

# Nutritional Antioxidants and Disease Prevention: Mechanisms of Action

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**DENNIS V. PARKE**

*Division of Molecular Toxicology, School of Biological  
Sciences, University of Surrey, Guildford, UK*

## Introduction

It has been known for 50 years or longer that much disease is caused by reactive oxygen species (ROS). At an international meeting of the World Health Organization in the mid-1960s, the distinguished Russian toxicologist, Professor Sanojki, presented a major treatise on the 'rusting diseases', a phrase not understood by many American and European delegates, but intended to mean diseases attributed to oxygen toxicity, such as rheumatoid arthritis. Since that time it has been increasingly realized that because of the ubiquitous biological toxicity of ROS, the similarity of ROS-induced pathology to that of many spontaneous diseases, and the relative ease with which ROS are produced, particularly by bacteria and other invasive organisms, much disease, from malignancy to cardiovascular disease and dementia, is associated with ROS. Indeed, ROS are also responsible for ageing and death, and were it not for the existence of a highly effective biological system for antioxidant defence, life as we know it could not exist.

## Sources of Reactive Oxygen

ROS, comprising the superoxide anion radical ( $O_2^{\cdot-}$ ), the peroxide anion ( $O_2^{2-}$ ), and singlet oxygen ( $^1O_2$ ), are highly reactive entities, produced from molecular oxygen ( $O_2$ ) by the gain of electrons, or the realignment of the electron spins. The hydroxyl radical ( $\cdot OH$ ), the most highly reactive species of ROS, is formed by dismutation of peroxide catalysed by  $Fe^{2+}$ . The hypochlorite ion ( $OCl^-$ ) is yet another highly reactive oxygen species which, together with the former ROS, is produced by leukocytes to kill invading microorganisms.

The free radical, nitric oxide (NO), identical with endothelium-derived relaxing factor (EDRF), is an important cytotoxic molecule, active in defence against malignant cells, fungi and protozoa; it results in vasodilation and inflammation (Valance and Moncada 1994). It is generated from L-arginine, and contributes to the endogenous nitrosation of secondary amines; its formation is decreased in old age, hence the increase in fungal infections and malignancies at that time.

ROS are formed spontaneously by many biological processes, and may be considered as a measure of biological inefficiency, since they are formed by electron leakage from membranes and inadequately coupled reactions (Table 1.1); the released electrons reduce molecular oxygen stepwise to superoxide anion, then peroxide. Electron leakage occurs continuously from the mitochondrial membranes and the endoplasmic reticulum; also from the futile cycling of the various cytochromes P450 in the catalysis of microsomal oxygenations, especially with CYP2E1 which acts primarily as a ROS generator to oxidize resistant chemicals such as benzene and ethanol. ROS are also produced by activated leukocytes and protect the organism against bacteria and viruses, and initiate the mechanism of the inflammation (Parke and Parke, 1995). Other ROS-generating systems include the reduction of tissue oxygen by iron and other redox metal systems, the redox cycling of quinones, and as a side-reaction in the conversion of PGG<sub>2</sub> to PGH<sub>2</sub> in prostaglandin biosynthesis (Parke and Parke, 1995).

**Table 1.1.** Origins of reactive oxygen species.

Origins	References
Homolytic scission of water by ionizing radiation	Halliwell and Gutteridge (1989)
Leaking of electrons from membranes and reduction of O <sub>2</sub>	Sohal <i>et al.</i> (1990)
Futile cycling of CYPs	Ekström and Ingelman-Sundgerg (1989)
Activation of CYP2E1	Ekström and Ingelman-Sundgerg (1989)
Reduction of tissue O <sub>2</sub> by Fe <sup>2+</sup> /Fe <sup>3+</sup> and other metal redox systems	Minotti and Aust (1989)
Activation of leukocytes in inflammation	Biamond <i>et al.</i> (1986)
Redox cycling of quinones	Powis <i>et al.</i> (1981)
Prostaglandin biosynthesis	Eling and Kraus (1985)

## Mechanisms of ROS Toxicity

ROS are the mediators of inflammation, and through this their interaction with platelets, neutrophils, macrophages and other cells can involve the synthesis of eicosanoids and the activation and release of various cytokines, propagating the inflammatory process from one organ system (liver) to another (kidney, lungs, etc.). This results in tissue oxidative stress and multiple-system organ failure (Parke and Parke, 1995). Generation of ROS in experimental animals by induction of CYP2E1 by fasting, or by exposure to ether anaesthesia, results in tissue oxidative stress by depletion of tissue glutathione (GSH), and restoration of the GSH can prevent the oxidative stress and tissue injury (Lui *et al.*, 1993).

ROS-mediated inflammation is involved in the pathogenesis of infectious disease, including tuberculosis and septic shock (Welbourn and Young, 1992), and in immune and autoimmune diseases such as rheumatoid arthritis and inflammatory bowel disease (Parke *et al.*, 1991). More recent studies have also implicated the involvement of ROS in cancer (Witz, 1991; Ames *et al.*, 1993), atherosclerosis (Halliwell, 1994), hepatitis (Elliot and Strunin, 1993), AIDS (Baruchel and Wainberg, 1992), Alzheimer's dementia (Evans, 1993), multiple-system organ failure (Fry, 1992; Parke and Parke, 1995) and respiratory distress syndrome (ARDS) (McLean and Byrick, 1993). Molecular mechanisms of ROS toxicity and ROS-mediated disease, include: (i) oxidation of vital thiol compounds to disulphides, (ii) loss of tissue GSH, (iii) impairment of energy generation (ATP, NADH, NADPH), (iv) inhibition of  $\text{Ca}^{2+}$  transport and electrolyte homeostasis, (v) oxidation of cytochromes, (vi) DNA strand cleavage, and (vii) the initiation and promotion of mutations and carcinogenesis (Parke, 1994a).

## Biological Defence Against ROS Injury

Biological defence against ROS comprises a complex array of endogenous antioxidant enzymes, numerous endogenous antioxidant factors including GSH and other tissue thiols, haem proteins, coenzyme Q, bilirubin and urates, and a variety of nutritional factors, primarily the antioxidant vitamins (Table 1.2).

Tissue GSH and other tissue thiols are the ultimate bastion against oxidative stress and tissue injury (Liu *et al.*, 1993), although these are maintained in the reduced state by the concerted action of tissue ascorbate, tocopherols and other reducing factors such as bilirubin and urates.

The cascade of endogenous antioxidant enzymes requires energy to maintain the living system in the reduced state. GSH reductase maintains tissue glutathione in the reduced state (GSH) at the expense of reduced NADP and FAD (Fig. 1.1). The GSH peroxidases reduce soluble peroxides (GSH peroxidase; GPX), and membrane-bound peroxides (phospholipid

**Table 1.2.** Antioxidant defence.

Endogenous factors	Endogenous enzymes	Nutritional factors
Glutathione and other thiols	GSH reductase	Ascorbic acid (vitamin C)
Haem proteins	GSH transferases	Tocopherols (vitamin E)
Coenzymes Q	GSH peroxidases (GPX and PHGPX)	$\beta$ -Carotene and retinoids
Bilirubin	Superoxide dismutase	Selenium – essential dietary component of peroxidase
Urates	Catalase	Methionine or lipotropes for choline biosynthesis

hydroperoxide GSH peroxidase; PHGPX) to the corresponding alcohols, at the expense of GSH which is oxidized to GSSG (Fig. 1.2). The enzyme, superoxide dismutase, catalyses the conversion of superoxide anion radical to peroxide and oxygen, and catalase converts the peroxide to water.

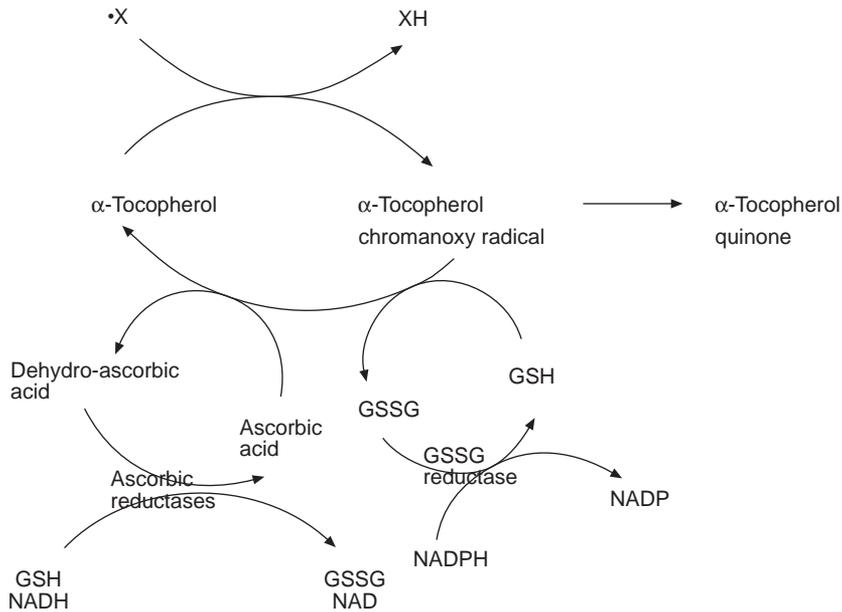
These endogenous antioxidant enzymes and other factors operate a number of repair systems which: (i) reduce toxic disulphides and quinones; (ii) scavenge ROS; and (iii) reduce soluble and membrane-bound peroxides, etc. (Box 1.1). The vital repair systems require constant replenishment of energy (NADH and NADPH) and antioxidant vitamins to function efficiently. Ascorbic acid,  $\alpha$ -tocopherol and GSH, interact as a complex system to reduce ROS and other oxidants (Fig. 1.1).

## Mechanisms of Nutritional Antioxidant Defence

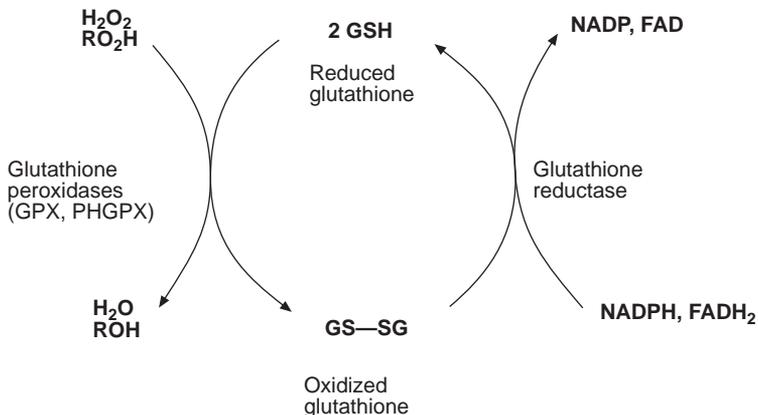
Among the various mechanisms for nutritional defence and disease prevention are: (i) ROS scavenging; (ii) reduction of peroxides and repair of peroxidized biological membranes; (iii) sequestration of iron to decrease ROS formation; (iv) utilization of dietary lipids (rapid energy production and ROS scavenging by short-chain fatty acids, ROS scavenging by cholesteryl esters); and (v) alternative biological pathways as occur in stomach cancer, multiple-system organ failure and diabetes.

### ROS Scavenging

Dietary vitamin C, vitamin E and retinoids provide an integrated antioxidant system, with tissue GSH scavenging ROS and protecting tissues from ROS-induced oxidative damage, characteristic of acute inflammation and chronic inflammatory diseases. This ascorbate–tocopherol–GSH antioxidant system



**Fig. 1.1.** The ascorbate–tocopherol–GSH antioxidant system. Biological oxidants and ROS (represented by  $\cdot X$ ) are reduced directly by the tocopherols. The oxidized tocopheryl chromanoxo radical is cyclically reduced back to the tocopherols by ascorbic acid or GSH. The dehydro-ascorbic acid is cyclically reduced back to ascorbic acid at the expense of GSH or NADH, and oxidized glutathione (GSSG) is reduced back to GSH by NADPH in the presence of glutathione reductase.



**Fig. 1.2.** Reduction of disulphides and peroxides by GSH reductase and GSH peroxidase. The GSH peroxidases are selenium-containing enzymes which reduce soluble peroxides by GPX, and reduce phospholipid membrane peroxides by PHGPX.

**Box 1.1.** Antioxidant nutrient repair systems.

Reduction of disulphides, peroxides, quinones, etc., by GSH, with GSH reductase and GSH peroxidase  
Radical scavenging by the GSH/ascorbate/tocopherol system  
Reduction of soluble peroxides by GSH peroxidase (GPX)  
Reduction of phospholipid membrane peroxides by GSH phospholipid peroxidase (PHGPX)  
Phospholipid membrane integrity, methylation of ethanolamine to choline, to prevent electron loss  
Defence against NO nitrosation in stomach by ascorbate

is self-regenerating, at the expense of energy (NADH, NADPH) (Fig. 1.1), so that as long as the subject is well-nourished, the integrated antioxidant system protects the individual against oxidative stress. Patients with systemic sclerosis had lower than normal concentrations of ascorbic acid, tocopherols, retinoids, folate and vitamin B<sub>12</sub>, and enjoyed lower dietary intakes of fruit and vegetables (Lundberg *et al.*, 1992). Similarly, rheumatoid arthritis patients had lower than normal levels of blood and joint ascorbate (Panush, 1991; Parke *et al.*, 1996).

Retinoids scavenge ROS and act directly on polymorphonuclear leukocytes to prevent their generation of hydroxyl radicals (Yoshioka *et al.*, 1986). In addition to ROS scavenging by the natural integrated antioxidant system, synthetic antioxidants, such as BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole) have long been used for this purpose as food additives, especially to prevent the peroxidation of lipids (Parke and Lewis, 1992).

### Reduction of peroxides

Reversal of oxidative damage is also affected by the antioxidant defence system, thereby arresting progression into oxidative stress and tissue damage. The dietary mineral element selenium is essential for this aspect of antioxidant protection and disease prevention, as it is a vital component of the two peroxidase enzymes, GPX, which reduces soluble peroxides and PHGPX, which removes lipid peroxides from biological membranes (Fig. 1.2). Dietary selenium, as selenomethionine, protects against ethanol-induced lipid peroxidation (Parke, 1994b), cirrhosis, cancer and cardiovascular disease. Selenium deficiency, through lower GPX and PHGPX activities, results in increased formation of thromboxanes (TXB<sub>2</sub>) at the expense of prostaglandin (PGI<sub>2</sub>) formation, leading to thrombotic and inflammatory episodes and vascular injury (Schoene *et al.*, 1986).

Selenium deficiency has also been associated with Kashin-Beck and Keshchin disease (Diplock, 1993). Dietary supplementation of rheumatoid

arthritis patients with L-selenomethionine restored levels of serum and erythrocyte selenium and GPX to normal after prolonged treatment, but had no effect on leukocyte GPX (Tarp *et al.*, 1992).

### Sequestration of iron

Among some of the most toxic substances known are the transitional metals, especially iron ( $\text{Fe}^{2+}/\text{Fe}^{3+}$ ), as these are potent generators of ROS from molecular  $\text{O}_2$ . Since iron catalyses ROS generation, it is also highly effective as a biological catalyst of oxidation reactions, and consequently is found in haemoglobin, the mitochondrial cytochromes responsible for energy production, and the microsomal cytochromes which catalyse the insertion of oxygen into biological compounds to form cholesterol, the steroid hormones, bile acids and many other essential molecules.

Therefore, iron, from the time of its absorption from the gut to its incorporation into haem and other stable organic iron complexes (transferrin, ferritin) has to be sequestered in biological systems to prevent toxicity through ROS generation. Inorganic iron, or simple complexes such as iron nitriloacetate, are highly toxic and carcinogenic (Preece *et al.*, 1989), and intraperitoneal injection of iron nitriloacetate into rodents results in hepatic and renal necrosis, malignancy and death. Intramuscular injection preparations of iron have been extensively prescribed for use in Africa and other developing countries for the treatment of hookworm anaemia; their prescription in more advanced countries would not have been countenanced because of the high incidence of tumours at the site of injection.

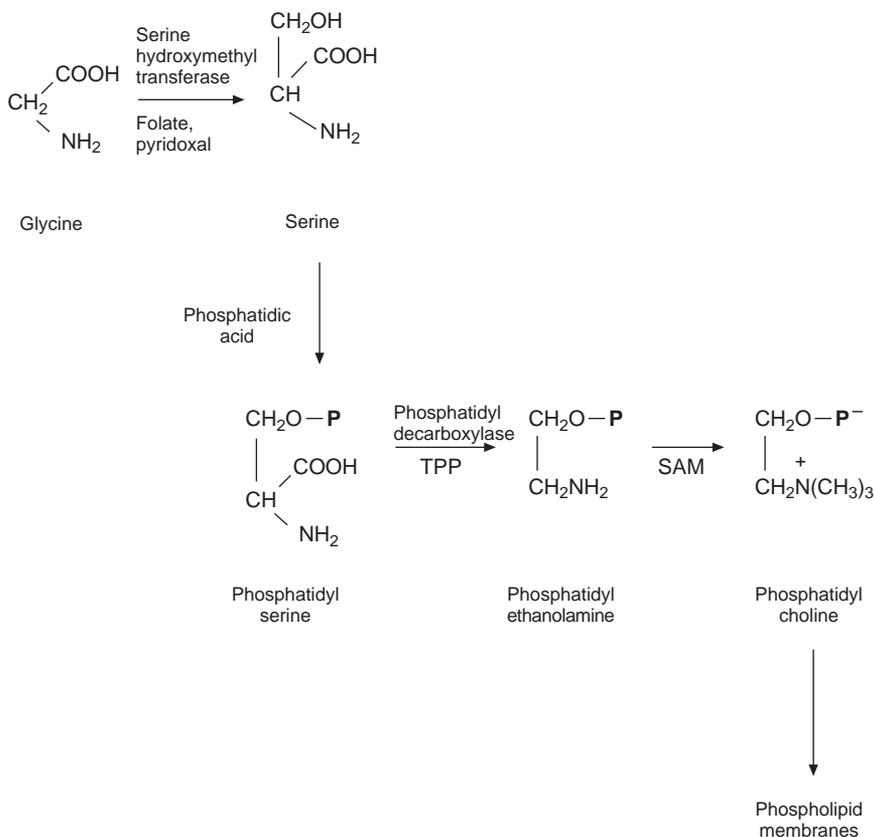
Silicic acid, a ubiquitously distributed component of cereals and other foods, forms complexes with inorganic iron, which enable the safe sequestration of iron in tissues, decreasing its ability to generate ROS, initiate membrane lipid peroxidation, and to mobilize leukocytes (Birchall, 1993). Iron, *per se*, as oxygen-bridged ferrous–ferric complexes may initiate lipid peroxidation (Minotti and Aust, 1989). Dietary supplementation of iron-enhanced dimethylhydrazine-induced colorectal cancer in rats, but this was reversed by phytic acid, a component of dietary fibre, probably due to the chelation of the iron by phytic acid, with the consequent decreased ability of the iron to generate ROS (Nelson *et al.*, 1989).

### Dietary lipids

The most common targets for autoxidative stress and oxidative tissue injury and disease are the biological membranes. These comprise a variety of lipids, including many different phospholipids and cerebroside, short-chain and long-chain saturated, unsaturated and polyunsaturated fatty acids. This diversity reflects the multiplicity of membrane functions. The short-chain fatty acid, butyrate, is known to protect against ROS damage, inflammation and cancer (Hill, 1995), possibly by ROS scavenging, or rapid production of

energy. However, the most fundamental membrane function is electron conductance, which appears to depend to a large extent on the presence of choline, a charged molecule, and a zwitterion  $[\text{OCH}_2\text{CH}_2\text{N}(\text{CH}_3)_3]$ ; which may facilitate membrane electron transport by Grotthüs conduction. Deficiency of dietary choline or dietary lipotropes (vitamin  $\text{B}_{12}$ , folate, pyridoxal, glycine,  $\text{PO}_4^{3-}$ , etc.) (Fig. 1.3) leads to ROS production, lipid peroxidation, tissue injury, malignancy and death (Vance, 1990; Lombardi *et al.*, 1991; Schragar and Newberne, 1993).

Although much attention has been focused on the causation of coronary heart disease (CHD), namely by dietary fats and cholesterol, it would appear that the mechanisms generally assumed by physicians are somewhat incorrect. Firstly, dietary cholesterol has little affect on blood cholesterol levels, and in any event probably leads to the decrease of cholesterol by



**Fig. 1.3.** Choline biosynthesis for membranes: choline occurs in the lecithins of membrane phospholipids. SAM, S-adenosylmethionine (folate + vitamin  $\text{B}_{12}$ ); TPP, thiamin pyrophosphate; P, phosphatidic acid.

enhancing cholesterol 7 $\alpha$ -hydroxylase activity and promoting bile acid production (Björkhem *et al.*, 1991). High dietary fibre keeps the bile acid in the stool and hence removes the negative feedback of cholic acid on cholesterol 7 $\alpha$ -hydroxylase. So for lower blood cholesterol dietary restriction is valueless, but a high fibre diet is highly efficacious (Anderson and Tietzen-Clark, 1986). Most cholesterol is synthesized in the body where it is needed, from fatty acids which may be derived from dietary carbohydrate (Lewis and Watts, 1997). However, reduction of low-density lipoprotein (LDL) cholesterol by 35% led to a 40% decrease in adverse CHD events (Shepherd *et al.*, 1995). Cholesterol is relatively inert, but some of its polyunsaturated fatty acid esters are highly peroxidisable, and form peroxy radicals capable of converting cholesterol to toxic oxidation products. Nutritional prophylaxis in CHD should focus on prevention of lipid peroxidation, by the use of antioxidants, instead of concentrating on cholesterol removal. Oestrogens also act as antioxidant cardioprotectants; and oxidative damage of LDL, which is implicated in atherogenesis, is inhibited by 17 $\beta$ -oestradiol (Wiseman and O'Reilly, 1997).

### Alternative pathways

Much disease is due to a sequential series of interactive phenomena, and disease prevention can best be achieved by studying the mechanisms involved and arresting one or more of the critical stages. For example, in multiple system organ failure, a disease syndrome seen in critical infections and accident trauma (Fry, 1992; Parke and Parke, 1995), systemic inflammation progresses from one organ to another, due primarily to the linking of these different systems by the actions of the cytokines, eicosanoids and ROS. A simple gut infection, or road traffic accident, or even elective minor surgery, may lead to liver involvement progressing to hepatic failure. When this is corrected by modern sophisticated medical procedures, inflammation may not be totally arrested but may progress to the kidneys, then the lungs, and even return again to the liver. The phenomena linking these organ failures are inflammation and the cytokines, and in addition to aggressive rescue and resuscitation by perfusion, nutrition and appropriate medication, the use of antioxidants to arrest ROS production is indicated.

A further example is gastric cancer, now attributed to *Helicobacter pylori* infections, but known to result also from surgical vagotomy, and from stress and trauma mediated by the cytokines (Fig. 1.4). A sequence of pathological events has been elucidated, progressing from stress to inflammation of the gastric mucosa, to achlorhydria, bacterial invasion of the gastric mucosal barrier, depletion of antioxidant protectants, formation of nitrosamines in the gastric lumen, mutations and malignancy (Parke, 1997). Although the *H. pylori* infection appears to be a most critical factor, this microorganism is highly resistant to antibiotic treatment. A more successful approach appears to be to administer ascorbic acid to inhibit nitrosation,

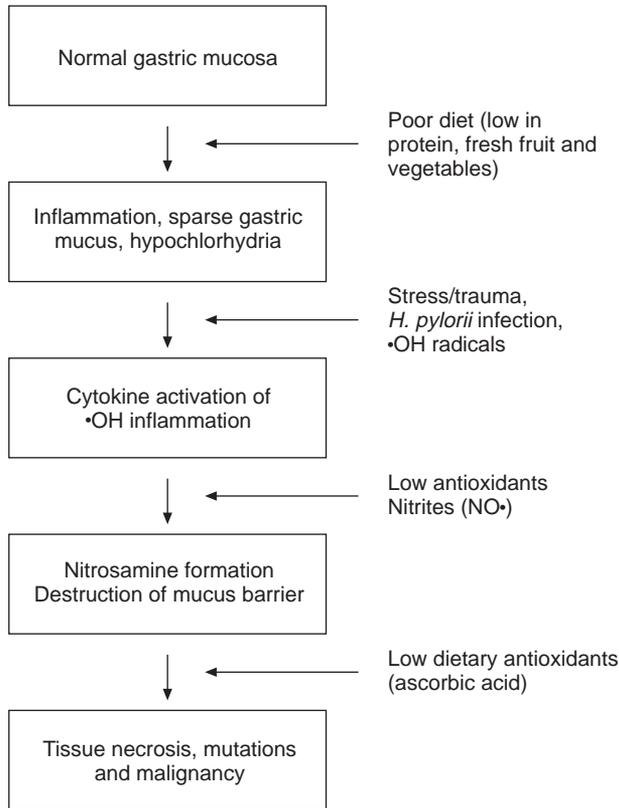


Fig. 1.4. Gastric cancer, stages in development.

and subsequently, with the aid of antibiotics, to eliminate the microbial overgrowth and restore the normal gastric equilibrium.

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