Antioxidant Adaptation:
A Unified Disease Theory

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Abstract

Free radical-mediated oxidative stress to cells and tissues can generate activated oxygen species and lipid peroxides, which mediate inflammatory/immune symptomatologies and are associated with degenerative disease states and possibly with environmental illness (Levine and Reinhardt, 1983. J. Orthomol. Psychiatry 12,166-183). Rats and mice experimentally exposed to high doses of chemical oxidant stressors can become tolerant as a consequence of antioxidant adaptation. Tolerance is characterized by increased activity of glutathione peroxidase (GP), a key antioxidant enzyme which detoxifies peroxide species, subject to adequate availability of its metal cofactor (selenium) and its cosubstrate (reduced glutathione). Increases in other enzymes of the glutathione pathway (glutathione reductase and glucose-6-phosphate dehydrogenase, among others) also are essential. Humans also may adapt biochemically to oxidative stress. In those inherited disease states characterized by abnormally elevated endogenous oxidative stress, peroxide production is enhanced in selected tissues and GP activity is correspondingly increased. GP activity is also abnormally altered (increased or decreased) in other disease states characterized by increased tissue lipid peroxide levels. The price of continued adaptation for humans, as for laboratory animals, may be premature progression to degenerative disease. Herein we hypothesize a "four-stage" clinical progression to degenerative disease, in individuals subject to sustained oxidative stress. This trend may be interrupted or reversed by the cessation of oxidative stress and dietary replenishment of essential antioxidant factors. We suggest that glutathione peroxidase activity (along with other biochemical indicators of oxidative stress) appears to be a valuable marker enzyme for monitoring the clinical response to nutritional antioxidant therapy.

In this article we review the evidence that laboratory animals and humans can adapt to acute oxidative stress, by augmenting

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protective antioxidant factors and/or by mobilizing them to the site(s) of acute oxidant attack. This ability for Antioxidant Adaptation often enables the experimental organism to survive severe oxidative stresses, but does have limits. In the face of sustained oxidative stress, the organism's composite antioxidant defenses may be taxed beyond their capabilities. The likely consequences include chronic degenerative changes foreshadowed by the development of acute chemical intolerances (food and chemical hypersensitivities) (Randolph, 1962; Rea, 1978).

Aerobic metabolism is a paradox of evolution. Air-breathing organisms have advanced beyond their anaerobic ancestors in the relative efficiency with which they can convert nutrient substrates (foods) to chemical energy by utilizing molecular oxygen in metabolism (aerobic metabolism). With this heightened efficiency came a liability: the potential toxicity of oxygen due to the relatively high reactivity and other unique properties of the oxygen molecule. Gerschman had proposed in 1964 that the initial event in oxygen poisoning was the generation of toxic free radicals. Since then it has become unquestionably clear that highly reactive free radicals are generated from molecular oxygen in living systems, as an unavoidable consequence of aerobic respiration. An antioxidant defense system has therefore evolved in air-breathing organisms to defend organelles, cells, and tissues against endogenous, free radical-mediated oxidative stress.

This antioxidant defense system is highly sophisticated. It utilizes nonenzymatic nutrient-derived antioxidants acting in concert with nutrient-modulated antioxidant enzymes, to neutralize and thereby detoxify oxygen radicals and other "activated" species derived from molecular oxygen (Chow, 1979; Forman and Fisher, 1981; Freeman and Crapo, 1983). The antioxidant defense system appears to be highly integrated and able to react adaptively in response to oxidative challenges, subject to regulation by the availability of nutrient-derived cofactors, cosubstrates, and other antioxidant compounds. Any substantial shift in local oxidation-reduction potential arising from intensified exogenous or endogenous oxidant stress threatens to impair the functioning and ultimately the viability of the cells and tissues affected; the antioxidant system attempts to resist such changes by a variety of inter-related, and inter-regulated mechanisms (Levine and Reinhardt, 1983; Forman and Fisher, 1981). Major sources of exogenous oxidative stress are environmental pollutant chemicals, drugs, physical trauma, and foods. Another possible major source is emotional stress, which appears capable of precipitating injury by oxidative mechanisms. (Schenkman et al., 1979).

Free Radical Attack, Biologic Damage, and Adaptation

Oxidative stress to the organism can be intensified beyond the basal level (Forman and Boveris, 1982) by a variety of endogenous and exogenous factors. Free radicals derived from circulating catecholamines are one possible endogenous source. Catecholamines can be converted to reactive oxidizing derivatives either by spontaneously decomposing in the presence of oxygen ("autoxida-tion"), or by Mixed-Function Oxidase activation (Misra and Fridovich, 1972; Schenck-man et al., 1979)

Oxidized derivatives of catecholamines have been implicated in schizophrenia (reviewed in Hoffer, 1983), and may (at least in part) mediate the immune suppression and other physiologic deterioration known to result from sustained emotional stress (reviewed in Vander, 1981).

Photochemical smog is a major exogenous source of oxidative stress (Levine and Reinhardt, 1983). Photochemical smog contains the potent oxidants ozone, nitrogen dioxide, peroxyacylnitrates, and numerous hydrocarbon-derived free radical species (Friedlander, 1977). Cigarette smoke, a major indoor air pollutant, also subjects the lungs (and other organs) to chronic oxidative stress (Small et al., 1983). Environmental pollutant chemicals and drugs (xenobiotic or "foreign" substances) are subject to metabolic transformation by the Mixed-Function Oxidase (MFO) enzyme complex located in the liver, lung and other organs. The metabolites of some xenobiotics can be extremely toxic, due either to their high reactivity as radicals (usually having a tendency to form covalent adducts with biomolecules) or to

their ability to reduce molecular oxygen to oxygen free radicals by "redox cycling" (Kappus and Sies, 1981). In the latter case superoxide anion and other oxygen radicals then mediate oxidative stress to the tissues.

Tolerance and Adaptation. The antioxidant defense system can adapt to severe, localized oxidative stress, whether acute or chronic, as evidenced by the augmentation of antioxidant factors in the tissue or organ subject to oxidant attack. Such localized antioxidant adaptation (tolerance) has been demonstrated particularly well in the lung tissue of rodents, in response to ozone, cigarette smoke and other airborne oxidant stressors (Stokinger, 1965). Laboratory animals exposed to a relatively high but sub-lethal dose of an airborne oxidant such as ozone, can become tolerant to this oxidant. Simultaneously they develop a cross-tolerance to other oxidant gases, i.e., those which attack lung tissue by free radical-mediated oxidative mechanisms. These include ketene, nitrogen dioxide, nitrosyl chloride, and phosgene (a chlorinated hydrocarbon) (Stokinger and Scheel, 1962). Upon reexposure to ozone, or de novo exposure to any of these other oxidant compounds, tolerant animals can survive levels of exposure that would otherwise be lethal.

The development of tolerance to ozone requires only a single, brief exposure (one hour or less) to concentrations of from 0.3-3 parts per million (Stokinger, 1965). Tolerance can develop rapidly (beginning within 30 minutes) and is manifested biochemically as increases in the activities of protective antioxidant enzymes in the lung tissue. The tolerant state can remain in effect for several weeks (i.e., up to 4 weeks in rats and 14 weeks in mice). Stokinger found that tolerance can be repeatedly reinstated by intermittent oxidant exposures. Alternatively, tolerance can be reduced by continual, repetitive oxidant stress.

Stokinger, a pioneer investigator of ozone toxicology, was the first to demonstrate tolerance by finding that continuous exposure of his experimental animals (mice) to high (sublethal) levels of ozone was more injurious than intermittent exposures to the same levels. Recently Chow (1979, 1982) and Mustafa et al. (1982) showed that the activities of the "front-line" antioxidant enzyme glutathione peroxidase (GP) and its "support" enzymes glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PD) became elevated in rat lung tissue as tolerance developed to ozone.* This linked triad of enzyme activities also becomes increased after exposure of rats to cigarette smoke (York et al., 1976).

The enzymatic adjustments which are manifested as increased tolerance to oxidant attack are subject to nutritional modulation, i.e., by the dietary availability of antioxidant cofactors and cosubstrates. Dietary sufficiency of the trace element selenium (Se, the metal cofactor of GP) and the sulfur amino acids cysteine and methionine (components of the antioxidant tripeptide reduced glutathione) is essential for GP to function. Thus dietary restriction of Se lowers the "baseline" activity of GP in the unstressed mouse lung, and upon subsequent exposure to ozone the usual adaptive increase in lung GP activity does not occur (Elsayed et al., 1982). Dietary supplementation with cysteine and methionine protects against the toxicity of hyperbaric oxygen, which is also an oxidative stressor (Balen-tine, 1982). Lung glutathione levels normally increase during the development of tolerance to hyperbaric oxygen; dietary deficiency in cysteine prevents this adaptive increase and results in higher mortality upon reexposure of the test group to this oxidant (Forman et al., 1983).

The development of tolerance appears to be essential for the survival of experimental animals exposed to high doses of oxidant chemicals, but Stokinger (1965) has cautioned against any "misimpression" that tolerance is all-encompassing. He stresses that degenerative effects may result from long-term exposure of tolerant animals to ozone. Stokinger recognized three categories of long-term degenerative effects in mice rendered tolerant to ozone: chronic pulmonary effects, premature aging, and lung-tumor acceleration in a cancer-

*GR reconverts oxidized glutathione to reduced glutathione, the cofactor required for GP; G6PD is the first enzyme in the hexose monophosphate shunt, the pathway through which reducing equivalents are made available for GP in the form of NADPH.

susceptible strain. Chronic bronchitis, bronchiolitis, and emphysematous and fibrotic changes developed in the pulmonary parenchyma of rats and mice exposed daily to 1 ppm ozone. In rabbits exposed to ozone just once per week for one hour the walls of the alveolar air sacs progressively collapsed, leading eventually to emphysematous changes. Stokinger concluded from these findings:

These irreversible changes develop in an animal tolerant to the acute inflammatory effects. Precisely how these two conditions exist side by side is unclear, but some indirect evidence on the relative ease of development of tolerance and chronic pulmonary change by various pulmonary irritants leads one to suspect that the two may be interrelated; indeed, tolerance may be the initiating mechanism (Stokinger, 1965, p. 724).

Humans continually exposed to oxidants may well be in an adapted state. Southern California residents suffer chronic exposure to ozone and the other oxidants present in the polluted air of the Los Angeles basin, whereas Canadians are not generally exposed to such high levels of photochemical oxidant pollutants. When experimentally exposed to ozone, a group of Canadian subjects exhibited greater clinical and physiologic reactivity than a group of "healthy" Southern Californians, who were only minimally reactive (Hackney et al., 1975). These findings suggest that the Californians may have been somewhat adapted to photochemical smog (i.e. "used to it"). In light of these preliminary findings and the more extensive animal studies which we have just reviewed, we suggest that more such controlled comparisons between human populations should be initiated.

The High Cost of Adaptation. Adaptation to ozone or other oxidant stressors (by the development of tolerance in the target tissue) has an obvious short-term advantage: survival. For Stokinger's experimental animals, adaptation to ozone by developing tolerance in their lung tissue meant the difference between life and death. Animals not pre-adapted to ozone, i.e., those which had not been previously exposed to sublethal levels, died on their initial exposure to high ozone levels. Adapted animals survived reexposure to high levels of ozone or oxidant gases. According to Stokinger, these adapted animals later paid a price: ongoing (repeated) exposure to oxidants led to the premature development of chronic degenerative diseases.

We suggest that the long-term limitations inherent in the development of tolerance by one organ system (in this case, the lung) to toxic airborne chemical exposures, must be placed in the context of adaptation by the whole organism to potentially fatal oxidant poisoning. The antioxidant defense system is inducible to confer tolerance on the exposed organ(s), but if the oxidative stress persists the organism's systemic antioxidant capacities must become compromised. A continued diminution in these composite antioxidant defense capacities could then lead to uncontrolled, free radical-mediated peroxidation of cell membrane lipids and the liberation of mediators of inflammation; crosslinking of membrane macromolecules, antigenic modification and immune dysfunction; and eventually to diverse degenerative symptomatologies (Levine and Reinhardt, 1983). Degenerative pathologies may be mediated systemically by circulating end-products from lipid peroxidation, such as malondialdehyde and lipid hydroperoxides, as reviewed in a recent international symposium (Yagi, 1982).

If severe exposure to stressful oxidants is sustained, at some point the organism's ability to adapt to oxidant stress (either local stress as to the lung or systemic stress as mediated by circulating oxidant factors) will reach an exhaustion stage, which is likely to be associated with further inflammatory symptomatology and immune depression. The long-term consequences of pulmonary degeneration, impaired respiratory function, premature aging and lung tumor acceleration observed in rodents by Stokinger are very likely just a few of many systemic changes which can result from deterioration of the antioxidant defense system.

Adaptation and Glutathione Peroxidase. Adaptation to oxidant attack apparently occurs by way of selective utilization of antioxidant factors by those tissues and organs currently subject to greater degrees of oxidative stress, at the expense of others less vulnerable. According to Tappel (1980),

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tissue distributions of antioxidant factors are heterogenous even in the "unstressed" state, possibly reflecting greater vulnerability of certain tissues to oxidative stress and therefore greater need for protective antioxidant factors. In the face of sustained oxidative stress (unless the body's systemic complement of antioxidant factors can be built up rapidly), we might expect to see a concomitant decrease in antioxidant defenses in certain less vulnerable (or less essential) tissues and organs. Glutathione peroxidase (GP) is a critical enzyme of the antioxidant defense system (Chow, 1979; Flohe, 1982). Stokinger's test animals responded to acute oxidant attack with markedly increased activity of GP in their lung tissues; this appears to be a characteristic feature of adaptation to oxidative stress (Witschi, 1977). The enzymes which "support" GP, i.e., GR and G6PD, often undergo a parallel increase in activity (Chow, 1982; Mustafa et al., 1982). Their activities may be feedback-regulated by GP, reflecting the increased demand for reduced glutathione and NADPH as the activity of GP increases. Hence, some tissues appear to have priority over others in synthesizing and deploying GP and other critical antioxidant defenses.

The patterns of distribution of selenium (Se) in tissues and organs may regulate their ability to adapt to oxidant attack. Se atoms are located at the active site of the GP tetramer and are essential for its activity (Flohe, 1982). Under conditions of dietary Se deficiency, this essential trace metal is selectively retained by certain tissues, particularly those of the immune (reticuloendothelial) system (Spallholz, 1981). Tissues of the spleen, lymph nodes, thymus, and adrenal selectively retain Se during dietary deprivation; Se falls to very low levels in red blood cells, liver, kidney, muscle, heart, pancreas, and thyroid. Since the increase in GP activity which occurs in lung tissue in response to oxidant attack appears to be modulated by Se availability (Elsayed et al., 1982), those tissues least able to retain Se (and by implication GP activity) are likely to be those which become most easily compromised by sustained oxidative stress.

Lipid Peroxide Attack and GP Response. Since the primary function of GP is to detoxify peroxides, the ability of tissues to respond adaptively to oxidant attack with increases in glutathione peroxidase activity can be tested directly by feeding lipid peroxides to test animals. Normally the GP of the intestinal mucosa can detoxify small amounts of lipid peroxides ingested as part of the normal diet, by reducing them to their corresponding nontoxic alcohols. In contrast, dietary administration of high levels of lipid peroxides can apparently overwhelm the detoxicative capacities of intestinal GP, thereby allowing significant quantities of peroxides to enter the circulation and eventually reach other tissues, particularly the liver. Reddy and Tappel (reviewed in Tappel, 1980) studied the effects of dietary Se on the ability of rats to detoxify dietary lipid peroxides (peroxidized corn oil). In the test group not fed peroxides, the specific activity of GP in the GI tract, liver, blood, and adipose tissue was higher with Se-supplementation than without Se-supplementation. Thus dietary Se availability limits (i.e., modulates) tissue GP activity in rats, even in the absence of oxidative stress. In two groups fed peroxides, those supplemented with Se showed no increase in GP activity above the basal levels while those not supplemented showed selectively increased GP activity in their red blood cells, plasma, liver, and adipose tissue. This finding suggests that Se- supplemented rats were capable of detoxifying the dietary levels of peroxides to which they were subjected, without a need for adaptive augmentation of GP in selected tissues. The group not supplemented with Se apparently were not similarly capable: peroxides accumulated in their adipose tissue in spite of an increased GP activity in this tissue. It seems that these non-supplemented rats attempted to react adaptively by way of marked augmentation of GP activity in selected tissues, but nonetheless suffered toxic effects from chronic ingestion of peroxidized lipids, as evidenced by their markedly lower weight gain over the course of the experiment.

In related studies, Tappel and his collaborators showed that the levels of GP and its support enzymes become increased in response to oxidative stress arising from deficiencies in other critical antioxidant factors (Tappel, 1980). One of these was alpha-tocopherol (vitamin E). In rats fed a tocopherol-depleted corn oil diet the activities

of GP, GR and G6PD increased in several adipose tissues and in muscle, concomitant with abnormal elevation of lipid peroxides in these tissues. The activities of these enzymes did not increase in the liver, lung, or kidney, those organs in which lipid peroxides did not accumulate. In reviewing these results, Tappel concluded:

The ability of animals to respond to oxidative stress by increasing the activity of glutathione peroxidase is the main feature of the protective system... The presence in various tissues of the glutathione peroxidase system supports the view that the protective mechanism is operative throughout the body. (Tappel, 1980, p. 44).

The interrelated functioning of glutathione peroxidase and the various other components of the antioxidant defense system has been particularly well studied in mammalian red blood cells, which are highly susceptible to lipid peroxidative damage.

Red Cell Oxidative Stress and Disease States

Red cells may not be the best "model" system for the study of cellular responses to oxidative stress, due (among other reasons) to their limited ability to repair and re-synthesize damaged constituents. Such studies are nonetheless useful because red cell membrane structure and function has been studied extensively and their metabolism is comparatively well understood. Peroxides are oxidizing, "activated" oxygen species. In experiments done in *vitro* by Wilkins (1979), sheep red cell glutathione peroxidase activity became increased in direct proportion to hydrogen peroxide added to the medium. This finding was a direct demonstration that red cells can adapt to oxidative stress by enhancement of the activity of the glutathione peroxidase which they contain. A number of our most common and well-understood genetic diseases appear to specifically affect the antioxidant defense capabilities of the red blood cell (Stanbury et al., 1983). These serve as genetic models with which to study the adaptability of the antioxidant defense system.

The circulating red cell is normally subject to a high level of oxidative stress (Chiu et al., 1982). It is bathed alternately in high oxygen concentrations in arterial blood and low oxygen concentrations in venous blood. The red cell has a high content of iron, a redox-active metal which facilitates oxidative electron transfer even under non-stressed conditions; and it has a limited ability to repair damaged macromolecular constituents, due to its lack of a nucleus. The red cell demonstrates the ability to respond adaptively to abnormal elevations in oxidative stress to which it is subject. These responses have been well studied biochemically in relationship to hereditary hemolytic diseases.

Reactions which occur under normal conditions between hemoglobin and oxygen in the red cell (catalyzed by Fe at the active site of hemoglobin) produce significant amounts of superoxide anion as a byproduct (Chiu et al., 1982). The accumulation of superoxide anion and other "activated" oxygen species presents a potential threat to the integrity of the red cell plasma membrane and therefore to its viability. The antioxidant enzyme superoxide dismutase (SOD), which detoxifies the superoxide anion, is thought to present "the first line of defense" against free radical damage in the red cell. SOD converts ("dismutates") the superoxide radical to hydrogen peroxide (Fridovich, 1982). Hydrogen peroxide is also potentially toxic, and must in its turn be detoxified by glutathione peroxidase, which under suitable conditions converts peroxides to alcohols (Forman and Fisher, 1981). The following examples serve to establish that GP increases adaptively in response to hereditary defects which increase oxidative stress on the red cell, subject to modulation by the availability of its metal cofactor selenium and its cosubstrate reduced glutathione.

Glutathione Peroxidase, NADPH, and G6PD Deficiencies. Glutathione peroxidase has an indirect requirement for NADPH as a source of reducing equivalents (electrons). NADPH is required to reconvert oxidized glutathione, an endproduct of the glutathione peroxidase reaction, to reduced glutathione for reuse by GP as necessary. Under conditions which limit NADPH availability the red cell becomes more susceptible to oxidative hemolytic destruction, resulting in acute or chronic hemolytic disease. The NADPH required for GP to function

optimally must be generated from glucose oxidation by way of the hexose monophosphate shunt (HM Shunt). Four enzymes make up this metabolic pathway, and abnormalities or deficiencies in any of these could compromise the pathway and limit NADPH availability. Hereditary abnormalities in glucose-6-phosphate dehydrogenase (G6PD), the first enzyme in the Shunt, are by far the most common group of conditions which limit NADPH production in human red cells; their usual clinical manifestation is chronic hemolytic anemia (Beutler, 1983). This disease state is characterized by red blood cell hemolysis, liberation of Heinz bodies (products of autoxidation of hemoglobin) into the plasma, and a rapid fall in hematocrit.

Most G6PD-deficient individuals are hematologically normal in the absence of abnormally high oxidative stress. Upon oxidative challenge, however, the red cell can undergo structural transformations which often result in its destructive lysis (hemolysis). Spectrin, a "peripheral" membrane protein which lies against the cytoplasmic face of the plasma membrane of the red cell, is linked with proteins deeply embedded in the membrane (Alberts et al., 1983). Spectrin is thought to regulate red cell deformability through these linkages. In G6PD-deficient cells, spectrin is particularly sensitive to oxidative stress, tending to aggregate and render the cell membrane abnormally rigid (Chiu et al, 1982). These changes eventually result in the premature hemolytic destruction of the cell. The degree of clinical expression of the G6PD-deficiency phenotype can vary markedly from patient to patient, but elevated GP activity is a consistent finding. Beutler (1977) surveyed red cell GP activity in 69 subjects with various hematologic disorders, including several with G6PD deficiencies. The subjects as a group averaged significantly higher GP activity than normal, but the G6PD subjects exhibited a more marked elevation in red cell GP activity.

Sickle Cell Anemia and the Thalassemias. Inherited hemoglobin abnormalities also can increase oxidative stress on the red cell. Sickle cell anemia arises from a hereditary defect which leads to altered structure of the B-globin chain of hemoglobin (Winslow and Anderson, 1983). Sickled hemoglobin has a tendency to aggregate upon deoxygenation of the red cell, thereby deforming the cell into a sickle shape. Upon reoxygenation, most cells resume their biconcave shape but some do not. Such "irreversibly sickled cells" (ISC) can become stuck in capillaries, thereby occluding them and often precipitating painful ischemic episodes for the afflicted individual. Lipid peroxidative damage may be important in sickle cell anemia (Chiu *et al.*, 1982). Approximately 3% of the hemoglobin in the red cell is normally converted to methemoglobin daily, generating superoxide anion which can peroxidize polyunsaturated fatty acids in the red cell membrane. Deficiency in the lipid-phase antioxidant alpha-tocopherol (vitamin E) exacerbates the tendency of sickle hemoglobin towards irreversible, peroxidative crosslink-ing.

The thalassemias are another family of inherited hemoglobin diseases, also characterized by defects in the globin chains which render them more susceptible to oxidation (Yan, 1983). As in sickle cell anemia, superoxide anion is produced in greater quantities in thalassemic conditions. Malondialdehyde (MDA) production is increased, this causes peroxidative crosslinking of PUFA's in the membrane, and eventually hemolysis occurs. Thalassemic patients are often overloaded with iron due to frequent transfusions; the presence of elevated quantities of Fe in the vicinity of the red cell may enhance the peroxidative hemolytic process due to its redox-active character (Demopoulos, 1982).

Red blood cell glutathione peroxidase levels are elevated both in sickle-cell anemia and in the thalassemias (Chiu et al., 1982). In the G6PD deficiencies, GP activity goes up even though the availability of reducing equivalents (NADPH) is limited. This observation can be explained from in *vivo* and *in vitro* experiments by Perona et al. (1978), which suggest that GP can become alloster-icaly activated by oxygen radicals generated via the superoxide anion. According to this model, glutathione peroxidase could "turn on" almost concurrently with increased oxidative stress on the red cell, subject to the availability of selenium, its trace metal

cofactor. Radioactive selenium injected into the bloodstream (as the Selenite salt) is readily taken up by the red cells, causing an almost instantaneous rise in their GP activity. This suggests immediate, almost instantaneous, activation of a Se-depleted GP precursor. According to this model developed by Perona and his co-workers, the net GP activity in the circulating red cell depends on the balance between Se availability, enzyme activation from oxidative stress, and enzyme decay during cellular aging.

The foregoing examples make it clear that GP activity increases adaptively in certain heredity defects, i.e., sickle cell anemia, G6PD deficiency, and the thalassemias (Stanbury et al., 1983). These inborn errors of metabolism are damaging to the organism largely by enhancing the susceptibility of the circulating red blood cell to oxidative stress. The red cell attempts to increase its production of NADPH with which to fuel the reduction of glutathione (the reduced form of which is an essential substrate for GP); in G6PD deficiency this attempt can be futile. Most individuals who have these genetic defects are generally asymptomatic except under conditions of elevated oxidative stress, which then act selectively to exacerbate the inherent metabolic defect.

These well-studied genetic models serve to illustrate our point. As we probe deeply into the mechanisms underlying these inherited disorders of the red blood cell, we see that as macromolecular structure is damaged so will function be impaired, and this impairment must ultimately make us more susceptible to oxidative damage. We should attempt to understand biological stress as that which increases some parameter of oxidative stress to the organism. The question then arises: Does red cell glutathione peroxidase activity increase adaptively in other disease states characterized by elevated oxidative stress?

Red Cell GP and Human Chemical Exposures. Studies on humans indicate that GP activity can be altered significantly as a result of environmental chemical exposure. "Hard data" on tissue GP activities and Se status is generally lacking for humans, but the information presently available is provocative and consistent with our thesis of Antioxidant Adaptation. Griffin and Lane

(1981) studied 229 subjects (with "unremarkable" medical histories) for red cell and plasma Se concentrations, and red cell GP activities. They compared normal subjects who were considered to have been occupationally exposed to chemicals (these individuals worked in an oil refinery) with others considered to have been less chemically exposed (these individuals were employed at schools and a hospital). The mean values for all three measures of selenium status were lower in the occupationally-exposed population*. Griffin and Lane also compared smokers with non-smokers from the same sample population. The group that smoked had lower mean erythrocyte selenium and GP activity. There were relatively high standard deviations in the data from both of these comparison studies. These high standard deviations reflect the possibility that the sampled subjects are experiencing varying degrees of adaptation to oxidative stress. Recall that GP and its support enzymes GR and G6PD become elevated in the lung tissue of rats exposed to cigarette smoke or ozone (reviewed in Lee et al., 1982). Chemical oxidant poisoning of these human subjects (as precipitated by exposure to oil refinery byproducts or cigarette smoke) may lead to an initial adaptive increase in pulmonary and red blood cell antioxidant defenses (i.e., tolerance), and to an eventual decrease in red cell antioxidant defenses when their systemic antioxidant capacities become sufficiently compromised. Red cell antioxidant defense status may be a valuable indicator of systemic antioxidant defense capability.

Abnormal GP Activity; in Systemic Disease. Data from other studies on human

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*We must emphasize that the alteration of GP activity in oil refinery workers presumably occurs in response to hydrocarbon byproducts. It is likely that the toxic species are not the parent hydrocarbon compounds but radical oxidizing derivatives of them. The "primary" (parent) hydrocarbons are oxidatively converted to free radical derivatives either in the atmosphere, the inhaled air, or intracellularly following absorption. It is these oxidizing (radical) derivatives of pollutant hydrocarbons which appear to mediate their toxic effects (Levine and Reinhardt, 1983; Mason and Chignell, 1982).

subjects indicate that GP activity is lower in certain systemic disease states. These findings cannot be taken as direct proof that the patients studied were in an adapted state; rather they suggest a trend and justify the need for further studies to directly test the Antioxidant Adaptation hypothesis.

Red cell GP activity and Se levels were significantly decreased in patients with untreated head and neck cancers (squamous cell carcinoma), and plasma Se levels were increased (Goodwin et al., 1983). Plasma Se levels were further increased in those patients with advanced disease and significant weight loss. The investigators suggested their patients might have impaired ability to transport Se from the plasma into the red cell for incorporation into the GP enzyme. Such impairment of Se transport could result from damage to red cell membrane transport proteins, which are known to be highly sensitive to oxidants (Chiu et al., 1982; Carafoli and Scarpa, 1982). A possible alternative explanation is that Se is liberated into the plasma from damaged subunits of the GP enzyme, which is itself susceptible to oxidative damage (Ganther and Kraus, 1981).

Glutathione peroxidase activity has also been measured lower in common skin diseases. A Swedish study of more than 500 patients with a broad range of skin disorders by Juhlin et al. (1982) found that (whole blood) GP activity was markedly reduced in the vast majority. Disorders which exhibited decreased GP activity included atopic dermatitis, eczema, psoriasis, vasculitis mycosis fungoides, and dermatitis herpetiformis. Treatment with Se and vitamin E for 6-8 weeks raised red cell GP levels markedly and had an overall beneficial effect, particularly on those patients with severe protracted seborrheic dermatitis.

Although there have been relatively few studies on human red cell GP and disease, the data currently available emphasizes that a broad spectrum of oxidative stressors, both endogenous and exogenous in origin, consistently affect red cell glutathione peroxidase activity. Alterations in GP and in other antioxidant factors, particularly the enzyme superoxide dismutase (SOD) are also evident in several common mental diseases.

Adaptation to Oxidative Mental Imbalances

Certain mental diseases display abnormal oxidant metabolism, accompanied by indications of antioxidant biochemical adaptation to the resulting oxidative stress. Down's Syndrome (DS) results from an inherited trisomy in chromosome 21, and is probably the single most common mental disorder in the United States (Lott, 1982). Down's Syndrome is consistently associated with premature aging: DS patients develop a progressive dementia which resembles that of Alzheimer's disease. Down's Syndrome is also associated with increased frequencies of cataracts, diabetes, pulmonary infections, leukemia, endocrine dysfunction, and neurotransmitter abnormalities, although atherosclerosis may occur less frequently in DS individuals (Patterson, 1982).

Increased activity of the antioxidant enzyme Cu,Zn-SOD (copper, zinc-dependent superoxide dismutate) (Fridovich, 1982) is a consistent characteristic of both non-nucleated and nucleated cell types from these trisomy-21 subjects (Sinet, 1982). This is presently accepted as a gene dosage effect: the SOD gene is carried on chromosome 21, and is therefore inherited by DS individuals as a third copy. However glutathione peroxidase and HM Shunt activities also are elevated in both the red and the white blood cells of DS individuals (Sinet, 1982). This finding cannot be readily explained as a gene dosage effect; rather it seems that the GP enzyme (and its supply of reducing equivalents by the HM Shunt) increases adaptively, possibly as a result of allosteric activation according to the model of Perona et al. (1978). This could result from increased availability of peroxide substrate (H_2O_2) due to the increased SOD activity manifested by trisomy 21 cells.

Down's Syndrome subjects, like individuals with Alzheimer's disease, show a tendency towards premature mental aging. This tendency could be attributed to accelerated peroxidative damage to their brain tissue. Brain neurons of DS subjects exhibit what may be premature degenerative changes, including lipofuscin pigment accumulation which is a known characteristic of peroxidative crosslinking of biomolecules (Sinet, 1982). The observation of accelerated

senescence in fibroblasts cultured from Down's patients, and the immune dysfunctional changes which occur in these individuals, also support the conclusion that DS subjects age faster. The various degenerative mental changes observed in DS subjects could result from the generation of abnormally increased quantities of hydrogen peroxide in brain neurons. Red cell GP activity and I.Q. are positively correlated in DS subjects — the higher the GP activity, the higher the subject's I.Q. (Sinet, 1982). If elevated GP activity in circulating red cells actually reflects elevated GP activity in brain tissue, this would suggest that adaptive increases in GP activity protect (at least partially) against the premature loss of I.Q. in Down's Syndrome.

Brain Tissue Particularly Sensitive to Oxidative Stress. The increased cellular production of hydrogen peroxide which occurs in Down's Syndrome may preferentially affect the brain precisely because neural tissue is particularly sensitive to oxidative stress. Ischemic or traumatic injuries damage the brain and spinal cord more readily and more extensively than other organs, and Demopoulos (1982) has suggested several reasons which might account for this finding:

1. Oxygen is seven to eight times more soluble in non-polar compartments, including the hydrophobic zone of the lipid bilayer of biological membranes, thus rendering lipid bilayers preferentially susceptible to peroxidative attack by activated oxygen species;

2. Neuronal membrane systems, especially mitochondria and myelin sheath membranes, are especially rich in polyunsaturated fatty acids, and may therefore be particularly sensitive to peroxidative attack;

3. Neurons (through their high mitochondrial content) have an unusually high rate of oxidative phosphorylation, presumably to support their large complement of ion pumps. Neurons are therefore enriched in ubiquinone (Coenzyme Q), a component of the respiratory electron transfer chain which can readily autoxidize in low oxygen tensions to produce super oxide anion (Forman and Boveris, 1982). Since neural tissue appears to be preferentially susceptible to oxidative stress, the most attractive interpretation of the

Down's Syndrome findings is that increased oxidative stress mediated by abnormally high endogenous production of hydrogen peroxide preferentially damages neurons in the brain. This results in deterioration of brain function (manifested as lowered I.Q.), derangement of neuronal organization (manifested as the dementia which resembles premature senility), and deterioration of neurons (manifested as lipofuscin accumulation). The elevated levels of GP and HM Shunt activity in blood cells of DS subjects suggests systemic antioxidant adaptation to their systemic genetic affliction.

Down's Syndrome as a Genetic Model of Adaptation. As evident in Down's Syndrome subjects, antioxidant adaptation occurs in response to inherited abnormalities which ultimately manifest as increased oxidative stress. The biochemical phenotype for DS appears to be elevated SOD activity, which results in elevated intracellular levels of hydrogen peroxide. This in turn results in an adaptive increase in GP-HM Shunt activity as the body attempts to compensate. The elevation of GP activity in this genetic model of elevated oxidative stress strongly supports our contention that red blood cell GP levels can be used as an indicator of systemic oxidant stress.

It seems likely that abnormally elevated red cell GP levels indicate some degree of ongoing adaptation to oxidative stress, whereas abnormally decreased levels may indicate exhaustion or impending failure of the antioxidant defense system. The existing literature suggests that ongoing oxidative stress eventually results in reduced red cell glutathione peroxidase activity. However the limited data presently available is likely to be an underestimate of the magnitude of alteration of GP activity in response to oxidative stress. We expect that the ability of the body to adapt would result in increased GP activity until just prior to the onset of antioxidant exhaustion, depending on the individual's systemic capacities. Though ongoing oxidative stress is likely to eventually depress red cell GP, as was evident from the example of untreated head and neck cancers (Goodwin et al., 1983), individual variability in the adaptive response

is likely to reduce the apparent significance of the data until the end-stage of exhaustion is approached, as was evident from the comparison between smokers and non-smokers (Griffin and Lane, 1981).

Data from other mental disorders also demonstrate a consistent relationship between elevated oxidative stress and abnormal red cell GP levels, further suggesting that oxidative stress is a primary mediator of mental degeneration and senility.

Oxidative Stress in Schizophrenia. Some 30 years ago Dr. Abram Hoffer and his collaborators suggested that oxidative stress was a causative factor in schizophrenia (reviewed in Hoffer, 1983). They proposed that adrenaline and related catecholamine neurotransmitters could become irreversibly oxidized *in vivo* to adrenochromes, highly reactive compounds with free radical properties and able to initiate peroxidative damage to brain tissues. Adrenochromes are hallucinogenic synaptic poisons, and are known to mediate damage to the brain from hyperbaric oxygen. Normally, oxidized catecholamines would be reconverted to their reduced forms in the presence of adequate levels of "reducing equivalents" carried in reduced pridine nucleotides (NADH or NADPH). Deficiencies in reducing equivalents in the brain could impair this re-reduction process or prevent it from occurring. Conversely, antioxidant factors in the brain (e.g. ascorbate at its normally high levels) would be expected to inhibit adrenaline oxidation.

Newer evidence lends support to Hoffer's hypothesis that schizophrenia is related to brain tissue damage from oxidized neurotransmitter derivatives.

Important findings on oxidative damage in schizophrenia were presented at the Academy *of* Orthomolecular Psychiatry (AOP) Meeting by Pecora and Shriftman in 1983. Chronic undifferentiated schizophrenics were found to be hypoglycemic, with a high fasting insulin level. The vast majority of these patients (some 79 percent) had significantly elevated serum lipid peroxide levels, indicative of systemic oxidative stress. The investigators suggested this was due to stimulation of fatty acid ligases via membrane-bound oxidases (perhaps as a consequence of hyperinsulinemia) with the

production of hydrogen peroxide, resulting in peroxidation of serum lipids. An even higher proportion of these patients (86 percent) had significantly lowered red cell glutathione peroxidase levels. Of the remaining 14 percent who did not exhibit significantly lowered red cell GP, half (7 percent) had significantly elevated red cell GP levels and only 7 percent fell within the normal range. These data indicate that chronic undifferentiated schizophrenics are under oxidative stress, as indicated by the high percentage who displayed elevated serum lipid peroxide levels. The comparative few (7 percent) who displayed elevated red cell GP may still be in the process of adapting to elevated levels of circulating peroxides. Thirdly, the great majority of schizophrenics with decreased red cell GP accompanying elevated serum lipid peroxide levels may no longer be capable of antioxidant adaptation. They may have exhausted their ability to maintain adaptively increased levels of GP to detoxify comparatively high levels of lipid peroxides in their serum. We speculate that the vast majority of these chronic undifferentiated schizophrenics may have over-stressed their systemic antioxidant capacities.

SOD and GP in Autism. Many infant development psychoses are characterized by absence of verbal communication, stereotyped behavior, and autism. Michelson (1982) reviewed the biochemistry of autism and suggested that the biochemical mechanism underlying this disease state may involve alterations in the synthesis of certain neurotransmitters. Autistic children have abnormally increased red cell and platelet Cu, Zn-SOD activity, as compared with healthy children. In contrast, their red cell glutathione peroxidase activity is abnormally decreased, to the extent that their red cell (SOD) to (GP) ratio is approximately twofold higher than normal. The elevated (SOD)/(GP) ratio in autism is analogous with Down's Syndrome, in which elevated SOD activity leads to greater production of hydrogen peroxide, a substrate for GP. By analogy with earlier examples, this should result in a compensatory adaptive increase in GP activity.

Autistic children appear to differ from schizophrenics in one important respect: in

the face of abnormally increased SOD activity, they have decreased red cell GP levels rather than normal or increased levels as measured in the schizophrenics. We might speculate that autistic children as a group have progressed further than schizophrenics towards loss of their ability to adapt to endogenous oxidative stress. Michelson sums up the present understanding of autism:

Whether this (perturbation of enzymatic protection against activated oxygen species) is a cause or a consequence of the cerebral dysfunction leading to infantile development psychosis remains to be established. It is nevertheless clear that the intermediary metabolism of molecular oxygen plays an important role in mental pathology. (Michelson, 1982, p. 279).

These consistent correlations between altered red cell GP activity and endogenous oxidative stress in inherited mental disease states further support our thesis that the GP activity of certain tissues varies with the composite oxidant stress experienced by the organism. Whereas reduced GP levels can result directly from reduced Se availability in normal subjects, elevated GP activity most likely indicates that the patient is in an adapted state. It is certainly possible that such elevated GP activity results from high levels of Se intake, but this would not adequately explain the data. The available findings on inherited mental deficiencies such as Down's Syndrome, undifferentiated schizophrenia, and autism strongly suggest that the vast majority of the subjects were under considerable oxidant stress which was a proximate mediator of their symptoms. From the work of Tappel and his collaborators on dietary peroxide administration, and Stokinger's work on inhaled oxidants, it seems likely that the levels of antioxidant enzymes in a selected tissue often directly correlate with the level of oxidative stress affecting that tissue. Thus elevated levels of GP in red cells are likely to indicate a systemic adaptive response to oxidative stress in the disease states reviewed above.

The major substrates for GP are peroxides. We might ask further: Are other disease states characterized by elevated serum lipid peroxide levels, thereby indicating a possible role for GP and other antioxidant enzymes in their management?

Elevated Lipid Peroxidation in Human Disease

There is little doubt that lipid peroxides mediate tissue injury in a variety of human disease states. In 1952, Glavind et al. had detected and measured lipid peroxides in human atherosclerotic plaques (atheromas), and reported that the degree of atheroma development correlated closely with the content of lipid peroxides. More recently, Yagi (1982) has shown that lipid peroxides injected into test animals can preferentially initiate vascular endothelial degeneration. Earlier assays for lipid peroxides in tissues involved fluorometry of thiobarbituric acid-reactive products (usually malondialde-hyde). A non-invasive method has been developed which involves measuring the levels of the volatile hydrocarbons ethane or pentane (breakdown products of ongoing lipid peroxidation *in vivo)* in air exhaled from the test subject. Tappel and various collaborators have used this method to estimate ongoing lipid peroxidation in experimental animals and human subjects. Dillard, Tappel, et al. (1978) found that lipid peroxidation in healthy human subjects, as estimated from hydrocarbon exhalation, is increased significantly during exercise, is further exacerbated by exposure to ozone, and can be minimized by vitamin E supplementation.

Elevated lipid peroxidation, as assessed noninvasively in experimental animals by the hydrocarbon exhalation test, can later be measured in selected tissues (following sacrifice of the animal) and correlated with oxidative damage to that specific tissue. Thus Hafeman and Hoekstra (1977) showed that carbon tetrachloride *(CCU)* increased ethane production in rats, caused lipid peroxides to accumulate in the liver, and produced extensive liver lesions. Dietary supplementation with selenium and the sulfur amino acid methionine protected against CCU-induced, lipid peroxidative damage by maintaining glutathione peroxidase activity and reduced glutathione at normal levels in the liver.

Recently, Yagi has refined the older fluorometric method for detecting and measuring lipid peroxides as malondialdehyde (MDA)

into a "micro-fluorometric" method for measuring MDA in small serum samples (Yagi, 1982). There was some age-dependent variation in healthy subjects, but patients with various degenerative diseases consistently exhibited abnormally increased tissue MDA levels. The availability of this relatively simple and accurate method for measuring lipid peroxides in small samples led to a proliferation of studies (particularly among Japanese investigators) which culminated in a symposium held in Japan in 1980 (edited by Yagi, 1982). Some of the results presented at this symposium are summarized below.

Lipid Peroxides Injure Arterial Endothelia. Yagi used his new method to investigate in rabbits the effects of intravenous administration of a lipid peroxide (linoleic acid hydroperoxide, with linoleic acid as control) (Yagi, 1982). After injection of the lipid peroxides, the serum lipid peroxide level immediately began to increase, and peaked at 24 hours. The rabbit was then sacrificed and several tissues were analyzed for their lipid peroxide content, namely the liver, lung, spleen, retina, the aorta, the pulmonary artery, and a major vein. The aorta was the only tissue with significantly elevated lipid peroxide levels. Scanning and transmission electron microscopy revealed widespread endothelial disruption in the aortas of the peroxide-treated rabbits, as well as adherence of platelets to zones of exposed subendo-thelium. Similar but less marked changes were observed in the pulmonary artery. Yagi attributed this specific insult to the experimentally-elevated serum lipid peroxide content, and stated "it is plausible that the damage to the intima of the aorta provoked with lipid peroxides is the initial event in the pathogenesis of atherosclerosis" (Yagi, 1982, p. 241).

Lipid Peroxides and Skin Burn Injury. Yagi (1982) also studied the time course of lipid peroxide production following skin burn injury, and the specific damage to the spleen which resulted. One hour after experimental skin burn injury to a rat, the lipid peroxide content of the burned skin was significantly higher than normal. Three hours after the injury, the skin peroxide content was six times higher than control levels. After one day the skin peroxide levels began to fall, returning to the control range by the seventh day. Lipid peroxides increased in the serum parallel with their decrease in the skin, suggesting release of peroxides from the skin into the circulation. On the seventh day the rats were sacrificed and the lipid peroxide content was determined for several organs, along with the serum activities of several organ-specific enzymes as independent indicators of damage. Those enzymes characteristic of spleen, kidney and liver were indeed significantly elevated in the serum, indicating they had leaked out of their parent organs due to peroxide-induced mejnbrane damage. Lipid peroxide levels were significantly elevated in the spleen but not in the other organs sampled. Yagi concluded from his findings that lipid peroxides entered the circulation following burn injury, mediated damage to the spleen, kidney, and liver, and accumulated preferentially in the spleen.

Lipid Peroxide Levels in Diabetes and Stroke. At the 1980 symposium Goto reviewed data from several studies which attempted to correlate degenerative disease states with elevations in serum lipid peroxide levels (Goto, 1982). Diabetic patients with angiopathy (blood vessel involvement) averaged 7.15 nanomoles of malondialdehyde per ml of plasma, versus 3.74 for healthy subjects ($p<0.001$). Diabetics without blood vessel involvement averaged 3.82 nmol/ml, not significantly different from healthy subjects. Goto also described a close correlation between events of cerebral ischemia or hemorrhage (stroke) and subsequent alterations in serum lipid peroxide levels, which might have prognostic value. Those individuals destined to die soon after their stroke had levels of lipid peroxides trending upward (away from the control range), while those who subsequently survived had lipid peroxide levels trending downward (towards the control range).

From the initial results sparked by Yagi's refinement of the fluorometric assay to detect and measure MDA in small serum samples, it appears that the levels of circulating lipid peroxides are indeed elevated in several degenerative disease states. Following injection of lipid peroxides into the test animal or the induction of lipid peroxidation in a tissue by a specific insult (as in skin burn injury), lipid peroxide levels become elevated

first in the bloodstream then selectively in various organs. This confirms earlier work by Tappel's group (Tappel, 1980,1982) and by Hafeman and Hoekstra (1977). Abnormally elevated levels of circulating lipid peroxides appear to correlate particularly well with damage to vascular endothelia, as in diabetic blood vessel disease and stroke in human subjects, and experimental atherosclerosis in rabbits. Future studies should attempt to better correlate findings on the degenerative effects of lipid peroxides with data on the availability of glutathione peroxidase and other antioxidant reserves in the tissues subjected to peroxidative damage. The antioxidant defense status of the organism locally and/or systemically may determine whether the damage is halted at an early stage, is subsequently reversed, or becomes progressively more severe.

Adaptation: Progression to Degenerative Disease

One of us (SL) had suggested earlier that the development of adaptive tolerance to oxidative stress in one organ can deplete or reduce antioxidant reserves elsewhere in the body (the hypothesis of Antioxidant Adaptation). A clinical corollary of this hypothesis is that, as a result of chronic (sustained) oxidative stress there would occur a progression from the adapted state, through an unstable state of health, to some early signs of clinical ill-health, which would manifest in inflammatory degenerative symptomatology.

Our individual health status is a consequence of many endogenous and exogenous interacting factors. Many stressful stimuli may manifest physiologically as oxidative stress. These include polluted air and water, dietary and occupational chemical exposure, physical trauma, and personal and job-related emotional stress. Many powerful pharmaceutical agents will contribute to oxidative stress upon their metabolic activation to reactive radical derivatives by Mixed-Function Oxidase and other enzyme systems. Individual genetic predispositions will likely dictate our unique resiliency to oxidative stress and determine which tissues and organ systems are particularly susceptible.

Glucose 6-phosphate dehydrogenase (GGPD) deficiency is the most common disease-producing enzyme deficiency of human beings (Beutler, 1983). G6PD provides a valuable genetic model wherein a hereditary aberration in the antioxidant defense system directly affects the ability of the organism to adapt to oxidant stress. This model graphically illustrates our conception of the Antioxidant Adaptation Hypothesis. Since G6PD is restricted to red blood cells, we can visualize a tissue-selective response to oxidant attack conditioned by inherent genetic predisposition and triggered by additional exogenous oxidant stressors.

Many exogenous oxidant stressors can stress the G6PD-deficient individual. According to Calabrese (1980), the possibility has been overlooked that G6PD-deficient individuals could be preferentially susceptible to high ambient levels of ozone in polluted air. Calabrese lists common substances which may precipitate hemolytic episodes in G6PD deficient individuals, and suggests that the use of oxidant chloramines or chlorine dioxide as biocides in our drinking water may be dangerous for G6PD-deficient individuals. In the "clinically-significant" G6PD phenotypes, clinical symptomatology is expressed upon provocation by oxidative stress, such as that which occurs when patients are exposed to oxidizing environmental chemicals and during infection.

Thus we see that G6PD deficiencies, Down's Syndrome, Sickle Cell disease and the thalassemias, these very common inherited abnormalities in humans, manifest biologic degeneration by increasing endogenous oxidative stress. We are also increasingly subject to exogenous oxidative stresses resulting from the generation or inclusion of reactive chemicals in our air, food, and water supplies. As environmental chemical pollution becomes increasingly more widespread, we can expect more individuals to develop symptoms of oxidant damage: inflammation, immune dysregulation, cardiovascular pathologies, cancers, and other degenerative diseases. These are predictable consequences of chronic, excessive oxidant stress. Witschi has suggested that the toxicity of oxidants to the lung is, in large part, a function of the individual's ability to adapt:

We may also speculate that it is not

necessarily the nature of the initial lesion (oxidation of NADPH) that distinguishes a toxic agent (paraquat) from one harmless to the lung, but the subsequent failure of the tissue to maintain an adaptive response (to the oxidant). (Witschi, 1977, p. 1632).

We believe also that the effectiveness of the individual's antioxidant defense capabilities will determine his initial response to oxidant stressors and his long-term resiliency, and that adaptive antioxidant capacities are critical in determining the individual's health status. The antioxidant defense system is of course limited in its capacity to resist oxidative stress — when the body is overwhelmed by sustained oxidative stress, disease must result.

Chronic, excessive oxidant stress may only be one side of the coin. The other side is acute, catastrophic oxidant stress precipitating an immediate biological "State of Siege". To muster an adaptive defense the individual may be forced to "borrow" reinforcements, in the form of reducing equivalents (GSH, NADPH and others), and protective antioxidant nutrients (Se, sulfur amino acids, vitamins A, C and E), from whatever systemic reserves are available. These antioxidant reserves would be essential for protecting those organs either inherently most susceptible or exposed to the most severe oxidant attack, i.e., the lung, liver, nervous system, or vascular system.

As seen from Stokinger's work on ozone toxicology (reviewed in Stokinger, 1965), the development of local antioxidant tolerance by systemic adaptation to oxidative stress may be a life-or-death issue. The organism must adapt to survive, even at the expense of compromising constitutional antioxidant reserves. If the oxidative stress is maintained, the individual's capacity to adapt will become progressively more compromised and eventually clinical illness will emerge. The timing and precise pattern of the emergence of disease will no doubt depend on the sum total and possible synergism of the oxidant stressors involved, and the genetic predispositions of the individual. We have arbitrarily categorized a four-stage clinical progression from health to degenerative disease.

Four-Stage Progression to Disease. At stage one of our hypothetical progression, an individual is in good health, comfortably protected against oxidant stressors, fully vibrant and resilient to oxidative stress. But with the rapidly accelerating rate of growth of modern technology has come a proliferation of chemicals totally unprecedented in human evolution. There are now thousands of chemicals dispersed in the ecosphere; their molecular structures are diverse, and their effects upon biological systems are bound to be varied. Though these chemicals will produce organ- and tissue- specific toxic effects, though they may differ in their physiochemical characteristics and in their mode of entry to the body, though other parameters associated with their biological effects will inevitably require thousands of toxicologists millions of hours to unravel, rest assured that the overriding theme of their toxicity is by some expression of oxidative stress to our bodies.

The disease-producing chemical stressors of our environment may be rivaled by the emotional stress of our 20th century life style. The intact nuclear family with both biological parents supporting the growth and development of hopeful and respectful children has been an anchor in previous generations. This is disappearing. Sexual roles are being redefined. The "gotta get enough" compulsion of our middle class during recessionary times must lead to alienation, frustration and confusion.

Stage *Two — Adaptation to Oxidative Stress.* With the rapid changing of social and economic structures and the massive environmental load of chemical stressors, the inevitable toll is stress, manifested biochemically as oxidative stress. At stage two, the individual has to compensate for various of life's stressors: he might be subject to oxidative stress from such everyday situations as breathing polluted Los Angeles air, living with a leaky gas stove or heating system, being exposed to chemicals at the work place (perhaps merely fumes from the copying machine), working in the garden with pesticides.

At stage two we step down a notch in our overall level of health. We are more susceptible to disease-producing pathogens, perhaps are using pharamaceutical aids to offset a stressful lifestyle. "Our mode of life is emerging as today's principal cause of illness,"

according to Joel Elkes, director of the Behavioral Medicine program at the University of Louisville. Emotional stress may well translate into oxidative stress through catecholamine oxidation. Most active individuals are likely to experience ongoing physiological and emotional stress as a normal part of their lives, and most are able to adapt without any encumbering signs of acute or chronic illness. Nonetheless, the energy (antioxidant defenses) required to handle life's stressors will eventually, to some degree, compromise our overall resistance to disease and our consequent sense of wellbeing.

Stage *Three—Losing the Struggle.* Stage 3 represents emerging clinical disease symptomatology. As our load of emotional and physical stressors accumulates, our waning ability to adapt impinges upon our overall reserves and so compromises our antioxidant defenses that normal metabolic functions begin to be compromised. At this stage, signs of chronic or acute illness appear as inflammatory damage in the more genetically-susceptible organs. The characteristics of the particular stressors which topple the load will only in part affect the specific symptomatology that results. At this stage individuals may notice that they have developed acute intolerances to various foods and environmental chemicals. The food and chemical hypersensitivities typical of ecological illness represent underlying pathology that results from the ongoing oxidative stress. Reactive oxidizing chemical species produced from numerous exogenous and endogenous sources attack cell membranes, leading to the liberation of arachidonic acid metabolites, kinins, histamine, and serotonin. These mediators of inflammation are liberated as a consequence of uncontrolled, oxidant-induced, membrane peroxidative damage. Atopic reactions to foods and autoimmune phenomena may follow as a secondary consequence.

At this stage many individuals may not yet be aware of their acute food and chemical reactivities. Though they are under considerable oxidative stress, their bodies make a grand attempt to bolster antioxidant defenses at target organs (those under the most oxidant attack or genetically most vulnerable) at the cost of the wiping out of their reserve antioxidant capacities. At this stage, that of "masking", the individual will begin to display inflammatory symptoms of a more chronic nature. Target organs are protected at the cost of the reduction of composite antioxidant defenses, such that chronic inflammation may begin. This is a very unstable state: nutrient absorption becomes diminished, antioxidant nutrients quickly become limiting*. The nutrients that are found to be lacking in ecologically ill patients are those most critical for the immune system, suggesting that individuals at this stage are prone to immune dysregula-tion. A list of such nutrients would include ascorbate (vitamin C), vitamin E, vitamin A, beta-carotene, selenium, zinc, and pantothenate. Thus any major breakdown of the antioxidant defenses (unaided or abetted with antioxidant supplements) would inevitably lead to immune depression and further deterioration of the protective antioxidant defenses.

When antioxidant defenses are damaged severely enough by oxidant stress and/or nutritional deficiency, a vicious cycle becomes established. There is a point at which the system further degenerates, rather than recuperating. This situation marks the progression to Stage 4. Glutathione peroxidase function exemplifies the compounding effect of severe oxidant damage at this stage of antioxidant deficiency.

Glutathione Peroxidase Vulnerable to Oxidants. Glutathione peroxidase activity is essential for the protection of cell membranes from oxidant damage, yet like many other critical redox enzymes GP is highly sensitive to oxidants and peroxidized lipids. Under conditions of excessive oxidative stress, adaptive increases in the production of reducing equivalents (based upon NADPH production from the HM Shunt) normally compensate for these alterations. However, reduced functioning of the antioxidant defense system becomes com-

*Reduced nutrient absorption may result from damage to the Na/K ATPase enzyme, the activity of which is linked also with glucose and amino acid uptake into the cell. Extracts from many common foods inhibit ATP-splitting activity by this enzyme in vitro (Harlan and Mann, 1982).

pounded as antioxidant enzymes, themselves preferentially sensitive to oxidants, are further damaged by continuing oxidant stress. Vulnerability to oxidative stress is an intrinsic feature of the GP enzyme (Ganther and Kraus, 1981; Flohe, 1982). Its peroxidase function depends on the adequate availability of reduced glutathione (GSH), but GSH itself functions as a primary nutrient-derived antioxidant. Reduced glutathione is oxidized to its disulfide form in the lung during exposure to nitrogen dioxide, ozone, hyperbaric oxygen, or a variety of other chemical species, including solvents and chlorinated hydrocarbons. As reduced glutathione becomes limiting, glutathione peroxidase function is further reduced, since the GP enzyme when not bound to reduced glutathione is more vulnerable to destruction from exposure to oxygen (autoxidation).

The inducibility of glutathione peroxidase activity is subject to the availability of several nutrient-derived factors. Under conditions of Se deficiency, the adaptability of the antioxidant system is severely compromised since increased GP activity is the cornerstone of the adaptive response and requires Se (Forman et al., 1983). Similarly, as glutathione precursors become limiting (as in cases of dietary insufficiency of sulfur-containing amino acids), supplies of GSH become insufficient for the conjugation reactions which normally serve to neutralize the many toxic free radical metabolites produced by enzymes of the Mixed-Function Oxidase system. Hundreds of potentially toxic chemicals are metabolized by the MFO systems in the liver, lung, kidney, skin, testes, and bone marrow, and the toxicity of many of these metabolites can be greatly amplified as a result of glutathione depletion. Reduction in liver glutathione levels resulting from the fasting of experimental animals has been directly associated with a greatly diminished tolerance to halogenated hydrocarbons (Pessayre et al., 1979). We suggest that the increased acute sensitivity of fasted patients to environmental toxins is in part due to depletion of their hepatic glutathione reserves.

Hypoxic Sequelae Transport Enzyme Breakdown. Paradoxically, in the oxygen-stressed state (induced by exposure to hyperbaric oxygen) laboratory animals often become hypoxic. They react adaptively to high oxygen tensions by physiologically reducing their blood oxygen tension (reviewed in Balentine, 1982). This phenomenon is familiar from studies in which the oxygen-stressed organism was shown to reduce its thyroid and other endocrine functions in order to reduce its basal metabolic rate. We suspect that, like animals exposed to hyperbaric oxygen, individuals in the oxidant-stressed state may adaptively reduce certain of their metabolic functions, to reduce in turn their endogenous production of activated oxygen species. The consequence is a functional hypothyroidism and reduced metabolic rate, which is even more dramatic during acute inflammatory and reactive states.

The sodium, potassium ATPase and the CA++ -ATPases are both critical membrane transport enzymes (Alberts et al., 1983). The Na+, K+ - ATPase regulates the intracellular concentrations of Na+ and K+ ions, thereby regulating cell volume and maintaining a transmembrane electrochemical gradient. Its function is directly linked with the transport of glucose and amino acids across the plasma membrane, a critical factor in the uptake of nutrients by the gut epithelium. This "integral" membrane enzyme is highly lipid-dependent and selectively susceptible to free radical-induced membrane damage (reviewed in Chiu et al., 1982). The immediate consequences are increased membrane permeability, breakdown of transmembrane ion gradients, and loss of the ability to carry out the transport (absorption) of amino acids and glucose which is linked with its pump activity. A functional hypoglycemia and amino acid deficiencies may result from compromise of this enzyme activity in the more susceptible tissues, even in situations where serum levels of glucose and amino acids are normal.

Beyond Stage Four: Progression to Degenerative Disease. Once the vicious cycle of oxidant attack and antioxidant weakening has been set into motion, under continued oxidative stress, the individual may eventually progress to an extreme degenerative state. His antioxidant defense mechanisms have become severely compromised, and significant adaptation is no longer possible. Further oxidant exposures

may precipitate acute inflammatory degenerative symptomatology, with the widespread derangement of arachidonate catabolism to produce a chaotic array of locally-acting tissue regulators (autacoids), many of which are inflammatory and some of which are immunosuppressive (Lewis, 1983). Unrestrained by a functioning antioxidant defense system, reactive oxidizing species are likely to damage free serum amino acids, oxidize serum lipids such as cholesterol, and conjugate with sulfhydryl and aromatic amino acids in cytosolic proteins as well as in membrane proteins. Peroxidized lipids can impair cellular membrane functioning and eventually disrupt the membrane, and auto-antigens derived from oxidized cellular macromolecules can become immunogenic with subsequent autoimmune effects (Levine and Reinhardt, 1983). Any of numerous other degenerative symptomatologies may eventually develop.

The Antioxidant Adaptation Hypothesis is well supported by some of our very best-studied inherited metabolic diseases. It offers a rational biochemical interpretation to numerous clinical symptomatologies (such as masking) in environmentally hypersensitive patients. The Antioxidant Adaptation Hypothesis is based upon the most recently-elaborated biophysical principles which underlie chemical toxicology and carcinogenesis. Inherent in the Antioxidant Adaptation Hypothesis is the assumption that the factors which precipitate biologic stress operate through avenues involving direct tissue damage by free radicals and other activated oxygen species. This theory is also strongly supported by the extraordinarily broad therapeutic potential attributed to key antioxidant nutrient factors such as beta-carotene, vitamins A, C, and E, selenium, and zinc.

Acknowledgement

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