B. Oliva, M.D.

Antioxidants and Stem Cells *for* Coronary Heart Disease



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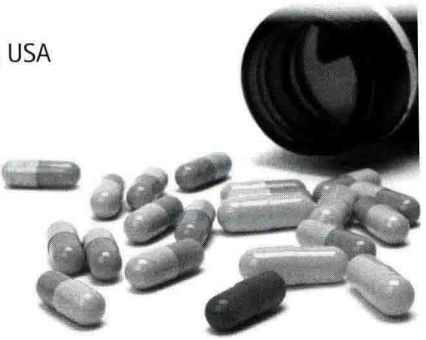

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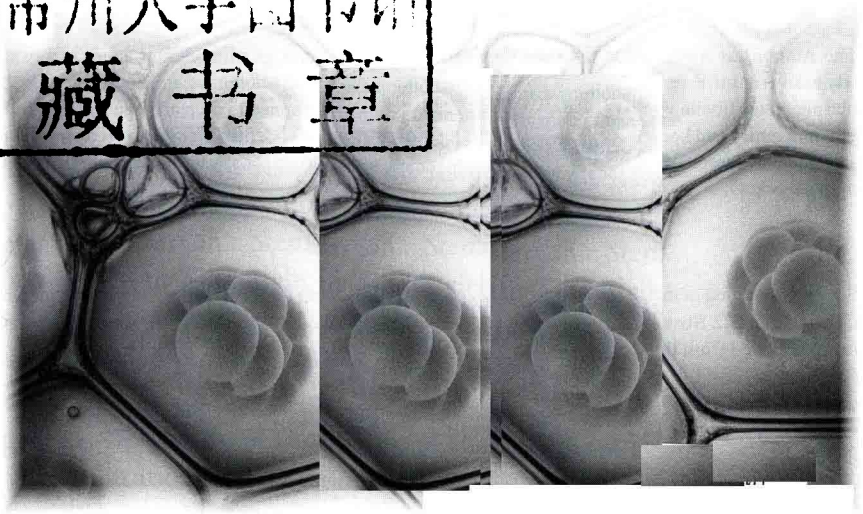

World Scientific
NEW JERSEY • LONDON • SINGAPORE • BEIJING • SHANGHAI • HONG KONG • TAIPEI • CHENNAI

Published by

World Scientific Publishing Co. Pte. Ltd.

5 Toh Tuck Link, Singapore 596224

USA office: 27 Warren Street, Suite 401-402, Hackensack, NJ 07601

UK office: 57 Shelton Street, Covent Garden, London WC2H 9HE

Library of Congress Control Number: **2013956293**

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

ANTIOXIDANTS AND STEM CELLS FOR CORONARY HEART DISEASE

ISBN 978-981-4293-44-0

Typeset by Stallion Press
Email: enquiries@stallionpress.com

Printed in Singapore by Fuisland Offset Printing (S) Pte Ltd

Antioxidants and Stem Cells *for* Coronary Heart Disease

PREFACE

Science and medicine need not be arcane. Both can be expressed in nonscientific terms. That is the aim of this book—to present in *plain English* information regarding vitamin antioxidants and stem cells *vis-à-vis* coronary heart disease, the leading cause of death in America, Canada, Europe, Southeast Asia, and Australia.

The title of this book is more apt to catch the eye of folks over 35 — the target audience — those under 35 being the invincible ones. That is because while older adults may experience the terrible outcomes of this devilish disease, its roots track back to infancy and all we ate during our childhood, adolescence and our younger adult years. It takes egg upon egg, hot dog upon hot dog, year upon year for plagues to insidiously narrow down coronary — and carotid — arteries, but just a few seconds of time for a blood clot to form leading to a heart attack or stroke. All those eat-whatever-we-want years are suddenly paid for as sirens wail on the way to the ER.

You may wonder, *What's the connection between antioxidants and stem cells*? The answer is that they're both hot topics, be it in print, television commentaries, or happy hour conversations. There's also a disjunctive relationship that will become apparent as time goes by. Anticipate the unexpected — or at least recognize the unexpected when it happens and figure out why it did.

No work such as this book is accomplished without the strong support of others. Many people have chipped in, in a variety of ways — particularly people with advanced computer skills. I am especially grateful to several individuals affiliated with the University of Colorado Medical School in Denver: *Ryan Laterzo,* then an undergraduate at the University of Colorado, now himself a cardiologist; Sergey Markovitch, a very smart graduate student from eastern Europe; and David H. Spodick, M.D., professor emeritus at the

University of Massachusetts in Worcester for his inspirational writing style over the years. Thanks are also due to copy editors Marta Victoria Colon, Shelley Chow, and Jihan Abdat of World Scientific Publishing Company for their useful comments and suggestions...and patience; a number of librarians in the Metro Denver region, the Mayo Clinic, and the NIH; and my son, John, and my daughter, Julie (now in their mid-30s and early-40s, respectively), for putting up with a dad who has holed up in science libraries for a big chunk of the last five years or so.

CONTENTS

Part I

1

THE FRENCH PARADOX

Three culturally distinct races — the French, the Japanese, and the North American (as well as Greenlandic) Eskimos — share a medical blessing: they have fewer heart attacks than the natives of many other countries. Their coronary resistance resides in what they eat and/or drink. In the case of the French, although they eat a lot of rich food, they also drink a lot of (red) wine. In the case of the Japanese, they eat a lot of low-cholesterol seafood and drink a lot of sake and beer. In the case of the Eskimos (or the Inuit[a]), they eat a lot of marine life — fish, whale, and seal[1] — and drink a lot of homemade or distilled spirits.

These dietary, cultural or medical facts spawned curiosity about compounds in food and/or alcoholic beverages that might protect against coronary heart disease. Advancement of the concept that low-density lipoprotein (LDL), the principal carrier of cholesterol in the blood, must be oxidized before it becomes dangerous[2] has led to intensive research on ways to slow or prevent its oxidation. And after wine was discovered to have antioxidant properties in 1993,[3] scientists conjectured that alcohol might link the three dissimilar cultures and their shared resistance to heart disease.

A trickle of publications regarding antioxidants and coronary heart disease appeared in the medical literature in the mid-1990s, and has now grown into a sea of papers throughout the scientific literature. The published material records the findings of a considerable body of research concerning the clinical use of antioxidants to prevent or treat coronary heart disease, and, to an even greater extent, it registers the results of an extraordinary volume of research by basic scientists pertaining to the biochemistry of how LDL is oxidized. The

[a]The term "Eskimo" means "he who eats (meat) raw." "Inuit" translates best as "real people." Most North American and Greenland Arctic Circle dwellers prefer the latter term.

last chapter of the antioxidant story has yet to be written. When it finally is put into print, it will be a memorable one. But, even today, the murky waters that encircled antioxidants just a few years ago have become much clearer. It's a tale worth telling even though its ending is still up in the air.

In the Beginning...

It all began in 1979, when a French physician published a paper in England's leading medical journal, showing that the mortality rate due to coronary heart disease (CHD) was lower in France — where wine consumption was higher — than in 17 other countries, including the U.S. (Fig. 1.1).[4]

At about the same time as the "French paradox" was being recognized, a group of researchers in the Department of Molecular Genetics at the University of Texas (in Dallas) came upon another seeming contradiction.

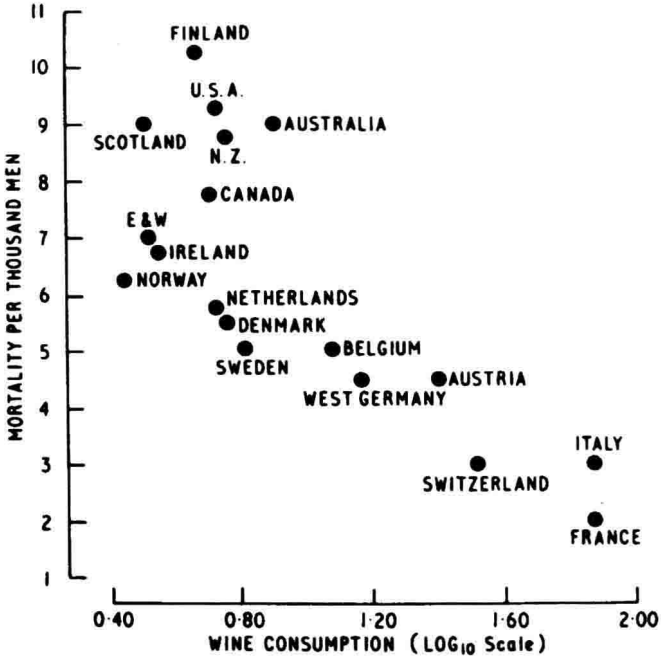

Fig. 1.1. The inverse relationship between mortality from coronary heart disease and wine consumption among men aged 55–64 in 17 countries.

(*Source:* Reproduced with permission from the publisher.) Reference 4.

They discovered that "bad cholesterol" (i.e. low-density lipoprotein)[b] was taken up by cells lining the walls of blood vessels at a rate too slow to result in any significant accumulation of cholesterol within their cytoplasm.[c] Yet blood vessels critically narrowed by cholesterol deposits had accumulated a huge amount of the lipid — a fat that, by definition, is not soluble in water. The scientists also learned that patients with familial hypercholesterolemia (elevated blood cholesterol level),[d] an inherited disorder of lipid metabolism leading to blood cholesterol levels often in the range of 400–1,000 mg/dL, similarly had vascular cells that did not take up any bad cholesterol. . .at least when they were incubated in a laboratory with their own blood containing very high levels of cholesterol. They were essentially invulnerable to harmful cholesterol. Yet the same type of cells clearly took up cholesterol when they were within the human body in direct contact with circulating blood, leading to severe atherosclerosis[e] even in the absence of any other risk factors. To

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[b]Low-density lipoprotein is a large compound made up of 22,000 cholesterol molecules and 2616 fatty acid molecules, which together constitute 79% of the compound's weight, attached to one protein molecule accounting for the remaining 21% of its weight. The lipoprotein is the principal carrier of cholesterol in the bloodstream. Cholesterol, being a lipid, is not soluble in the watery plasma, which constitutes 55% of the blood volume. Therefore, a carrier is needed to transport cholesterol from its origin in the liver or digestive tract to its destination at cells throughout the body. The modifier "low-density" refers to the compactness of the lipoprotein, expressed as the ratio of mass to volume. In the case of cholesterol-carrying lipoproteins, such as LDL and HDL, the units of density are g/cm^{-1}, with 1.063 g/cm^{-1} separating low- from high-density lipoproteins. The range is 1.019–1.210 g/cm^{-1}.

[c]Such cells are known collectively as the intima — the innermost coat of arteries and veins — though only the intima of arteries takes up cholesterol. The *single* layer of intimal cells in direct contact with blood is called the endothelium.

[d]Individuals with this rare condition affecting one in one million persons inherit a defective gene governing LDL receptors (to be discussed in Chap. 3) from each parent. The defective gene usually leads to a very high cholesterol level (600–1000 mg/dL), which, in turn, results in a heart attack during childhood.

[e]The term "atherosclerosis" refers to the disease process that causes the deposition of LDL in arteries. It describes the mushy consistency of cholesterol in the wall of arteries during the early and intermediate stages of the disease. The term comes from the Greek words "*athero*" (meaning "porridge") and "*sclerosis*" (meaning "hardening"). The term "arteriosclerosis" refers to the more advanced stage of the disease, when the arteries become brittle. That stage of the disease process is often not reversible. "Arteriosclerosis" comes from the Greek words "*arterio*" and "*sclerosis*" (meaning "artery" and "hardening," respectively). The phrase "hardening of the arteries" is often applied to such vessels. In this book, "Atherosclerosis" is used almost exclusively to discuss (for the most part) the early and mid-phases of the disease.

explain the paradox of how fat-filled cells in the lining of such cholesterol-narrowed blood vessels became overloaded with the lipid in spite of taking it up too slowly in the laboratory to result in a buildup, and to account for why LDL was also taken up too slowly to cause atherosclerosis — even in individuals *without* a genetic predisposition to develop the disease — it was suggested that LDL must be changed into a form that could both penetrate cells and be taken up more rapidly than "native" LDL.[f] Only then could massive deposits of LDL occur throughout the body. In 1984, that related form was shown to be "oxidized" LDL.[5]

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[f]The term "native LDL," rather than "natural LDL," is traditionally used in science because LDL does not occur "naturally," i.e, it does not occur in nature. The adjective "native" is used to indicate that the LDL exists in its original (i.e, its unoxidized) form. Native (unoxidized) LDL is harmless. It does not damage cells until it undergoes oxidation.

2

LDL OXIDATION — BRIEFLY

But just what is "oxidized LDL"? How does native (unoxidized) LDL become oxidized? How does its oxidation accelerate the deposition of cholesterol in the intima of arteries? How do antioxidants affect those deposits, particularly those in the coronary arteries? And, perhaps most importantly, can taking an antioxidant prevent a buildup — and perhaps, one day, a heart attack?

We bandy the term "oxidized cholesterol" around as if it were a well-known compound, a common dietary substance — like flour or rice. We use the term "oxidation" (with reference to LDL) as if it were a straightforward process — like boiling an egg. We even throw around phrases related to oxidized LDL — such as "free radicals," "omega-3 fatty acids," "cytokines," "superoxide ions," and "trans fats" — at cocktail parties and during dinner table conversations as if we were talking about last Sunday's NFL games or today's stock market activities. But our ease with these technical terms belies the complexity of the LDL oxidation process.[a] That process will be put in a nutshell to encourage

[a] Understanding the process of LDL oxidation gets confusing right off the bat, because the process doesn't necessarily require oxygen.

The term "oxidation" was used originally to describe a process during which a substance, such as iron, chemically combined with oxygen to form an altered compound, — in the case of iron, rust. However, the definition of "oxidation" was broadened when it was learned that at the atomic level it was the loss of an (negative) electron from the substance being oxidized that "oxidized" it, irrespective of whether oxygen was available or not. In the case of iron changing to rust, oxygen is present and it converts reduced, ferrous iron (Fe^{++}) into its oxidized ferric (Fe^{+++}) form. But at an even more fundamental level, it's the loss of one electron from the ferrous (Fe^{++}) ion that converts reduced iron into its more positive state, the oxidized Fe^{+++} ion, which becomes rust — ferric oxide. Thus, oxidation is now defined as a loss of one or more electrons, while reduction is defined as a gain of one or more electrons, whether or not oxygen is involved in the reaction. The Mnemonic "oil rig" is a convenient way to remember this oxidation/reduction, electron-number relationship, "oil rig" standing for "oxidation is loss, reduction is gain."

continued reading, before going into more detail and losing whatever reader interest exists at this point. Readers bored by biochemistry, even if condensed, should turn to Chapter 10.

LDL Oxidation. . .Briefly

Unoxidized LDL is harmless as it circulates in the bloodstream. It would not lead to any accumulation of cholesterol in the walls of the blood vessels through which it flows if it remained *within* the bloodstream. It would not lead to an artery being blocked by plaque if it stayed in solution within the channel of the artery, just as iron in water would not lead to a pipe being clogged by rust if the iron remained in solution i.e. if it did not react with oxygen in the air above/around the water or dissolved in it). But (unoxidized) LDL *does* cross from blood within the lumen of a blood vessel into cells making up the blood vessel's wall, simply because the concentration of LDL in the blood is higher than its concentration in the wall.

At first, the unoxidized lipoprotein is confined to a narrow space within the wall of arteries where it remains innocuous, until it is acted on by certain waste products — known as free radicals — made by cells in the arteries' wall. The byproducts are, in essence, biological (potentially) toxic wastes generated by arterial cells as they burn oxygen to use nutrient food molecules. . .just as potentially toxic automotive wastes are generated by cylinders in a car engine as they burn oxygen to use gasoline. These (human) oxidative waste products now oxidize LDL (even though the cells' oxygen has been burned and none is left to oxidize LDL) by removing electrons from the LDL molecule, thereby

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The majority of oxidative processes in the human body are not dependent on the immediate presence of oxygen. They are accomplished by the enzymatic removal of a hydrogen atom or atoms (each hydrogen atom having one electron) from a molecule, such as the successive removal of several hydrogen atoms from adenosine triphosphate (ATP) during the enzymatic conversion of glucose to pyruvate or lactic acid during exercise.[1] The highly efficient Krebs cycle, the hub of the metabolic system, is another example of oxidation without oxygen. Three of the eight sequential steps in the cycle involve oxidation achieved by the transfer of hydrogen atoms from one compound to another, with energy released at each step. As a result, 11 times more energy is produced by utilizing the Krebs cycle than would have been produced by burning glucose directly. Oxygen is finally converted to water by nicotinamide dinucleotide (NAD) in its reduced form, NADH, but only after much energy for vital functions has been released or stored through the metabolism of fats, carbohydrates, and proteins — the raw materials for the cycle.[2] NAD itself is formed by cells from niacin, also known as vitamin B3.

meeting the modern definition of oxidation. Oxidizing LDL is the physiologic equivalent of rusting.

A Prelude to a More Detailed Description of LDL Oxidation in Chap. 9

That's the nutshell version of how LDL is oxidized. The expanded version picks up the LDL molecule as it is diffusing passively — simply due to its higher concentration in the bloodstream — across the exceedingly thin lining of an artery into the space immediately beyond; that is to say, immediately underneath the innermost lining — the subendothelial space. But, unlike outer space, which is filled with asteroids, comets, and tens of millions of stars — not to mention objects we can't see, such as ultraviolet rays and quasars — the subendothelial space is (normally) devoid of biological bodies, such as cells. Instead, it is filled with a gelatin-like substance that appears clear under a conventional microscope, although strands of connective tissue (collagen) become visible under an electron microscope. The tangled collagen strands make the subendothelial space look more like the crawlspace under a house in the Louisiana bayou filled with (plumbing) pipes and (electrical) wires. And, akin to a crawlspace, the subendothelial space is largely ignored until something goes wrong.

The Endothelium and the Subendothelial Space

The term "endothelium" describes a delicate tissue lining the inside of the heart, arteries, and veins, as well as the abdominal and thoracic cavities. The prefix "endo-" means "inside," while the suffix "-thelium" refers to a tissue that covers the surface of or lines a body cavity. The endothelium, then, lines the extensive lining of the entire cardiovascular system, an estimated distance — including capillaries — of 62,000 miles, or more than twice the circumference of Earth.[3]

Before the 1960s, scientists tended to ignore the endothelium and the subendothelial space (Fig. 2.1). The vascular endothelium was viewed as simply a "sheet of cellophane"[5] between the blood and the more peripheral layers of a blood vessel's wall, while the subendothelial space was looked on as if it were an extraterrestrial "black hole" that might be interesting to explore. . .someday. But the technology wasn't on hand for subendothelial-space exploration in the 1960s, though it was available for outer-space exploration, as many