Biochemical-Pathology Initiated by Free Radicals, Oxidant Chemicals, and Therapeutic Drugs in the Etiology of Chemical Hypersensitivity Disease

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Dedication

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Abstract

Free radicals formed during normal cellular metabolism or produced in response to environmental chemicals and certain therapeutic drugs are highly reactive molecular species which initiate and propagate the formation of toxic metabolites. Normally, the cellular antioxidant protective system operates to inactivate excess free radicals and protect susceptible cell membranes, enzymes, and nucleic acids. When antioxidant molecules, which are derived from essential nutrients, are deficient, the protective antioxidant system is functionally compromised and free radical compounds accumulate in local tissue environments. The excess free radicals and toxic metabolites react rapidly with cellular constituents to initiate diverse biochemical and immunologic pathologies which progress to the clinical manifestations of ***Chemical Hypersensitivity*** *disease. Treatment of this multifaceted syndrome requires an understanding of the molecular nature of free radical induced biochemical-pathologies as a basis for the therapeutic use of nutritional antioxidants.*

Introduction

Effective treatment of Chemical Hypersensitivites requires an understanding of the diverse biochemical pathologies resulting from exposure to the chemicals encountered in the home, environment, and on the job. Individuals exposed to these chemicals are susceptible to oxidant stresses which can overwhelm the body's protective, antioxidant mechanisms leading to neuropsychiatric effects, liver and lung pathology, and immune disorders. The protective antioxidant mechanisms maintain the cellular oxidation-reduction potentials required for normal

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metabolism and to prevent free radical attack of amino acids, proteins, and the lipid components of cell membranes necessary for functional and structural integrity of cells and tissues. (Demopoulos, 1973; Pryor, 1973; Feldman et al., 1980).

Identifying the chemical or chemicals involved in each case is a prerequisite for diagnosis and counseling patients to avoid contact with both the specific chemical(s) involved and with other chemicals which may cause additional oxidant stress. Because free radicals generated by these environmental chemicals and certain therapeutic drugs are ultimately responsible for the acute and chronic pathologic effects seen in Chemical Hypersensitivity disease, treatment requires a clear rationale which links the underlying biochemical mechanisms of Chemical Hypersensitivity with the clinical picture presented by the patient.

Inert foreign chemicals can be converted to more chemically reactive metabolites by drug metabolizing enzymes in the liver, lung, kidney, and skin (Conney and Burns, 1972; Coon, 1978). These enzymatically activated metabolites can cause clinical hypersensitivity reactions and cellular necrosis. (Gillette, 1974). The classes of chemicals encountered in today's environment which may be activated in this manner are shown in Table 1. Each of these chemical compounds is capable of causing tissue damage either directly by oxidant mechanisms which are corrosive to tissue **or** indirectly by their metabolic conversion to more reactive molecules (toxic metabolites) and free radicals. Metabolic conversion, to the more reactive state is catalyzed by the drug metabolizing or "xenobiotic" enzymes which include cytochrome P450 oxidases, NADPH-cytochrome c reductase, aryl hydrocarbon hydroxylase, or a wide variety of metabolic oxido-reductases [dehydrogenases] (Coon, 1978; Coon, 1981). Normally, these enzymes function to detoxify metabolic waste products or exogenous chemicals and drugs. Paradoxically, this detoxification process may also generate free radical intermediates which are more damaging to the cells and tissue than the original chemical or drug (Smuckler, 1977).

The activated metabolites produced by the "xenobiotic" enzymes can also lead to increased drug toxicity with loss of therapeutic effectiveness, changes in the biologic activities of steroid hormones and thyroxin, decreased serum bilirubin concentrations, and symptoms of vitamin D and vitamin K deficiency (Conney, 1967; Conney and Burns, 1972).

When either certain nutritional deficits or frank deficiencies exist, or high concentrations of these oxidant chemicals or drugs are present, or whenever both conditions exist simultaneously, more free radical intermediates are produced than the localized, antioxidant protective mechanisms can effectively process. This saturates or overwhelms the antioxidant system. The excess free radical compounds formed react very rapidly in the immediate cellular micro-environment with the fat soluble vitamins and the unsaturated fatty acids in cell membranes (Chow, 1979; Slater, 1979). Excess free radicals or toxic metabolites can also react with amino acid residues in enzymes and structural proteins such as the sulfhydryl groups of both cysteine and methionine, histidine, and tyrosine, or with the nucleic acid bases in DNA and RNA (Roubal and Tappel, 1966; Myers, 1973). These damaged molecules must be repaired or removed before normal cellular function can be restored. If the damaged cellular molecules are not removed or repaired, they may act as persistent, low level stimuli for abnormal metabolism, altered protein synthesis, mutations, production of auto-immune antibodies, and chronic inflammation (Tappel, 1973).

The purpose of this paper is to describe the components and function of the antioxidant protective system and to illustrate how chemicals found in air, water, food, and therapeutic agents interact with this system to produce the biochemical and immuno-pathologies which underlie the signs and symptoms of Chemical Hypersensitivity.

Free Radicals in Biologic Systems

Free radical intermediates are continuously produced by all living cells in aerobic environments, (Haugaard, 1968; Fridovich, 1981). The processes of cellular respiration (oxidative phosphorylation to produce ATP), intermediary metabolism (oxidase and dehydrogenase enzymes), phagocytosis by

TABLE 1
CLASSES OF CHEMICALS IMPLICATED IN CHEMICAL HYPERSENSITIVITY

Cases	Inorganic Chemicals	Organic Chemicals			
		Aliphatic Hydrocarbons	Halogenated Aliphatic Hydrocarbons	Aromatic Hydrocarbons	Therapeutic Agents
Carbon disulfide	Antimony	Acetaldehyde	Bromoform	Aldrin	Acetaminophen
Ethylene oxide	Arsenic	Acetonitrile	Carbon tetrachloride	Benzene	Chlordiazepoxide **(Librium)**
Nitrogen dioxide	Beryllium	Acrolein	Chloroform	3,4-Benz(a)pyrene	Chlorpromazine
Nitrous oxide	Cadmium	Amyl nitrite	Dibromochloropropane **(DBCP)**	Caffeine	Glutethimide
Ozone	Chromium (Chromic acid)	1,3-Butadiene	Dibromoethane	Chlordane	Halothane
Phosgene	Mercury	Butyl nitrite	Dichloromethane	Dieldrin	Meprobamate
Sulfur dioxide	Nickel	Dimethyl formamide	Ethylene dibromide **(EDB)**	Erythrosine **(Red Dye No.** 3)	Methoxyflurane
	Thallium	Ethyl alcohol	Epichlorohydrin	Heptachlor	Metronidazole **(Flagyl)**
		Formaldehyde	Hexachloro-1,3 butadiene	Nicotine	Phenacetin
		Methyl alcohol	Methylene chloride	Paraquat	Phenobarbitol
			1,1,1 -Trichloroethane	Phenols	Theophylline
			1,1,2-Trichloroethylene	Salicylates	Tolbutamide
			Tetrachloroethylene	Tartrazine **(Yellow Dye No.** 5)	
			Vinyl chloride monomer **(VCM)**	Toluene	
				Toluene di-isothio-cyanate	
				Xylene	

macrophages and neutrophils, and the production of inflammatory mediators (prostaglandins, thromboxanes, and prostacyclins) are examples of cellular metabolic mechanisms which generate free radicals in both health and disease (McCord and Fridovich, 1978; Fridovich, 1981). The free radicals produced during cellular metabolism are described in Table 2.

Free radicals are also produced by ionizing radiation, during photochemical sensitization, by exposure to oxidant chemicals and ozone, and via metabolism of certain therapeutic drugs (Demopoulos, 1973).

If free radicals generated by either the endogenous metabolic processes or by exogenous chemicals and drugs are not inactivated by local antioxidant mechanisms, then immediate tissue damage ensues. This biochemical pathology is produced by peroxidation of unsaturated cell membrane lipids, Inactivation of enzymes, and modification of nucleic acid bases. The most significant and well understood aspect of cellular injury by free radicals is lipid peroxidation (Slater, 1979).

Excess free radicals (*FR) produced by metabolism, radiation, oxidant chemicals and gases, or drugs can initiate lipid peroxidation by removing a proton from a membrane polyunsaturated fatty acid (PUFAH) to form a fatty acid radical (*PUFA) as shown below:

•FR + PUFAH ---------► FRH + *PUFA (1)

This initiation step normally occurs on the external surfaces of cell membranes when the water soluble antioxidants (vitamin C, sulfhydryl amino acids, and reduced glutathione) are either absent from or depleted in the interstitial fluids. Initiation may also occur intracellularly when the cytoplasmic surfaces of the endoplasmic reticulum, microsomes, or mitochondria are attacked by free radicals following exposure to toxic levels of chemicals and drugs and to a lesser extent during prolonged hypoxia or severe ischemia (Kong

and Davison, 1980).

The initial peroxidation of unsaturated fatty acids at the membrane surface may be propagated within the membrane if the fat soluble, membrane antioxidants(B-carotene, vitamin A, and vitamin E) are depleted. Inner membrane peroxidation is essentially a self-perpetuating process which can be blocked by the fat soluble antioxidants and the critical membrane enzyme glutathione peroxidase. Glutathione peroxidase, a selenium containing enzyme located in membranes, requires reduced glutathione (GSH) and vitamin E for its catalytic action in converting peroxy fatty acid free radicals (*PUFA02) to non-toxic hydroxy fatty acid alcohols (Tappel, 1974; Flohe, 1979).

In the absence of membrane antioxidants, propagation of membrane lipid peroxidation occurs in a sequence of chain-reaction steps following the initial formation of fatty acid radical (*PUFA) shown in reaction (1). The steps in propagation are shown in reactions (2) and (3); the first event is the formation of the peroxy fatty acid radical (*PUFAOa) in reaction (2):

$$\bullet PUFA + 0_2 \text{ ----------►} \quad *PUFA0_2 \quad (2)$$

The peroxy fatty acid radical (•PUFAO2) formed in reaction (2) immediately attacks another membrane polyunsaturated fatty acid (FUFAH) to produce a second fatty acid radical (•PUFA) as shown in reaction (3):

$$\bullet PUFA0_2 + PUFAH \text{ --►} \quad PUFA0_2H + 'PUFA \quad (3)$$

The continued regeneration of fatty acid radicals (*PUFA) in this manner continues until the propagation process is blocked by a membrane antioxidant which must be at the site of free radical generation at the time the

TABLE 2

FREE RADICALS DERIVED FROM METABOLISM

Free Radical	Radical Name	Antioxidants	Products
•O2	Superoxide	S.O.D. Vitamin C Glutathione (red.)	H_20_2 + O2 H_20_2 H_20_2
•OH	Hydroxyl	PUFA (red.) Methionine Guanine Cytosine Uracil Formate	Lipid peroxides
$^1O_2$	Singlet Oxygen	B-carotene ~-Tocopherol PUFA (red.) Glutathione (red.)	0_2 Lipid hydroperoxides
•PUFA	Polyunsaturated Fatty Acid Radical	B-carotene ~-Tocopherol	PUFA
•PUFAO2	Polyunsaturated Fatty Acid Peroxyl Radical	Glutathione peroxidase Glutathione (red.) Selenium Glutathione reductase NADPH	Hydroxy-PUFA

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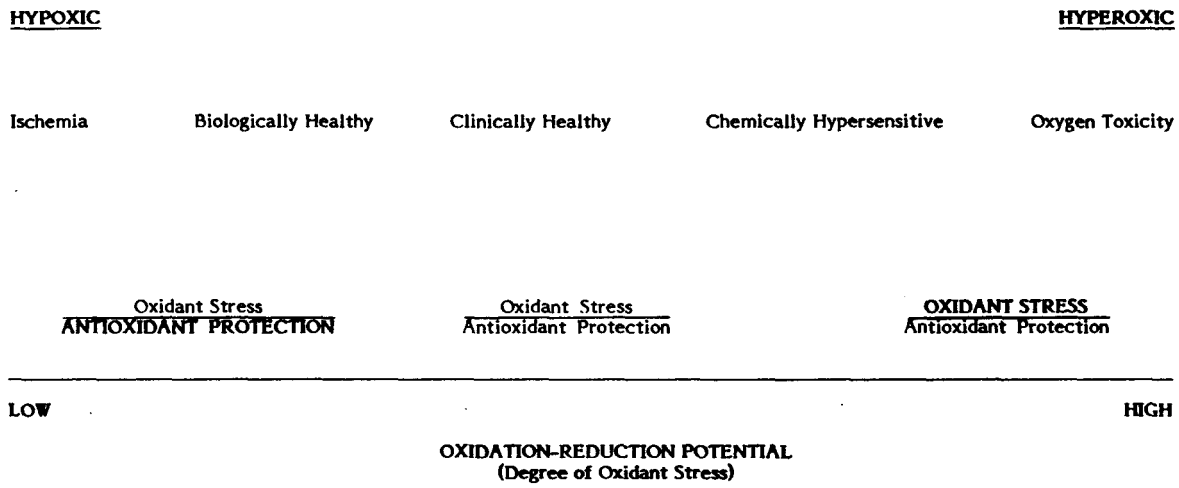
FIGURE I O
XIDANT-ANTIOXIDANT BALANCE IN HEALTH AND DISEASE



fatty acid radical (*PUFA) is produced. Adequate membrane levels of B-carotene, vitamin A, vitamin E, selenium, and glutathione peroxidase are thus absolutely necessary to protect cells from the deleterious effects of progressive lipid peroxidation.

The Antioxidant Protective System

In intact cells and healthy tissue, free radicals and the highly reactive chemical intermediates produced by oxidative metabolism, biological oxidations, and chemical and drug detoxification mechanisms are normally isolated from susceptible molecules and enzymes by cell membrane barriers which contain numerous antioxidant molecules ultimately derived from nutrients. These antioxidant molecules act in concert with several important, protective enzyme systems and function to maintain the cellular oxidation-reduction potentials necessary for achieving balance between oxidative stresses and the antioxidant defense capacity of the cell (Chow, 1979). Any substantial shift in the cellular oxidation-reduction potential in response to either external chemical exposure or metabolic oxidant stresses will directly affect the viability and function of cells, tissue, and organ systems. The status of a person's health is, therefore, related to the degree of oxidant stress imposed on the individual's antioxidant protective system by environmental chemicals, drug metabolism, and radiation; this dynamic relationship is portrayed diagrammatically in Figure 1.

The molecules and enzyme cofactors necessary for proper function of the antioxidant defense system are derived from essential nutrients which must either be obtained from the diet or be provided as nutritional supplements. These antioxidant nutrients and enzymes function concertedly to prevent local formation of membrane lipid peroxides by free radicals in the membrane. The nutrients and enzymes required for the protective, antioxidant defense system are shown in Table 3.

Free radical induced pathology is also one of the primary biochemical events in the alteration of cellular macromolecules and membranes associated with the aging process (Leibovitz and Siegel, 1980); the initial phases of atherosclerosis (Hartroft, 1965; Perkins, Joh and Kummerow, 1965; Tappel, 1973), chlorinated hydrocarbon hepatotoxicity (DiLuzio, 1967, 1973; Smuckler, 1977), ethanol-induced liver injury (DiLuzio, 1968; Hartman and DiLuzio, 1968; Porta, Koch, and Hartroft, 1970); photochemical oxidant damage to cells and lung tissue by air pollutants (Witschi, 1977; Menzel, 1979); high pressure oxygen and ozone toxicity (Goldstein, 1979; Huber and Drath, 1981), and ionizing radiation injury (Greenstock, 1981).

Chemicals in Drinking Water

Chlorination of drinking water (which con-

TABLE 3

ESSENTIAL ENZYMES AND NUTRIENTS REQUIRED FOR MAINTAINING THE PROTECTIVE ANTIOXIDANT SYSTEM

Enzymes	Minerals	Vitamins	Amino Acids-Peptides	Lipids
Catalase	Copper	Ascorbic acid	Glutathione, reduced	Phosphatidyl choline **(Lecithin)**
Glucose-6~phosphate dehydrogenase	Iron	Riboflavin **(FAD, FMN)**	L-Cysteine	Phosphatidyl ethanolamine
Glutathione peroxidase	Manganese	Niacinamide **(NAD, NADP)**	L-Methionine	Phosphatidyl inositol
Glutathione reductase	Molybdenum	Vitamin A or B-Carotene		
Glutathione S-transferase	Selenium	Vitamin E		
Superoxide dismutase	Zinc			

tains small amounts of organic material), leads to the formation of a class of chlorinated hydrocarbons known as trihalomethanes (THM's) which include chloroform and dichlorobromomethane in addition to many other halogenated hydrocarbons that may occur in water supplies (Federal Register, 1980; American Water Works Association, 1980; Munson et al., 1982; Pereira et al., 1982). Chemical Hypersensitivity patients who either drink or shower with water containing these halogenated hydrocarbons may, therefore, experience an exacerbation of their signs and symptoms by exposure to parts per billion levels of these chemicals in water.

Another source of chemicals in drinking water results from discharges of industrial acids and solvents into waste sites which may subsequently leak into ground waters; the principal chemicals found in ground waters vary depending on the industrial activity and discharges in a local area. Over twenty-five thousand different chemicals are used by industry and dumped into approximately eleven thousand waste sites around the country (Maugh, 1981). Total domestic production of the eleven halogenated aliphatic hydrocarbons listed in Table 3 was 10 billion pounds in 1973 and is estimated to be approximately 15 billion pounds currently (Fishbein, 1976). Typical examples of chemicals frequently encountered in ground water in industrial areas include parts per million to parts per trillion concentrations of chromic acid, hydrochloric acid, sulfuric acid, benzene, toluene, xylene, chlorobenzenes, isopropyl alcohol, methyl ethyl ketone, 1,1,1-trichloroethane, 1,1,2-trichloroethylene, chloroform, and methylene chloride (Fishbein, 1976; Droull - National Academy of Sciences, 1980; Keith and Telliard, 1979; Page and Greenberg, 1980). A more detailed list of chemicals found in ground water supplies is presented in Table 4.

People who drink water contaminated with these chemicals for prolonged periods may be predisposed to develop Chemical Hypersensitivity. Extended periods of exposure to these chemicals may also exacerbate existing disease, or lead to damage of the liver, kidney, and pulmonary alveoli, decrease spermatogenesis, and contribute to deleterious effects on endocrine function. Consumption of drinking water containing low levels of these chemicals is also associated with an increased incidence of rectal, bladder, and colon cancer (Council on Environmental Quality, 1980; Munson et al., 1982; Pereira et al., 1982).

Vinyl chloride or vinyl chloride monomer (VCM) is perhaps the most ubiquitous of the halogenated aliphatic hydrocarbons listed in Table 4. Vinyl chloride monomer is used extensively in the plastics industry to produce polyvinyl chloride, vinyl chloride copolymer (Saran Wrap®), and as a chemical intermediate in large scale production of other chlorinated hydrocarbons (Wheeler, 1981). Vinyl chloride is also found throughout the United States in drinking water (Council on Environmental Quality, 1980). Vinyl chloride is a well known chemical carcinogen which causes liver angiosarcomas in exposed

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TABLE * REPRESENTATIVE HYDROCARBON CHEMICALS IN GROUND WATER SUPPLIES

Aliphatics
Acetonitrile
Acrolein
1,3-Butadiene
Dimethylformamide
Formaldehyde
Methyl alcohol

Chlorinated Aliphatics
bis-Chloromethyl ether
(BCME)
Carbon tetrachloride
Chloroform
Dibromochloropropane
(DBCP)
Dichloroethylene
Methylene chloride
Methyl ethyl ketone
Perchloroethylene
Trihalomethanes (THM)
1,1,1 -Trichloroethane
1,1,2,-Trichloroethylene
Vinyl chloride monomer
(VCM)

Aromatics
Anthracene
Aldrin
Benzo (a) Pyrene
Benzene
Bis-(2-ethylhexyl)-phthalate
Butyl benzyl phthalate
Chlordane
Chlorobenzenes
Dieldrin
Dimethyl phenols
Ethyl benzene
Heptachlor
Lindane
Naphthalene
Nitrophenols
Pentachlorophenol
Phenanthrene
Polychlorinated Biphenyls
(PCB's)
Pyrene
Toluene
Trichlorophenols
Xylene

workers (Fishbein, 1976; Vianna, Brady and Harper, 1981) and cancers of the brain and lung (Infante, 1981).

Low levels of exposure to vinyl chloride over prolonged periods of time produce a multifaceted syndrome recently described as Vinyl Chloride Disease in which patients present with pulmonary dysfunction, sclerotic changes of the skin, Raynaud's phenomenon, hepatic portal fibrosis, thrombocytopenia with T-cell depression, and endothelial vasculitis with circulating immune complexes associated with activation of the complement pathway via C_3 and C_5. The biochemical pathology of Vinyl Chloride Disease is initiated by oxidation of vinyl chloride monomer in the liver and lung to form the free radical metabolites chloroe-thylene oxide and a cyclic chloroethyl dioxide (Ward et al., 1976). These free radical metabolites of vinyl chloride initiate peroxidation of membranes and react to form tissue adducts (haptenic complexes) which lead to the immuno-pathologic sequelae characteristic of this disease. The various pathologies and related symptoms of Vinyl Chloride Disease may be a representative model for

the specific types of free radical damage at the cellular and organ level produced by other chlorinated hydrocarbons shown in Table 3 (Pessayre et al., 1979a; Kluwe and Hook, 1980; Chu and Milman, 1981). Recent studies in experimental animals have also shown that ethanol potentiates the carcinogenicity of vinyl chloride (Radike et al., 1981).

Liver Disease — Alcohol, Carbon Tetrachloride and Drug Metabolism

Acute and chronic ingestion of ethanol in man leads to alcoholic cirrhosis with fatty liver and a decreased protein and caloric intake (Patek, 1979; Russell, 1979). The classical histopathologic changes in the liver are increases in hepatocyte mitochondrial size, disruption of mitochondrial membranes, disarrangement of the endoplasmic reticulum and marked triglyceride deposition in the hepatocyte (Porta et al., 1967; Lieber, 1978). Prolonged consumption of alcohol also leads to a metabolic tolerance for ethanol accompanied by an increased rate of oxidation of ethanol with production of acetal-dehyde by the zinc-containing, liver enzyme alcohol dehydrogenase which is associated with the liver microsomal, cytochrome P_{450} oxidase system (Thurman, 1977; DiLuzio, 1973). The histopathologic changes observed in alcoholic cirrhosis and the oxidative enzyme responses to chronic ingestion of ethanol are consistent with free radical initiated peroxidation of the hepatocyte membranes as the primary patho-physiologic event in chronic alcoholism.

The oxidative metabolism of ethanol, which generates acetaldehyde and free radical intermediates, thus leads to increased oxidant stress and enhanced peroxidation of membrane lipids in the liver. Chronic ingestion of alcohol is also associated with potentiation of hepatotoxic drugs such as acetaminophen, elevations of liver pyridine nucleotides (NADH and NADPH), decreases in the levels of water soluble and fat soluble anti-oxidant vitamins in the liver cells, lowered concentrations of serum selenium and zinc, and the accumulation of uric acid leading to hyperuricemia (Leevy and Ten-Hove, 1967; DiLuzio, 1973; Thurman, 1977; Aaseth et al., 1981, Faller and Fox, 1982).

Both carbon tetrachloride and chloroform produce cellular abnormalities in the liver similar to those seen in alcoholic cirrhosis. Studies with carbon tetrachloride, formerly used as an anesthetic, antihelminthic agent, and industrial solvent have provided a classic model for halogenated, aliphatic hydrocarbon toxicity. Ingestion or inhalation of toxic amounts of carbon tetrachloride leads to centrolobular, hepatic necrosis with deposition of neutral fats in the hepatocyte, significant alterations of the rough endoplasmic reticulum and plasmalemma membranes, and altered protein synthesis by the hepatic parenchymal cells (Smucker, 1976). All of these changes are associated with destruction of hepatocyte cytochrome P450 oxidase enzyme activity, decreased liver content of NADPH, enzymatic dehalogenation of the carbon tetrachloride to form a trichlorome-thane free radical (CCl_3), and peroxidation of membrane lipids in both the liver and lung by CCl_3O_2 (Chen et al., 1977; Weddle et al., 1976).

Numerous therapeutic drugs have also been implicated as direct or proximate causes of hepatotoxicity associated with peroxidation of liver cell membrane lipids. The peroxidation of the membrane lipids is, in some cases, associated with increased cytochrome P_{450} oxidase enzyme activity. Drugs thought to produce hepatotoxicity by mechanisms involving cytochrome P450 oxidase to form a toxic metabolite include acetaminophen, adriamycin, amitriptyline, chlorpromazine, chloroinipramine, dantrolene, dilantin, halothane, imipramine, methotrexate, phenobarbital, probenecid, and tetracycline (Smuckler, 1977; Plaaand Witschi, 1976; Plaa and Hewitt, 1982). Paradoxically, the detoxification of these drugs, and other metabolic toxins requires oxidative biotransformation by the same cytochrome P_{450} oxidase system prior to conjugation and elimination in the bile or excretion by the kidneys. The initial steps in the metabolism of many other chemicals, in addition to the drugs listed above, is also catalyzed by the cytochrome P450 oxidases and mixed function oxidase enzymes located in the hepatic endoplasmic reticulum, and glutathione S-transferases, a liver cytoplasmic enzyme (Biaglow, 1981).

The cytochrome P_{450} and mixed function

oxidase systems hyroxylate these chemicals by a variety of mono and di-oxygenation reactions (Gillette, 1979; Hill, 1979). Hydroxylation by this enzyme system is required for the detoxification of many of the aliphatic and aromatic hydrocarbon molecules listed in Table 1. After these chemical pollutants or drugs have been hydroxylated by the cytochrome P_{450} enzymes, the hydroxyl group formed serves as the site for subsequent conjugation with glutathione, glucuronic acid, sulfate, taurine and glycine moieties, or methyl and acetyl groups. The conjugated toxin is less toxic than the original molecule and more water soluble, and is thus more readily excreted by the kidney (Bentley and Oesch, 1982). The conjugating substances described above are normally available in the liver at varying concentrations; however, their synthesis from precursors or their availability as preformed molecules is dependent on adequate nutritional intake by the individual.

Prolonged fasting decreases serum and cellular levels of reduced glutathione, the coenzyme for the protective enzymes glutathione peroxidase and the conjugate for glutathione S-transferase (Pessayre et al., 1979b; Pessayre et al., 1980). Restricted protein consumption or fasting may, therefore, increase oxidant stresses in Chemical Hypersensitivity patients by reducing their dietary intake of L-Cysteine necessary for the synthesis of adequate tissue levels of glutathione. Low protein intakes may also decrease the levels of glycine or taurine available for conjugation reactions (Pessayre et al., 1979b). Thus, fasting or low protein diets may lead to exacerbation of the signs and symptoms of Chemical Hypersensitivity in ecologically ill patients by impairing the detoxification and excretion of the toxic metabolites formed by cytochrome P_{450} oxidase activation of environmental chemicals or drugs.

Ethanol, Carbon Tetrachloride and Acetaminophen Liver Toxicity Prevented by Antioxidants

The enzymatic activity of the "xenobiotic" cytochrome P450 oxidase system in the liver is induced in response to the presence of foreign chemicals and drugs (Bentley and Oesch, 1982). The free radical metabolites produced by this adaptive response are the ultimate toxic molecules which bind irreversibly to the hepatocyte lipid membranes, proteins, and nucleic acids. Frequently there is an additive or synergistic toxicity associated with exposure to more than one of these oxidant chemicals; this effect is well documented for the enhanced hepatotoxicity which results from exposure to ethanol and carbon tetrachloride (Plaa and Hewitt, 1982) and ethanol and acetaminophen (LeBrecque and Mitros, 1980; McLain et al., 1980).

Studies of the vitamin content of livers from experimental animals exposed to carbon tetrachloride have demonstrated decreases in the B vitamins (B1, B2, and B3), vitamin A, vitamin E, and ascorbic acid. (Shils et al., 1951; Bernheim, 1961). Oral or parenteral administration of the fat soluble vitamins A, E, and ubiquinone (CoQ_{10}), and the water soluble vitamins C, B_{12}, and choline prevent the enhanced lipid peroxidation and cellular pathology produced by ethanol and carbon tetrachloride in rat liver (Hove and Hardin, 1951; DiLuzio, 1967, 1973; DiLuzio and Costales, 1965; Gallagher, 1961, 1964; Russell, 1979; Slater, 1979).

Acetaminophen, which is found in many over-the-counter analgesic formulations, also causes hepatotoxicity when used for long periods or at high dosages (Mitchell and Jollows, 1975; Ockner, 1979; Black, 1980). Acetaminophen induced liver toxicity is also significantly potentiated by ethanol, possibly by mechanisms similar to those observed with carbon tetrachloride and ethanol (LeBrecque and Mitros, 1980; McLain et al., 1980).

In the liver, most of the acetaminophen is conjugated with either glucuronic acid or sulfate for excretion by the kidney; small amounts are, however, converted by the microsomal cytochrome P_{450} oxidase system to N-acetylimidoquinone, the ultimate toxic metabolite. In the presence of adequate hepatocyte levels of glutathione and the enzyme glutathione S-transferase, this toxic metabolite is detoxified and no significant cellular damage occurs. If high dosages (10 to 15 grams), of acetaminophen are ingested or if liver stores of glutathione are depleted, the toxic metabolite, N-acetylimidoquinone, or other free radical intermediate metabolites

bind covalently to hepatocyte proteins and initiate peroxidation of the membrane lipids leading to hepatocellular necrosis and cell death (Black, 1980).

Acute and chronic acetaminophen toxicities in humans are effectively treated and may be prevented by the antioxidants glutathione, N-acetylcysteine, cysteamine, L-methionine, and vitamin E (Walker et al., 1974; Mitchell et al., 1974; Prescott et al., 1979).

Liver Cytochrome P_{450} Oxidase Enzyme Activity Regulated by Vitamin E

Cytochrome P_{450} oxidases, localized in the endoplasmic reticulum of the hepatocyte, may be regulated by levels of corticosteroids and vitamin E (Diplock, 1974; Carpenter and Howard, 1974). Carpenter's studies in rats indicated that alpha-tocopherol (Vitamin E) has a direct role in regulating the activity of phenobarbital induced liver microsomal cytochrome P_{450} oxidases and NADPH-cytochrome c reductase; however, the cliniunable to hydroxylate phenobarbital and other drugs administered to induce the P450 enzyme activity. This hydroxylation deficiency prolongs the exposure of the body to the action of these compounds. When these vitamin E deficient animals were given supplemental vitamin E, they were then able to hydroxylate the drugs as the obligatory first step required for their rapid conjugation and elimination.

The therapeutic implications of these studies in experimental animals for treatment of **Chemical Hypersensitivity** patients is that vitamin E has an apparent role in maintaining the microsomal membrane integrity required for cytochrome P_{450} oxidase activity and also functions to prevent lipid peroxidation by the toxic metabolite formed during the hydroxylation of toxic drugs and environmental chemicals.

Pulmonary Effects of Environmental Chemicals and Drugs

Environmental chemicals and drugs are delivered to the lungs by two primary routes: the bronchial airways and the pulmonary vasculature (Witschi, 1977). The biochemical events associated with exposure to these agents via the respiratory tract are similar to those that occur in the liver during detoxification by the "xenobiotic" enzymes cytochrome P_{450} oxidases and NADPH-cytochrome c reductase; however, the clinical manifestations produced by chemical exposures to the lung are frequently acute and may threaten life by interfering with gas exchange and/or by triggering acute hypersensitivity reactions.

Gaseous oxidants such as ozone, nitrogen dioxide, and cigarette smoke destroy pulmonary cytochrome P_{450} activity and promote peroxidation of membrane lipids with concomitant destruction of reduced glutathione leading to the development of both acute symptoms and chronic disease (Amdur, 1971; Fishbein, 1976b, Kleinerman, 1977; Witschi, 1977; Kaya et al., 1980). The herbicide paraquat is also metabolically activated in the lung to form a toxic metabolite of paraquat and the superoxide radical which initiates peroxidation of alveolar membranes leading to pulmonary fibrosis and death (Copland et al., 1974; Fisher et al., 1973). The antineoplastic drugs bleomycin and busulfan may also cause pulmonary disease by similar free radical initiated lipid peroxidation mechanisms.

Airborne fungal, grass, or plant allergens also cause acute pulmonary edema and respiratory distress by a complex series of events involving increased oxidative metabolism and membrane peroxidation which precede the release of histamine and serotonin. Membrane peroxidation also stimulates the "arachadonic cascade" which leads to the release of prostaglandins E_2 and F_2^{0C}, release of leukotrienes C and D and slow reacting substance (SRS-A), bradykinin, and the thromboxanes which act as initiators of the complement pathway. This complex chain of events, which is triggered initially by free radical lipid peroxidation of membranes of alveolar mast cells, macrophages, and epithelial cells precedes the appearance of clinical signs and symptoms of classical respiratory allergy (Lichtenstein, 1977; Hocking and Golde, 1979; Brain, 1980; Kadowitz et al., 1980; Newcombe et al., 1980; Soter and Austen, 1977, Wasserman, 1980).

TABLE 5
CHEMICAL COMPOSITION OF PHOTOCHEMICAL SMOG

Acetaldehyde	Hydrogen sulfide
Acetone	Methyl ethyl ketone
Acetylene	Nitrogen dioxide
Acrolein	Nitrous oxide
Ammonia	Ozone
n-Butene	Peroxyacetylnitrile
Carbon dioxide	Propylene
Carbon monoxide	Sulfuric acid
Cyclopentane	Sulfur dioxide
Ethylene	Toluene
Formaldehyde	Xylene
Hydrogen peroxide	

Air Pollutants, Photochemical Smog and Ozone

Some of the airborne pollutants arising from automobile exhaust, industrial discharges and photochemical reactions are listed in Table 5. All of these compounds, with the exception of carbon dioxide, are oxidants which may act as irritants to the conjunctival and respiratory epithelium and cause injury to lung tissue upon prolonged exposure (Friedlander, 1977).

The characteristic tissue response to these airborne pollutants is also typical of free radical pathology; alterations in alveolar membrane integrity with release of acid hydrolases, histamine, bradykinin and SRS-A are typical observations which precede the development of clinical signs of edema, hemorrhage, and acidosis associated with pulmonary congestion following exposure to air pollutants (Stokinger, 1965).

The components of smog interact to cause a wide variety of additive and/or synergistic effects which are difficult to define and ascribe to a single cause. Therefore, ozone, which is one of the principal components of photochemical smog derived from the action of sunlight on nitrogen dioxide, has been used in a number of studies to model these complex photochemical pollutant effects. Ozone is also produced by high intensity carbon arc and heliarc sources for generating ultraviolet light and is found in airplane cabins at high altitudes. In Europe, ozone is produced by ozonation equipment during the purification of drinking water.

Although extrapulmonary effects of ozone exposure such as defective desaturation of oxyhemoglobin, loss of mental acuity, and altered taste sensations with loss of appetite have been noted, the primary toxicity occurs in the lung. The early stages of pulmonary toxicity have been shown to occur concomitantly with the oxidation of lung glutathione and vitamin E coupled with the Inactivation of sulfhydryl containing enzymes (McCay et al., 1976). Destruction of these antioxidants and enzymes by ozone leads to the formation of both superoxide anion and peroxidation of the membrane lipids in the alveoli (Stokinger, 1965; Menzel, 1971; Roehm, Hadley and Menzel, 1971). Extensive lipid peroxidation following ozone inhalation is followed by pulmonary congestion and death.

The normal lung response to increased production of lipid peroxides formed during acute ozone exposure is an increase in the synthesis of the selenium dependent enzyme, glutathione peroxidase which detoxifies the lipid peroxides formed. Glutathione peroxidase also protects other sensitive cellular membrane components from further oxidative damage (Chow, Dillard and Tappel, 1974). If the exposure to ozone is prolonged or the nutritional antioxidant levels of glutathione, selenium, and vitamin E are depleted, progressive oxidative damage to the alveolar membranes occurs leading to pulmonary edema and functional respiratory problems characterized as the acute respiratory

distress syndromes (Alpert and Lewis, 1971; Huber and Drath, 1981).

Tolerance to ozone has also been demonstrated after intermittent, short term exposures (Stokinger and Scheel, 1962; Stokinger, 1965). Ozone tolerance is an adaptive response to ozone by the pulmonary antioxidant protective system which increases the lungs' ability to protect susceptible membranes from repeated subsequent exposures to potentially lethal concentrations of ozone or other oxidant gases including ketene, nitrogen dioxide, nitrosyl chloride, and phosgene [a chlorinated hydrocarbon] (Stokinger and Scheel, 1962). Tolerance to all of these oxidant gases is also associated with increases in lung glutathione levels and enhanced activity of the protective antioxidant enzymes glutathione peroxidase and superoxide dismutase (Huber and Drath, (1981). These induced biochemical responses function to minimize the oxidative damage to the alveolar and vascular endothelial membranes by superoxide free radicals and the lipid peroxides generated by these oxidant gases (Forman and Fisher, 1981). These adaptive mechanisms also provide the increased antioxidant defenses necessary to achieve the cross-tolerance to the other oxidant gases described by Stokinger.

Chemical Hypersensitivity patients are uniquely sensitive to air pollutants and other airborne chemicals such as those found in perfumes and lotions. This hypersensitivity is normally associated with the immediate manifestation of pulmonary signs and symptoms of bronchio-constrictive, respiratory distress during or immediately after exposure. Rapid reactions of this severity suggest that the adaptive enzyme responses associated with tolerance induction are not operative or that the nutritional antioxidants ascorbic acid, glutathione, selenium, /3-carotene, and vitamin E are deficient due to restricted dietary intakes or the inability of the patient to regenerate the biologically protective, reduced forms of these antioxidant nutrients. The absence of the protective, antioxidant defense system in the Chemical Hypersensitivity patients exposed to airborne oxidant chemicals thus predisposes them to rapid, extensive free radical peroxidation of lipids followed by the liberation of inflammatory mediators of immediate hypersensitivity. Numerous drugs and environmental chemicals may produce these responses which resemble the classical asthmatic reactions seen in atopic patients (Burrell, 1975; Silverman, 1979).

Acute and chronic exposures to ozone have additional effects on the immune system which are related to changes in white cell membranes (Peterson, 1978). Experimental studies in humans have demonstrated a decreased ability of B lymphocytes to form rosettes which is related to alterations in lymphocyte membrane receptors (Savino et al., 1978; McCombs et al., 1982); autoimmune parathyroiditis with concomitant hypoparathyroidism has been demonstrated in rabbits exposed to ozone (Atwal et al., 1975).

Prolonged low level exposure to oxidant gases in air pollution, the chlorinated hydrocarbons in water, and other environmental chemicals, all acting in concert, may produce cumulative deleterious effects on the immune system which lead to chronic Chemical Hypersensitivity, symptoms such as immunosuppression, autoimmune reactions, and lowered resistance to infection which are frequently seen in Chemical Hypersensitivity disease patients (Loose et al., 1978; Ehrlich, 1980; Loose et al., 1981; Marsh, 1981; Silkworth and Loose, 1981; Heise, 1982). Each of these responses to chemical exposure may be related to the metabolic generation of a toxic metabolite and the production of free radicals which impair the production, maturation, differentiation and specific functions of the white cell populations which function in both humoral and cellular immunity (Ercegovich, 1973; Sharma, 1980; Bick, 1982).

Mechanisms of Pulmonary Disease

Exogenous oxidant chemicals or free radicals generated metabolically can react rapidly with pulmonary membrane lipids to trigger the release of the inflammatory mediators histamine, serotonin, prostaglandins E_2 and $F_2{}^{\alpha c}$, SRS-A, and thromboxane B_2 from the lung mast cells and pulmonary macrophages (Brain, 1980; Kadowitz et al., 1980; Wasserman, 1980). Free radicals can also react chemically with cellular constituents leading to the formation of immunogenic molecules

(haptene-protein complexes) or modified self-proteins with alterations of the specific recognition sequences in the antigenic determinant sites (Perucca and Richens, 1980).

Two different reactions to environmental chemicals are, therefore, possible. The first is strictly chemical in nature, does not involve sensitization, and is dependent upon the oxidant nature or free radical generating capacity of the chemical and the functional competence of the exposed individual's anti-oxidant protective system. Phosgene, COCI2, used formerly as a war gas and currently in the preparation of organic chemicals, provides an example of the insidious nature of this type of immediate, chlorinated hydrocarbon induced oxidant reaction. The initial inhalation of phosgene may not produce immediate signs of irritation in all cases; however, exposure to phosgene is followed rapidly by major symptoms related to the direct chemical action of phosgene on the pulmonary vascular bed as evidenced by immediate constriction of the pulmonary capillaries, rapid and progressive pulmonary edema leading to hypovolemic shock, and death within thirty minutes to several hours (Seaton and Morgan, 1975). The course of this pulmonary congestive response can best be explained by the rapid and extensive formation of free radicals (»COCl and «C1) throughout the airways, within the alveoli, and in the pulmonary capillaries. Propagative free radical attack of the cellular membrane lipids in the pulmonary and vascular endothelium produces putative high levels of toxic lipid peroxides which stimulate the release of endogenous histamine and serotonin and activate the "arachadonic cascade" leading to the liberation of the inflammatory prostaglandins. These events produce the clinical signs and symptoms of phosgene toxicity described earlier.

In addition to the rapid and direct effects seen with phosgene, oxidant chemicals and free radicals can covalently bond to either polysaccharides or protein molecules in the respiratory tract to form haptenic antigens. This leads to chemical allergy mediated by a Type 1 immune response involving the production of haptene specific IgE. Occupational asthma due to toluene di-isocyanate (found in correct-type fluids) and tetrachloroph-thalic anhydride are examples of this type of chemically induced (haptenic) immediate hypersensitivity disease (Seaton, 1975; Schlueter et al., 1978; Perucca and Richens, 1980).

In immune-mediated chemical or drug allergy, there is a latent period between the initial, sensitizing exposure to the oxidant chemical, drug, or to their free radical metabolites. During the initial exposure, haptene-protein conjugates are formed which stimulate the production of the haptene specific IgE which subsequently binds to lung mast cells and basophils. The typical asthmatic response is elicited by re-exposure to the chemical, drug, or free radical; acute respiratory hypersensitivity signs and symptoms ensue. This clinical response is not related to the pharmacologic or oxidant properties of the drug or chemical and may be precipitated by exposure to very small quantities of the sensitizing agent (Burrell, 1975).

Inhalation of minute amounts of fumes, gases, or vapors also produce major symptoms in most Chemical Hypersensitivity patients. This response may be similar to the chemical or drug induced allergic reactions described above. If this is the case, then the respiratory responses in sensitized Chemical Hypersensitivity patients would begin similarly with degranulation of sensitized mast cells following re-exposure to the original chemical or drug, or by direct chemical induction of membrane changes resulting from lipid peroxidation. Degranulation of the mast cells releases histamine, serotonin, prostaglandins E_2 and F_2 , and leukotrienes C and D which form SRS-A. These inflammatory mediators amplify the local inflammatory process and alter vascular permeability; this facilitates the migration of polymorphonuclear leucocytes (PMNL) into the area of the focal tissue injury. The infiltrating phagocytic leucocytes respond with the classical respiratory burst which adds to the oxidant stress (Badwey, Curnutte and Karnovsky, 1979). This produces further depletion of the antioxidant protective system, accelerates the accumulation of lipid hydroperoxides, and stimulates the production of additional inflammatory mediators. This acute inflammatory reaction may progress and produce the lysis of pulmonary macrophages, leucocytes, and respiratory

endothelial cells with the liberation of their lysosomal enzymes and other necrotic breakdown products (Mathe, et al., 1977a, 1977b). The untreated progression of these events further depletes the antioxidant protective system over a larger area of tissue and may result in the development of chronic, inflammatory foci which then predispose individual patients to either cytotoxic (Type III) allergic manifestations of chemical or drug allergy or the development of delayed hypersensitivity (Type IV) reactions (Perucca and Richens, 1980).

Thus, the respiratory symptoms seen in Chemical Hypersensitivity may be initiated by either direct exposure to strong chemical oxidants such as ozone, phosgene, or other air pollutants **or** by immune mediated mechanisms similar to those seen in chemical or drug pulmonary hypersensitivity reactions. Both responses have a common biochemical event; that is, the initial formation of free radicals and the peroxidation of membrane lipids which increases the oxidant stress on the antioxidant protective system (Chow, 1976). Therapeutic use of the nutritional antioxidants to trap the free radicals and prevent or moderate the degree of peroxidation of both alveolar and mast cell membrane lipids should have positive clinical effects in the treatment of Chemical Hypersensitivity disease. This approach has been reported recently by Wold and Seeger who employed high dosages of alpha-tocopherol (vitamin E) to treat chemically induced respiratory distress syndrome (Wold and Seeger, 1981) and by Menzel and Diplock who used vitamin E to prevent peroxidation of membrane lipids in experimental animals exposed to ozone and nitrogen dioxide (Menzel, 1979; Diplock, 1981).

Summary

We have described the fundamental biochemical events which occur in response to exposure to air pollutants, environmental chemicals, and certain therapeutic drugs. The evidence cited has focused on free radical mechanisms and cell membrane lipid peroxidation in the liver and the lung as the initial pathologic events which we speculate occurs in all cells and tissues in response to oxidant stresses induced by these chemicals.

The biochemical rationale predicts that Chemical Hypersensitivity disease will present as a multifaceted syndrome manifesting great variability depending upon the interaction of numerous factors including: the nature of the chemical the patient was exposed to; the amount and duration of exposure; the primary route of exposure; the functional competence of the patient's antioxidant protective system; dietary factors; pre-existing disease states; possible interactions and potentiation by therapeutic drugs and alcohol; and genetic factors.

Effective treatment of Chemical Hypersensitivity therefore requires minimizing future exposure to the specific chemical, drug, or environmental pollutants which produce this free radical pathology while restoring the patient's antioxidant defense mechanisms with the nutrients and co-factors listed in Table 3.

Speculations

1. Chemical Hypersensitivity is a disease manifestation of free radical induced membrane peroxidation and is reversible by the reconstitution of the antioxidant protective system.
2. The acute and chronic hypersensitivity reactions seen in Chemical Hypersensitivity are associated with peroxidation of membranes followed by the release of prostaglandins and other inflammatory mediators.
3. The functional repletion of the selenium dependent, glutathione peroxidase enzyme activity will parallel the recovery of Chemical Hypersensitivity patients; glutathione peroxidase may be a valuable marker enzyme for monitoring the clinical response to nutritional antioxidant therapy.
4. The immunologic sequelae associated with Chemical Hypersensitivity are also symptoms of free radical pathology and can be expected to resolve as the antioxidant protective system and membrane integrity are restored.
5. The immune suppression and susceptibility to infections seen in Chemical Hypersensitivity disease patients will improve with treatment using antioxidant nutrients in conjunction with immuno-stimulation therapy.
6. The combined use of thymus hormones and antioxidant nutrients will be effective in

stimulating T lymphocyte populations and restoring those T-lymphocyte functions which are depressed in most Chemical Hypersensitivity patients.

7. The free radical pathology which underlies the development of Chemical Hypersensitivity disease may also play a primary role in the development of malignancies and in the metastatic spread of cancers.

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