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Dr. Janet Woodcock
Director, CDER
Food and Drug Administration
Dockets Management Branch (HFA-305)
5630 Fishers Lane, Room 1061
Rockville, MD 20852

Dr. Woodcock:

Thank you for encouraging us to complete the enclosed literature review cysteine/glutathione deficiency in HIV and a wide range of other diseases in man. I am pleased to have the opportunity to submit this review in support of a citizens' petition that John James (AIDS Treatment News) and I filed some time ago, asking that acetaminophen be labeled with a warning for HIV-infected people.

In the review submitted here, I have listed studies in which glutathione measurements directly demonstrate cysteine/glutathione deficiency and/or in which positive clinical response to treatment with N-acetylcysteine (a cysteine pro-drug) provides a surrogate marker that reflects this deficiency. References to over thirty placebo-controlled trials are included in this latter category.

I have heard "through the grapevine" that the Agency is extensively reviewing acetaminophen labeling. My colleagues and I strongly applaud this effort and hope that bright and clear warnings about the dangers of overdose become part of the labeling procedure. Through collaborative work with the Pediatrics Intensive Care Unit, I have become acutely aware of the number of times that children have arrived in the unit near death because of inadvertent acetaminophen overdose, often because parents did not (could not) read the fine print of the labels of cold remedies and other medications in which acetaminophen is included.

Relative to this issue, we recently sent responded to an article discussing on the dangers of acetaminophen, which appeared in the British Medical Journal, by sending the enclosed letter to the journal. (It appeared on their Website and is now apparently waiting for "space" to be published in the Journal.) In essence, in this letter, we propose packaging acetaminophen with its antidote (NAC) to prevent inadvertent poisoning. We have been in touch with a reputable NAC supplier (BioAdvantex, Canada and US) about this idea and have had a favorable response from them. We have now urged them to advise you of their interest.

I hope that the material included here will be of some help. Please do not hesitate to get in touch with me if I can be of further help. I can be reached by phone at 650-723-5054 and by email as LeeHerz@darwin.stanford.edu.

Sincerely,

Leonore A. Herzenberg
Professor of Genetics

97P-0102

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Review of cysteine/glutathione deficiency in diseases in man

presented in support of a citizen petition requesting labeling of acetaminophen with a warning for HIV-infected people

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Figures and Tables

Introductory comment

We are submitting this document in support of a citizens' petition, filed some time ago by John James and Leonore. A. Herzenberg, requesting that the Agency require inclusion of a warning for HIV-infected individuals on acetaminophen package inserts. Dr. Janet Woodcock recently informed us that acetaminophen labeling is under review at the Agency and suggested that we update our earlier petition with additional information, which we had informally advised the Agency that we have collected.

We are pleased to update this material here with a review of current evidence demonstrating the prevalence of glutathione GSH deficiency in HIV disease and supporting idea that this deficiency makes HIV-infected individuals more susceptible to acetaminophen toxicity. This review also summarizes evidence demonstrating GSH deficiency in a wide variety of diseases other than HIV. We include this material partly to draw attention to potential problems posed by acetaminophen usage for individuals with these diseases, and partly to point out that acetaminophen may be additionally dangerous for HIV-infected people who have other diseases in which GSH deficiency occurs.

The evidence we present demonstrating GSH deficiency in HIV and other diseases is derived from two types of studies: those that rely on direct measurement of GSH levels; and those that demonstrate clinical improvement following treatment with a N-acetylcysteine (NAC), the well-known cysteine pro-drug used to treat acetaminophen overdose. This clinical response to NAC is an effective surrogate marker for GSH deficiency, since orally ingested NAC is rapidly deacetylated and principally provides a source of cysteine, the limiting precursor for GSH synthesis.

Notation

NAC treatment actually replenishes both cysteine and GSH. Therefore, a positive clinical response to NAC could reflect correction of either a cysteine or a GSH deficiency. However, since GSH deficiency is clearly a consequence of cysteine deficiency, and since GSH is central to many more key metabolic processes than cysteine itself, we discuss this cysteine/GSH deficiency here in terms of its effect on GSH levels and refer to it simply as a GSH deficiency in the sections that follow.

Bibliographic note

We have included the full citation for each article to which we refer and have added the abstract for the article to the citation. We will be pleased to rapidly submit the full text for any or all cited articles on request, either in electronic or printed version, since we have all of the articles in hand. Note that we have omitted literature citations in the Overview section and have included these citations instead in the more detailed sections that follow the Overview.

Overview

Glutathione (GSH) is a vital cysteine-containing intracellular tripeptide that plays key roles in energy metabolism, cell division and many other physiological processes. Oxidants produced during normal metabolism are scavenged by GSH, which is oxidized in the process and released from cells as the GSH disulfide (GSSG). GSH is also oxidized to GSSG and released from cells when disease processes, e.g., TNF production, induce systemic or localized oxidative stress and when GSH participates in oxidant-generating drug detoxification mechanisms. In all cases, the intracellular GSH is readily replenished as long as there is a sufficient supply of cysteine to enable GSH resynthesis.

Detoxification of acetaminophen and certain other drugs (e.g., cyclophosphamide) depletes GSH. These drugs are well tolerated as long as doses do not exceed limits defined by the availability of cysteine to replenish the depleted GSH. However, ingestion of the drugs at dose levels that cause marked GSH deficiency results in massive cell death and ultimate loss of liver and other organ function.

Treatment with N-acetylcysteine (NAC), a cysteine pro-drug that provides the cysteine necessary for GSH repletion, is the accepted therapy for replenishing GSH depleted due to acute drug overdose. Accomplished in a timely manner, NAC treatment of acetaminophen overdose is one of the most successful therapies for accidental or intentional poisoning. In contrast, failure to treat rapidly to replenish GSH within 24 hours commonly results in irreversible loss of liver function and often in the death of the patient.

Acetaminophen dose levels that can be safely tolerated by normal healthy individuals are well known. However, putatively safe doses of acetaminophen can be well above toxic levels for individuals in whom GSH is depleted due to ingestion of alcohol and/or other GSH-depleting drugs, to chemical oxidants or even to large amounts of cigarette smoke. Acetaminophen package inserts now caution people who routinely consume relatively large amounts of alcohol to seek medical advice concerning acetaminophen usage. However, no caution is yet suggested for people with other behaviors that may put them at risk.

Individuals who have diseases or conditions in which GSH deficiency is common are also at greater risk for encountering acetaminophen toxicity at putatively safe doses. This deficiency has been documented in a surprising number of diseases, either directly or via clinical improvement following NAC treatment (as indicated above, a surrogate marker of GSH deficiency). In HIV infection, GSH deficiency has been shown to occur quite frequently and tends to become progressively worse as the disease progresses. It is important to recognize that in diseases where GSH deficiency, acetaminophen toxicity may be manifest not only in hepatic damage but in exacerbation of the disease itself, since there is substantial evidence (see below) to indicate that increased GSH deficiency is associated with poor prognosis whereas correction of GSH deficiency by NAC treatment is associated with improved prognosis.

In the sections that follow, we first survey the literature demonstrating GSH deficiency in a broad array of diseases, ranging from chronic bronchitis to sepsis and Parkinson's disease. We then consider detailed evidence concerning GSH deficiency in HIV disease and the potential dangers of uncontrolled acetaminophen use by HIV-infected people.

I. GSH deficiency in disease

Background

Glutathione (GSH) is a cysteine-containing tripeptide (γ-glutamylcysteinylglycine) that plays a wide variety of physiological roles, including regulation of signal transduction[1, 2] intracellular defense against oxidative stress[3, 4] and systemic defense against drug toxicity[5, 6]. It is central to the regulation of metabolic and cell cycle related functions in all animal cells[7-11]. In addition, it regulates T cell and NK cell function, e.g., low GSH in T cells impairs IL-2 production, interleukin (IL)-2 responses and cytotoxic T cell activity[12-17]; low GSH in NK cells impairs killing activity[18, 19]; and low GSH in antigen presenting cells (APC) impairs IL-12 production and favors T helper (TH)2 over TH1 responses[20].

Although GSH deficiency is not commonly recognized as an important aspect of disease, GSH deficiency and oxidative stress have been associated with a large number of debilitating diseases, ranging from Parkinson's disease to HIV infection (see table 1 in the next section). This lack of specific association with individual diseases, or even with classes of disease, is damaging in an era where physicians and scientists tend to consider aspects of disease that are not unique to the disease as unimportant to the disease process. However, because all cells require maintenance of substantial levels of intracellular GSH, and because minimal changes in GSH can have highly important metabolic consequences, decreases in intracellular GSH can result in serious functional alterations in cells of all types.

The mechanisms through which GSH deficiency exerts its influence in diseases vary, as do the biochemical consequences of GSH deficiency in different cell types. Low GSH, for example, favors the induction of apoptosis in some cells (e.g., lymphocytes) but can prevent apoptosis induction in others (e.g., cells in the skin). Similarly, the mechanisms responsible for GSH deficiency vary in different diseases. This striking diversity in both the causes and the effects of GSH deficiency in disease perhaps accounts for the current failure to recognize its broad influence on disease processes.

GSH deficiency has been demonstrated in some diseases by direct measurement of GSH levels. However, it has also been recognized by clinical evidence of improvement in patient condition following treatment with NAC or other cysteine-replenishing agents in placebo-controlled trials[21-60] and observational studies[5, 18, 21, 22, 61-69]. Based on the role of NAC in replenishing GSH in acetaminophen overdose, the success of NAC treatment in these circumstances has commonly been interpreted as indicating a lack of sufficient cysteine to maintain adequate GSH synthesis in the face of a disease-related GSH drain. However, of importance with respect to our interest here, the success obtained with NAC treatment can also be interpreted as indicating a greater potential sensitivity to acetaminophen toxicity.

In HIV-infected patients, we have directly demonstrated GSH deficiency both by HPLC analysis of erythrocytes and by Fluorescent Activated Cell Sorting (FACS) or Flow Cytometry analysis of peripheral blood mononuclear cells[70]. Further, we have demonstrated GSH repletion due to NAC treatment in a placebo-controlled trial and have evidence of patient improvement in an observational trial. Similarly, our colleagues have demonstrated massive sulfur loss and GSH deficiency in HIV-infected subjects and have presented placebo-controlled evidence demonstrating that NAC treatment replenishes GSH and improves T cell function in these subjects[22, 29, 71, 72]. We present a detailed discussion of these findings in Section II of this report, after first presenting a series of summary tables demonstrating GSH deficiency in disease and a relatively brief discussion of the findings in these tables.

Demonstrations of GSH deficiency in disease

Diseases and conditions in which GSH deficiency has been directly demonstrated are listed in Table 1 (on the following page). The causes of the deficiency in these diseases are largely unknown. Acetaminophen usage, alcohol consumption or other medications may contribute to lowering GSH in some patients, particularly in conditions where acetaminophen is used to lower fever. However, except for deficiencies due to overdose of GSH-depleting drugs, there is little reason to expect that exposure to toxic agents accounts for the overall GSH loss.

Much of the observed GSH depletion in these diseases is likely due to oxidative stress or other causes of GSH depletion, coupled with a failure to obtain sufficient cysteine for GSH resynthesis. TNF-α, which is produced in many inflammatory conditions[73], may be a particularly central to GSH deficiency since it is known to cause intracellular peroxide production, release of oxidized GSH (GSSG) from cells and, in excessive amounts to cause severe toxicity reversible by NAC administration[74]. Similarly, treatment with acetaminophen or other GSH-depleting drugs may be an important cause of GSH deficiency. In sepsis, for example, the production of TNF-α likely to contribute GSH deficiency[75-78]. Furthermore, the reliance on parenteral nutrition when necessary may exacerbate the deficiency, since the common formulae are not designed to provide enough cysteine to overcome GSH loss in disease. The liberal use of acetaminophen, which is common in the treatment of sepsis, is also likely to play a major role in decreasing GSH levels.

Table 1
Diseases and conditions in which GSH deficiency has been demonstrated

	Disease or Condition	Citation(s)
Neurodegenerative	Alzheimer's disease	[79, 80]
	Multiple sclerosis	[81]
	Parkinson's disease	[66, 79, 82]
	Freidrich's disease	[62]
	Epilepsy	[63-65]
	Amyothropic Lateral Sclerosis	[67, 79]
Gastrointestinal	Crohn's disease	[8385]
	Inflammatory bowel disease	[86]
	Barrett's esophagus	[87]
	Post-surgical trauma	[88]
	Liver cancer	[18]
	Liver disease	[68, 74]
	Cirrhosis	[68]
Endocrine	Diabetes	[49, 50, 89-99]
Pulmonary	Cystic fibrosis	[47, 100-110]
	Idiopathic pulmonary fibrosis	[111-114]
	Bronchiolitis Obliterans Syndrome	[115]
	Emphysema, Chronic Bronchitis	[116]
Cardiovascular	Atherosclerosis	[117]
	Dilated cardiomyopathy	[118]
	Acute myocardial infarction	[119]
Optic	Cataract	[120]
	Eale's disease	[121]
Kidney	Chronic kidney failure	[57, 122]
Urogenital	Chronic prostatitis	[123]
Skin	Psoriasis	[124]
Immune system	Rheumatoid arthritis	[125, 126]
	Common variable immunodeficiency	[127]
Critical care	Patients in intensive care	[128]
	Malnutrition	[129, 130]
	Sepsis	[76, 78]
	Acute Respiratory Distress Syndrome	[43, 131, 132]
Infection	HIV	[12, 21, 22, 28, 69-71, 133-154]
	Hepatitis	[155157]
	Helicobacter pylori	[158]
Perinatal	Pre-eclampsia	[159]
	Neonates ventilated with oxygen	[160]
Aging		[161-165]
Metabolism	Phenylketonuria	[166]
Toxic agents	Acetaminophen	[6, 167, 168]
	Alcohol	[59, 74, 169171]
	Other chemicals and medications	[73, 172],

The question of whether the GSH deficiency is important in the diseases listed in table 1 essentially resolves partly to theoretical considerations that are beyond the scope of this discussion. However, substantial insight can be gained from data that indicate whether NAC treatment results in positive clinical benefit(when such have been published) . We discuss these data in the next sections and, for HIV disease, in part II of this report.

GSH deficiency and NAC treatment

NAC occupies a peculiar place in clinical medicine. Although well recognized as the antidote to acetaminophen and cyclophosphamide overdose, there appears to be little appreciation for the broad range of diseases and conditions for which its use has been shown to be beneficial. Thus, while treatment with NAC is well accepted as the method of choice for detoxification of acetaminophen and other drugs[5, 6, 73, 167, 168, 173], there are (to our knowledge) no significant reviews that have critically collected reports of health improvement in NAC-treated subjects.

Despite the lack of such reviews, there is widespread evidence demonstrating health benefits for NAC treatment in a panoply of diseases, many recorded in placebo-controlled studies (table 2-4 and table 6 in section II) and still more recorded in uncontrolled studies (table 5). Since the principal effect of NAC therapy known to date is the restoration of the cysteine supply and the replenishment of intracellular and extracellular GSH, it is reasonable to conclude that relief of cysteine/GSH deficiency is responsible for the observed improvements in the condition of the treated patients. As a corollary, then, it is also reasonable to conclude that patients with GSH deficiency are at greater risk and that exacerbation of this deficiency is likely to exacerbate the symptoms of their disease and hence that medications, including but not limited to acetaminophen, must be used with caution in such patients.

The randomized, placebo-controlled, mostly double-blind clinical studies in which treatment with NAC or other GSH-replenishing drugs has been shown to be beneficial are listed in tables 2-4. Table 2 (page 9) lists findings from a rather large series of studies testing NAC efficacy in treatment of chronic bronchitis[30-41, 48, 106, 174, 175]. The focus on bronchitis in NAC studies originates with evidence indicating that NAC can act as a mucolytic agent *in vitro*. Results from clinical trials showing modest but significant efficacy in bronchitis treatment supported the idea that NAC might similarly act as a mucolytic agent *in vivo*. However, current understanding of the many pathways in which GSH replenishment and relief of oxidative stress may contribute to lessening of disease leave this interpretation open to question. For example, NAC has been shown to decrease negative effects of TNF[176] and thus its positive benefit in bronchitis could trace to prevention of TNF toxicity in patients in whom the bronchial infection induces TNF production.

Table 3 (page 10-11) collects a series of reports of placebo-controlled trials in which NAC treatment resulted in relief of symptoms in an assortment of diseases or conditions (see table for citations). This broad efficacy is sometimes doubted in a research climate where specificity in therapy is the dominant paradigm. However, the ubiquitous presence and multifunctional activity of GSH in virtually all living cells provides sufficient grounds for explaining the wide variety of diseases in clinical benefits have been obtained with NAC treatment. Except for a few parasites, which have evolved redox-active substitutes, GSH is present at carefully regulated levels in plant and animal cells and serves functions that range from detoxification to maintenance of energy supplies. These include important metabolic roles in nucleotide synthesis, cell division, electron transport and protein synthesis and folding. In addition, they include key roles in the protection against oxidative stress and induction of apoptosis, favoring the latter process in some cells and preventing its induction in others. Reflecting the many intracellular and extracellular roles that GSH plays, the mechanisms responsible for clinicial benefits of NAC treatment most likely differ from disease to disease, the common feature being reliance on some aspect of GSH function that deteriorates when GSH levels drop below normal.

Table 2. Placebo-controlled studies: chronic bronchitis

n	Study length	NAC Dosage	Findings
29	4 weeks	600mg daily	Oral NAC changes the consistency of sputum with resultant ease of expectoration and the expectoration of increased volumes of sputum; peak expiratory flow rate was increased **Aylward, 1980**[32].
215	10 days	600mg daily + antibiotics	NAC was effective in decreasing sputum volume and viscosity **Brocard, 1980**[33].
566	6 months	*	**Meta-analysis:** Treating chronic bronchitis patients with NAC during the winter months is cost-effective both from the payer's and social point of view. This report is based on retrospective analysis of eight published placebo-controlled studies (presented immediately below). **Grandjean, 2000**[40]
69	6 months	300mg every 12hr for 3d/wk	The frequency of exacerbations was significantly lower in the NAC-treated group. (Included in Grandjean et al.) **Grassi and Morandini, 1976**[30]
744	6 months	400mg daily	Oral NAC was associated with decreased thickness of sputum, improved ease of expectoration, and decreased incidence of exacerbations. (Included in Grandjean et al.) **Grassi, 1980**[31]
259	6 months	400mg daily	The exacerbation rate was significantly lower in the NAC-treated group. (Included in Grandjean et al.) **Boman, 1983**[34]
155	3 months	600mg daily	Although improvement in subjective symptoms (sputum viscosity and character, difficulty in expectoration and cough severity) occurred in both treatment groups, improvements in difficulty in expectoration and cough severity were greater in patients receiving NAC. (Included in Grandjean et al.) **Jackson,** 1984[35]
181	5 months	600mg daily	Although the differences did not reach conventional levels of statistical significance, there was a trend toward significantly reduced number of exacerbations in the NAC-treated group. (Included in Grandjean et al.) **British Thoracic Society, 1985**[36]
526	6 months	600mg daily	There was not a statistically significant difference in the number of exacerbations between the two groups although there was a slight trend towards improvement in the NAC group during the first 3 months. NAC-treated subjects had a significantly reduced number of days on which they were incapacitated. (Included in Grandjean et al.) **Parr and Huitson, 1987**[37]
116	6 months	600mg daily	NAC-treated group had reduced number of sick-leave days caused by exacerbations of chronic bronchitis. (Included in Grandjean et al.) **Rasmussen and Glennow, 1988**[38]
153	22 weeks	1200mg daily	The glutathione concentration in plasma is increased after oral administration of NAC. Efficacy not tested. (Included in Grandjean et al.)**Hansen, 1994**[39].
-	12-24 weeks	400 – 600mg daily	**Meta-analysis:** Eleven previously published NAC studies were reviewed. Based on this analysis, the authors conclude that with treatment periods of approximately 12-24 weeks, oral NAC reduces the risk of exacerbations and improves symptoms in patients with chronic bronchitis compared with placebo, without increasing the risk of adverse effects. They suggest that further studies are needed to determine whether this benefit is sufficient to justify the routine and long-term use of NAC **Stey, 2000**[41].

* Cost analysis – difficult to determine dosages.

Table 3. Placebo-controlled studies: assorted diseases and conditions

Disease or condition	*n*	Study length	NAC Dosage	Findings
ARDS	61	72 hours	40 mg/kg/d intravenous	IV NAC treatment improved systemic oxygenation and reduced the need for ventilatory support in patients presenting with mild-to-moderate acute lung injury subsequent to a variety of underlying diseases. GSH replenishment not reported. **Suter, 1994**[42].
	48	10 days	70mg/kg NAC intravenous; 63mg/kg OTZ intravenous.	Findings suggest that repletion of GSH may safely be accomplished with NAC or OTZ in patients with acute lung injury/ARDS. **Bernard, 1997**[43]
Bronchoalveolar lavage	27	5 days	600mg daily	Oral NAC prior to bronchoalveolar lavage resulted in increases in the concentration of cysteine in plasma and of reduced (not decreased) glutathione in plasma and the airways, which thus temporarily increases the anti-oxidant capacity of the lung. **Bridgeman, 199**[44]
Diaphragm dysfunction	4	3 weeks	150 mg/kg in 250ml D_5W	Preadministration of IV NAC attenuates the development of diaphragm fatigue in normal subjects breathing against high inspiratory loads. (Relevant to treatment of patients with ventilatory failure secondary to respiratory muscle fatigue.) GSH replenishment not reported. **Travaline , 1997**[45]
Influenza	262	6 months	1200mg daily	Long term treatment with oral NAC during the cold-season appears to significantly attenuate the frequency and severity of influenza or influenza-like episodes in elderly subjects and/or patients suffering from chronic non-respiratory diseases. GSH replenishment not reported. **De Flora, 1997**[48]
Pancreatitus and cystic fibrosis	20	20 weeks	Not stated	An antioxidant cocktail of Selenium, β-carotene, Vitamin C, Vitamin E, and methionine at levels that can supply cysteine to restore GSH resulted in absence of clear-cut attacks of pancreatitis during treatment (3 attacks occurred in the placebo group) and a reduction in background pain. There was also a reduction in free radical activity. **Uden, 1990**[47]
Renal function	83	48 hours	1200 mg daily	Prophylactic oral administration of NAC, along with hydration, prevents the reduction in renal function induced by iopromide, a nonionic, low-osmolality contrast agent in patients with chronic renal insufficiency. **Tepel, 2000**[46]
Sepsis/ hyperoxic ventilation	38	72 hours	150ml/kg NAC in D_5W over a period of 15 min.	NAC helped preserve whole body oxygen uptake, oxygen extraction ratio, and gastric intramucosal pH during brief hyperoxic ventilation. (Findings are relevant to treatment of patients with impaired tissue oxygenation during hyperoxic ventilation.) **Reinhart, 1995**[52]
Septic shock	58	2 hrs	150 mg/kg NAC for 15 min then 12.5 mg/hr over 90 min.	NAC provided a transient improvement in tissue oxygenation in about half of the septic shock patients, as indicated by the increase in $V0_2$ and gastric intramucosal pH and decrease in veno-arterial Pco_2. The NAC responders had a higher survival rate. **Spies, 1994**[53]

Disease or condition	*n*	Study length	NAC Dosage	Findings
	22	24 hours	150 mg/kg bolus followed by 50 mg/kg over 4 h.	NAC improved oxygenation and static lung compliance at 24h. NAC had no significant effect on plasma TNF, IL-6 or IL-10 levels, but acutely decreased IL-8 and sTNFR-p55 levels. Mortality was similar in both groups, but survivors who received NAC had shorter ventilator requirement and were discharged earlier from the ICU. **Spapen, 1998**[54]
Colonic polyps	64	12 weeks	800mg NAC daily	NAC lowered proliferative index in the colonic crypt epithelium of human volunteers who previously had adenomatous polyps. **Estensen, 1999**[51]
Cardio-vascular	96	24 hours	1800mg IV bolus followed by 20μg per min.	Thrombolytic treatment for acute myocardial infarction resulted a significant increase in plasma malondialdehyde (MDA) levels, a sign of abnormally high production of free radical substances likely due to ischemia reperfusion injury. Patients receiving a 24-hour intravenous infusion of GSH in addition to thromolytic therapy had a significant decrease in plasma MDA levels. **Altomare,** 1996[177]
	11	14 days	4000 mg daily	NAC has no effect on plasma Lp(a) levels while the reduction in homocysteine is considerable. **Wiklund, 1996**[55]
	48	4 hours	4.5g OTC	Oral OTC improves brachial artery flow-mediated dilation and endothelium-derived nitric oxide (ENDO) action in human atherosclerosis. **Vita, 1998**[56]
Peritoneal dialysis	20	14 days	1500mg OTZ daily	OTC increases blood GSH levels in patients with chronic renal failure treated by dialysis. **Moberly, 1998**[57]
Diabetes	15	1 month	1200 mg NAC daily	Oral NAC treatment increased erythrocyte GSH concentrations and the GSH:GSSG ratio, and decreased plasma soluble vascular cell adhesion molecule (VCAM-1), a potential measure of vascular damage in non-insulin dependent diabetes. **De Mattia, 1998**[50]
	20	2 hours	($1.35 g \cdot m^2 \cdot min^{-1}$) GSH	IV administration of GSH increased intra-erythrocytic GSH/GSSG ratio and total glucose uptake in both non-insulin dependent diabetic patients and controls. Significant correlations were found between GSH/GSSG ratio and total glucose uptake. **De Mattia, 1998**[49]
	29	3 months	100 IU/day Vitamin. E	Oral Vitamin E administration to type I diabetic patients resulted in a significant increase in red cell GSH and a significant decrease in red cell malondialdehyde. **Jain, 2000**[58]
Otitis media with effusion	75	39 months	.05ml Mucomyst solution at 1, 3 and 7 days.	Instillation of NAC into one ear at the time of bilateral insertion of ventilation tubes (VTs), and on days 3 and 7 afterwards, resulted in reduced recurrence of otitis media with effusion, reduced re-insertion of VTs, and increased time until VT extrusion. The number of episodes of ear problems and visits to the ENT clinic were reduced. **Ovesen, 2000**[24]

Table 4. Placebo-controlled studies: toxic agents

Disease or condition	*n*	Study length	NAC Dosage	Findings
Acetaminophen toxicity	50	21 days	150 mg/kg in 200 ml 5% dextrose for 15 m. followed by 50mg/kg in 500 ml. for 4 h. then 100mg/kg in 1 litre over 16 h.	Patients with fulminant hepatic failure who had not previously received NAC (late presentation) were randomized to IV NAC or IV dextrose. NAC-treated subjects had increased survival and lower incidence of cerebral edema. Rates of deterioration and recovery of liver function, however, were similar in both groups. GSH replenishment not reported. **Keays, 1991**[60]
Alcoholism	40	15 days	S-adenosyl-methionine (SAM-e) rather than NAC used as a cysteine source. 2g daily in 250ml 0.15 M NaCl	Erythrocyte cysteine was significantly decreased in alcoholics with and without cirrhosis. After parenteral treatment with S-adenosyl-methionine (SAM-e), erythrocyte GSH was significantly increased in the group of alcoholics with cirrhosis. **Loguerico,** **1994**[59]

NAC treatment following ingestion of toxic agents

Although NAC administration is the standard approved treatment for accidental or intentional acetaminophen (Paracetomol, Tylenol) overdose, we were not able to locate placebo-controlled trials demonstrating the efficacy of this treatment. An extensive uncontrolled trial by Smilkstein and colleagues[178] demonstrates clearly that lethality is prevented, regardless of the blood level of acetaminophen, if NAC is administered within 8 hours of acetaminophen overdosage and that no deaths occurred among any of the subjects in their study to whom NAC was administered. Jones and colleagues[179] concur but indicate the need for further study with respect to whether NAC administration is valuable if administered later than 8 hours after acetaminophen ingestion. To this, the placebo-controlled acetaminophen trial listed in Table 4 (above) responds that NAC treatment can be of benefit even among subjects not treated with NAC early enough to prevent onset of fulminant liver failure in that NAC-treated patients show improved survival and decreased the incidence of pulmonary edema.

We did not find placebo-controlled studies for other toxic agents. However, animal (and some clinical) studies indicate a potential role for NAC in treating heavy metal poisoning and a variety of other toxic agents, e.g., [180-186].

GSH replenishment: uncontrolled studies

Data from a series of uncontrolled studies provide further support for the idea that GSH deficiency is widespread in disease. Uncontrolled data is always difficult to deal with. Nevertheless, it is difficult to ignore the large number of such studies that report beneficial effects of NAC treatment in various diseases (table 5, page 13). Results from these kinds of studies have not generally resulted in acceptance of NAC as therapy for the indicated disease. The clear exception, however, is the use of NAC for treatment of acetaminophen overdose (see refs [5, 173] at the end of the table 5), where retrospective analysis of outcomes in a large number of patients has led to the standard acceptance and approval of this therapy.

Table 5. Uncontrolled studies reporting clinical improvement following treatment with GSH-replenishing drugs

Classification	Disease or Condition
Neurodegenerative	Freidreich's disease[62] Progressive myoclonic epilepsy[6365] Parkinson's disease[66] Amyothropic lateral sclerosis[67]
Gastrointestinal	Cirrhosis[68] Hepatocellular carcinoma[18]
Endocrine	Diabetes[90]
Pulmonary	Cystic fibrosis[109] Chronic Bronchitis[175] Idiopathic pulmonary fibrosis[111, 114] Chronic obstructive pulmonary disease[174, 187]
Kidney	Hepatorenal syndrome[61]
Cardiovascular	Arteriosclerosis[188] Nitrate tolerance[189] Homocysteine levels[190, 191]
Skin	Lamellar ichthyosis[192]
Infection	HIV[21, 22, 69] Hepatitis[193]
Critical care	Sepsis (animal studies)[75, 77]
Malignancy	Adults at high risk for recurrence or development of cancer[194]
Toxic agents	Acetaminophen[5, 169, 173, 195-212] Mercury and metal poisoning (some are animal studies)[180, 213-216] Alpha-Amanitin (Death cap)[217]

Conclusion: part I

Data from a large number of clinical trials, both placebo-controlled and uncontrolled, indicate that GSH deficiency is common in a wide variety of diseases. The existence of such deficiency perhaps argues for NAC therapy in these diseases but certainly makes a strong case for cautious administration of acetaminophen and other medications whose toxicity is increased when GSH is depleted.

II. GSH deficiency in HIV disease.

This section deals in depth with GSH deficiency in HIV disease, both with the direct demonstration of that deficiency and with evidence that replenishing GSH by treating HIV-infected people with NAC is beneficial. Collectively, this evidence argues strongly against the unguarded use of acetaminophen or other therapies that may exacerbate the GSH deficiency in these people.

HIV-infected individuals frequently have low levels of GSH, a cysteine-containing tripeptide central to the metabolism of all cells and known to be require. for optimal T cell function[27-29, 140-148, 218]. We and others have shown clearly that GSH is deficient in HIV-infected people[22, 70, 133-139, 142, 148]. Droge and colleagues were the first to demonstrate lower GSH levels, as measured by HPLC with peripheral blood samples from HIV-infected subjects[133, 134]. Consistent with these findings, our initial studies demonstrate that GSH deficiency in HIV disease is detectable either by FACS measurement of glutathione-S-bimane (GSB) in monoclorobimane-stained T cell subsets or by the more classical HPLC-determination of GSH in whole blood, which is principally a measure of GSH in erythrocytes[22, 70, 135-139], and that the two measures are correlated[22]. This GSH deficiency in HIV-infected people is due to a shortage of cysteine, which is required for GSH synthesis[71, 142]. Table 6 summarizes four placebo-controlled NAC studies in which GSH replenishment, efficacy or both were measured in HIV-infected subjects.

Our initial studies also showed that the GSH deficiency becomes progressively more pronounced as HIV disease progresses[22]. Further, several studies show that the deficiency is associated with impaired T cell function[12, 29, 140, 151, 219] and impaired survival in HIV-infected subjects[21, 22, 220]. Part of this survival impairment may be explained by evidence showing that GSH deficiency is also associated with a wide variety of clinical conditions that may contribute to impaired survival, both in uninfected or HIV-infected individuals (see above).

Table 6. Placebo-controlled studies: GSH replenishment in HIV infection

n	Length of study	NAC Dosage	Findings
69	7 months	0.6 - 3.6g depending on GSH levels.	Two studies are reported: both show that NAC consistently causes a marked increase in improvement in immunological function and restores plasma albumin levels. Subject numbers: 40 w/ART; 29 wo/ART. **Breitkreutz, 2000**[29].
45	4 months	800 mg; 2 200mg NAC effervescent tablets twice daily.	NAC treatment resulted in normalization of plasma cysteine levels and a lower rate of decline in the CD4 count. **Akerlund, 1996**[27]
81	8 weeks	3,200 – 8,000 mg NAC, supplied as 800mg effervescent tablets.	NAC treatment safely replenishes whole blood GSH and T cell GSH in HIV infected individuals. Replenishment is associated with increased survival in the open-label portion of the study. **De Rosa, 2000**[21], **Herzenberg, 1997**[22]
37	4 weeks	734 ± 234 nmol/mL of OTC at the highest dosage studied.	A significant increase in whole blood GSH was seen after oral administration of OTC in the 1,500mg and the 3,000mg dose groups **Barditch-Crovo, 1998**[28].

The placebo-controlled double-blind randomized clinical trial that we conducted (Herzenberg, De Rosa and colleagues)[21] was designed to determine whether NAC would replenish GSH in HIV-infected individuals and indeed shows clearly that oral NAC administration is both safe and effective for this purpose. These findings are confirmed by results from two additional placebo-controlled double-blind trials, conducted by Breitkreutz and colleagues[29], which also show that NAC administration improves T cell function in HIV-infected individuals. Based on the totality of this evidence, which is summarized in table 5 and discussed in detail below, we submit that treating HIV-infected people with NAC to replenish and/or maintain adequate GSH levels is beneficial to the HIV-infected patient and hence that uncontrolled use of acetaminophen or other GSH-replenishing drugs poses a danger to HIV-infected individuals.

Causes of GSH deficiency in HIV disease

The GSH deficiency in HIV disease may be caused by production and release of an HIV protein (HIV TAT) which has been shown to decrease GSH both in cultured human cells[221, 222] and *in vivo* in HIV TAT transgenic mice[223] in which GSH-synthesizing enzymes (and possibly cysteine-transport enzymes) are down-regulated. In addition, infections and conditions that upregulate the expression and release of cytokines that contribute to oxidative stress may thereby result in GSH loss. All of these activities, plus consumption of acetaminophen, alcohol and other GSH-depleting agents, may be important in the HIV immunodeficiency.

To avoid confounding loss of GSH due to external factors with GSH deficiency intrinsic to the disease in our GSH replenishment studies, we excluded all subjects who were found, in an initial interview, to routinely consume excessive amounts of alcohol, acetaminophen or other GSH-depleting drugs or to routinely consume NAC or other drugs that could significantly increase GSH levels (see trial protocol, Appendix B). Blood samples were thus obtained only from subjects, who were not, to our knowledge consuming drugs or other agents that would significantly deplete or replenish GSH. The dramatic GSH deficiency that we uncovered by analyzing these samples, therefore demonstrates clearly that GSH deficiency in HIV infection is in some way intrinsic to the disease.

GSH deficiency in the HAART era

We have recently extended the findings discussed above by showing that HIV-infected subjects on HAART show a similar GSH deficiency to the subjects in our earlier study. In both cases, the median value for HIV-infected subjects is essentially equivalent to the 25th percentile for unifected subjects (De Rosa, Green et al, unpublished).

These studies, which are still in progress, demonstrate that GSH deficiency is clearly detectable in HAART-treated HIV-infected subjects (Figure 1). We have not yet determined whether this deficiency has clinical correlates. However, preliminary evidence from the small number of subjects in these studies suggests that patients with the lowest GSH levels are at greater health risk.

That is: we have only measured GSB levels at one time-point in this study. However, longitudinal CD4 count and viral load data are available for the subjects we examined, as are physician evaluations for the clinical status of the patients. Survey of patient disease status at the time GSB was measured and roughly 6 months later indicates that therapy was/is successful in most of patients. However, therapy in several patients is currently considered to be "failing". Examination of viral loads when GSB was measured and at the latest analysis confirms the "failure" of therapy in these patients and also reveals several additional patients who had high viral loads when GSB was measured but whose viral loads were brought under control by therapy in the ensuing 6 months. Interestingly, although there are too few data points to be conclusive, the patients who "failed" had GSB levels below the control median whereas among patients who responded to therapy during this period, all had GSB levels above the control median.

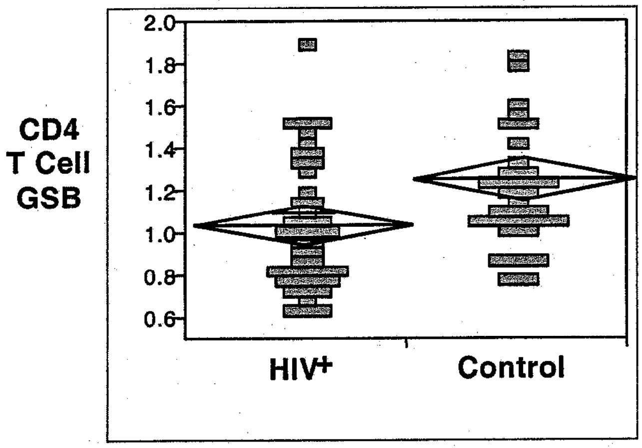

Figure 1. GSH (GSB) deficiency in subjects treated with modern antiretroviral drugs. Data shown were collected for 39 relatively healthy HIV-infected outpatients seen in the Stanford Positive Care Clinic in 1999 and 2000.

Consistent with these findings, Breitkreutz and colleagues demonstrated GSH deficiency and "massive sulfur loss" in HAART-treated and ART-treated HIV-infected subjects[71]. In addition, these workers demonstrated T cell functional deficiencies within this group of subjects and have further shown that NAC treatment restores the deficient T cell function[29].

GSH replenishment in HIV disease

NAC replenishment of GSH in HIV-infected subjects

Several laboratories have conducted randomized, placebo-controlled studies to test this hypothesis[21, 22, 27, 29, 141, 142]. Our trial[21] provides the most extensive replenishment data, i.e., more subjects; both T cell GSH (table 7) and whole blood GSH (not shown) outcomes. However, all trials yielded comparable results, i.e., NAC administration replenishes GSH in GSH-deficient HIV-infected subjects.

Table 7
NAC administration increases T cell GSH (GSB) in HIV-infected subjects

Variables	P^{**}	Adjusted mean GSB change*	
		NAC	Placebo
Trial arm	0.04	0.038	0.009
Baseline β_2-microglobulin	0.04	*na*	*na*
Baseline CD4 T cells	0.02		

**Standard least squares model fit. GSB data were available for all (81) trial subjects. Four outliers were excluded from the analysis; three on the basis of GSB change and one on the basis of baseline β_2-microglobulin values. p= significance of the indicated variable in the model; p for the entire model <0.004. Except for the two baseline variables shown in the table, none of the baseline variables measured in this study were significant covariates in this model.

* Change in GSB (CD4/50) is computed as the difference between the GSB values (area-under-the-curve) obtained prior to initiation of treatment and GSB values (area-under-the-curve) obtained until the subject completed or left the trial. Statistical computations are weighted according to the number of trial visits (equal to the number of data points acquired).

Consistent with these findings, later studies by Jahoor et al[142] introduce pharmacokinetic evidence directly demonstrating that increasing cysteine availability increases the rate of GSH synthesis in HIV-infected subjects. These authors conclude that the GSH deficiency in subjects with AIDS is "due in part to a reduced synthesis rate secondary to a shortage in cysteine availability" and argue, as we do, for treating HIV-infected subjects with a cysteine source to prevent or reverse GSH deficiency.

Association between GSH deficiency and impaired survival in HIV disease

Several studies collectively show that GSH levels in erythrocytes, individual T cell subsets, plasma and other body fluids[69, 133, 142-144, 148, 149, 224, 225] are low in a high proportion of HIV-infected people and tend to decrease as the disease progresses[22, 70, 138, 139, 142]. The clinical significance of this HIV-associated GSH deficiency is reflected by the strong association between decreased survival and either low thiol levels in serum[220] or low GSB levels in CD4 T cells[22]. This association, we find, is at least as strong as the association of survival with CD4 T cell count in the same analysis. In essence, among HIV-infected individuals with CD4 T cell counts below 200/ml blood, individuals with T cell GSH (GSB) levels below the GSB ROC (Receiver Operating Characteristic) value, 1.05, are roughly twice as likely to die within 2-3 years as subjects with GSB levels above the ROC value (figure 2 and table 8).

No survival data is available for our current studies with HAART-treated patients nor from the recent studies conducted by Breitkreutz and colleagues[29].

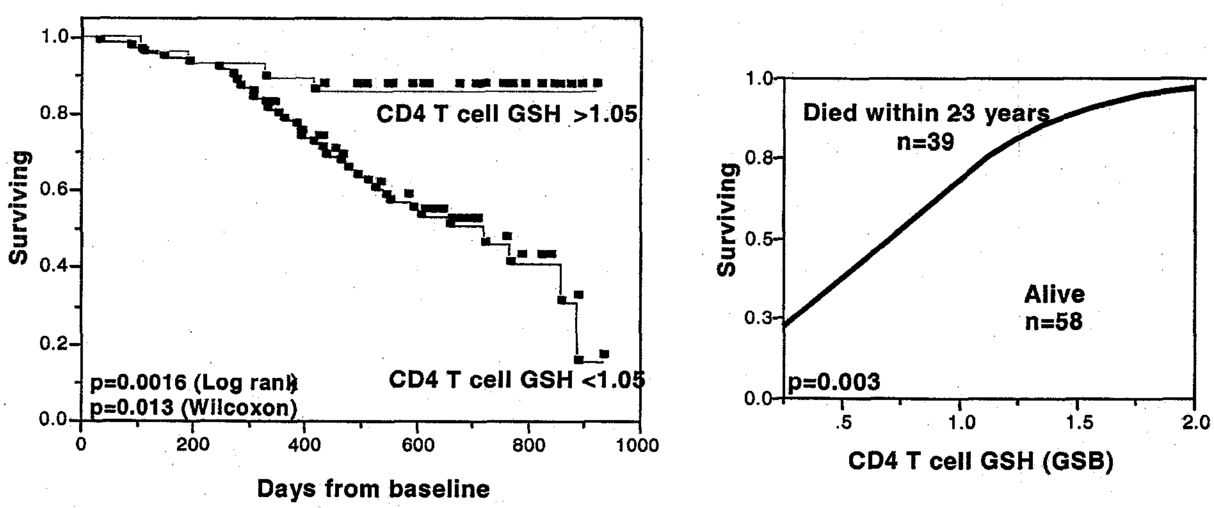

Figure 2. Kaplan-Meier and logistic regression survival analyses. Data are shown for all subjects with CD4 T cell counts below 200/ml blood (n=97) who were screened for entrance into the NAC replenishment trial.

Table 8
T cell GSH is a significant predictor of survival in HIV disease even after adjustment for CD4 T cell count, NAC treatment and reverse transcriptase inhibitor usage

Proportional Hazard Analysis			
Variable	**Threshold**	***p***	**Relative risk of dying**
CD4 T cell GSB (FACS GSH)	<1.05	0.001	2.1 (1.3-3.9)
CD4 T cell count	<100	0.003	1.7 (1.2-2.7)
NAC treatment	none	0.01	1.7 (1.1-2.6)
Reverse transcriptase inhibitors*	none	0.06	1.4 (.99-1.9)

*Subjects were either untreated or treated with nucleoside analogs. Protease inhibitors and non-nucleoside analogs were introduced after this study was completed

Opposing evidence

Opposing evidence: GSH replenishment.

Several studies[23, 26, 226-229] report findings that are interpreted as indicating that NAC treatment does not replenish GSH in HIV-infected people. However, these apparent contradictions are resolved by examination of the evidence presented in these reports. In essence, the conclusions in these reports are based on misinterpretation of pharmacokinetic data[226-228], administration of NAC to too few patients for too little time[229] or the use of questionable GSH measurement methods[23] that do not conform to current standards[230]. Therefore, we conclude that none of these earlier studies rule out the use of NAC or its effectiveness in replenishing GSH in HIV disease.

Table 9. Negative evidence for GSH replenishment by NAC treatment and/or efficacy of NAC treatment in HIV disease

n	Study length	Findings
41	6 months	**Clotet, 1995** [26] The combination of NAC and AZT were compared with placebo and AZT in antiretroviral naïve patients started on AZT at the same time as they started NAC or placebo. The authors found no differences in CD4 counts and neopterin values between the two groups. Further, they report a high incidence of adverse reactions (not necessarily associated with NAC treatment, see text).
24	24 weeks	After 12 weeks of oral treatment with NAC and Sodium selenite, there was a significant increase in the CD4/CD8 ratio and a significant decrease in CD8/CD38 cells compared to the untreated group (not placebo-treated). However, these observations were not paralleled in the control group after crossing over to treatment at 12 weeks and continuing treatment to 24 weeks. In addition, findings were confounded by the inclusion of selenite in the study. **Look, 1998** [23].

Opposing evidence: GSH and survival.
Two studies failed to find significant survival differences associated with GSH deficiency. One, a brief communication[231], uses methods of storing blood samples for GSH analysis (to identify GSH-deficient subjects) that are not considered satisfactory by current standards[230]. The second[232] study used the well-known genetic deficiency for glucose-6-phosphate dehydrogenase (G6PD), which markedly lowers erythrocyte GSH and lowers lymphocyte GSH to a lesser extent, as a selection tool to identify subjects whose GSH levels were putatively lower than levels in a putatively similar population of HIV-infected subjects who were not deficient for G6PD. When retrospective comparison of the survival of these two groups of subjects failed to reveal a significant survival difference between them, the authors of the study concluded that low GSH levels do not impact survival in HIV disease.

This study was published in a highly reputable journal (the Lancet). However, careful examination of the data presented reveal several flaws, any one of which is sufficient to undermines the conclusion drawn by the study authors. First, although there was no significant survival difference between the two groups of subjects, the data presented reveal a trend suggesting that significance could well be attained if sufficient numbers of subjects were studied. In addition, interpretation of the findings presented is marred by potential differences in the study groups, e.g., GSH levels were only assumed to be different between the groups; the groups were not matched for CD4 T cell count at the beginning of the study; treatment may have been different since Dapsone treatment for PCP prophylaxis is contra-indicated in G6PD-deficient subjects.

Finally, even if the findings as presented in this study are valid, the conclusion that GSH deficiency is not relevant to disease progression and survival in HIV-infected people is too strong, since genetic and adaptive mechanisms that help G6PD-deficient subjects cope with lifelong GSH deficiency may selectively decrease the importance of low GSH levels in HIV disease progression in these subjects. Thus, although this study received considerable attention, its conclusions remain to be confirmed.

Benefits of GSH replenishment for HIV-infected individuals

T cell function.
In a recent pair of randomized, placebo-controlled studies with HIV-infected individuals, Breitkreutz, Droge and colleagues showed clearly that NAC treatment improves T and NK cell function[29]. In these studies, T cell and NK cells functions were evaluated after 7 months of NAC treatment by standard *in vitro* assays detecting NK lysis of target cells and T cell proliferation stimulated by anti-CD3/CD28, phytohemagglutinin (PHA) or tetanus toxoid. In all cases, significant functional improvement was observed in the NAC-treated subject group.

These findings are supported by data from *in vitro* T cell function studies and from *in vitro* studies of NAC-supplemented T cells[17, 127, 151, 233-236]. Improvements in T cell cytokine production and proliferation have been demonstrated[20]. In addition, both *in vitro* and *in vivo* studies with mice show that Th2-type function is dominant under conditions of oxidative stress whereas Th1-type function predominates at normal GSH levels[20].

General health.
We have reported a strong association between NAC administration and increased survival in two publications in which we analyzed data from the same overall cohort[21, 22]. The survival portion of the studies reported in these publications were not conducted under placebo-controlled conditions and thus do not meet the Agency's rigorous standards for proof that NAC treatment improves survival in HIV infection. Nevertheless, the strength of the observed association, whether considered for all subjects screened for entry into the trial ($p=0.002$)[22] or for those subjects actually recruited into the

trial (p<0.0001)[21], suggests that these findings merit some consideration, particularly since there are strong reasons to expect that NAC treatment would favor survival in HIV-infected subjects.

The impairment in T cell function in the GSH-deficient subjects in the Breitkreutz et al studies, and the improvement in their T cell function following treatment with NAC[29], may be sufficient to explain the putatively improved survival that we observed in NAC-treated subjects. However, the beneficial effects of NAC treatment demonstrated for other clinical conditions may also have helped to improve the survival of these subjects. Since neither T cell functionality nor the cause of death was determined in our study, we cannot directly evaluate these hypotheses.

Nevertheless, since HIV-infected subjects typically have impaired T cell function, we can expect that the NAC-treated subjects in our study benefited from the improved T cell function observed in NAC treated subjects in the Breitkreutz study. Furthermore, these subjects may have benefited from the overall health improvement implied by the many clinical situations in which NAC treatment brings improvement. Thus, we submit that the collective beneficial effects attributable to NAC treatment may be sufficient to account for the association between improved survival and NAC-treatment that we have observed.

Conclusion: part II

GSH deficiency is prevalent in HIV disease and is associated with poor prognosis. NAC treatment replenishes GSH and is associated with improved prognosis. These associations do not prove causation. However, since there is no known toxicity associated with NAC treatment, they suggest that NAC treatment could prove a valuable adjunct to conventional therapy in HIV disease.

In any event, a cautious approach to HIV counseling and treatment suggests that HIV-infected people would do well to avoid behaviors (e.g., excessive alcohol consumption) and where possible, medications that deplete GSH. Acetaminophen, therefore, should be held to a minimum dosage and should probably be taken in conjunction with NAC.

Overall conclusion

Current data demonstrate that improvement in T cell function, overall physiological health and in the ability to detoxify many agents can be expected in HIV-infected individuals treated with NAC. This may translate to improved survival, to resistance to development of diseases and conditions with which GSH deficiency is associated or to slowing the progress of such diseases and conditions. In the past, most of these diseases and conditions were not considered relevant when HIV infection was present. However, the efficacy of today's anti-retroviral drugs has brought other life-threatening diseases and conditions well back into the picture. Therefore, preventing or removing the GSH deficiency associated with HIV disease has now become more imperative. In particular, with reference to our purposes here, assuring that adequate physician advice is given to HIV-infected patients concerning the use of acetaminophen is an important, albeit minimal, step in this direction.

Similarly, the recognition that GSH depletion is a common feature in a wide variety of diseases and that NAC therapy has been shown to have beneficial effects in many cases suggests that medications and behaviors that deplete GSH should be avoided in these diseases whenever possible. Furthermore, greater attention should be paid to the idea of using NAC as adjunct therapy in these diseases and to treating with NAC when potentially GSH-deficient individuals are exposed to acetaminophen or other toxic agents that deplete GSH.

Citations with Abstracts

1. **Staal, F. J. T., M. Roederer, L. A. Herzenberg and L. A. Herzenberg (1990). "Intracellular thiols regulate activation of nuclear factor kappaB and transcription of human immunodeficiency virus." Proc. Natl. Acad. Sci. USA 87(Dec): 9943-9947.**

The activation of nuclear factor kappa B (NF-kappa B) has been implicated in the regulation of transcription of a variety of genes and has been shown to be essential for the expression of genes controlled by the long terminal repeat of human immunodeficiency virus (HIV LTR). We show here that intracellular thiol levels play a key role in regulating this process. That is, stimulation with tumor necrosis factor alpha and/or phorbol 12-myristate 13-acetate activates NF-kappa B and markedly decreases intracellular thiols; N-acetyl-L-cysteine, an efficient thiol source, prevents this thiol decrease and blocks the activation of NF-kappa B; and the lack of activated NF-kappa B prevents the activation of the HIV LTR and the transcription of genes under its control. These findings reveal a previously unrecognized genetic regulatory mechanism in which cytokine-induced shifts in intracellular thiol levels are crucial in the control of NF-kappa B activity and thereby influence the spectrum of genes expressed by cytokine- stimulated cells.

2. **Schreck, R., P. Rieber and P. A. Baeuerle (1991). "Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappaB transcription factor and HIV-1." EMBO J. 10(8): 2247-2258.**

Hydrogen peroxide and oxygen radicals are agents commonly produced during inflammatory processes. In this study, we show that micromolar concentrations of H2O2 can induce the expression and replication of HIV-1 in a human T cell line. The effect is mediated by the NF-kappaB transcription factor which is potently and rapidly activated by an H2O2 treatment of cells from its inactive cytoplasmic form. N-acetyl-L-Cysteine (NAC), a well characterized antioxidant which counteracts the effects of reactive oxygen intermediates (ROI) in living cells, prevented the activation of NF-kappaB by H2O2. NAC and other thiol compounds also blocked the activation of NF-kappaB by cycloheximide, double-stranded RNA, calcium ionophore, TNF-alpha, active phorbol ester, interleukin-1, lipopolysaccharide and lectin. This suggests that diverse agents thought to activate NF-kappaB by distinct intracellular pathways might all act through a common mechanism involving the synthesis of ROI. ROI appear to serve as messengers mediating directly or indirectly the release of the inhibitory subunit I-kappaB from NF-kappaB.

3. **Meister, A. (1994). "Glutathione ascorbic acid antioxidant system in animals." J Biol Chem 269(13): 9397-9400.**

4. **Shan, X., T. Y. Aw and D. P. Jones (1990). "Glutathione-dependent protection against oxidative injury." Pharmac. Ther. 47: 61-71.**

5. **Smilkstein, M. J., G. L. Knapp, K. W. Kulig and B. H. Rumack (1988). "Efficacy of oral N-acetylcysteine in the treatment of acetaminophen overdose. Analysis of the National Multicenter Study (1976 to 1985)." N. Engl. J. Med. 319: 1557-1562.**

During the investigational use of oral N-acetylcysteine as an antidote for poisoning with acetaminophen, 11,195 cases of suspected acetaminophen overdose were reported. We describe the outcomes of 2540 patients with acetaminophen ingestions treated with a loading dose of 140 mg of oral N-acetylcysteine per kilogram of body weight, followed four hours later by 70 mg per kilogram given every four hours for an additional 17 doses. Patients were categorized for analysis on the basis of initial plasma acetaminophen concentrations and the interval between ingestion and treatment. Hepatotoxicity developed in 6.1 percent of patients at probable risk when N-acetylcysteine was started within 10 hours of acetaminophen ingestion and in 26.4 percent of such patients when therapy was begun 10 to 24 hours after ingestion. Among patients at high risk who were treated 16 to 24 hours after an acetaminophen overdose, hepatotoxicity developed in 41 percent--a rate lower than that among historical controls. When given within eight hours of acetaminophen ingestion, N-acetylcysteine was protective regardless of the initial plasma acetaminophen concentration. There was no difference in outcome whether N-acetylcysteine was started zero to four or four to eight hours after ingestion, but efficacy decreased with further delay. There were 11 deaths among the 2540 patients (0.43 percent); in the nine fatal cases in which aminotransferase was measured before treatment, values were elevated before N-acetylcysteine was started. No

deaths were clearly caused by acetaminophen among patients in whom N-acetylcysteine therapy was begun within 16 hours. We conclude that N-acetylcysteine treatment should be started within eight hours of an acetaminophen overdose, but that treatment is still indicated at least as late as 24 hours after ingestion. On the basis of available data, the 72-hour regimen of oral N-acetylcysteine is as effective as the 20-hour intravenous regimen described previously, and it may be superior when treatment is delayed.

6. **Thomas, S. H. (1993). "Paracetamol (acetaminophen) poisoning." Pharmacol. Ther. 60: 91-120.**

Paracetamol poisoning caused by intentional overdose remains a common cause of morbidity. In this article the mechanism of toxicity and the clinical effects and treatment of poisoning, including specific antidotal therapy, are reviewed. Areas for further research directed at reducing morbidity and mortality from paracetamol poisoning are considered

7. **Vina, J., J. R. Vina and G. T. Saez (1986). "Glutathione: metabolism and physiological functions." Life Chem. Rep. 4: 1-35.**

8. **Dolphin, D., O. Avramovic and R. Poulson, Eds. (1989). Glutathione: Chemical, Biochemical, and Medical Aspects. Coenzymes and Cofactors. NY, John Wiley & Sons.**

9. **Taniguchi, N., T. Higashi, Y. Sakamoto and A. Meister, Eds. (1989). Glutathione Centennial: Molecular Perspectives and Clinical Applications. NY, Academic Press.**

Glutathione S-transferase was found to be a good substrate of Ca++- phospholipid-dependent protein kinase in vitro. Of 6 isozymes of glutathione transferase purified from rat liver cytosol (1-1, 1-2, 2-2, 3-3, 3-4, 4-4), only isozymes 1-1, 1-2 and 2-2 were significantly phosphorylated by the kinase purified from rabbit brain. Phosphorylation was more pronounced in subunit 1 than in subunit 2, and the degree of the phosphorylation was similar in all three homo- and heterodimers, where 1 mol of phosphoryl group per mol subunit was transferred to the subunit 1. The phosphorylated transferase 1-1 showed decreased affinity for bilirubin, suggesting that the phosphorylation affects the function of glutathione S-transferase in an isozyme- specific manner.

10. **Poot, M., H. Teubert, P. S. Rabinovitch and T. J. Kavanagh (1995). "De novo synthesis of glutathione is required for both entry into and progression through the cell cycle." J. Cell. Physiol. 163(3): 555-560.**

To study the putative role of de novo synthesis of glutathione (GSH) in the regulation of the cell cycle, we exposed NIH-3T3 cells to buthionine sulfoximine (BSO) and analysed cell cycle kinetics with continuous bromodeoxyuridine (BrdU) labeling and bivariate Hoechst 33258/ethidium bromide flow cytometry. Treating quiescent cells, which themselves had a low GSH content, with BSO did not affect subsequent entry into and progression through the cell cycle. Adding BSO during serum stimulation, however, provoked a dose-dependent inhibition of cell growth and a delayed increase in GSH level. The cell kinetic mechanism underlying BSO-induced growth inhibition is a diminished entry into the cell cycle and a permanent arrest in the S and G2 phase of the cell cycle. Our results are consistent with the hypothesis that GSH de novo synthesis is required for cell activation and proper S and G2 phase transit. (C) 1995 Wiley-Liss, Inc.

11. **Adamson, D. C., B. Wildemann, M. Sasaki, J. D. Glass, J. C. McArthur, V. I. Christov, T. M. Dawson and V. L. Dawson (1996). "Immunologic NO synthase: elevations in severe AIDS dementia and induction by HIV-1 gp41." Science 274: 1917-1919.**

Indirect mechanisms are implicated in the pathogenesis of the dementia associated with human immunodeficiency virus-type 1 (HIV-1) infection. Proinflammatory molecules such as tumor necrosis factor alpha and eicosanoids are elevated in the central nervous system of patients with HIV-1-related dementia. Nitric oxide (NO) is a potential mediator of neuronal injury, because cytokines may activate the immunologic (type II) isoform of NO synthase (iNOS). The levels of iNOS in severe HIV-1- associated dementia coincided with increased expression of the HIV-1 coat protein gp41. Furthermore, gp41 induced iNOS in primary cultures of mixed rat neuronal and glial cells and killed neurons through a NO- dependent

mechanism. Thus, gp41-induced NO formation may contribute to the severe cognitive dysfunction associated with HIV-1 infection.

12. **Droge, W., H. P. Eck and S. Mihm (1992). "HIV-induced cysteine deficiency and T-cell dysfunction - a rationale for treatment with N-acetylcysteine." Immunol. Today 13(6): 211-214.**

Markedly decreased plasma cystine and cysteine concentrations have been found in HIV-infected patients at all stages of the disease and in SIV-infected rhesus macaques. The elevated glutamate levels found in the same patients aggravate the cysteine deficiency by inhibiting the membrane transport activity for cystine. The intact immune system appears to require a delicate balance between pro-oxidant and antioxidant conditions, maintained by a limited and well-regulated supply of cysteine. This balance is obviously disturbed in HIV infection and may contribute to the pathogenesis of AIDS.

13. **Yim, C.-Y., J. B. Hibbs Jr, J. R. McGregor, R. E. Galinsky and W. E. Samlowski (1994). "Use of N-acetyl cysteine to increase intracellular glutathione during the induction of antitumor responses by IL-2." J. Immunol. 152(12): 5796-5805.**

IL-2 therapy can induce marked oxidative stress via reactive oxygen and nitrogen intermediates. Glutathione, the major intracellular reductant, may become rate limiting to cytotoxic lymphocyte activation and proliferation under these circumstances. N-Acetyl cysteine (NAc-cys) was used to increase intracellular glutathione levels during lymphokine-activated killer (LAK) cell activation by IL-2. Incubation of splenocytes with NAc-cys (0.6 to 1.0 mM) resulted in significant changes in intracellular reduced and total glutathione (92% and 58% increase, respectively) at 96 h. These levels correlated with markedly enhanced cell proliferation (threefold) and cytolytic effector cell generation (>fivefold increase in LU/10(6) cells) induced by the combination of NAc-cys with IL-2. IL-2 exposure by itself unexpectedly increased intracellular reduced glutathione by 43%. IL-2 and NAc-cys were synergistic in increasing glutathione levels (reduced glutathione: 292% increase; total: 251% increase). Inhibition of glutathione synthesis, using L-buthionine-(S,R)-sulfoximine reversed the effects of NAc-cys on intracellular glutathione, as well as cellular proliferation and cytotoxicity. This experiment established that the effects of NAc-cys required de novo glutathione synthesis. In conjunction with IL-2/LAK treatment, oral NAc-cys administration (260 to 900 mg/kg/day for 7 days) significantly decreased tumor progression in a refractory s.c. tumor model. A small fraction of mice (11 to 17%) had complete tumor regressions. NAc-cys may be useful as an adjunct to increase the antitumor activity of IL-2/LAK therapy.

14. **Hargrove, M. E., J. Wang and C. C. Ting (1993). "Regulation by glutathione of the activation and differentiation of IL-4- dependent activated killer cells." Cell Immunol 149(2): 433-43.**

Glutathione (GSH) was shown to regulate the generation of IL-2- dependent activated killer cells. Generation of alpha CD3-activated killer cells CD3-AK was regulated by both IL-2 and IL-4. In the present study the role of GSH in the regulation of IL-4-dependent CD3-AK cells was examined. After initial activation of mouse splenocytes by alpha CD3, subculturing the CD3-AK cells in IL-4 resulted in the production of IL-4-dependent killer cells whose proliferative and cytolytic activities were abrogated by alpha IL-4 antibody 11B11. Adding graded doses of BSO, a GSH synthetase inhibitor, into CD3-AK cells culturing in IL-4 resulted in the reduction of their proliferative and cytotoxic responses. Adding exogenous GSH reversed the inhibitory effect of BSO and restored the proliferation and cytolytic activity of IL-4-dependent CD3-AK cells. The dose requirement for BSO to affect the IL-4-dependent CD3-AK cells was similar to that for the IL-2-dependent CD3-AK cells. These findings indicate that GSH also regulates the function of IL-4 in the activation and differentiation of CD3-AK cells. To further study the mechanism for the GSH regulation of the cytolytic activity of CD3- AK cells, we found that BSO did not reduce the production of BLT- esterase which contained mostly the cytolytic granules; in fact, BLT- esterase production was often increased by BSO. Furthermore, the exocytosis and effector function of cytolytic granules were also not affected by BSO. Thus it appears that reduction of cellular GSH may result in the accumulation of defective cytolytic granules which accounts for the reduction of killer cell cytolytic activity.

15. **Iwata, S., T. Hori, N. Sato, Y. Ueda-Taniguchi, T. Yamabe, H. Nakamura, H. Masutani and J. Yodoi (1994). "Thiol-mediated redox regulation of lymphocyte proliferation. Possible involvement of adult T cell leukemia-derived factor and glutathione in transferrin receptor expression." J. Immunol. 152(12): 5633-5642.**

The proliferative response of PBMC to PHA, Con A, OKT3 mAb and IL-2-dependent proliferation of PHA-blasts was examined in a thiol-free environment (cultured in a L-cystine- and GSH-free medium). [H-3]TdR incorporation assay and cell cycle analysis revealed that stimulated PBMC could not enter the S phase when deprived of these thiol compounds. In thiol-free cultures, an increase in intracellular free Ca2+ concentration and IL-2R alpha-chain/p55 (Tac) induction was still observed, whereas transferrin receptor induction was markedly reduced, suggesting that the proliferative response of mitogenically stimulated PBMC was arrested in the late G(1) phase in which transferrin receptor is induced. In GSH-depleted cultures, a similar reduction of the proliferative response of PBMC and PHA-blasts was observed when the concentration of L-cystine was lowered, in a dose-dependent manner. Each reduction or loss of proliferative response was partially restored by supplementation of 2-ME or adult T cell leukemia-derived factor (ADF)/human thioredoxin which is considered to be an endogenous dithiol-reducing factor. L-Cystine transport analysis showed that mitogenically stimulated PBMC and PHA blasts incorporated L-cystine, whereas resting PBMC did not. Furthermore, ADF as well as 2-ME exhibited an enhancing activity on the L-cystine transport in PHA blasts. Together with the fact that L-cystine transport is a limiting step in glutathione synthesis, these findings suggest that GSH and ADF might cooperate in the thiol-mediated redox regulation process and might also play key roles in cell cycle (late G(1) to S) progression of activated lymphocytes.

16. Chen, G., S. H. Wang and C. A. Converse (1994). "Glutathione increases interleukin-2 production in human lymphocytes." Int J Immunopharmacol 16(9): 755-60.

It is known that glutathione (GSH) has an immunological effect on several features of the immune system. The present study investigated the effects of GSH on interleukin-2 (IL-2) production from normal human peripheral blood lymphocytes (PBL). The results showed that both exogenous GSH and 2-mercaptoethanol (2-ME) significantly increased intracellular GSH levels after PBL were incubated with both agents. IL- 2 production from PBL was markedly increased at the presence of exogenous GSH (0.5-8 mmol/l) or 2-ME (12.5-50 mumol/l) which corresponded to 1.57-2.82 nmol/10(6) cells and 1.41 - 1.80 nmol/10(6) cells of intracellular concentrations of GSH, respectively. However, IL- 2 production seemed to reach a steady level when exogenous GSH concentrations in cell culture were between 2 and 8 mmol/l. The findings also showed that there was a positive correlation between the IL-2 concentrations and intracellular GSH levels. This study indicated that both exogenous GSH and 2-ME were able to elevate intracellular GSH levels and the increased intracellular GSH could increase IL-2 production in vitro. It is suggested that GSH may exert its effects on the immune system via the regulation of IL-2 synthesis.

17. Jeannin, P., Y. Delneste, S. Lecoanet-Henchoz, J.-F. Gauchat, P. Life, D. Holmes and J.-Y. Bonnefoy (1995). "Thiols decrease human interleukin (IL) 4 production and IL-4-induced immunoglobulin synthesis." J. Exp. Med. 182(6): 1785-1792.

N-Acetyl-L-cysteine (NAC) is an antioxidant precursor oi intracellular glutathione (GSH), usually given in humans as a mucolytic agent. In vitro, NAC and GSH have been shown to act on T cells by increasing interleukin (IL) 2 production, synthesis and turnover of IL-2 receptors, proliferation, cytotoxic properties, and resistance to apoptosis. We report here that NAC and GSH decrease in a dose-dependent manner human IL-4 production by stimulated peripheral blood T cells and by T helper (Th) 0- and Th2-like T cell clones. This effect was associated with a decrease in IL-4 messenger RNA transcription. In contrast, NAC and GSH had no effect on interferon gamma and increased IL-2 production and T cell proliferation. A functional consequence was the capacity of NAC and GSH to selectively decrease in a dose-dependent manner IL-4-induced immunoglobulin (Ig) E and IgG4 production by human peripheral blood mononuclear cells. Interestingly, NAC and GSH also acted directly on purified tonsillar B cells by decreasing the mature epsilon messenger RNA, hence decreasing IgE production. In contrast, IgA and IgM production were not affected. At the same time, B cell proliferation was increased in a dose-dependent manner. Not all antioxidants tested but only SH-bearing molecules mimicked these properties. Finally, when given orally to mice, NAC decreased both IgE and IgG1 antibody responses to ovalbumin. These results demonstrate that NAC, GSH, and other thiols may control the production of both the Th2-derived cytokine IL-4 and IL-4-induced Ig in vitro and in vivo.

18. Tsuyuki, S., A. Yamauchi, H. Nakamura, K. Kinoshita, T. Gomi, K. Tanaka, T. Inamoto and Y. Yamaoka (1998). "Possible availability of N-acetylcysteine as an adjunct to cytokine therapy for hepatocellular carcinoma." Clin. Immunol. Immunopathol. 88(2): 192-198.

To examine the possibility of immunotherapy for activating liver-associated mononuclear cells (liver MNC) in hepatocellular carcinoma (HCC), we evaluated the cytotoxicity of liver MNC and peripheral blood mononuclear cells (PBMNC) in HCC patients and examined how they can be activated by cytokines and how this activation is modulated by reduction/ oxidation. Cytotoxicity of liver MNC but not PBMNC in HCC patients was significantly decreased compared with that of controls, despite no alteration in the subpopulation of liver MNC between the two groups. We next measured intracellular glutathione (GSH), which is required for the enhancement of the cytotoxicity by interleukin-2 (IL-2). Intracellular GSH levels of liver MNC in HCC were significantly lower than that of controls. In vitro administration of N-acetylcysteine (NAC) not only restored intracellular GSH levels but also enhanced the IL-2-stimulated cytotoxicity of liver MNC in HCC patients. This suggests that intracellular GSH of liver MNC in HCC may participate in the modulation of cytotoxicity of liver MNC in vitro and that NAC may be effective as an adjunct to immunotherapy for HCC. (C) 1998 Academic Press.

19. **Tsuyuki, S., A. Yamauchi, H. Nakamura, Y. Nakamura, K. Kinoshita, T. Gomi, Y. Kawai, T. Hirose, K. Furuke, I. Ikai, K. Ohmori, Y. Yamaoka and T. Inamoto (1998). "N-acetylcysteine improves cytotoxic activity of cirrhotic rat liver-associated mononuclear cells." International Immunol. 10(10): 1501-1508.**

Liver cirrhosis, which is associated with decreased plasma and hepatic glutathione (GSH), has been reported to cause the suppression of NK activity in peripheral blood mononuclear cells. Since low GSH levels in lymphocytes are known to alter lymphocyte function, we examined the correlation between intracellular GSH levels and the cytotoxic activity of liver-associated mononuclear cells (liver MNC), We show here that rat liver contains a highly active population of NK cells (CD3(-) NKR-P1(+) cells) that kill Yac-l in vitro and that the cytotoxic activity of this NK population is directly proportional to liver MNC GSH. This proportionality is independent of the methods used to alter GSH level. Thus, in vitro treatment of liver MNC with buthionine sulfoximine to lower GSH levels lowers the cytotoxic activity. MNC from cirrhotic liver, in which implanted tumor cells grow faster, have both low GSH levels and low cytotoxicity, and supplementation of cirrhotic liver MNC with N-acetylcysteine raises GSH levels and increases cytotoxicity. These findings suggest a physiologic mechanism, i.e. Decreased GSH, may be causally associated with the increased incidence of hepatoma in cirrhotic individuals and the increased growth of hepatoma cells in cirrhotic animals. Thus, we suggest that the GSH is important to the optimal functioning of the hepatic immunity that protects against hepatoma development.

20. **Peterson, J. D., L. A. Herzenberg, K. Vasquez and C. Waltenbaugh (1998). "Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns." Proc. Natl. Acad. Sci. USA 95(6): 3071-3076.**

Current thinking attributes the balance between T helper 1 (Thl) and Th2 cytokine response patterns in immune responses to the nature of the antigen, the genetic composition of the host, and the cytokines involved in the early interaction between T cells and antigen-presenting cells. Here we introduce glutathione, a tripeptide that regulates intracellular redox and other aspects of cell physiology, as a key regulatory element in this process. By using three different methods to deplete glutathione from T cell receptor transgenic and conventional mice and studying in vivo and/or in vitro responses to three distinct antigens, we show that glutathione levels in antigen-presenting cells determine whether Thl or Th2 response patterns predominate. These findings present new insights into immune response alterations in HIV and other diseases. Further, they potentially offer an explanation for the well known differences in immune responses in "Thl" and "Th2" mouse strains.

21. **DeRosa, S. C., M. D. Zaretsky, J. G. Dubs, M. Roederer, M. Anderson, A. Green, D. Mitra, N. Watanabe, H. Nakamura, I. Tjioe, S. C. Deresinski, W. A. Moore, S. W. Ela, D. Parks, L. A. Herzenberg and L. A. Herzenberg (2000). "N-acetylcysteine (NAC) replenishes glutathione in HIV infection." European Journal of Clinical Investigation 30: 841-856.**

Background. Glutathione (GSH) deficiency is common in HIV-infected individuals and is associated with impaired T cell function and impaired survival. N-acetylcysteine (NAC) is used to replenish GSH depleted by acetaminophen overdose. Studies here, test oral administration of NAC for safe and effective GSH replenishment in HIV infection.

Design. Oral NAC administration in a randomized, 8-week double-blind, placebo-controlled trial followed by optional open-label drug for up to 24 weeks. Subjects: HIV-infected, low GSH, CD4 T cells <500/ml, no active opportunistic infections or other debilitation; n=81. Study conducted prior to introduction of protease inhibitors,

Results. Whole blood GSH levels in NAC arm subjects significantly increased from 0.88 mM to 0.98 mM, bringing GSH levels in NAC-treated subjects to 89% of uninfected controls. Baseline GSH levels in the placebo group (0.91) remained essentially the same during the 8 week placebo-controlled trial (p= 0.03). T cell GSH, adjusted for CD4 T cell count and b2-microglobulin levels, also increased in the NAC-treated subjects (p=0.04). Adverse effects were minimal and not significantly associated with NAC ingestion.

Conclusion. NAC treatment for 8 weeks safely replenishes whole blood GSH and T cell GSH in HIV-infected individuals. Thus, NAC offers useful adjunct therapy to increase protection against oxidative stress, improve immune system function and increase detoxification of acetaminophen and other drugs. These findings suggest that NAC therapy could be valuable in other clinical situations in which GSH deficiency or oxidative stress plays a role in disease pathology, e.g., rheumatoid arthritis, Parkinson's disease, hepatitis, liver cirrhosis, septic shock and diabetes.

22. Herzenberg, L. A., S. C. De Rosa, J. G. Dubs, M. Roederer, M. T. Anderson, S. W. Ela, S. C. Deresinski and L. A. Herzenberg (1997). "Glutathione deficiency is associated with impaired survival in HIV disease." Proc. Nat. Acad. Sci. 94: 1967-1972.

Glutathione (GSH), a cysteine-containing tripeptide, is essential for the viability and function of virtually all cells. In vitro studies showing that low GSH levels both promote HIV expression and impair T cell function suggested a link between GSH depletion and HIV disease progression. Clinical studies presented here directly demonstrate that low GSH levels predict poor survival in otherwise indistinguishable HIV- infected subjects. Specifically, we show that GSH deficiency in CD4 T cells from such subjects is associated with markedly decreased survival 2-3 years after baseline data collection (Kaplan-Meier and logistic regression analyses, $P < 0.0001$ for both analyses). This finding, supported by evidence demonstrating that oral administration of the GSH prodrug N-acetylcysteine replenishes GSH in these subjects and suggesting that N-acetylcysteine administration can improve their survival, establishes GSH deficiency as a key determinant of survival in HIV disease. Further, it argues strongly that the unnecessary or excessive use of acetaminophen, alcohol, or other drugs known to deplete GSH should be avoided by HIV-infected individuals.

23. Look, M. P., J. K. Rockstroh, G. S. Rao, S. Barton, H. Lemoch, R. Kaiser, B. Kupfer, T. Sudhop, U. Spengler and T. Sauerbruch (1998). "Sodium selenite and N-acetylcysteine in antiretroviral-naive HIV-I-infected patients: a randomized, controlled pilot study." Eur. J. Clin. Invest. 28(5): 389-397.

Background The aim of this work was to study the effects of combined oral administration of N-acetylcysteine (NAC) and sodium selenite (Se) on plasma glutathione (GSH), lymphocyte subpopulations and viral load in asymptomatic human immunodeficiency virus (HIV)-infected patients. Methods We used a prospective, randomized and controlled therapy trial with partial crossover. Twenty-four antiretroviral-naive HIV-infected outpatients at Centers for Disease Control (CDC) '93 stages I and II were randomized to receive the antioxidant combination NAC 600 mgt.i.d. And Se 500 mu g per day for either 24 weeks (group A, n = 13) or from the end of week 12 (group B, n = 11) until the end of week 24. Thus, group B served as untreated control during the first 12 weeks. Results There was (a) a trend towards an increase in the percentage of CD4(+) lymphocytes after 6 weeks (P=0.08); (b) an increase in the CD4/CD8 ratio after 6 and 12 weeks (P = 0.02 and P = 0.04 respectively); and (c) a decrease in the absolute CD8/CD38 count and percentage of lymphocytes after 6 weeks (P = 0.002 and P = 0.033 respectively) and 12 weeks (P = 0.033, P = 0.1 respectively) in group A compared with the control period of group B. The effects observed in group A were, however, not paralleled to the same extent by group B after crossing-over to treatment after 2 weeks. In addition, erythrocyte glutathione peroxidase (GSH-Px) activity and GSH, glutathionedisulphide (GSSG) concentrations and the reduced/total GSH ratio were not affected by the treatment. Serum selenium levels increased significantly (P<0.001) upon treatment. Viral load was not altered. Conclusions The changes in lymphocyte subsets after NAC/Se treatment were not comparable to those after standard antiretroviral drug therapy. This, however, does not preclude per se possible benefits of antioxidant supplementation in HIV disease.

24. Ovesen, T., J. U. Felding, B. Tommerup, L. P. Schousboe and C. G. Petersen (2000). "Effect of N-acetylcysteine on the incidence of recurrence of otitis media with effusion and re-insertion of ventilation tubes." Acta Oto - Laryngol.: 79-81.

Previous studies have demonstrated the anti-inflammatory, anti-oxidant, and mucolytic nature of N-acetylcysteine (NAC). Theoretically, these properties make the substance ideal for therapeutic use against otitis media with effusion (OME). The disease is characterized as a sustained non-specific inflammation of the middle ear mucosa with secretory. Transformation of the epithelium resulting in accumulation of fluid in the middle ear space. To investigate the effects of instillation of NAC in the middle ear, a double-blind, placebo-controlled, randomized trial was carried out. A total of 75 children who were undergoing their first bilateral insertion of ventilation tubes (VT) due to OME were randomized to Mucomyst (NAC) or placebo, (the Vehicle) on one ear in relation to the VT insertion. The contralateral ear underwent VT insertion exclusively. Instillation of Mucomyst or placebo was repeated 3 and 7 days afterwards. The children were followed regularily for 11-39 months. Episodes of otorrhea the recurrence of OME after VT extrusion and re-insertion of VTs were registered as primary outcome parameters. The results demonstrated that Mucomyst significantly reduced the recurrence of OME: and re-insertion of VTs ($p < 0.025$) and significantly increased the time until VT extrusion ($p < 0.0167$). In addition, the number of episodes of ear problems and visits at the ENT clinic were reduced significantly by NAC ($p < 0.0383$).

25. Kinscherf, R., T. Fischbach, S. Mihm, S. Roth, E. Hohenhaus-Sievert, C. Weiss, L. Edler, P. Bartsch and W. Droge (1994). "Effect of glutathione depletion and oral N-acetyl-cysteine treatment on CD4+ and CD8+ cells." FASEB J. 8(6): 448-451.

HIV-infected individuals and SIV-infected rhesus macaques have, on the average, decreased plasma cysteine and cystine concentrations and decreased intracellular glutathione levels. We show that the cysteine supply and the intracellular glutathione levels have a strong influence on the T cell system. A study of healthy human subjects revealed that persons with intracellular glutathione levels of 20-30 nmol/mg protein had significantly higher numbers of CD4(+) T cells than persons with either lower or higher glutathione levels. Persons who moved during a 4-week observation period from the optimal to the suboptimal range (10-20 nmol/mg) experienced, on the average, a 30% decrease in CD4(+) T cell numbers. This decrease was prevented by treatment with N-acetyl-cysteine (NAC). NAC caused this relative increase of CD4(+) T cell numbers in spite of decreasing glutathione levels and not by increasing the glutathione level. Our studies suggest that the immune system may be exquisitely sensitive not only against a cysteine and glutathione deficiency but also against an excess of cysteine.

26. Clotet, B., M. Gomez, L. Ruiz, G. Sirera and J. Romeu (1995). "Lack of short-term efficacy of N-acetyl-L-cysteine in human immunodeficiency virus-positive patients with CD4 cell counts <250/mm(3) {Letter}." J. Acq. Immun. Defic. Synd. Hum. Retrov. 9(1): 98-99.

27. Akerlund, B., C. Jarstrand, B. Lindeke, A. Sonnerborg, A.-C. Akerblad and O. Rasool (1996). "Effect of N-acetylcysteine (NAC) treatment on HIV-1 infection: a double-blind placebo-controlled trial." Eur. J. Clin. Pharmacol. 50(6): 457-461.

Objective: In a double-blind placebo-controlled trial, human immunodeficiency virus (HIV)seropositive patients with a CD4 lymphocyte cell count of more than 200 x 10(6) . l(-1) were randomised to receive either 800 mg N-acetylcysteine (NAC) or placebo for 4 months. Before treatment low plasma cysteine levels, high free radical activity in neutrophils in the presence of autologous plasma - measured by the nitroblue tetrazolium (NBT) test - and increased tumor necrosis factor (TNF)-alpha levels were found in the HIV positive patients.

Results: After treatment the low plasma cysteine level in the NAC group increased to normal, and the decline of the CD4+ lymphocyte count before the study start, was less steep in the NAC group than in the placebo group after treatment, There was also a reduction in TNF-alpha level. However NAC had no effect on the radical production by neutrophils, and although it did not increase the CD4+ cell count, it may have decreased the decline in CD4+ cells.

Conclusion: Further controlled trials with NAC are needed to determine whether it has a beneficial effect in the treatment of asymptomatic HIV-infected individuals.

28. Barditch-Crovo, P., D. Noe, G. Skowron, M. Lederman, R. C. Kalayjian, P. Borum, R. Buier, W. B. Rowe, D. Goldberg and P. Lietman (1998). "A phase I/II evaluation of oral L-2-oxothiazolidine-4-carboxylic acid in asymptomatic patients infected with human immunodeficiency virus." J. Clin. Pharmacol. 38(4): 357-363.

A randomized double-blind, placebo-controlled study was conducted in 37 asymptomatic HIV-infected individuals (mean CD4 count 707 cells/mm(3)) to characterize the safety, pharmacokinetics, and effect on blood thiols of three dosage levels of a cysteine prodrug, L-2-oxothiazolidine-4-carboxylic acid (OTC; Procysteine; Clintec Technologies, Deerfield, IL). Single-dose administration of OTC resulted in measurable plasma levels at all dosages, with a mean peak plasma concentration of 734 +/- 234 nmol/mL at the highest dosage studied. After 4 weeks of administration three times daily, a statistically significant increase was seen in whole blood glutathione in the 1,500 mg and 3,000 mg dose groups compared with the placebo group. A significant increase in whole blood cysteine and peripheral blood mononuclear cell (PBMC) glutathione was not seen during the study period. (C)1998 The American College of Clinical Pharmacology.

29. **Breitkreutz, R., N. Pittack, C. T. Nebe, D. Schuster, J. Brust, M. Beichert, V. Hack, V. Daniel, L. Edler and W. Droge (2000). "Improvement of immune functions in HIV infection by sulfur supplementation: Two randomized trials." J. Molecular Med. - JMM 78(1): 55-62.**

To determine the therapeutic effect of sulfur amino acid supplementation in HIV infection we randomized 40 patients with antiretroviral therapy (ART; study 1) and 29 patients without ART (study 2) to treatment for 7 months with N-acetyl-cysteine or placebo at an individually adjusted dose according to a defined scheme. The main outcome measures were the change in immunological parameters including natural killer (NK) cell and T cell functions and the viral load. Both studies showed consistently that N-acetyl-cysteine causes a marked increase in immunological functions and plasma albumin concentrations. The effect of N-acetyl-cysteine on the viral load, in contrast, was not consistent and may warrant further studies. Our findings suggest that the impairment of immunological functions in HIV+ patients results at least partly from cysteine deficiency. Because immune reconstitution is a widely accepted aim of HIV treatment, N-acetyl-cysteine treatment may be recommended for patients with and without ART. Our previous report on the massive loss of sulfur in HIV-infected subjects and the present demonstration of the immunoreconstituting effect of cysteine supplementation indicate that the HIV-induced cysteine depletion is a novel mechanism by which a virus destroys the immune defense of the host and escapes immune elimination.

30. **Grassi, C. and G. C. Morandini (1976). "A controlled trial of intermittent oral acetylcysteine in the long-term treatment of chronic bronchitis." Eur J Clin Pharmacol 09(5-6): 393-6.**

In 69 out-patients with chronic bronchitis in 6 centres the effects of acetylcysteine 600 mg daily, 3 days a week for 6 months, and a placebo have been compared in a double-blind controlled trial. Thirty-five patients were treated with the mucolytic and 34 with the dummy preparation. In the former the clinical course of the chronic bronchitis improved to a greater extent and a significantly lower number of exacerbations was observed. The advantages of long-term oral treatment with the mucolytic in chronic bronchitis suggest that it may be useful as an alternative to long-term antibiotic prophylaxis, or to complement brief courses of antibiotics, in addition to the usual physiotherapy.

31. **Grassi, C. (1980). "Long-term oral acetylcysteine in chronic bronchitis. a double-blind controlled study." Eur J Respir Dis Suppl 111: 93-108.**

32. **Aylward, M., J. Maddock and P. Dewland (1980). "Clinical evaluation of acetylcysteine in the treatment of patients with chronic obstructive bronchitis: a balanced double-blind trial with placebo control." Eur J Respir Dis Suppl 111: 81-9.**

33. **Brocard, H., J. Charpin and J. Germouty (1980). "[Multicenter, double-blind study of oral acetylcysteine vs. placebo]." Eur J Respir Dis Suppl 111: 65-9.**

The mucolytic activity of acetylcysteine (NAC) was evaluated in a double-blind, placebo controlled, clinical trial performed in three pneumology centres and involving a total of 215 patients with the following diagnoses: 84 acute bronchitis, 95 superinfections of chronic bronchitis, 36 complicated bronchitis in patients with severe chronic respiratory insufficiency. Treatment consisted of 1 sachet of 200 mg NAC t.i.d. for 10 days. Standard antibiotic therapy (amoxycillin 1.5 g/day) was concurrently administered for 7 days. Statistical analysis comparing sputum volume and viscosity, sedation of cough and improvement of PEFR in 108 NAC and in 107 placebo treated patients, showed that NAC was very significantly more effective than placebo. The effect of NAC was negligible in the 36 patients with complicated bronchitis, whereas it was evident and remarkable in patients with acute and chronic bronchitis.

34. **Boman, G., U. Backer, S. Larsson, B. Melander and L. Wahlander (1983). "Oral acetylcysteine reduces exacerbation rate in chronic bronchitis: report of a trial organized by the Swedish Society for Pulmonary Diseases." Eur J Respir Dis 64(6): 405-15.**

This multicentre trial was undertaken to confirm previous results indicating that long-term treatment with oral acetylcysteine reduces the exacerbation rate in patients with chronic bronchitis. Two hundred and eighty-five patients, smokers or ex-smokers, with chronic bronchitis started a pre-trial placebo-period of 1 month. After this run-in period 259

patients were included in the trial and randomized into two parallel groups. The patients were treated in a double-blind way either with acetylcysteine 200 mg b.i.d. or placebo b.i.d. for 6 months. The trial was completed by 98 patients in the acetylcysteine group and by 105 patients in the placebo group. Initially, there were no significant differences between the groups. Twice weekly, the patients filled in a diary card concerning symptoms. The number of exacerbations was assessed from these cards and at visits 2, 4 and 6 months after institution of therapy. The exacerbation rate was significantly lower in the acetylcysteine group in which 40% of the patients remained free from exacerbations compared to 19% in the placebo group. Sick-leave due to acute exacerbation was significantly less common in the acetylcysteine group. The drug was well tolerated.

35. Jackson, I. M., J. Barnes and P. Cooksey (1984). "Efficacy and tolerability of oral acetylcysteine (Fabrol) in chronic bronchitis: a double-blind placebo controlled study." J Int Med Res 12(3): 198-206.

This multicentre, double-blind, placebo controlled, between-patient study in general practice in the United Kingdom examined the effect of oral N-acetylcysteine (Fabrol) on the symptomatology of patients with chronic bronchitis over a 3-month period. Although improvement in subjective symptoms (sputum viscosity and character, difficulty in expectoration and cough severity) occurred in both treatment groups over the trial period, improvements in difficulty in expectoration and cough severity were greater in patients receiving N-acetylcysteine compared to matching placebo. Trial medication was well tolerated, with a greater number of side-effects attributed to therapy occurring in patients receiving placebo.

36. British Thoracic Society Research Committee (1985). "Oral N-acetylcysteine and exacerbation rates in patients with chronic bronchitis and severe airways obstruction. British Thoracic Society Research Committee." Thorax 40(11): 832-5.

The influence of oral N-acetylcysteine on the exacerbation rate in patients with chronic bronchitis and severe airways obstruction has been studied. Two hundred and forty four patients entered the study during October and November 1983 and took placebo sachets for a run in month. One hundred and eighty one who completed this month satisfactorily were randomised to receive either active (acetylcysteine 200 mg three times a day) or matching placebo sachets for five months in a double blind parallel group study. The two groups were well matched. Patients kept detailed daily symptom diaries and were assessed monthly. At the end of the five months' study the outcome in the group taking acetylcysteine appeared a little better, but the differences did not reach conventional levels of statistical significance for the mean (SD) number of exacerbations (2.1 (0.2) for acetylcysteine, 2.6 (0.2) for placebo; $p = 0.08$); total days taking an antibiotic (13.5 (1.7), 18.0 (2.8); $p = 0.17$); total days spent in bed (4.8 (0.8), 5.1 (1.1); $p = 0.9$); number of withdrawals (13 (15%), 20 (21%); $p = 0.4$); incidence of side effects (which were few); drug compliance (which was good); and the patients' assessment of the treatment.

37. Parr, G. D. and A. Huitson (1987). "Oral Fabrol (oral N-acetyl-cysteine) in chronic bronchitis." Br J Dis Chest 81(4): 341-8.

Five hundred and twenty-six patients suffering from chronic bronchitis were randomized to receive either N-acetylcysteine (NAC) or placebo during a 6-month period. The aim was to compare the number of acute exacerbations in the two groups. General practitioners were asked to enter patients with a diagnosis of chronic bronchitis, based on the MRC criteria. We failed to find any statistically significant difference in the number of exacerbations between the two treatment groups although there was a slight trend towards improvement in the NAC group during the first 3 months of the trial. The tolerability was similar for both treatments. Patients taking NAC showed a reduction in number of days on which they were incapacitated and this result was statistically significant.

38. Rasmussen, J. B. and C. Glennow (1988). "Reduction in days of illness after long-term treatment with N- acetylcysteine controlled-release tablets in patients with chronic bronchitis." Eur Respir J 1(4): 351-5.

The clinical effect of N-acetylcysteine (NAC) controlled-release tablets, 300 mg b.i.d., and placebo, in chronic bronchitis was investigated. The study was performed as a double-blind six month comparison between active drug and placebo in two parallel groups, with statistical evaluation after four and six months. The patients were chosen from nine centres. One hundred and sixteen out-patients were included and ninety one of them completed the six month study. The acetylcysteine-treated group had a significantly reduced number of sick- leave days caused by exacerbations of chronic bronchitis after the four winter months December-March compared with the control group (NAC 173, placebo 456). The number of exacerbation days was also very much reduced, however, not significantly (NAC 204, placebo 399). At the end of the six month trial, including also two spring months, the absolute numbers of sick-leave days and exacerbation days were still fewer in the acetylcysteine-treated group, (NAC 260, placebo 739) and (NAC 378, placebo 557) respectively. This study demonstrates a significant reduction in sick-leave days after four months of NAC-treatment. A constant tendency to reduction in the number of exacerbations and exacerbation days was also registered after four and six months. The differences in these parameters were, however, not statistically significant. This was probably due to the small number of patients participating.

39. Hansen, N. C. G., A. Skriver, L. Brorsen-Riis, S. Balslov, T. Evald, N. Maltbaek, G. Gunnersen, P. Garsdal, P. Sander, J. Z. Pedersen, T. B. Ibsen and F. V. Rasmussen (1994). "Orally administered N-acetylcysteine may improve general well-being in patients with mild chronic bronchitis." Resp. Med. 88(7): 531-535.

Oral N-acetylcysteine (NAC) exerts a beneficial action in chronic bronchitis by reducing the number of exacerbations. There have been few studies of the effect of NAC (or of any other drug) on general well-being in chronic bronchitis. We used an established psychiatric instrument (General Health Questionnaire; GHQ) and a visual analogue scale (VAS) to measure well-being in a 22-week, placebo-controlled, double-blind, parallel-group study of NAC administered as sustained release tablets 600 mg b.i.d., including during the winter months, to patients with mild chronic bronchitis. One hundred and fifty-three patients were accepted for randomized treatment, 129 finished the study (59 NAC, 70 placebo), and well-being was measured in 105 (46 NAC, 59 placebo). The number of observed exacerbations was unexpectedly low in both groups. The number was lowest in the NAC group, however, the difference did not reach statistical significance in the present study (P = 0.08). There were no statistically significant differences between NAC and placebo in subjective symptom scores, FEV1 or FVC. The distribution of GHQ score at baseline was uneven, but NAC was significantly superior to placebo in terms of a favourable effect on GHQ score. GHQ score correlated with the number of exacerbations, and VAS correlated with GHQ score. This study therefore demonstrates the validity of measuring general well-being in patients with mild chronic bronchitis. Future studies of the treatment of chronic bronchitis should use a battery of more specifically adapted instruments which are now becoming available to measure well-being.

40. Grandjean, E. M., P. Berthet, R. Ruffmann and P. Leuenberger (2000). "Cost-effectiveness analysis of oral N-acetylcysteine as a preventive treatment in chronic bronchitis." Pharmacol. Res. 42(1): 39-50.

Chronic bronchitis has a prevalence of approximately 11% in the population aged over 35 years and its frequent acute exacerbations (AECBs) are an important cause of morbidity and costs in health-care resources. Oral N-acetylcysteine (NAC) is administered during the winter months as a way of reducing AECBs. This cost-effectiveness analysis was done from the payers' point of view in the Swiss health-care system, based on a retrospective analysis of published placebo-controlled studies. The pooled data show that continuous administration of 400 mg day(-1) per os of NAC leads to a significant reduction in the number of AECBs (NAC: 16.2 vs 25.2% AECBs per month); a significantly smaller percentage of days of sick leave (NAC: 3.6 us 5.3%) and a lower rate of hospitalizations (NAC: 1.5 vs 3.5% over a period of 6 months). Taking into account the poor compliance of these patients, calculations assumed a compliance of 80%. Direct costs were those of an NAC treatment, the management of an AECB (biological tests in 59%, X-rays in 65% and pulmonary function tests in 45%; antibiotics 70%, bronchodilators in 89%, corticosteroids in 24% and 'others' in 25% of the patients), and of hospitalizations (estimated at 10 days per case). Based on these figures, the mean direct costs of an untreated patient were CHF 869 us CHF 700 in the NAC-treated patient. Univariate sensitivity analysis indicated that cost neutrality is reached with 0.6 (< 0.25-1.94, 95% CI) AECBs per 6 months. Indirect costs (based on sick leave) were also significantly different; the mean in untreated patients was CHF 1324 vs CHF 779 in the NAC-treated patients. Conclusion: Treating chronic bronchitis patients with NAC during the winter months is cost-effective both from the payer's and a social point of view. (C) 2000 Academic Press.

41. Stey, C., J. Steurer, S. Bachmann, T. C. Medici and M. R. Tramer (2000). "The effect of oral N-acetylcysteine in chronic bronchitis: a quantitative systematic review [In Process Citation]." Eur Respir J 16(2): 253-62.

The role of N-acetylcysteine (NAC) in the treatment of chronic bronchitis is unclear. Since a number of studies have been published on this topic, a systematic review of published studies seems justified. A systematic search (Medline, Embase, Cochrane Library, bibliographies, no language restriction) for published randomized trials comparing oral NAC with placebo in patients with chronic bronchitis was performed. Dichotomous data on prevention of exacerbation, improvement of symptoms and adverse effects were extracted from original reports. The relative benefit and number-needed-to-treat were calculated for both individual trials and combined data. Thirty-nine trials were retrieved; eleven (2,011 analysed patients), published 1976-1994, were regarded as relevant and valid according to preset criteria. In nine studies, 351 of 723 (48.5%) patients receiving NAC had no exacerbation compared with 229 of 733 (31.2%) patients receiving placebo (relative benefit 1.56 (95% confidence interval (CI) 1.37-1.77), number-needed-to-treat 5.8 (95% CI 4.5-8.1). There was no evidence of any effect of study period (12-24 weeks) or cumulative dose of NAC on efficacy. In five trials, 286 of 466 (61.4%) patients receiving NAC reported improvement of their symptoms compared with 160 of 462 (34.6%) patients receiving placebo (relative benefit 1.78 (95% CI 1.54-2.05), number-needed-to-treat 3.7 (95% CI 3.0-4.9)). With NAC, 68 of 666 (10.2%) patients reported gastrointestinal adverse effects compared with 73 of 671 (10.9%) taking placebo. With NAC, 79 of 1,207 (6.5%) patients withdrew from the study due to adverse effects, compared with 87 of 1,234 (7.1%) receiving placebo. In conclusion, with treatment periods of approximately 12-24 weeks, oral N-acetylcysteine reduces the risk of exacerbations and improves symptoms in patients with chronic bronchitis compared with placebo, without increasing the risk of adverse effects. Whether this benefit is sufficient to justify the routine and long-term use of N- acetylcysteine in all patients with chronic bronchitis should be addressed in further studies and cost-effectiveness analyses.

42. **Suter, P. M., G. Domenighetti, M.-D. Schaller, M.-C. Laverriere, R. Ritz and C. Perret (1994). "N-acetylcysteine enhances recovery from acute lung injury in man. A randomized, double-blind, placebo-controlled clinical study." <u>Chest</u> 105: 190-194.**

OBJECTIVE: To determine the effects of intravenous N-acetylcysteine (NAC) on the development of severe adult respiratory distress syndrome (ARDS) and mortality rate in patients with mild-to-moderate acute lung injury and to analyze the duration of ventilatory support and FIO2 required as well as the evolution of the lung injury score.

SETTING: Three university hospital ICUs and one regional ICU in Switzerland.

PATIENTS: Sixty-one adult patients presenting with mild-to-moderate acute lung injury and various predisposing factors for ARDS received either NAC, 40 mg/kg/d, or placebo intravenously for 3 days.

MEASUREMENTS: Respiratory dysfunction was assessed daily according to the need for mechanical ventilation and FIO2, the evolution of the lung injury score, and the PaO2/FIO2 ratio. The cardiovascular state, liver function, and kidney function were also monitored. Data were collected at admission (day 0), during the first 3 days, and on the day of discharge from the ICU.

RESULTS: The NAC and placebo groups (32 and 29 patients, respectively) were comparable at ICU admission for severity of illness assessed by the simplified acute physiology score (SAPS) (10.8 +/- 4.6 vs 10.9 +/- 4.8) and lung injury score (LIS) (1.39 +/- 0.95 vs 1.11 +/- 1.08) (mean +/- SD). Three patients in each group developed ARDS. The 1-month mortality rate was 22 percent for the NAC group and 35 percent for the placebo group (difference not statistically significant). At admission, 22 of 32 patients (69 percent) in the NAC group were mechanically ventilated compared with 22 of 29 (76 percent) in the placebo group. At the end of the treatment period (day 3), 5 of 29 (17 percent) in the NAC group and 12 of 25 (48 percent) in the placebo group were still receiving ventilatory support ($p = 0.01$), The FIO2 was 0.37 less than admission value (day 0) in the NAC group, and 0.20 less in the placebo group ($p < 0.04$); the oxygenation index (PaO2/FIO2) improved significantly ($p < 0.05$) from day 0 to day 3 only in the NAC-treated group. The LIS showed a significant regression ($p = 0.003$) in the NAC-treated group during the first 10 days of treatment: no change was observed in the placebo group. No adverse effects were observed during the treatment with NAC.

CONCLUSIONS: Intravenous NAC treatment during 72 h improved systemic oxygenation and reduced the need for ventilatory support in patients presenting with mild-to-moderate acute lung injury subsequent to a variety of underlying diseases. Development of ARDS and mortality were not reduced significantly by this therapy.

43. **Bernard, G. R., A. P. Wheeler, M. M. Arons, P. E. Morris, H. L. Paz, J. A. Russell, P. E. Wright, G. R. Bernard, M. M. Arons, A. P. Wheeler, L. C. Carmichael, P. E. Morris, S. B. Higgins, W. D. Dupont, T. R. Edens, B. B. Swindell, J. A. Russell, H. L. Paz, P. E. Wright and K. P. Steinberg (1997). "A trial of antioxidants N-acetylcysteine and procysteine in ARDS." <u>Chest</u> 112(1): 164-172.**

Objective: To determine the levels of glutathione and cysteine in patients with ARDS and examine the effect of treatment with N-acetylcysteine (NAC) and L-2-oxothiazolidine-1-carboxylate (Procysteine; Clintec Technologies Inc; Chicago [OTZ]) on these levels and on common physiologic abnomalities, and organ dysfunction associated with ARDS. Design: Randomized, double-blind, placebo-controlled, prospective clinical trial. Setting: ICUs in five clinical centers in the United States and Canada. Patients: Patients meeting a predetermined definition of ARDS and requiring mechanical ventilation. Intervention: Standard care for ARDS and IV infusion, every 8 h for 10 days, of one of the following: NAC (70 mg/kg, n = 14), OTZ (63 mg/kg, n = 17), or placebo (n = 15). Main results: Both antioxidants effectively repleted RBC glutathione gradually over the 10-day treatment period (47% and 49% increases from baseline values for NAC and OTZ, respectively). There was no difference in mortality among groups (placebo, 40%: NAC, 36%; OTZ, 35%). However, the number of days of acute lung injury was decreased and there was also a significant increase in cardiac index in both treatment groups (NAC/OTZ[+]14%; placebo[-]6%). Conclusions: Our findings suggest that repletion of glutathione may safely be accomplished with NAC or OTZ in patients with acute lung injury/ARDS. Such treatment may shorten the duration of acute lung injury, but larger studies are needed to confirm this.

44. Bridgeman, M. M. E., M. Marsden, W. Macnee, D. C. Flenley and A. P. Ryle (1991). "Cysteine and glutathione concentrations in plasma and bronchoalveolar lavage fluid after treatment with N-acetylcysteine." Thorax 46(1 (Jan)): 39-42.

N-acetylcysteine (600 mg/day) was given to patients by mouth for five days before bronchoscopy and bronchoalveolar lavage to determine whether N-acetylcysteine could increase the concentrations of the antioxidant reduced glutathione in plasma and bronchoalveolar lavage fluid. Bronchoalveolar lavage was performed 1-3 hours (group 2, n = 9) and 16-20 hours (group 3, n = 10) after the last dose of N- acetylcysteine and the values were compared with those in a control group receiving no N-acetylcysteine (group 1, n = 8). N-acetylcysteine was not detected in plasma or lavage fluid. Plasma concentrations of cysteine, the main metabolite of N-acetylcysteine and a precursor of reduced glutathione, were greater in the groups receiving treatment (groups 2 and 3) than in group 1. Cysteine concentrations in lavage fluid were similar in the three groups. Concentrations of reduced glutathione were greater in both plasma and lavage fluid in group 2 than in group 1. These data suggest that N-acetylcysteine given by mouth is rapidly deacetylated to cysteine, with resulting increases in the concentrations of cysteine in plasma and of reduced glutathione in plasma and the airways, which thus temporarily increase the antioxidant capacity of the lung.

45. Travaline, J. M., S. Sudarshan, B. G. Roy, F. Cordova, V. Leyenson and G. J. Criner (1997). "Effect of N-acetylcysteine on human diaphragm strength and fatigability." Amer. J. Respir. Crit. Care Med. 156(5): 1567-1571.

Free radical injury is believed to be important in diaphragm dysfunction. N-Acetylcysteine (NAC) is a potent free radical scavenger shown in animal models to attenuate diaphragm fatigue; however, its effects on human diaphragm function are unknown. We assessed diaphragm function by electrophrenic twitch stimulation (Pdi(T)) and twitch occlusion (to yield Pdi(max)) in four healthy subjects 35 +/- 3 yr of age (mean +/- SD). We intravenously administered NAC (150 mg/kg in 250 ml D5W) or placebo (CON) (250 ml D5W) in a randomized manner after subjects were premedicated with antihistamines. There were no significant side effects with the infusion. After infusion, we measured baseline Pdi(max) and Pdi(T) at FRC. Diaphragm fatigue was then induced by subjects breathing through an inspiratory resistive load. Pdi(max) and Pdi(T) were then measured at 15 to 30 min and 1, 2, 3, 4, and 20-25 h after fatigue. Times to fatigue were 13 +/- 4 min (CON) and 21 +/- 6 min (NAC) (p = 0.04). At 15 min after fatigue, Pdi(T) was reduced to 40% (CON) compared with 30% (NAC) initial Pdi(T) value (p = 0.05). Other twitch characteristics (maximal rate of relaxation and maximal contraction rate) were reduced to a greater degree after placebo compared with NAC. There were no significant differences in the rate of recovery between CON and NAC. Pdi(max) at 30 min after fatigue was significantly greater with NAC; however, at 1 h after fatigue, Pdi(max) for CON and NAC were not different, suggesting similar rates of recovery in high-frequency fatigue. These data suggest that NAC may attenuate low-frequency human diaphragm fatigue.

46. Tepel, M., M. van der Giet, C. Schwarzfeld, U. Laufer, D. Liermann and W. Zidek (2000). "Prevention of radiographic-contrast-agent-induced reductions in renal function by acetylcysteine." N. Eng. J. Med. 343((20 July)): 180-184.

BACKGROUND: Radiographic contrast agents can cause a reduction in renal function that may be due to reactive oxygen species. Whether the reduction can be prevented by the administration of antioxidants is unknown.

METHODS: We prospectively studied 83 patients with chronic renal insufficiency (mean [+/-SD] serum creatinine concentration, 2.4+/- 1.3 mg per deciliter [216+/-116 micromol per liter]) who were undergoing computed tomography with a nonionic, low-osmolality contrast agent. Patients were randomly assigned either to receive the antioxidant acetylcysteine (600 mg orally twice daily) and 0.45 percent saline intravenously, before and after administration of the contrast agent, or to receive placebo and saline.

RESULTS: Ten of the 83 patients (12 percent) had an increase of at least 0.5 mg per deciliter (44 micromol per liter) in the serum creatinine concentration 48 hours after administration of the contrast agent: 1 of the 41 patients in the acetylcysteine group (2 percent) and 9 of the 42 patients in the control group (21 percent; P=0.01; relative risk, 0.1; 95 percent confidence interval, 0.02 to 0.9). In the acetylcysteine group, the mean serum creatinine concentration decreased significantly (P<0.001), from 2.5+/-1.3 to 2.1+/-1.3 mg per deciliter (220+/-118 to 186+/-112 micromol per liter) 48 hours after the administration of the contrast medium, whereas in the control group, the mean serum creatinine concentration increased nonsignificantly (P=0.18), from 2.4+/-1.3 to 2.6+/-1.5 mg per deciliter (212+/-114 to 226+/-133 micromol per liter) (P<0.001 for the comparison between groups).

CONCLUSIONS: Prophylactic oral administration of the antioxidant acetylcysteine, along with hydration, prevents the reduction in renal function induced by contrast agents in patients with chronic renal insufficiency.

47. Uden, S., D. Bilton, P. M. Guyan, P. M. Kay and J. M. Braganza (1990). "Rationale for antioxidant therapy in pancreatitis and cystic fibrosis." Adv Exp Med Biol 264: 555-72.

The overlapping features of the acquired diseases acute pancreatitis and chronic pancreatitis on the one hand, and of chronic pancreatitis and pancreatic involvement in the congenital condition cystic fibrosis on the other, suggest that the basic mechanism of pancreatic injury may be the same in each illness. We propose that pancreatic oxidant stress is the common denominator and, furthermore, that this is facilitated by a shortfall of micronutrient antioxidants in the face of heightened free radical activity through different sources. If so antioxidant supplements should alleviate symptoms. This deduction was supported by an exploratory dose-seeking study that spanned five years in 20 patients with recurrent (non-gall stone) acute or chronic pancreatitis and confirmed by a 20-week double-blind placebo-controlled crossover trial of the successful combination (daily doses of 600 micrograms organic selenium, 0.54 g vitamin C, 9000 IU B-carotene, 270 IU vitamin E and 2 g methionine) in a further 20 cases. A randomised trial of glutathione precursors, given intravenously for 24 hours after admission in patients with a first attack of acute pancreatitis, is in progress. Long-term trials of oral antioxidant formulas are planned in patients with cystic fibrosis.

48. De Flora, S., C. Grassi and L. Carati (1997). "Attenuation of influenza-like symptomatology and improvement of cell-mediated immunity with long-term N-acetylcysteine treatment." Eur. Resp. J. 10(7): 1535-1541.

N-acetylcysteine (NAC), an analogue and precursor of reduced glutathione, has been in clinical use for more than 30 yrs as a mucolytic drug. It has also been proposed for and/or used in the therapy and/or prevention of several respiratory diseases and of diseases involving an oxidative stress, in general. The objective of the present study was to evaluate the effect of long-term treatment with NAC on influenza and influenza-like episodes. A total of 262 subjects of both sexes (78% greater than or equal to 65 yrs, and 62% suffering from non-respiratory chronic degenerative diseases) were enrolled in a randomized, double-blind trial involving 20 Italian Centres. They were randomized to receive either placebo or NAC tablets (600 mg) twice daily for 6 months. Patients suffering from chronic respiratory diseases were not eligible, to avoid possible confounding by an effect of NAC on respiratory symptoms. NAC treatment was well tolerated and resulted in a significant decrease in the frequency of influenza-like episodes, severity, and length of time confined to bed. Both local and systemic symptoms were sharply and significantly reduced in the NAC group. Frequency of seroconversion towards A/H1N1 Singapore 6/86 influenza virus was similar in the two groups, but only 25% of virus-infected subjects under NAC treatment developed a symptomatic form, versus 79% in the placebo group. Evaluation of cell-mediated immunity showed a progressive, significant shift from anergy to normoergy following NAC treatment. Administration of N-acetylcysteine during the winter, thus, appears to provide a significant attenuation of influenza and influenza-like episodes, especially in elderly high-risk individuals, N-acetylcysteine did not prevent A/H1N1 virus influenza infection but significantly reduced the incidence of clinically apparent disease.

49. De Mattia, G., M. C. Bravi, O. Laurenti, M. Cassone-Faldetta, A. Armiento, C. Ferri and F. Balsano (1998). "Influence of reduced glutathione infusion on glucose metabolism in patients with non-insulin-dependent diabetes mellitus." Metabolism 47(8): 993-997.

To evaluate the relationship between oxidative stress and glucose metabolism, insulin sensitivity and intraerythrocytic reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio were measured in 10 non-insulin-dependent diabetes mellitus (NIDDM) patients and 10 healthy subjects before and after the intravenous administration of GSH. In particular, after baseline insulin sensitivity was assessed by a 2-hour euglycemic hyperinsulinemic clamp, either glutathione (1.35 g.m(2).min(-1)) or (saline) were infused over a period of 1 hour. The same protocol was repeated at a 1-week interval, in cross-over, according to a randomized, single-blind design. In healthy subjects, baseline intraerythrocytic GSH/GSSG ratio (P <.0005) and total glucose uptake (P <.005) were significantly higher than in NIDDM patients. In the same subjects, GSH infusion significantly increased total glucose uptake (from 37.1 +/- 6.7 mu mol.kg(-1).min(-1) to 39.5 +/- 7.7 mu mol.kg(-1).min(-1), P <.05), whereas saline infusion was completely ineffective. In addition, the mean intraerythrocytic GSH/GSSG ratio significantly increased after GSH infusion (from 21.0 +/- 0.9 to 24.7 +/- 1.3, P <.05). Similar findings were found in diabetic patients, in whom GSH infusion significantly increased both total glucose uptake (from 25.3 +/- 9.0 mu mol.kg(-1).min(-1) to 31.4 +/- 10.0 mu mol.kg(-1).min(-1), P <.001) and intraerythrocytic GSH/GSSG ratio (from 14.8 +/- 4.1 to 21.7 +/- 6.7, P <.01). Pooling diabetic patients and controls, significant correlations were found between intraerythrocytic GSH/GSSG ratio and total glucose uptake (r = .425, P <.05), as well as between increments of the same variables after GSH infusion (r = .518, P <.05). In conclusion, our data support the hypothesis that abnormal intracellular GSH redox status plays an important role in reducing insulin sensitivity in NIDDM patients. Accordingly, intravenous GSH infusion significantly increased both intraerythrocytic GSH/GSSG ratio and total glucose uptake in the same patients. Copyright (C) 1998 by W.B. Saunders Company.

50. **De Mattia, G., M. C. Bravi, O. Laurenti, M. Cassone-Faldetta, A. Proietti, O. De Luca, A. Armiento and C. Ferri (1998). "Reduction of oxidative stress by oral N-acetyl-L-cysteine treatment decreases plasma soluble vascular cell adhesion molecule-1 concentrations in non-obese, non-dyslipidaemic, normotensive, patients with non-insulin-dependent diabetes." Diabetologia 41(11): 1392-1396.**

To assess in vivo effects of antioxidants on vascular cell adhesion molecule (VCAM)-1 expression, circulating soluble VCAM-1 and intraerythrocytic reduced glutathione (GSH) and GSH disulphide (GSSG) concentrations were evaluated in non-insulin-dependent diabetic patients without complications (9 men, 6 women, 48 +/- 6 years old) before and after 1 month of either oral N-acetyl-L-cysteine (1.200 mg/day) or placebo treatments, given in randomized, cross-over, double-blind fashion. Ten healthy subjects (7 men, 3 women, 52 +/- 3 years old) served as control subjects. Baseline plasma VCAM-1 concentrations were higher (p = 0.007) in non-insulin-dependent diabetic patients (707.9 +/- 52.5 ng/ml) than in control subjects (627.3 +/- 84.6 ng/ml). Intraerythrocytic GSSG content was higher (non-insulin dependent diabetic patients: 0.618 +/- 0.185 mu mol/g Hb; control subjects: 0.352 +/- 0.04 mu mol/g Hb, p = 0.0002), whereas intraerythrocytic GSH concentrations were lower (p = 0.001) in non-insulin dependent diabetic patients (6.0 +/- 0.7 mu mol/g Hb) than in control subjects (7.1 +/- 0.5 mu mol/g Ib). The mean GSH:GSSG ratio was also lower (p = 0.0001) in the first (10.9 +/- 4.5) than in the second group (20.2 +/- 1.4). Circulating VCAM-1 and intraerythrocytic GSH concentrations were negatively correlated in non-insulin diabetic patients (r = 0.605, p = 0.01). Treatment with N-acetyl-L-cysteine decreased plasma VCAM-1 (p = 0.01) and intraerythrocytic GSSG (p = 0.006) but increased GSH concentrations (p = 0.04) and the GSH:GSSG ratio (p = 0.004) in non-insulin dependent diabetic patients. Our data indicate that the vascular endothelium is activated in non-insulin dependent diabetes. Antioxidant treatment counterbalanced such endothelial activation. Thus, antioxidant agents might protect against oxidant-related upregulation of endothelial adhesion molecules and slow down the progression of vascular damage in non-insulin dependent diabetes.

51. **Estensen, R. D., M. Levy, S. J. Klopp, A. R. Galbraith, J. S. Mandel, J. A. Blomquist and L. W. Wattenberg (1999). "N-acetylcysteine suppression of the proliferative index in the colon of patients with previous adenomatous colonic polyps." Cancer Lett 147(1-2): 109-14.**

This investigation is part of an effort to develop chemoprevention for carcinogenesis of the large bowel. The agent investigated is N- acetylcysteine (NAC). We used as a predictive biomarker, the proliferative index (PI), in a short-term human study. Patients with previous adenomatous colonic polyps are a cohort with increased risk for colon cancer and an increased PI of colonic crypts. They were randomly assigned to an experimental group given 800 mg/day of NAC for 12 weeks or a placebo group. Using proliferative cell nuclear antigen immunostaining, the PI of colonic crypts was measured prior to and after the treatments. The PI of the NAC group was decreased significantly (P < 0.02) while the placebo group showed no difference (P > 0.45). Since this decrease in PI may be an indicator of decreased risk of colon cancer, more extensive studies of the potential of NAC as a chemopreventive agent for colon cancer appear warranted.

52. **Reinhart, K., C. D. Spies, A. Meier-Hellmann, D. L. Bredle, L. Hannemann, M. Specht and W. Schaffartzik (1995). "N-acetylcysteine preserves oxygen consumption and gastric mucosal pH during hyperoxic ventilation." Am. J. Respir. Crit. Care Med. 151(3 Pt 1): 773-779.**

Hyperoxic ventilation, used to prevent hypoxemia during potential periods of hypoventilation, has been reported to paradoxically decrease whole body oxygen consumption (VO2). Reduction in nutritive blood flow due to oxygen radical production is one possible mechanism. We investigated whether pretreatment with the sulfhydryl group donor and O2 radical scavenger N-acetylcysteine (NAC) would preserve whole body VO2 and prevent deterioration of oxygenation in gastric mucosal tissue during hyperoxia. Thirty-eight patients, requiring hemodynamic monitoring (radial and pulmonary artery catheters) due to sepsis syndrome, were included in this randomized experiment. All patients exhibited stable clinical conditions (hemodynamics, body temperature, hemoglobin, FIO2 < 0.5). A gastric tonometer was placed to measure the gastric intramucosal pH (pHi), which indirectly assesses nutritive blood flow to the mucosa. Cardiac output was determined by thermodilution and VO2 by cardiovascular Fick. After baseline measurements, patients randomly received either 150 mg.kg-1 NAC (n = 19) or placebo (n = 19) in 250 ml 5% dextrose intravenously over a period of 15 min. Measurements were repeated 30 min after starting NAC or placebo infusion, 30 min after starting hyperoxia (FIO2 = 1.0), and 60 min after resetting the original FIO2. There were no significant differences between groups in any of the measurements before treatment and after the return to baseline FIO2 at the end of the study. NAC, but not placebo infusion, caused a slight but significant increase in cardiac output and decrease in systemic vascular resistance.(ABSTRACT TRUNCATED AT 250 WORDS)

53. Spies, C. D., K. Reinhart, I. Witt, A. Meier-Hellmann, L. Hannemann, D. L. Bredle and W. Schaffartzik (1994). "Influence of N-acetylcysteine on indirect indicators of tissue oxygenation in septic shock patients: results from a prospective, randomized, double-blind study." Crit. Care Med. 22(11): 1738-46.

OBJECTIVES: Deactivation of endothelium-derived relaxing factor due to an increased oxygen radical load during sepsis may contribute to an impairment in microcirculatory blood flow. We investigated whether treatment with the sulfhydryl donor and oxygen radical scavenger, N-acetylcysteine, would improve whole-body oxygen consumption (VO2), gastric intramucosal pH, and veno-arterial CO2 gradient (veno-arterial PCO2) during septic shock.

DESIGN: Prospective, randomized, double-blind study conducted over 2 yrs. SETTING: Septic shock patients admitted to the intensive care unit.

PATIENTS: Fifty-eight patients requiring hemodynamic monitoring (radial and pulmonary artery catheters) due to septic shock, were included in this study. All patients were examined within 72 hrs after the onset of sepsis. They were optimally resuscitated by conventional means with volume and inotropic agents, and exhibited stable clinical conditions (hemodynamic values, body temperature, hemoglobin, FIO2).

INTERVENTIONS: A gastric tonometer was inserted to measure the gastric intramucosal pH. Subjects randomly received either 150 mg/kg of intravenous N-acetylcysteine or placebo over a 15-min period, then a continuous infusion of 12.5 mg/hr of N-acetylcysteine or placebo over approximately 90 mins. MEASUREMENTS: Infusion measurements were begun 60 mins after the beginning of infusion and lasted approximately 30 mins. The infusion was then discontinued and 2 hrs later the final measurements were taken.

MAIN RESULTS: Basic patient characteristics (age, sex, Acute Physiology and Chronic Health Evaluation [APACHE] II scores, Multiple Organ Failure scores) did not differ significantly, nor did pre- and 2-hr postinfusion measurements differ between any of the groups. Thirteen (45%) patients responded (i.e., showed an increase in VO2 > 10%, reaching a mean of 19%) to the N-acetylcysteine infusion. The N-acetylcysteine responders also showed an increase in gastric intramucosal pH, a decrease in veno-arterial PCO2, an increase in oxygen delivery, cardiac index, stroke index, and left ventricular stroke work index, as well as a significant decrease in systemic vascular resistance in comparison to baseline. The N-acetylcysteine nonresponders, as well as the patients in the placebo group, did not show any significant changes in any of these variables. The N-acetylcysteine responders had a higher survival rate (69%) than the non-responders (19%) and were studied earlier after onset of sepsis (37 hrs) than the nonresponders (61 hrs). The only significant difference between the entire N-acetylcysteine group (which included responders plus nonresponders) and the placebo group was an increased VO2 in the entire N-acetylcysteine group during infusion measurements.

CONCLUSIONS: N-acetylcysteine provided a transient improvement in tissue oxygenation in about half of the septic shock patients, as indicated by an increase in VO2 and gastric intramucosal pH and a decrease in veno-arterial PCO2. The higher survival rate in the N-acetylcysteine responders and the fact that half of the patients receiving N-acetylcysteine did not respond, suggests that, in some patients, sepsis irreversibly damages the microvasculature to the extent that N-acetylcysteine has no effect. If analyzed by intention to treat, the N-acetylcysteine did not produce effects that were significantly different from the placebo. Whether the N-acetylcysteine challenge was merely diagnostic or whether N-acetylcysteine can be effective in the treatment of sepsis deserves further investigation.

54. Spapen, H., H. B. Zhang, C. Demanet, W. Vleminckx, J.-L. Vincent and L. Huyghens (1998). "Does N-acetyl-L-cysteine influence cytokine response during early human septic shock?" Chest 113(6): 1616-1624.

Study objective: To assess the effects of adjunctive treatment with N-acetyl-L-cysteine (NAC) on hemodynamics, oxygen transport variables, and plasma levels of cytokines in patients with septic shock. Design: Prospective, randomized, double-blind, placebo-controlled study. Setting: A 24-bed medicosurgical ICU in a university hospital. Patients: Twenty-two patients included within 4 h of diagnosis of septic shock. Interventions: Patients were randomly allocated to receive either NAC (150 mg/kg bolus, followed by a continuous infusion of 50 mg/kg over 4 h; n = 12) or placebo (n = 10) in addition to standard therapy. Measurements: Plasma concentrations of tumor necrosis factor-alpha (TNF), interleukin (IL)-6, IL-8, IL-10, and soluble tumor necrosis factor-alpha receptor-p55 (sTNFR-p55) were measured by sensitive immunoassays at 0, 2, 4, 6 and 24 h, Pulmonary artery catheter-derived hemodynamics, blood gases, hemoglobin, and arterial lactate were measured at baseline, after infusion (4 h), and at 24 h. Results: NAC improved oxygenation (PaO2/FIo(2) ratio, 214+/-97 vs 123+/-86; $p<0.05$) and static lung compliance (44+/-11 vs 31+/-6 L/cm H2O; $p<0.05$) at 24 h. NAC had no significant effects on plasma TNF, IL-6, or IL-10 levels, but acutely decreased IL-8 and sTNFR-p55 levels. The administration of NAC had no significant effect on systemic and pulmonary hemodynamics, oxygen delivery, and oxygen consumption. Mortality was similar in both groups (control, 40%; NAC, 42%) but survivors who received NAC had shorter ventilator requirement (7+/-2 days vs 20+/-7 days; $p<0.05$) and were discharged earlier from the ICU (13+/-2 days vs 32+/-9 days; $p<0.05$). Conclusion: In this small cohort of patients with early septic shock, short-term IV infusion of NAC was well-tolerated, improved respiratory function, and shortened ICU stay in survivors. The attenuated production of IL-8, a potential mediator of septic lung injury, may have contributed to the lung-protective effects of NAC.

55. Wiklund, O., G. Fager, A. Andersson, U. Lundstam, P. Masson and B. Hultberg (1996). "N-acetylcysteine treatment lowers plasma homocysteine but not serum lipoprotein(a) levels." Atherosclerosis 119(1): 99-106.

High levels of lipoprotein(a) (Lp(a)) or homocysteine in plasma have both been associated with an increased risk for premature cardiovascular disease. For both components, the plasma levels are primarily genetically determined, and they have been very restistant to therapeutic approaches. It has been suggested that N-acetylcysteine (NAC) breaks disulphide bonds in Lp(a) as well as between homocysteine and plasma proteins. In the present study we analyze if this mechanism, in vivo, could be used to lower plasma concentrations of Lp(a) and homocysteine. Treatment with NAC and placebo was performed in a double blind cross over design with 2 weeks wash-out between treatments. Eleven subjects with high plasma Lp(a) (> 0.3 g/l) were recruited from the Lipid Clinic at Sahlgren's Hospital, Goteborg, Sweden. Main outcome measures were treatment effects on plasma Lp(a) and plasma amino thiols (homocysteine, cysteine and cysteinyl glycine), There was no significant effect on plasma Lp(a) levels. Plasma thiols were significantly reduced during treatment with NAC: homocysteine by 45% ($P < 0.0001$), cysteinyl glycine by 24% ($P < 0.0001$) and cysteine by 11% ($P = 0.0002$). The high dose of NAC was well tolerated. In conclusion NAC has no effect on plasma Lp(a) levels while the reduction in homocysteine is considerable and might be of clinical significance in cases with high plasma homocysteine levels.

56. Vita, J. A., B. Frei, M. Holbrook, N. Gokce, C. Leaf and J. F. Keaney Jr (1998). "L-2-oxothiazolidine-4-carboxylic acid reverses endothelial dysfunction in patients with coronary artery disease." J. Clin. Invest. 101(6): 1408-1414.

The effective action of endothelium-derived nitric oxide (EDNO) is impaired in patients with atherosclerosis. This impairment has been attributed in part to increased vascular oxidative stress. EDNO action is improved by administration of ascorbic acid, a water-soluble antioxidant. Ascorbic acid is a potent free-radical scavenger in plasma, and also regulates intracellular redox state in part by sparing cellular glutathione. We specifically investigated the role of intracellular redox state in EDNO action by examining the effect of L-2-oxo-4-thiazolidine carboxylate (OTC) on EDNO-dependent, flow-mediated dilation in a randomized double-blind placebo-controlled study of patients with angiographically proven coronary artery disease. OTC augments intracellular glutathione by providing substrate cysteine for glutathione synthesis. Brachial artery flow-mediated dilation was examined with high-resolution ultrasound before and after oral administration of 4.5 g of OTC or placebo in 48 subjects with angiographically documented coronary artery disease. Placebo treatment produced no change in flow-mediated dilation (7.0+/-3.9% vs. 7.2+/-3.7%), whereas OTC treatment was associated with a significant improvement in flow-mediated dilation (6.6+/-4.4% vs, 11.0+/-6.3%; P = 0.005). OTC had no effect on arterial dilation to nitroglycerin, systemic blood pressure, heart rate, or reactive hyperemia. These data suggest that augmenting cellular glutathione levels improves EDNO action in human atherosclerosis. Cellular redox state may be an important regulator of EDNO action, and is a potential target for therapy in patients with coronary artery disease.

57. **Moberly, J. B., J. Logan, P. R. Borum, K. O. Story, L. E. Webb, S. V. Jassal, L. Mupas, H. Rodela, G. A. Alghamdi, J. E. Moran, M. Wolfson, L. Martis and D. G. Oreopoulos (1998). "Elevation of whole-blood glutathione in peritoneal dialysis patients by L-2-oxothiazolidine-4-carboxylate, a cysteine prodrug (Procysteine(R))." J. Amer. Soc. Nephrol. 9(6): 1093-1099.**

Glutathione is a major cellular antioxidant that protects protein thiols and inhibits cellular damage due to oxygen free radicals. It has been reported previously that patients undergoing dialysis have low levels of blood glutathione, which may lead to increased susceptibility to oxidant stress. L-2-oxothiazolidine-4-carboxylic acid (OTZ) is a cysteine prodrug that raises cellular glutathione levels by increasing delivery of cysteine, the rate-limiting substrate for glutathione synthesis. This study investigates the effect of OTZ on blood glutathione in a blinded, placebo-controlled study of patients with chronic renal failure treated by peritoneal dialysis. Twenty patients were randomly selected to receive OTZ (0.5 g three times a day orally with meals) or placebo for 14 d. Patients visited the clinic for predose blood collection and safety evaluation at baseline (days 3, 7, and 14 and again at 14 d from the last dose [follow-up]). Glutathione concentrations were determined in whole blood by HPLC. OTZ resulted in a significant rise in whole-blood glutathione at days 7 (594 +/- 129 mu mol/L) and 14 (620 +/- 108 mu mol/L) compared with baseline (544 +/- 139 mu mol/L) ($P < 0.01$ and $P < 0.05$, respectively). Glutathione was also significantly increased at days 7 and 14 when normalized by hematocrit (Hct) or hemoglobin to correct for anemic status (e.g., 20.7 +/- 5.7 mu mol/L per % Hct [day 7] and 20.9 +/- 4.0 mu mol/L per % Hct [day 14] versus 18.0 +/- 4.2 mu mol/L per % Hct [baseline]; $P < 0.05$). Glutathione levels did not change in the placebo group at any patient visit, and levels in the OTZ-treated group returned to baseline at follow-up. There were no serious adverse events attributable to OTZ, and the drug appeared to be well tolerated by patients with renal failure treated by continuous ambulatory peritoneal dialysis. Our results show that OTZ increases blood glutathione levels, which may improve antioxidant status in dialysis patients.

58. **Jain, S. K., R. McVie and T. Smith (2000). "Vitamin E supplementation restores glutathione and malondialdehyde to normal concentrations in erythrocytes of type 1 diabetic children [In Process Citation]." Diabetes Care 23(9): 1389-94.**

OBJECTIVE: This study examined the relationship between cellular glutathione and vitamin E concentrations and the effect of vitamin E (alpha-tocopherol) supplementation on glutathione and lipid peroxidation product concentrations in the erythrocytes of type 1 diabetic patients.

RESEARCH DESIGN AND METHODS: We obtained written informed consent to participate in this study from diabetic patients ($n = 29$) and their age-matched nondiabetic siblings ($n = 21$) according to the guidelines of the Institutional Review Board on Human Experimentation. Diabetic patients were supplemented with a DL-alpha- tocopherol (vitamin E) capsule (100 IU/orally) or placebo for 3 months in a double-blind clinical trial. Fasting blood samples were collected from each diabetic patient before the start of and after the 3 months of vitamin E or placebo supplementation. Glutathione, malondialdehyde (which is a product of lipid peroxidation), and alpha-tocopherol were determined using high-performance liquid chromatography A total of 5 diabetic patients were excluded after randomization from the data analyses. Data were analyzed statistically using a paired Students t test to compare 12 diabetic patients taking vitamin E with 12 diabetic patients receiving placebo supplementation and to compare diabetic patients with healthy nondiabetic subjects.

RESULTS: Erythrocytes of diabetic patients had 21% higher ($P<0.001$) malondialdehyde and 15% lower ($P<0.05$) glutathione concentrations than healthy subjects. Vitamin E in erythrocytes had a significant correlation with the glutathione concentrations in the erythrocytes ($r = 0.46$, $P<0.02$). Vitamin E supplementation increased glutathione concentrations by 9% ($P<0.01$) and lowered concentrations of malondialdehyde by 23% ($P<0.001$) and of HbA1c by 16% ($P<0.02$) in erythrocytes of diabetic patients. No differences were evident in these parameters before versus after placebo supplementation.

CONCLUSIONS: Glutathione level is significantly related to vitamin E level, and supplementation with vitamin E (100 IU/day) significantly increases glutathione and lowers lipid peroxidation and HbA1c concentrations in the erythrocytes of type 1 diabetic patients.

59. Loguercio, C., G. Nardi, F. Argenzio, C. Aurilio, E. Petrone, A. Grella, C. Del Vecchio Blanco and M. Coltorti (1994). "Effect of S-adenosyl-L-methionine administration on red blood cell cysteine and glutathione levels in alcoholic patients with and without liver disease." Alcohol Alcohol 29(5): 597-604.

We measured glutathione and cysteine concentrations in erythrocytes of chronic alcohol misusers with (20 subjects) and without liver cirrhosis (20 subjects). Glutathione levels were decreased, whereas those of cysteine were increased in all patients. Parenteral treatment with S- adenosylmethionine (SAME); (2 g daily in 250 ml 0.15 M NaCl for 15 days) corrected the erythrocyte thiol alterations. We conclude that parenteral treatment with SAME affects the metabolism of SH compounds in erythrocytes of alcoholic patients.

60. Keays, R., P. M. Harrison, J. A. Wendon, A. Forbes, C. Gove, G. J. Alexander and R. Williams (1991). "Intravenous acetylcysteine in paracetamol induced fulminant hepatic failure: a prospective controlled trial." Bmj 303(6809): 1026-9.

OBJECTIVE--To see whether intravenous acetylcysteine would improve outcome in patients with fulminant hepatic failure after paracetamol overdose. DESIGN--A prospective randomised controlled study.

SETTING-- The Institute of Liver Studies, King's College Hospital, London.

PATIENTS--50 consecutive patients (21 male) aged 16-60 with fulminant hepatic failure after paracetamol overdose who had not previously received acetylcysteine.

INTERVENTIONS--Conventional intensive liver care plus either acetylcysteine (25 patients) in the same dose regimen as used early after a paracetamol overdose, except that the infusion was continued until recovery from encephalopathy or death, or an equivalent volume of 5% dextrose (25 patients).

MAIN OUTCOME MEASURES-- Survival; incidence of cerebral oedema, renal failure, and hypotension requiring inotropic support; liver function as assessed by prolongation of the prothrombin time; and degree of encephalopathy.

RESULTS--The rate of survival was significantly higher in the acetylcysteine treated group than in the controls (48% (12/25 patients) v 20% (5/25); $p = 0.037$, 95% confidence interval for difference in proportions surviving 3% to 53%). Acetylcysteine treated patients had a lower incidence of cerebral oedema (40% (10/25) v 68% (17/25); $p = 0.047$, 95% confidence interval for difference in incidence 2% to 54%), and fewer developed hypotension requiring inotropic support (48% (12/25) v 80% (20/25); $p = 0.018$, 95% confidence interval 7% to 57%). Rates of deterioration and recovery of liver function, however, were similar in the two groups. No adverse reactions to acetylcysteine were seen.

CONCLUSIONS-- Acetylcysteine is safe and effective in fulminant hepatic failure after paracetamol overdose.

61. Holt, S., D. Goodler, R. Marley, D. Patch, A. Burroughs, B. Fernando, D. Harry and K. Moore (1999). "Improvement in renal function in hepatorenal syndrome with N-acetylcysteine." Lancet 353(9149): 294-295.

The hepatorenal syndrome (HRS) is the development of renal failure in patients with severe liver disease in the absence of any other identifiable cause. Patients with hepatorenal syndrome have a mortality approaching 95% and a mean survival of 1·7 weeks from the time of diagnosis.

1.There is often a reluctance to institute renal replacement therapy, since the outcome is not improved unless there is a realistic chance of hepatic recovery or liver transplantation. One study observed 100% mortality in cirrhotics with HRS despite renal support.

2 Despite the dismal prognosis continuous-mode haemofiltration is still instituted in many cases as these patients are unsuitable for intermittent haemodialysis. Thus, any therapy that can prevent or ameliorate this condition before renal replacement therapy is required would have considerable clinical benefit for this group of patients. We have previously shown that N-acetylcysteine (NAC) can improve renal function in an experimental model of acute cholestasis and renal failure.

3 When given to patients with acute paracetamol-induced liver and renal failure, NAC treatment showed a trend towards improved renal function.

4 However, there have been no studies on the effect of NAC on patients with hepatorenal syndrome.

62. **Helveston, W., J. E. Cibula, R. Hurd, B. M. Uthman and B. J. Wilder (1996). "Abnormalities of antioxidant metabolism in a case of Friedreich's disease." Clin. Neuropharmacol. 19(3): 271-275.**

We report a patient with Friedreich's disease (FD) who exhibited abnormalities of antioxidant metabolism, including decreased levels of glutathione peroxidase, glutathione reductase, and selenium, and an increased lipid peroxide index. These abnormalities became normal after treatment with N-acetylcysteine, selenium, and low-dose vitamin E therapy. Treatment was associated with a decreased rate of clinical decline. FD is a neurodegenerative disorder that may be related to disturbed antioxidant metabolism; the disorder may be treatable with antioxidant compounds.

63. **Hurd, R. W., B. J. Wilder, W. R. Helveston and B. M. Uthman (1996). "Treatment of four siblings with progressive myoclonus epilepsy of the Unverricht-Lundborg type with N-acetylcysteine." Neurology 47(5): 1264-1268.**

The finding of increased activity of the enzyme extracellular superoxide dismutase in four siblings with progressive myoclonus epilepsy of the Unverricht-Lundborg type (PME-UL) prompted the addition of antioxidants to these patients' treatment regimen. After 6 months treatment with vitamin E, selenium, riboflavin, and zinc, there was some improvement in patient awareness and speech. N-acetylcysteine (NAC) is a sulfhydryl antioxidant that increases cellular glutathione and the activity levels of several antioxidant enzymes and has additional actions that contribute to its demonstrated efficacy in preventing or decreasing damage in models of neuronal toxicity. We treated the affected siblings with 4 to 6 grams a day of NAC in addition to the other antioxidants and magnesium. There has been a marked decrease in myoclonus and some normalization of somatosensory evoked potentials with NAC treatment. The patients were treated with NAC for up to 30 months with continued beneficial effects. NAC may prevent further deterioration in the clinical course of patients with PME-UL and may be indicated in other neurodegenerative conditions where excess free radical activity may contribute to disease progression.

64. **Ben-Menachem, E., M. Kyllerman and S. Marklund (2000). "Superoxide dismutase and glutathione peroxidase function in progressive myoclonus epilepsies." Epilepsy Res. 40(1): 33-39.**

Progressive myoclonic epilepsies (EPM) are difficult to treat and refractory to most antiepileptic drugs. Besides epilepsy, EPMs also involve continuous neurological deterioration. Oxidative stress is thought to be an important factor in this process. We therefore analyzed a series of antioxidant enzymes in the blood of patients and compared with healthy age matched controls. In addition patients were given high doses of N-acetylcysteine (NAC), a glutathione percursor to determine if symptoms of EPM would improve. Five patients, four with EPM 1 (Unvericht-Lundborg disease) and one patient with EPM2 (Lafora body disease) were treated with 6 g/day of NAC. Before treatment, plasma samples were analyzed for glutathione peroxidase activity, catalase activity, extracellular superoxide dismutase (SOD) and CuZn-SOD and compared with the controls. Erythrocyte CuZn-SOD was significantly lower in the EPM patients compared to controls. NAC improved markedly and stabilized the neurological symptoms in patients with EPM 1 but had a doubtful effect in the patient with EPM 2.

65. **Selwa, L. M. (1999). "N-acetylcysteine therapy for Unverricht-Lundborg disease." Neurology 52(2): 426-427.**

Unverricht–Lundborg disease is an autosomal recessive degenerative disorder (21q22.3) that begins age 6 to 15 with tonic-clonic seizures and myoclonus, and gradually progresses to include dysarthria, cognitive dysfunction, and ataxia. Most patients live long, disabled adult life spans with the average onset of disability 5 years after diagnosis.

In response to an earlier case report suggesting dramatic improvement in tremor, somatosensory evoked potentials, and overall disability in a sibship with Unverricht–Lundborg disease, we treated a genetically confirmed patient who had moderately advanced disease with N-acetylcysteine (NAC) and were able to replicate similar improvements in ambulation and myoclonus. In this patient, we also documented a significantly reduced generalized seizure frequency and improved verbal output. The benefits have been sustained for 10 months.

66. Sechi, G., M. G. Deledda, G. Bua, W. M. Satta, G. A. Deiana, G. M. Pes and G. Rosati (1996). "Reduced intravenous glutathione in the treatment of early Parkinson's disease." Prog. Neuro-Psych. Biol. Psych. 20(7): 1159-1170.

1. Several studies have demonstrated a deficiency in reduced glutathione (GSH) in the nigra of patients with Parkinson's Disease (PD). In particular, the magnitude of reduction in GSH seems to parallel the severity of the disease. This finding may indicate a means by which the nigra cells could be therapeutically supported.

2. The authors studied the effects of GSH in nine patients with early, untreated PD. GSH was administered intravenous, 600 mg twice daily, for 30 days, in an open label fashion. Then, the drug was discontinued and a follow-up examination carried-out at 1-month interval for 2-4 months. Thereafter, the patients were treated with carbidopa-levodopa.

3. The clinical disability was assessed by using two different rating scale and the Webster Step-Second Test at baseline and at 1-month interval for 4-6 months. All patients improved significantly after GSH therapy, with a 42% decline in disability. Once GSH was stopped the therapeutic effect lasted for 2-4 months.

4. Our data indicate that in untreated PD patients GSH has symptomatic efficacy and possibly retards the progression of the disease.

67. Vyth, A., J. G. Timmer, P. M. M. Bossuyt, E. S. Louwerse and J. M. B. V. Dejong (1996). "Survival in patients with amyotrophic lateral sclerosis, treated with an array of antioxidants." J. Neurol. Sci. 139(Suppl.): 99-103.

Between 1983 and 1988 we treated 36 patients with sporadic amyotrophic lateral sclerosis (ALS) by an array of antioxidants and added other drugs to the regimen whenever a patient reported deterioration. Our customary prescription sequence was N-acetylcysteine (NAC); vitamins C and E; N-acetylmethionine (NAM); and dithiothreitol (DTT) or its isomer dithioerythritol (DTE). Patients with a history of heavy exposure to metal were also given meso-2,3-dimercaptosuccinic acid (DMSA). NAC, NAM, DTT, and DTE were administered by subcutaneous injection or by mouth or by both routes; the other vitamins and DMSA by mouth alone. The hospital pharmacy supplied NAC and NAM injections fluid as 100 mi bottles of 5.0 and 5.85% solutions, respectively. DTT was delivered in special double-walled capsules of 200 mg. DTT/DTE injection fluid was added to the NAC and NAM bottles, the final DTT/DTE concentrations never exceeding 0.5%. DMSA was provided in 250 mg capsules. All of the 36 patients used NAC and DTT/DTE; 29 also used vitamins C and E; 21 also used NAM; and 7 also used DMSA. DMSA, NAM, vitamins C and E were tolerated well. In many patients, DTT, DTE, NAC and NAM induced pain, redness and swelling at the injection sites in that order of decreasing frequency. DTT and DTE did often and NAC did sometimes cause gastric pain, nausea and other abdominal discomfort. Comparison of survival in the treated group and in a cohort of untreated historical controls, disclosed a median survival of 3.4 years (95% confidence interval: 3.0-4.2) in the treated and of 2.8 (95% confidence interval 2.2-3.1) years in the control patients. This difference may be explained by self-selection of our highly motivated treated group and by its initial survival of diagnosis for an average of 8.5 months before onset of treatment. We conclude that antioxidants neither seem to harm ALS patients, nor do they seem to prolong survival.

68. Pena, L. R., D. B. Hill and C. J. McClain (1999). "Treatment with glutathione precursor decreases cytokine activity." J. Parenter. Enteral Nutr. 23(1): 1-6.

BACKGROUND: Inflammatory cytokine activity is increased in many forms of experimental and clinical liver injury including alcoholic liver disease (ALD). Monocytes and Kupffer cells produce cytokines such as tumor necrosis factor (TNF), interleukin (IL)-8, and IL-6 in response to stimuli such as endotoxin (lipopolysaccharide [LPS]). This cytokine production is regulated by the oxidative stress-sensitive transcription factor NFkappaB. Glutathione (GSH) prodrugs such as oxathizolidine-4- carboxylic acid (OTZ) can inhibit activation of NFkappaB and subsequent cytokine production in monocytes and Kupffer cells in vitro. The objective of this study was to treat stable cirrhotic patients with OTZ in vivo to evaluate its effects on monocyte cytokine production (TNF, IL-8, and IL-6) and whole blood GSH levels.

METHODS: Nine patients with stable cirrhosis received OTZ (70 mg/kg IV every 8 hours) for 9 days. Peripheral blood monocytes were obtained on study days 1 and 9, using density gradient centrifugation and adherence to plastic, and were stimulated with LPS (5 microg/mL). TNF, IL-8, and IL-6 were measured in culture supernatants by enzyme-linked serum immunosorbent assay. Whole blood GSH levels were measured by high-performance liquid chromatography.

RESULTS: There was a significant decrease in monocyte TNF, IL-8, and IL-6 production after OTZ therapy. Patients with cirrhosis had significantly lower admission whole blood GSH levels compared with controls and GSH normalized with OTZ administration.

CONCLUSIONS: Treatment with the GSH prodrug OTZ inhibited monocyte cytokine production and increased whole blood GSH. This may have important therapeutic implications for multiple cytokine-mediated disease processes.

69. **de Quay, B., R. Malinverni and B. H. Lauterburg (1992). "Glutathione depletion in HIV-infected patients: role of cysteine deficiency and effect of oral N-acetylcysteine." AIDS 6(8): 815-819.**

Objective: To determine whether a single oral dose of N-acetylcysteine corrects the deficiency of cysteine and glutathione in plasma and mononuclear cells of HIV-infected patients. Design: Pharmacokinetic and pharmacodynamic study. Methods: Cysteine and glutathione were measured in plasma and peripheral blood mononuclear cells of patients at different stages of HIV infection before and after a single oral dose of N-acetylcysteine. Results: At baseline, the plasma concentrations of glutathione and cysteine were significantly lower in HIV-infected patients than in healthy controls. The intracellular concentration of glutathione correlated with the absolute CD4 lymphocyte counts: the concentration of glutathione in mononuclear cells was significantly lower in patients with more advanced immunodeficiency. A single oral dose of N-acetylcysteine increased the concentration of cysteine in plasma and mononuclear cells of HIV-infected patients. Four hours after N-acetylcysteine administration, intracellular glutathione concentrations in the patients were moderately higher than at baseline and at 2 h. Conclusions: Oral N-acetylcysteine transiently increases the concentrations of cysteine and glutathione in mononuclear cells of patients with HIV infection. A sustained increase in intracellular cysteine may be necessary to normalize intracellular glutathione. This may be accomplished by repeat administration of N-acetylcysteine.

70. **Roederer, M., F. J. T. Staal, H. Osada, L. A. Herzenberg and L. A. Herzenberg (1991). "CD4 and CD8 T cells with high intracellular glutathione levels are selectively lost as the HIV infection progresses." Intl. Immunol. 3(9): 933-937.**

Maintenance of intracellular glutathione (GSH) levels has been implicated in blocking cytokine-stimulated HIV replication in vitro, in both acute and latent infection models. We demonstrate here that subsets of human peripheral blood mononuclear cells differ substantially in mean GSH levels, as measured on a cell-by-cell basis with the fluorescence-activated cell sorter (FACS): B cells have the lowest GSH levels; T cells are intermediate; and monocytes and macrophages have the highest levels. Furthermore, GSH levels subdivide the CD4 and CD8 T cell subsets into two classes each: high- and low-GSH cells, which cannot be distinguished by cell size or by currently known surface markers. Significantly, the high-GSH T cells are selectively depleted early during the HIV infection, and are effectively missing in all ARC and AIDS patients.

71. **Breitkreutz, R., S. Holm, N. Pittack, M. Beichert, A. Babylon, J. Yodoi and W. Droge (2000). "Massive loss of sulfur in HIV infection." AIDS Res Hum Retroviruses 16(3): 203-9.**

Skeletal muscle tissue from SIV-infected macaques was previously found to contain abnormally high sulfate and low glutathione levels indicative of an excessive cysteine catabolism. We now confirm the peripheral tissue as a site of massive cysteine catabolism in HIV infection and have determined the urinary loss of sulfur per time unit. The comparison of the sulfate concentrations of the arterial and venous blood from the lower extremities of 16 symptomatic HIV+ patients and 18 HIV- control subjects (study 1) revealed (1) that the peripheral tissue of HIV+ patients with or without highly active antiretroviral therapy (HAART) releases large amounts of sulfate and (2) that plasma sulfate, thioredoxin, and interleukin-6 levels are elevated in these patients. A complementary investigation of 64 asymptomatic HIV+ patients and 65 HIV- subjects (study 2) revealed increased plasma sulfate levels in the asymptomatic patients. The analysis of the daily urinary excretion of sulfate and urea of another group of 19 HIV+ patients and 22 healthy HIV- subjects (study 3) confirmed (1) that HIV+ patients experience a massive loss of sulfur and (2) that this loss is not ameliorated by HAART. The sulfur loss of asymptomatic patients was equivalent to a mean loss of about 10 g of cysteine per day. If extrapolated, this would correspond to an alarming negative balance of approximately 2 kg of cysteine per year under the assumption that the normal sulfate excretion equivalent to approximately 3 g of cysteine per day is balanced by a standard Western diet. The abnormally high sulfate/urea ratio suggests that this process drains largely the glutathione pool.

72. **De Rosa, S. C., M. D. Zaretsky, J. G. Dubs, M. Roederer, M. Anderson, A. Green, D. Mitra, N. Watanabe, H. Nakamura, I. Tjioe, S. C. Deresinski, W. A. Moore, S. W. Ela, D. Parks**

and L. A. Herzenberg (2000). "N-acetylcysteine replenishes glutathione in HIV infection." Eur J Clin Invest 30(10): 915-29.

BACKGROUND: Glutathione (GSH) deficiency is common in HIV-infected individuals and is associated with impaired T cell function and impaired survival. N-acetylcysteine (NAC) is used to replenish GSH that has been depleted by acetaminophen overdose. Studies here test oral administration of NAC for safe and effective GSH replenishment in HIV infection. DESIGN: Oral NAC administration in a randomized, 8-week double-blind, placebo-controlled trial followed by optional open-label drug for up to 24 weeks. SUBJECTS: HIV-infected, low GSH, CD4 T cells < 500 micro L(-1), no active opportunistic infections or other debilitation; n = 81. Study conducted prior to introduction of protease inhibitors. RESULTS: Whole blood GSH levels in NAC arm subjects significantly increased from 0.88 mM to 0.98 mM, bringing GSH levels in NAC-treated subjects to 89% of uninfected controls (P = 0.03). Baseline GSH levels in the placebo group (0.91) remained essentially the same during the 8 week placebo-controlled trial. T cell GSH, adjusted for CD4 T cell count and beta2-microglobulin levels, also increased in the NAC-treated subjects (P = 0.04). Adverse effects were minimal and not significantly associated with NAC ingestion. CONCLUSION: NAC treatment for 8 weeks safely replenishes whole blood GSH and T cell GSH in HIV- infected individuals. Thus, NAC offers useful adjunct therapy to increase protection against oxidative stress, improve immune system function and increase detoxification of acetaminophen and other drugs. These findings suggest that NAC therapy could be valuable in other clinical situations in which GSH deficiency or oxidative stress plays a role in disease pathology, e.g. rheumatoid arthritis, Parkinson's disease, hepatitis, liver cirrhosis, septic shock and diabetes.

73. **Calabresi, P. and J. Parks, R.E. (1985). Antiproliferative agents and drugs used for immunosuppression. Goodman and Gilman's Pharmacological Basis of Therapeutics. L. S. G. A. G. Goodman, T. W. Rall and F. Murad. New York., Macmillan: 1247-1306.**

74. **Altomare, E., G. Vendemiale and O. Albano (1988). "Hepatic glutathione content in patients with alcoholic and non alcoholic liver disease." Life Sci. 43: 991-998.**

Reduced and oxidized hepatic glutathione was evaluated during alcoholic and non alcoholic liver injury. We studied 35 chronic alcoholics, 20 patients with non alcoholic liver diseases, 15 control subjects. Hepatic glutathione was measured in liver biopsies and correlated with histology and laboratory tests. Alcoholic and non alcoholic patients exhibited a significant decrease of hepatic glutathione compared to control subjects (controls: 4.14 +/- 0.1 mumol/g liver; alcoholics: 2.55 +/- 0.1, p less than 0.001; non alcoholics 2.77 +/- 0.1, p less than 0.001). Oxidized glutathione was significantly higher in the two groups of patients compared to controls (controls: 4.4 +/- 0.2% of total; alcoholics 8.2 +/- 0.3, p less than 0.001; non alcoholics: 8.5 +/- 0.8, p less than 0.001). The decreased hepatic glutathione levels in patients with alcoholic and non alcoholic liver diseases may represent a contributing factor of liver injury and may enhance the risk of toxicity in these patients.

75. **Villa, P. and P. Ghezzi (1995). "Effect of N-acetyl-L-cysteine on sepsis in mice." Eur J Pharmacol 292(3-4): 341-4.**

The effect of the antioxidant N-acetyl-L-cysteine was studied in a model of polymicrobial sepsis induced in CD-1 mice by cecal ligation and puncture. N-Acetyl-L-cysteine significantly improved survival during the 6 days following sepsis induction and caused lower liver toxicity. This effect was not related to free radicals generated by xanthine oxidase which was significantly induced in liver after cecal ligation and puncture. A specific inhibitor of xanthine oxidase, allopurinol, significantly reduced this enzyme and reduced the early survival rate. The effect of N-acetyl-L-cysteine was not related either to a reduction in tumor necrosis factor production or to a modulation of nitrites or to liver glutathione content. These results show that the induction of xanthine oxidase is not deleterious in this model of sepsis and suggest that N-acetyl-L-cysteine works as a direct antioxidant and scavenger of free radicals generated from other sources.

76. **Kretzschmar, M., L. Pfeiffer, C. Schmidt and W. Schirrmeister (1998). "Plasma levels of glutathione, alpha-tocopherol and lipid peroxides in polytraumatized patients; evidence for a stimulating effect of TNF alpha on glutathione synthesis." Exp Toxicol Pathol 50(4-6): 477-83.**

Prognosis and outcome of polytraumatized patients are determined by the possible development of multiple organ failure (MOF). Among the direct traumatic organ damage, it is caused by a systemic inflammatory reaction. This might be

triggered by an activation of the inflammatory mediator cascade following hemorrhagic-traumatic shock as well as by oxygen-derived free radicals (ROS). The aim of our present study was to answer the following questions: 1. Is the "oxidative stress" measurable during the development of MOF after polytraumatic injury? 2. Is there a relation between the activation of the inflammatory mediator cascade and changes of the organism's antioxidative system? The study group included 26 patients (15 survivors, 11 non-survivors) suffering from severe polytraumatic injury (Hannover Polytrauma Score 12-63 points). Plasma levels of reduced (GSH) and oxidized (GSSG) glutathione alpha- tocopherol (TOC), lipid peroxides (expressed in terms of thiobarbituric acid reagible substances = TBARS), and tumor necrosis factor alpha (TNF) were measured each day from the point of admission on the ICU until the discharge or death of the patients. The following results were obtained: Independent from the outcome, we observed a continuous loss of plasma sulfhydryl groups and TOC. In the patients developing a MOF score > 5 on 10th day after injury (n = 6), a significant increase in plasma GSSG level was measurable. Additionally, a total loss of plasma GSH was seen in some of these patients indicating the collapse of the GSH-dependent antioxidative system. Similar changes were never observed in patients with MOF score < or = 5 on 10th day after injury (n = 15). In this group, a significant correlation between plasma TNF peaks and short time GSH boosts was obtained as a possible indicative for a stimulating effect of TNF on GSH synthesis. It can be concluded that processes of oxidative stress in connection with a consumption of endogenic antioxidants might be able to promote the development of MOF after polytraumatic injury.

77. Zhang, H., H. Spapen, D. N. Nguyen, M. Benlabed, W. A. Buurman and J. L. Vincent (1994). "Protective effects of N-acetyl-L-cysteine in endotoxemia." Am J Physiol 266(5 Pt 2): H1746-54.

Because oxygen free radicals have been implicated in the endothelial cell damage and in the myocardial depression occurring during severe sepsis, we investigated whether N-acetyl-L-cysteine (NAC) could influence the oxygen extraction capabilities during an acute reduction in blood flow induced by cardiac tamponade after endotoxin challenge. Sixteen anesthetized, saline-infused, and ventilated dogs received Escherichia coli endotoxin (2 mg/kg) 30 min before tamponade was induced by repeated bolus injections of warm saline into the pericardial space. Thirty minutes before endotoxin administration, nine dogs received NAC (150 mg/kg, followed by a 20 mg.kg-1.h-1 infusion); the other seven dogs served as a control group. The NAC group maintained higher cardiac index, oxygen delivery (DO2), and left ventricular stroke work index, but lower systemic and pulmonary vascular resistance, than the control group. The oxygen uptake (VO2) levels at critical DO2 (DO2crit) were identical in the two groups. However, DO2crit was significantly lower in the NAC than in the control group (8.1 +/- 1.7 vs. 10.8 +/- 1.8 ml.kg-1.min-1, P < 0.01). Critical oxygen extraction ratio and the slope of the VO2-to-DO2-dependent line were higher in the NAC than in the control group (72 +/- 14 vs. 53 +/- 15% and 0.80 vs. 0.56, respectively; both P < 0.05). The peak lactate and the maximal tumor necrosis factor (TNF) levels were lower in the NAC than in the control group (5.2 +/- 0.4 vs. 7.6 +/- 0.4 mM, and 0.14 +/- 0.03 vs. 1.21 +/- 0.58 ng/ml, respectively; both P < 0.01). NAC significantly increased glutathione peroxidase activity.(ABSTRACT TRUNCATED AT 250 WORDS)

78. Seema, R. Kumar, R. N. Mandal, A. Tandon, V. S. Randhawa, G. Mehta, S. Batra, G. N. Ray and A. K. Kapoor (1999). "Serum TNF-alpha and free radical scavengers in neonatal septicemia." Indian J Pediatr 66(4): 511-6.

Tumor necrosis factor-alpha (TNF-alpha) and free radicals have been implicated in the pathogenesis of neonatal septicemia and its complications. This case control study was conducted between November 1996 to July 1997 to determine the levels of TNF-alpha and free radical scavengers viz. superoxide dismutase (SOD) and glutathione peroxidase (GPX) in the serum of 30 septic neonates and 20 healthy controls. Patients with neonatal sepsis registered significantly higher levels of TNF-alpha, SOD and GPX in comparison to controls (p < 0.05). The neonates with septic shock had five fold increase in TNF-alpha levels (2262 +/- 605.8 pg/ml) as compared to those without shock (738.8 +/- 728.8 pg/ml). There was no statistically significant difference in levels of antioxidant enzymes between neonates with shock and without shock. The levels of TNF-alpha and antioxidant enzymes were not affected by the type of organism isolated in blood culture.

79. Bains, J. S. and C. A. Shaw (1997). "Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death." Brain Res. Rev. 25(3): 335-358.

Oxidative stress has been implicated in both normal aging and in various neurodegenerative disorders and may be a common mechanism underlying various forms of cell death including necrosis, apoptosis, and excitotoxicity. In this review, we develop the hypothesis that oxidative stress-mediated neuronal loss may be initiated by a decline in the antioxidant molecule glutathione (GSH). GSH plays multiple roles in the nervous system including free radical scavenger, redox modulator of ionotropic receptor activity, and possible neurotransmitter. GSH depletion can enhance oxidative stress and may also increase the levels of excitotoxic molecules; both types of action can initiate cell death in distinct neuronal populations. Evidence for a role of oxidative stress and diminished GSH status is presented for Lou Gehrig's disease (ALS), Parkinson's disease, and Alzheimer's disease. Potential links to the Guamanian variant of these diseases (ALS-PD complex) are discussed. In context to the above, we provide a GSH-depletion model of neurodegenerative disorders, suggest experimental verifications of this model, and propose potential therapeutic approaches for preventing or halting these diseases. (C) 1997 Elsevier Science B.V.

80. Ramassamy, C., D. Averill, U. Beffert, L. Theroux, S. Lussier-Cacan, J. S. Cohn, Y. Christen, A. Schoofs, J. Davignon and J. Poirier (2000). "Oxidative insults are associated with apolipoprotein E genotype in Alzheimer's disease brain." Neurobiol. Disease 7(1): 23-37.

The epsilon 4 allele of the apolipoprotein E gene (APOE) is associated with sporadic and familial late-onset Alzheimer's disease (AD). Oxidative stress is believed to play an important role in neuronal dysfunction and cell death in AD. We now provide evidence that in the hippocampus of AD, the level of thiobarbituric acid-reactive substances (TBARS) and the APOE genotype are linked. Within AD cases, the levels of TBARS were found to be higher among epsilon 4 carriers while the apoE protein concentrations were lower. The relationship between the levels of TBARS and apoE proteins was corroborated by the results from the APOE-deficient mice, in which the levels of TBARS were higher than those in wild-type mice. Among AD cases, tissues from patients with the E4 allele of APOE displayed lower activities of catalase and glutathione peroxidase and lower concentration of glutathione than tissues from patients homozygous for the epsilon 3 allele of APOE. Together these data demonstrate that, in AD, the epsilon 4 allele of APOE is associated with higher oxidative insults. (C) 2000 Academic Press.

81. Langemann, H., A. Kabiersch and J. Newcombe (1992). "Measurement of low-molecular-weight antioxidants, uric acid, tyrosine and tryptophan in plaques and white matter from patients with multiple sclerosis." Eur. Neurol. 32(5): 248-252.

The levels of the antioxidants ascorbic acid, cysteine, reduced glutathione and alpha-tocopherol, of the free-radical marker uric acid and of the amino acids tyrosine and tryptophan were measured by means of high-pressure liquid chromatography in plaques, adjacent white matter and distant white matter from patients with multiple sclerosis, and in central nervous system tissue from patients without neurological diseases. Cholesterol and DNA were also determined, to check demyelination and cellularity. Uric acid was increased and glutathione correspondingly decreased in plaques; alpha-tocopherol was lowest in plaques and highest in distant white matter in all cases. Ascorbic acid, cysteine, tyrosine and tryptophan were not significantly changed in any tissue. The results provide evidence supporting the involvement of free radicals in multiple sclerosis.

82. Sofic, E., K. W. Lange, K. Jellinger and P. Riederer (1992). "Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease." Neurosci. Lett. 142(2): 128-130.

Reduced and oxidized glutathione concentrations in post-mortem brain tissue from the substantia nigra of control subjects and patients with neuropathologically confirmed Parkinson's disease were measured by a coulometric method using high-pressure liquid chromatography and electrochemical detection. Reduced glutathione concentrations were decreased in the substantia nigra of parkinsonian patients compared with controls. Differences in the concentration of oxidized glutathione and in the percentage of oxidized glutathione of the total glutathione were not observed between parkinsonian and control subjects. The finding that oxidized glutathione is not decreased in Parkinson's disease suggests that the decrease in reduced glutathione is not exclusively the consequence of neuronal loss in the substantia nigra but may indicate a state of oxidative stress.

83. Iantomasi, T., P. Marraccini, F. Favilli, M. T. Vincenzini, P. Ferretti and F. Tonelli (1994). "Glutathione metabolism in Crohn's disease." Biochem. Med. Metab. Biol. 53(2): 87-91.

A statistically significant decrease of glutathione (GSH) and an increase of GSH disulfide (GSSG) both in healthy and ill ileum of patients with Crohn's disease in comparison with the controls (without this pathology) is demonstrated. However, the lowering of these levels was more remarkable in ill ileum in which high levels of GSSG were detected, too. These alterations may be in part explained by the changes obtained in GSH-related enzyme levels. Finally, considering the results that others and we obtained by studies on GSH oral absorption in rat intestine, an oral therapy of GSH in Crohn's disease is suggested.

84. Ruan, E. A., S. Rao, J. S. Burdick, S. J. Stryker, G. L. Telford, M. F. Otterson, E. C. Opara and T. R. Koch (1997). "Glutathione levels in chronic inflammatory disorders of the human colon." Nutr. Res. 17(3): 463-473.

Glutathione depletion has been described in tissues obtained from several chronic diseases. Increased free radical production by inflammatory cells occurs in inflammatory bowel disease. We hypothesized that this could induce depletion of gut antioxidants. In this study, we examined the potential relationship between chronic inflammation and colonic glutathione levels. Using a validated assay, glutathione levels were determined in the mucosal-submucosal layer and the muscularis externa layer in surgical colonic specimens from 26 patients with ulcerative colitis, 14 patients with Crohn's colitis, and 10 patients who underwent partial colectomy for non-obstructive neoplasia. Inflammation was graded histologically. Glutathione levels were decreased in the muscularis externa and in the mucosal-submucosal layers from both ulcerative colitis and Crohn's colitis (both $p<0.05$). There were parallel declines of glutathione levels in the muscularis externa layer compared to the mucosal-submucosal layer from individual colonic specimens. In ulcerative colitis, glutathione levels were reduced in histologically active disease compared to inactive disease (in the mucosal-submucosal layers: Mean+/-SEM were 214+/-68 nmol/g wet tissue and 808+/-30, respectively; in the muscularis externa layers: 333+/-97 and 890+/-340; both $p<0.05$). In Crohn's colitis, there were no significant differences between histologically active and inactive disease (in the mucosal-submucosal layers: 114+/-53 and 461+/-206; in the muscularis externa layers: 105+/-59 and 553+/-211; both $p>0.05$). This study provides evidence that chronic inflammatory disorders of the colon are associated with glutathione depletion. In ulcerative colitis, there was a relationship between the severity of inflammation and glutathione depletion. By contrast, this relationship was not significant in Crohn's colitis. The results suggest that there could be a primary defect in glutathione production in Crohn's colitis, or a difference in the relative levels of free radical production by inflammatory cells present in these two disorders of colonic inflammation. Copyright (C) 1997 Elsevier Science Inc.

85. Miralles-Barrachina, O., G. Savoye, L. Belmonte-Zalar, P. Hochain, P. Ducrotte, B. Hecketsweiler, E. Lerebours and P. Dechelotte (1999). "Low levels of glutathione in endoscopic biopsies of patients with Crohn's colitis: the role of malnutrition." Clin. Nutr. 18(5): 313-317.

Background and aims: During active Crohn's disease, generation of free radicals is increased, and nutritional depletion is frequent. We investigated the glutathione concentration of the colonic mucosa in biopsies from patients with active Crohn's colitis depending on nutritional status. Methods: Endoscopic biopsies were taken in 10 well-nourished control patients, and 18 patients with active Crohn's disease (11 well-nourished, seven malnourished with a recent weight loss > 10 %). Colonic biopsies were taken from healthy and inflamed mucosa and analysed for total glutathione concentration. Results: Mucosal glutathione concentration (nmol/mg wet tissue) was lower in patients with active colitis both in diseased and healthy mucosa as compared with controls (1.89 +/- 0.39, 2.08 +/- 0.4 and 6.69 +/- 4.94, respectively, $P< 0.05$). Mucosal glutathione was lower in healthy mucosa from malnourished versus well-nourished patients: 1.8 +/- 0.2 vs 2.3 +/- 0.37 ($P= 0.02$). Conclusion: Mucosal glutathione is markedly lower in active Crohn's colitis, even in healthy mucosa; glutathione depletion tends to be more severe in malnourished patients. Glutathione depletion may be related in part to malnutrition and contribute to a prolonged evolution of disease and could be a target for pharmacological and nutritional support. (C) 1999 Harcourt Publishers Ltd.

86. Sido, B., V. Hack, A. Hochlehnert, H. Lipps, C. Herfarth and W. Droge (1998). "Impairment of intestinal glutathione synthesis in patients with inflammatory bowel disease." Gut 42(4): 485-492.

Background-Reactive oxygen species contribute to tissue injury in inflammatory bowel disease (IBD). The tripeptide glutathione (GSH) is the most important intracellular antioxidant. Aims-To investigate constituent amino acid plasma levels and the GSH redox status in different compartments in IBD with emphasis on intestinal GSH synthesis in Crohn's disease. Methods-Precursor amino acid levels were analysed in plasma and intestinal mucosa. Reduced (rGSH) and oxidised glutathione (GSSG) were determined enzymatically in peripheral blood mononuclear cells (PBMC), red blood cells (RBC), muscle, and in non-inflamed and inflamed ileum mucosa. Mucosal enzyme activity of gamma-glutamylcysteine synthetase (gamma GCS) and gamma-glutamyl transferase (gamma GT) was analysed. Blood of healthy subjects and normal mucosa from a bowel segment resected for tumour growth were used as controls. Results-Abnormally low plasma cysteine and cystine levels were associated with inflammation in IBD (p<10(-4)). Decreased rGSH levels were demonstrated in noninflamed mucosa (p<0.01) and inflamed mucosa (p=10(-6)) in patients with IBD, while GSSG increased with inflammation (p=0.007) compared with controls. Enzyme activity of gamma GCS was reduced in noninflamed mucosa (p<0.01) and, along with gamma GT, in inflamed mucosa (p<10(-4)). The GSH content was unchanged in PBMC, RBC, and muscle. Conclusions-Decreased activity of key enzymes involved in GSH synthesis accompanied by a decreased availability of cyst(e)ine for GSH synthesis contribute to mucosal GSH deficiency in IBD. As the impaired mucosal antioxidative capacity may further promote oxidative damage, GSH deficiency might be a target for therapeutic intervention in IBD.

87. Peters, W. H., H. M. Roelofs, M. P. Hectors, F. M. Nagengast and J. B. Jansen (1993). "Glutathione and glutathione S-transferases in Barrett's epithelium." Br. J. Cancer 67(6): 1413-7.

Glutathione content, enzyme activity and isoenzyme composition of glutathione S-transferases were assayed in normal and Barrett's esophageal epithelium of ten patients with Barrett's esophagus. In addition, gastric and duodenal specimens from the same patients were also investigated. Glutathione content, glutathione S-transferase enzyme activity as well as glutathione S-transferase pi content were all significantly lower in Barrett's epithelium as compared to normal esophageal mucosa. In contrast, glutathione S-transferase class alpha enzymes are markedly expressed in Barrett's epithelium, whereas only low amounts are present in normal esophageal epithelium. Glutathione and glutathione S-transferase composition in Barrett's epithelium show striking similarities with gastric epithelium, whereas duodenal epithelium is provided with considerable higher amounts of glutathione and glutathione S-transferases, except for levels of glutathione S-transferase class pi, which are lower. A significant negative correlation exists between glutathione S-transferase enzyme activity in the mucosa along the gastrointestinal tract, and the tumour incidence. Since glutathione and glutathione S-transferase are correlated with protection against cellular or cytogenetic damage, the low content of glutathione and glutathione S-transferases in the Barrett's esophagus may be a factor of relevance for the increased tumour risk in this tissue.

88. Luo, J. L., F. Hammarqvist, K. Andersson and J. Wernerman (1996). "Skeletal muscle glutathione after surgical trauma." Ann. Surg. 223(4): 420-427.

Objective The authors investigate the effect of surgical trauma on skeletal muscle concentrations of glutathione in patients undergoing selective abdominal surgery. Summary Background Data The posttraumatic state is accompanied by characteristic changes in the pattern of free amino acids and a decline of protein synthesis in human skeletal muscle. Glutathione has multiple metabolic functions that are involved in cellular homeostasis. It is unknown how surgical trauma affects the glutathione metabolism of skeletal muscle in surgical patients. Methods Eight patients undergoing elective abdominal surgery were investigated. Percutaneous muscle biopsies and blood samples were taken before operation and at 6, 24, and 48 hours after operation. The concentrations of glutathione were determined in muscle tissue, plasma, and whole blood, as well as the concentrations of the related amino acids in muscle and plasma. Results In skeletal muscle, the levels of both reduced and total glutathione decreased by 40% ($p < 0.01$) at 24 hours and remained low at 48 hours after operation compared with the preoperative values. The glutathione concentration in plasma was 20% lower after operation compared with the concentration before operation ($p < 0.05$). There were no changes at the whole blood levels of glutathione. Tissue glutamate and glutamine decreased significantly after operation ($p < 0.001$), whereas intracellular cysteine and glycine remained unchanged. Conclusions Skeletal muscle glutathione deficiency occurs after surgical trauma. This may lead to an increase in the susceptibility to intracellular oxidative injury.

89. Jain, S. K. and R. McVie (1999). "Hyperketonemia can increase lipid peroxidation and lower glutathione levels in human erythrocytes in vitro and in type 1 diabetic patients." Diabetes 48(9): 1850-1855.

Recent studies have suggested that elevated cellular lipid peroxidation may play a role in the development of cellular dysfunction and other complications of diabetes. People with type 1 diabetes frequently encounter elevated levels of the ketone bodies acetoacetate (AA), beta-hydroxybutyrate (BHB), and acetone (ACE). This study was undertaken to test the hypothesis that ketosis might increase lipid peroxidation and lower glutathione (GSH) levels of red blood cells (RBCs) in diabetic patients. This study demonstrates that incubation of AA with normal RBCs in phosphate-buffered saline (37 degrees C for 24 h) resulted in marked GSH depletion, oxidized glutathione accumulation, hydroxyl radical generation, and increased membrane lipid peroxidation. Increases in oxygen radicals and lipid peroxidation and depletion of GSH in RBCs were not observed with BHB or ACE treatments. Similarly, there was a significant generation of superoxide ion radicals even in a cell-free buffer solution of AA, but not in that of BHB. The presence of BHB together with AA did not influence the capacity of AA to generate oxygen radicals in a cell-free solution or the increase in lipid peroxidation of RBCs incubated with AA. The antioxidants vitamin E and N-acetylcysteine (NAC) blocked increase in lipid peroxidation in AA-treated RBCs. To examine the effects of ketone bodies in vivo, studies were performed that showed a significant decrease in GSH and an increase in lipid peroxidation levels in RBCs of hyperketonemic diabetic patients, but not in normoketonemic type 1 diabetic patients, when compared with age-matched normal subjects. This study demonstrates that elevated levels of the ketone body AA can increase lipid peroxidation and lower GSH levels of RBCs in people with type 1 diabetes.

90. Martina, V., G. A. Bruno, A. Pannocchia, E. Zumpano, M. Tagliabue, F. Trucco, A. Giorgianni, S. Stella and G. P. Pescarmona (1996). "PAI-1 reduction after treatment with glutathione in NIDDM." Fibrinolysis 10(Suppl. 2): 63-65.

The increase of the plasminogen activator inhibitor 1 (PAI-1) is considered a biological risk factor of coronary heart disease and is observed in patients with non-insulin-dependent diabetes mellitus (NIDDM). In vitro, hyperglycaemia increases PAI-I production by generating free radicals and the antioxidant defences reduce this phenomenon. In order to evaluate the effect of glutathione (GSH) on PAI-1, 10 patients with NIDDM underwent a treatment with GSH i.m. for 10 days. The plasma PAI-1 levels were reduced significantly after the treatment (80.1 +/- 5.2 vs 68.4 +/- 5.9 ng/ml, p < 0.02) and the red blood cell GSH concentration increased (1.53 +/- 0.2 vs 1.99 +/- 0.1 mu mol/10(10) RBC, p < 0.02). In conclusion our data suggest that the GSH may be useful to improve the fibrinolytic state in NIDDM.

91. Aaseth, J. and G. Stoa-Birketvedt (2000). "Glutathione in overweight patients with poorly controlled type 2 diabetes." J. Trace Elem. Exp. Med. 13(1): 105-111.

Increased lipid peroxidation due to an altered intracellular ratio between free radicals and antioxidant systems has been associated with development of diabetic complications. This report explores the biochemical reliability of this hypothesis by measuring glutathione (GSH) in overweight patients with poorly controlled type 2 diabetes. GSH, a crucial antioxidant and cofactor for the selenium-dependent glutathione peroxidase (GSHPx), was analyzed in red blood cells. Ten overweight and poorly controlled type 2 diabetic patients (6 women and 4 men, age 45-60 years, body mass index (BMI) 26-32 kg/m(2), HbA1c > 9.4%) and 13 healthy normal weight controls (7 women and 6 men, age 40-60 years, BMI 20-25 kg/m(2), HbA1c < 6.0%) were included in the study. The intracellular level of GSH in red blood cells (mean 1.54 mmol/l) of the diabetic patients was reduced to 60% of reference values (mean 2-6 mmol/l). Reduced activity of GSHPx and increased levels of peroxides in diabetic patients have been found previously. Discussed are several mechanisms that contribute to the depletion of GSH in poorly controlled type 2 diabetic patients; involving reduced levels of NADPH that is essential for the regeneration of GSH in vivo. The probability of direct trapping of GSH to sugar aldehydes that invade the intracellular space in diabetic states should also be taken into account. Therapeutic trials with antioxidants that can regenerate the intracellular level of GSH are scarce but promising. An attractive hypothesis is that intracellular excesses of glucose inhibit the antioxidant systems primarily by its ability to cause depletion of the crucial protector GSH. The ultimate effects of such derangement of the protective systems against free radicals may involve vascular and neurological complications. J. Trace Elem. Exp. Med. 13:105-111, 2000. (C) 2000 Wiley-Liss, Inc.

92. Bravi, M. C., P. Pietrangeli, O. Laurenti, S. Basili, M. Cassone-Faldetta, C. Ferri and G. De Mattia (1997). "Polyol pathway activation and glutathione redox status in non-insulin-dependent diabetic patients." Metabolism 46(10): 1194-1198.

The current study aimed to evaluate whether nicotinamide adenine dinucleotide phosphate (NADPH) alteration in erythrocytes from patients with non-insulin-dependent diabetes mellitus (NIDDM) is responsible for the impaired glutathione (GSH) redox status, and to assess if short-term inhibition of the polyol pathway normalizes NADPH levels and GSH redox status via an amelioration of the NADPH/total NADP (tNADP) ratio. For this purpose, erythrocyte NADPH and GSH levels were measured in 18 NIDDM patients at baseline and then after 1 week of random double-blind assignment to treatment with either tolrestat (an aldose reductase inhibitor, 200 mg daily) (n = 12) or placebo (n = 6), A group of 16 healthy volunteers served as the control, In the basal condition, mean GSH (P <.0001) and NADPH (P <.0001) levels and NADPH/tNADP (P <.0001) and GSH/glutathione disulfide (GSSG) (P <.005) ratios were lower in NIDDM patients than in control subjects. Tolrestat treatment increased GSH levels (P <.05 v placebo and baseline) and the NADPH/tNADP ratio (P <.05 v placebo and baseline). Interestingly, tolrestat-induced changes in GSH and NADPH levels and in GSH/GSSG and NADPH/tNADP ratios were significant only in patients who showed a decreased NADPH/tNADP ratio at baseline (n = 8). In these latter patients, we also found a direct correlation between percentage increments in GSH levels and NADPH/tNADP ratios after tolrestat treatment (r = .71, P <.05). In conclusion, our findings support the hypothesis that polyol pathway activation decreases NADPH and GSH levels. Accordingly, short-term inhibition of this enzymatic route increased both the GSH level and the NADPH/tNADP ratio. These changes were observable only in the subgroup of patients with an abnormal NADPH/tNADP ratio at baseline. Polyol pathway inhibition could be useful for decreasing oxidative stress in NIDDM.

93. Chen, L. H., Y. de Osio and J. W. Anderson (1999). "Blood antioxidant defense system and dietary survey of elderly diabetic men." Arch. Gerontol. Geriatr. 28(1): 65-83.

Levels of antioxidants, activities of free radical scavenging enzymes and extent of lipid peroxidation were determined in the blood of 37 elderly diabetic men and 30 control elderly men, 16 without cardiovascular disease (CVD) and 14 with CVD. The mean +/- S.D. Of the ages of the diabetic men was 66 +/- 5 and those of the control men was 69 +/- 5, while serum glucose levels of diabetic men were 213 +/- 81 mg/dl and that of control subjects were 95 +/- 14 mg/dl:dl. Among the diabetic men, 13 men were obese with body mass index > 30, 26 men had poor control of diabetes (glycohemoglobin > 7%) and 25 men had retinopathy. The diets of the control and diabetic men were evaluated. Blood samples were collected and analyzed for major endogenous antioxidant defense parameters and lipid peroxidation. The results show that diabetic men had significantly lower blood reduced glutathione levels (p < 0.001) and erythrocyte (RBC) CuZn-superoxide dismutase activity (p < 0.001) when compared to control groups with or without CVD. There was no significant differences in plasma vitamin E levels and the activities of catalase and glutathione peroxidase in RBC among the three groups. The extent of lipid peroxidation was highest in diabetic patients, intermediate in controls with CVD, and lowest in controls without CVD. The results suggest that a decline of endogenous antioxidant defense capability contributes to oxidative stress in the diabetic elderly patients. Dietary survey showed that there were no differences in the nutrient intakes of diabetic and control groups. It appears that individual dietary advice is needed for a large portion of diabetic patients in view of their poor glycemic control, hypertriglyceridemia and obesity.

94. Dominguez, C., E. Ruiz, M. Gussinye and A. Carrascosa (1998). "Oxidative stress at onset and in early stages of type 1 diabetes in children and adolescents." Diabetes Care 21(10): 1736-1742.

OBJECTIVE - In diabetes, the persistence of hyperglycemia has been reported to cause increased production of oxygen free radicals through glucose autooxidation and nonenzymatic glycation. The aim of this study was to determine whether oxidative cellular damage occurs at the clinical onset of diabetes and in later stages of the disease in young patients.

RESEARCH DESIGN AND METHODS - Indicative parameters of lipoperoxidation, protein oxidation, and changes in the status of antioxidant defense systems were evaluated in single blood samples from 54 diabetic children, adolescents, and young adults and 60 healthy age- and sex-matched control subjects.

RESULTS - Malondialdehyde and protein carbonyl group levels in plasma were progressively higher in diabetic children and adolescents than in control subjects (P < 0.0001). The highest erythrocyte superoxide dismutase (SOD) activity was found in diabetic children at onset of clinical diabetes. In diabetic adolescents, SOD was also significantly higher (P < 0.0001) than in control subjects. Erythrocyte glutathione peroxidase was significantly lower in diabetic children and adolescents compared with control subjects (P < 0.002), A significant decline in blood glutathione content at the recent onset of diabetes was found (P < 0.0001), Furthermore, our results demonstrated progressive glutathione depletion during diabetes evolution. The plasma alpha-tocopherol/total lipids ratio and beta-carotene levels during diabetes development (P < 0.001) were low

CONCLUSIONS - This cross-sectional study in young diabetic patients showed that systemic oxidative stress is present upon early onset of type 1 diabetes and is increased by early adulthood. Decreased antioxidant defenses may increase the susceptibility of diabetic patients to oxidative injury. Appropriate support for enhancing antioxidant supply in these young diabetic patients may help prevent clinical complications during the course of the disease.

95. Graber, R., J. C. Farine, I. Fumagalli, V. Tatti and G. A. Losa (1999). "Apoptosis and oxidative status in peripheral blood mononuclear cells of diabetic patients." Apoptosis 4(4): 263-270.

We have compared the concentrations of intracellular glutathione (GSH), glutathione-dependent antioxidative enzymes, the cell death rate and immunophenotype profile of peripheral blood mononuclear cells (PBMC) from healthy donors and from patients with insulin-dependent type I (IDDM) or non insulin-dependent type II (NIDDM) diabetes mellitus. The

IDDM and NIDDM patients had above-normal absolute lymphocyte counts, whereas the percentages of CD3, CD4 and CD8 T lymphocytes were significantly reduced. In contrast, the absolute number and percentage of B lymphocytes was higher in diabetic patients than in healthy donors. The low intracellular reduced glutathione (GSH) and the unbalanced profile of key enzymes involved in GSH metabolism, gamma glutamyltransferase (gamma-GT) and glutathione-S-transferase (GST), account for the increased oxidative status of PBMC from diabetic patients. The plasma membranes of PBMC from diabetic patients were less permeable to propidium iodide than those of PBMC from healthy donors, indicating that the apoptotic cell death rate was lower in the cells from diabetic patients. These differences are potentially useful markers of pathogenic metabolic changes which occur during clinical diabetes and if they are confirmed could be used to identify the onset of diabetes.

96. Konukoglu, D., T. Akcay, Y. Dincer and H. Hatemi (1999). "The susceptibility of red blood cells to autoxidation in type 2 diabetic patients with angiopathy." Metabolism 48(12): 1481-1484.

We examined the in vitro susceptibility of red blood cell (RBC) lipids to oxidation in type 2 diabetic patients with or without angiopathy. Lipid peroxidation was assessed by quantifying thiobarbituric acid (TBA) reactivity as malondialdehyde (MDA). We also examined the RBC antioxidant status by determining glutathione (GSH) levels. Before in vitro oxidation, RBC MDA levels were significantly higher in both diabetic groups than in the controls (P <.001), and a significant difference was found between the two diabetic groups (P <.05). Alter in vitro treatment of RBCs with hydrogen peroxide, the degree of lipid peroxidative damage was significantly higher in diabetic patients with angiopathy versus diabetics without angiopathy (P <.001). Diabetic patients have low RBC GSH levels compared with controls, and after in vitro oxidation, the levels were significantly decreased in diabetics (P <.001). There was not a significant correlation between RBC MDA levels and glycated hemoglobin (GHb), plasma cholesterol, and triglyceride. The correlation between RBC MDA and GSH was weak (P <.001). We suggest that the results of this study might help to clarify the role of oxidative mechanisms as an in vitro model of degenerative damage in type 2 diabetic angiopathic complications. Copyright (C) 1999 by W.B. Saunders Company.

97. Konukoglu, D., H. Hatemi, E. M. Ozer, S. Gonen and T. Akcay (1997). "The erythrocyte glutathione levels during oral glucose tolerance test." J. Endocrinol. Invest. 20(8): 471-475.

Erythrocytes glutathione (GSH) levels were measured in erythrocytes from 33 subjects, at baseline and after 2-hour glucose loading in order to investigate the effect of glucose ingestion on the erythrocyte GSH. According to the World Health Organisation criteria 18 subjects had normal glucose tolerance (NGT) (mean age 48+/-10 years, 10 women, 8 men), 15 subjects had impaired glucose tolerance (IGT) (mean age 52+/-8 years, 9 women, 6 men). After 12-hour fasting, erythrocyte GSH levels were 40.5+/-8.06 and 39.27+/-10.26 mg/dl hemolisate in subjects with NGT and IGT, respectively (p=N.S). After 2-hour glucose loading, erythrocyte GSH levels decreased to 36.01+/-9.4 (p<0.05) and 32.36+/-5.7 (p<0.005) in subjects with NGT and IGT, respectively. The decrease in erythrocyte GSH levels in subjects with IGT was greater than in NGT individuals (p<0.001). There was negative correlation between glucose, insulin, C-peptide, and erythrocyte GSH levels after glucose loading (p<0.005). Our results suggest that glucose loading induce an oxidative stress in all subjects but this oxidative stress is greater in subjects with IGT than with NGT. (C) 1997, Editrice Kurtis.

98. Murakami, K., T. Kondo, Y. Ohtsuka, Y. Fujiwara, M. Shimada and Y. Kawakami (1989). "Impairment of glutathione metabolism in erthrocytes from patients with diabetes mellitus." Metab. Clin. Exp. 38: 753-758.

The metabolism of glutathione and activities of its related enzymes were studied in erythrocytes from patients with non-insulin-dependent diabetes mellitus (NIDDM). A decrease in the levels of the reduced form of glutathione and an increase in the levels of glutathione disulfide were found in erythrocytes of diabetics. To elucidate these changes in the levels of glutathione, synthetic and degradative processes were studied. The activity of gamma-glutamylcysteine synthetase was significantly lower in diabetics than in normal controls. The activity of glutathione synthetase of each group was the same. The rate of outward transport of glutathione disulfide in diabetics decreased to approximately 70% of that of normal controls. The activity of glutathione reductase decreased in diabetics. These data suggest that the decrease in the levels of reduced form of glutathione in erythrocytes of diabetics is brought about by impaired glutathione synthesis and that the increase in the levels of glutathione disulfide is brought about by the decreased transport activity of glutathione disulfide through the erythrocyte membrane together with a decrease in the activity of glutathione reductase. These data also suggest that the impairment of glutathione metabolism weakens the defense mechanism against oxidative stress in erythrocytes of diabetics.

99. Yoshida, K., J. Hirokawa, S. Tagami, Y. Kawakami, Y. Urata and T. Kondo (1995). "Weakened cellular scavenging activity against oxidative stress in diabetes mellitus: regulation of glutathione synthesis and efflux." Diabetologia 38(2): 201-210.

Glutathione functions to scavenge oxidants or xenobiotics by covalently binding them and transporting the resulting metabolites through an adenosine 5'-triphosphate-dependent transport system. It has been reported that the intracellular concentration of glutathione decreases in diabetes mellitus, In order to elucidate the physiological significance and the regulation of anti-oxidants in diabetic patients, changes in the activity of the glutathione-synthesizing enzyme, gamma-glutamylcysteine synthetase, and transport of thiol [S-(2,4-dinitrophenyl)glutathione] were studied in erythrocytes from patients with non-insulin-dependent diabetes and K562 cells cultured with 27 mmol/l glucose for 7 days. The activity of gamma-glutamylcysteine synthetase, the concentration of glutathione, and the thiol transport were 77%, 77% and 69%, respectively in erythrocytes from diabetic patients compared to normal control subjects, Treatment of patients with an antidiabetic agent for 6 months resulted in the restoration of gamma-glutamylcysteine synthetase activity, the concentration of glutathione, and the thiol transport. A similar impairment of glutathione metabolism was observed in K562 cells with high glucose levels. The cytotoxicity by a xenobiotic (1-chloro-2,4-dinitrobenzene) was higher in K562 cells with high glucose than in control subjects (50% of inhibitory concentration, 300+/-24 mu mol/l vs 840+/-29 mu mol/l, p<0.01). Expression of gamma-glutamylcysteine synthetase protein was augmented in K562 cells with high glucose, while enzymatic activity and expression of mRNA were lower than those in the control subjects. These results suggest that inactivation of glutathione synthesis and thiol transport in diabetic patients increases the sensitivity of the cells to oxidative stresses, and these changes may lead to the development of some complications in diabetes mellitus.

100. Shapiro, B. L., Q. T. Smith and W. J. Warwick (1973). "Red cell glutathione and glutathione reductase in cystic fibrosis." Proc Soc Exp Biol Med 144(1): 181-3.

101. Shapiro, B. L., Q. T. Smith and W. J. Warick (1975). "Serum glutathione reductase and cystic fibrosis." Pediatr Res 9(12): 885-8.

Serum glutathione reductase (NADPH-GSSG oxidoreductase, EC. 1.6.4.2 (GR)) has been examined in cystic fibrosis subjects (CF), obligate CF heterozygotes, and control subjects. Serum protein concentration was similar in the three groups. Regardless of the units used to express activity (milligrams of protein or milliters of serum) or whether or not samples were dialyzed against water or phosphate buffer, mean serum GR in CF was greater than in control subjects (P less than or equal to 0.002) in all series over several years. Under the above assay conditions no difference in serum GR between control subjects and carriers was detected. Calculated and assayed values of combined control and CF sera agreed as did expected and observed 50% activity in 1:2 sera dilutions in CF, control subjects, and carriers. Addition of FAD to incubation media did not effect enzyme activity in the three groups. Differences between CF and control subjects persisted after dialysis in membranes permitting passage of molecules of approximately 12,000 mol wt or less. These findings would tend to exclude the effect of extraneous serum factors in explaining the diffferences between CF and control subjects. The percentage of initial GR activity after four days storage (0-4 degrees) was significantly greater in CF than in control subjects (P less than 0.025). The effect of heparin on serum GR was recorded as the percentage of activity after incubation with heparin vs. activity in the standard assay for individual subjects. The effect of incubation with 5 mug/ml heparin on serum GR activity was greater in control subjects than in carriers (P less than 0.0005) and CF (P less than 0.0005). Mean serum GR activity in CF and carriers was unaffected by heparin, whereas mean activity in control subjects was decreased. In no control was the percentage of initial activity with heparin greater than the mean of CF and carrier groups. Only 3 of 20 CF and 4 of 20 carrier individuals had percentages lower than the control mean. The CF and carrier distributions were clearly different from the control distribution. Serum GR was determined in seven non-CF individuals with chronic obstructive pulmonary disease (COPD). Activity in the COPD was different from CF and no different from control subjects. In none of these controls or COPD was serum GR as great as the CF mean. Serum GR in no CF was as low as the mean of control subjects or COPD. It is concluded that serum GR activity is greater in CF than in control subjects, carriers, and non-CF COPD subjects; that the difference in activity is not attributable to an extraneous serum factor, that the activity difference is not secondary to chronic respiratory disease; that in comparison with control subjects, GR from CF serum behaves differently after storage; and that serum GR from CF and carriers behaves differently from control GR in the presence of heparin.

102. **Ward, K. P., J. R. Arthur, G. Russell and P. J. Aggett (1984). "Blood selenium content and glutathione peroxidase activity in children with cystic fibrosis, coeliac disease, asthma, and epilepsy." Eur J Pediatr 142(1): 21-4.**

Long-term selenium status in children from the North-East of Scotland was estimated using whole blood selenium content (BSe) and glutathione peroxidase activity (BGSH-Px). BSe was significantly lower than the reference range in children with cystic fibrosis, coeliac disease and in older patients with phenylketonuria. Whereas BGSH-Px of all the children with coeliac disease and those with cystic fibrosis aged over 6 years matched the reference range, it was reduced in younger patients with cystic fibrosis and in children with dietetically treated phenylketonuria. No child had clinical features of selenium deficiency. BSe in treated epileptics and asthmatics conformed to the reference range, but BGSH-Px in both groups was increased significantly; this was most evident in those receiving corticosteroid preparations.

103. **Roum, J. H., R. Buhl, N. G. Mcelvaney, Z. Borok and R. G. Crystal (1993). "Systemic deficiency of glutathione in cystic fibrosis." J. Appl. Physiol. 75(6): 2419-2424.**

Cystic fibrosis (CF), a disorder characterized by mutations of the CF transmembrane regulator gene, is characterized in the lung by chronic inflammation, leading to progressive damage to the airway epithelium, bronchiectasis, and chronic obstructive lung disease. One process contributing to the airway derangement is the chronic burden of oxidants released by inflammatory cells on the respiratory epithelial surface. With this background, we hypothesized that glutathione in respiratory epithelial lining fluid (ELF) in CF patients might be oxidized and/or diminished in amount compared with that in normal subjects. Recovery of ELF by bronchoalveolar lavage from young adults with CF ($n = 21$) and normal subjects ($n = 25$) demonstrated marked neutrophil-dominated inflammation in ELF in CF patients. As predicted, ELF in CF patients was characterized by a deficiency of glutathione ($P < 0.001$), but this was secondary to a reduction in reduced glutathione ($P < 0.001$), inasmuch as there were no differences in ELF levels of oxidized glutathione ($P > 0.2$). Unexpectedly, there was also a marked deficiency of reduced glutathione in plasma ($P < 0.02$); i.e., the glutathione "deficiency" observed in ELF in CF patients is not limited to the site of the inflammation but is systemic. Although the etiology of this generalized deficiency of extracellular glutathione is unknown, it is important in considering options for treating the concomitant and devastating lung pathology in this disorder.

104. **Mangione, S., D. D. Patel, B. R. Levin and S. B. Fiel (1994). "Erythrocytic glutathione in cystic fibrosis. A possible marker of pulmonary dysfunction." Chest 105(5): 1470-3.**

To evaluate the role of red blood cell (RBC) antioxidants as clinical markers of oxidative exposure, we measured RBC glutathione (GSH) concentrations in 32 adult patients with cystic fibrosis (CF), and 8 healthy age-matched control subjects. We chose patients with CF because this disease is characterized by severe bronchial inflammation and marked oxidant-antioxidant imbalance. Although the GSH concentration of the two study groups was not significantly different, the RBC GSH concentration of patients with CF had a greater variability ($p = 0.01$) and was also inversely and significantly correlated to tests of pulmonary function ($p < 0.05$). These data indicate a large and significant interindividual variability of erythrocytic antioxidants in patients with CF, with a compensatory, but probably inadequate, increase in patients with more severe respiratory deterioration. Red blood cell GSH concentration may thus provide a biologic marker for disease severity and a rationale for antioxidant manipulation in these patients.

105. **Dominguez, C., S. Gartner, S. Linan, N. Cobos and A. Moreno (1998). "Enhanced oxidative damage in cystic fibrosis patients." Biofactors 8(1-2): 149-53.**

Antioxidant depletion and increased free radical production by inflammatory cells have been described in cystic fibrosis (CF) patients. To evaluate oxidative damage intensity, we measured plasma concentrations of malondialdehyde, hydroperoxides and protein carbon groups as markers of oxidative injury to lipids and proteins in a group of 101 CF patients free of acute exacerbation, and in 43-112 controls. Moreover, we estimated antioxidant function by measuring activities of erythrocyte superoxide dismutase, glutathione reductase and vitamin E concentrations. In CF patients, malondialdehyde and hydroperoxide plasma levels were significantly higher than in controls ($p < 0.001$). Increased lipid peroxidation was documented by these two markers. Parallel rises in protein carbonyls in plasma of CF patients were observed ($p < 0.0001$). These patients presented biochemical but not clinical vitamin E deficiency. Glutathione reductase and superoxide dismutase activities were significantly higher than in controls. These results show a serious imbalance of CF patients between oxidant- antioxidant status leading to oxidative stress.

106. Gillissen, A. and D. Nowak (1998). "Characterization of N-acetylcysteine and ambroxol in anti-oxidant therapy." Resp. Med. 92(4): 609-623.

Reactive free oxygen radicals are known to play an important role in the pathogenesis of various lung diseases such as idiopathic pulmonary fibrosis (IPF), adult respiratory distress syndrome (ARDS) or cystic fibrosis (CF). They can originate from endogenous processes or can be part of exogenous exposures (e.g. Ozone, cigarette smoke, asbestos fibres). Consequently. Therapeutic enhancement of anti-oxidant defence mechanisms in these lung disorders seems a rational approach. In this regard, N-acetyl-L-cysteine (NAC) and ambroxol have both been frequently investigated. Because of its SH group, NAC scavenges H2O2 (hydrogen peroxide), OH. (hydroxyl radical), and HOCl (hypochlorous acid). Furthermore, NAC can easily be deacetylated to cysteine, an important precursor of cellular glutathione synthesis, and thus stimulate the cellular glutathione system. This is most evident in pulmonary diseases characterized by low glutathione levels and high oxidant production by inflammatory cells (e.g. In IPF and ARDS). NAC is an effective drug in the treatment of paracetamol intoxication and may even be protective against side-effects of mutagenic agents. In addition NAC reduces cellular production of pro-inflammatory mediators (e.g. TNF-a, IL-1). Also, ambroxol [trans-4-(2-amino-3,5-dibromobenzylamino)-cyclohexane hydrochloride] scavenges oxidants (e.g. . OH, HOCl). Moreover, ambroxol reduces bronchial hyperreactivity, and it is known to stimulate cellular surfactant production. In addition, ambroxol has anti-inflammatory properties owing to its inhibitory effect on the production of cellular cytokines and arachidonic acid metabolites. For both substances effective anti-oxidant and antiinflammatory function has been validated when used in micromolar concentrations. These levels are attainable in vivo in humans. This paper gives an up-to-date overview about the current knowledge of the hypothesis that oxidant-induced cellular damage underlies the pathogenesis of many human pulmonary diseases, and it discusses the feasibility of anti-oxidant augmentation therapy to the lung by using NAC or ambroxol.

107. Kelly, F. J. (1999). "Gluthathione: in defence of the lung." Food Chem Toxicol 37(9-10): 963-6.

Oxidative stress is implicated in the pathology of numerous diseases of the lung. These include cystic fibrosis, chronic obstructive airway disease and asthma. All these conditions are characterised by an imbalance between the amounts of reactive oxygen species (ROS) and available antioxidant defences. In the lung, ROS arise from endogenous sources, such as the influx of inflammatory cells or exogenous sources, such as from air pollution and cigarette smoke. When ROS production increases the redox balance of the airways alters, and this can lead to bronchial hyperactivity and further inflammation. The lung, like many other tissues, has a range of antioxidant defences which help to maintain a balanced redox status. These antioxidants are present in the intracellular, the vascular and extracellular respiratory tract lining fluid (RTLF) compartments. The reduced glutathione (GSH) content of RTLF is particularly high and new findings are beginning to reveal the role that the RTLF GSH pool plays in defending the lung.

108. Lands, L. C., V. L. Grey and C. Grenier (2000). "Total plasma antioxidant capacity in cystic fibrosis." Pediatr Pulmonol 29(2): 81-7.

Several studies have demonstrated ongoing oxidative stress in cystic fibrosis (CF). With the complexity of the antioxidant network, measurement of individual antioxidants does not necessarily assess how they work in combination. One measure that has been proposed as a gauge of total plasma antioxidant capacity is the Trolox-equivalent antioxidant capacity (TEAC) of plasma. We decided to look at plasma TEAC levels in children with CF, and relate this measure to their nutritional status, lung function, and blood measurements of several known antioxidants. We hypothesized that values in general would be lower than healthy control values, especially during acute pulmonary exacerbations. Twenty-nine children were evaluated, five of whom were during an acute pulmonary exacerbation. Height and weight, expiratory spirometry, and lung volumes were assessed, as were serum concentrations of vitamins A and E, uric acid, albumin, and lymphocyte glutathione (GSH) concentrations. TEAC values for nonhospitalized patients (1.40 +/- 0. 20 mmol/L) were not different from laboratory control values (1.35 +/- 0.11 mmol/L), but greater than values for hospitalized patients (1.09 +/- 0.17 mmol/L). TEAC correlated with anthropometric values (height: $r = 0.39$, $P < 0.03$; weight: $r = 0.50$, $P < 0.01$; body mass index: $r = 0.47$, $P < 0.01$), and pulmonary function (forced expiratory volume in 1 sec: $r = 0.43$, $P < 0.02$; residual volume/total lung capacity: $r = -0.42$, $P < 0.03$), but not with age. Univariate correlation with blood measurements demonstrated a significant correlation of TEAC with uric acid ($r = 0.49$, $P < 0.02$), but not with albumin, vitamins A or E, or lymphocyte GSH. Multiple regression analysis demonstrated a correlation between TEAC and uric acid, albumin, and lymphocyte GSH in the non-hospitalized group ($r(2) = 0.38$, $P < 0.03$). We conclude that TEAC appears to represent a mixed antioxidant response, rather than response to a single antioxidant. While being responsive to oxidative stress, the mechanism of the response may differ between clinical situations, such that the clinical significance of changes in plasma TEAC remains to be defined. Copyright 2000 Wiley-Liss, Inc.

109. **Roum, J. H., Z. Borok, N. G. McElvaney, G. J. Grimes, A. D. Bokser, R. Buhl and R. G. Crystal (1999). "Glutathione aerosol suppresses lung epithelial surface inflammatory cell-derived oxidants in cystic fibrosis." J. Appl. Physiol. 87(1): 438-443.**

Cystic fibrosis (CF) is characterized by accumulation of activated neutrophils and macrophages on the respiratory epithelial surface (RES); these cells release toxic oxidants, which contribute to the marked epithelial derangements seen in CF. These deleterious consequences are magnified, since reduced glutathione (GSH), an antioxidant present in high concentrations in normal respiratory epithelial lining fluid (ELF), is deficient in CF ELF. To evaluate the feasibility of increasing ELF GSH levels and enhancing RES antioxidant protection, GSH aerosol was delivered (600 mg twice daily for 3 days) to seven patients with CF. ELF total, reduced, and oxidized GSH increased ($P < 0.05$, all compared with before GSH therapy), suggesting adequate RES delivery and utilization of GSH. Phorbol 12-myristate 13-acetate-stimulated superoxide anion (O-2(-).) release by ELF inflammatory cells decreased after GSH therapy ($P < 0.002$). This paralleled observations that GSH added in vitro to CF ELF inflammatory cells suppressed O-2(-). Release ($P < 0.001$). No adverse effects were noted during treatment. Together, these observations demonstrate the feasibility of using GSH aerosol to restore RES oxidant-antioxidant balance in CF and support the rationale for further clinical evaluation.

110. **Winklhofer-Roob, B. M. (2000). "Cystic fibrosis: nutritional status and micronutrients [In Process Citation]." Curr Opin Clin Nutr Metab Care 3(4): 293-7.**

Recent studies have focused on the current dietary intake of cystic fibrosis patients, the impact of nutritional support on both the nutritional status and clinical outcome variables, and the effects on the nutritional status of antibiotic therapy and surgical treatment of meconium ileus. In addition to weight and height, skinfold measurements, bioelectrical impedance analysis and dual energy X-ray absorptiometry have been employed for the determination of nutritional status. A proton pump inhibitor has been used successfully along with pancreatic enzymes for the improvement of fat absorption. Attention has been paid to resting energy expenditure during pulmonary exacerbations, to vitamin K function in bone mineralization and to risk factors for low bone mineral density in cystic fibrosis. The relationships between glutathione and nutritional status have been studied, along with possible interactions with albumin, a potent antioxidant. Finally, a beneficial effect of docosahexaenoic acid on cystic fibrosis pathology has been suggested, but this requires further critical evaluation.

111. **Meyer, A., R. Buhl and H. Magnussen (1994). "The effect of oral N-Acetylcysteine on lung glutathione levels in idiopathic pulmonary fibrosis." Eur. Resp. J. 7(3): 431-436.**

Idiopathic pulmonary fibrosis (IPF) is characterized by an increased oxidant burden and by a deficiency of glutathione, a major antioxidant, in the lung epithelial lining fluid (ELF). Therefore, a rational therapeutic approach is to reverse the imbalance between oxidants and antioxidants in the lung by enhancing the antioxidant screen. With this background, the aim of our study was to evaluate oral N-acetylcysteine (NAC) as a strategy to augment lung glutathione levels in patients with IPF. Concentrations of total glutathione in bronchoalveolar lavage fluid (BALF) were quantified spectrophotometrically, before and following oral therapy with 3 x 600 mg NAC per day for 5 days, in 17 nonsmoking patients with biopsy-proven IPF The volume of ELF recovered by BAL was determined using the urea method. Pretherapy, total glutathione levels in ELF in IPF patients were significantly less than normal (187+/-36 vs 368+/-60 muM), in contrast to levels in BALF (0.99+/-0.12 vs 1.18+/-0.19 muM). Following therapy with oral NAC, glutathione levels in BALF were 1.54+/-0.24 muM (a significant increase compared to pretherapy), whereas the increase in ELF levels (319+/-92 muM) did not reach significance. The therapy was well tolerated, and all routine clinical and bronchoscopic parameters remained unchanged It is thus feasible and safe to augment deficient lung glutathione levels in patients with IPF; thereby, potentially augmenting pulmonary antioxidant protection.

112. **Cantin, A. M., H. R.C. and R. G. Crystal (1989). "Glutathione deficiency in the epithelial lining fluid of the lower respiratory tract in idiopathic pulmonary fibrosis." Am. Rev. Respir. Dis. 139: 370-372.**

Glutathione (L-gamma-glutamyl-L-cysteinyl-glycine, GSH), a sulfhydryl- containing tripeptide produced by most mammalian cells, is an efficient scavenger of toxic oxidants, including hydrogen peroxide, an oxidant that plays a major role in the oxidant burden placed on the epithelial surface of the lower respiratory tract in chronic inflammatory states. GSH is present in the epithelial lining fluid of the normal lower respiratory tract, where it is thought to play a major role in providing antioxidant protection to the epithelial cells. In this regard, we hypothesized that the lower respiratory tract

of patients with IPF may be chronically depleted of this antioxidant, thus leading to an increased susceptibility of lung epithelial cells to oxidant injury. To evaluate this concept, the concentration of glutathione was determined in the epithelial lining fluid of the lower respiratory tract of 15 patients with IPF and compared to that of 19 normal subjects. Strikingly, whereas ELF glutathione concentrations were high in normal subjects (429 +/- 34 microM), a fourfold decrease was found in patients with IPF (97 +/- 18 microM, p less than 0.001). In the context of the known oxidant burden present in the lower respiratory tract of patients with IPF, these observations of a "GSH deficiency" in IPF ELF suggest that there is a marked oxidant-antioxidant imbalance at the alveolar surface of these persons, thus increasing the susceptibility to the severe epithelial cell damage characteristic of this disease

113. Rahman, I., E. Skwarska, M. Henry, M. Davis, C. M. O'Connor, M. X. FitzGerald, A. Greening and W. MacNee (1999). "Systemic and pulmonary oxidative stress in idiopathic pulmonary fibrosis." Free Radical Biol. Med. 27(1-2): 60-68.

An oxidant/antioxidant imbalance has been proposed in patients with idiopathic pulmonary fibrosis (IPF). We tested this hypothesis by measuring various parameters of the oxidant/antioxidant balance in the plasma of 12 patients with IPF (7 nonsmokers and 5 smokers); in the bronchoalveolar lavage fluid (BALF) of 24 patients with IPF (17 nonsmokers and 7 smokers) and 31 healthy subjects (23 nonsmokers and 8 smokers). The trolox equivalent antioxidant capacity (TEAC) in plasma and BALF was lower in nonsmoking patients with IPF (plasma 0.55 +/- 0.1 mM, p < .001; BALF 4.8 +/- 1.2 mu M, mean +/- SEM, p < .01), compared with healthy nonsmokers (plasma 1.33 +/- 0.03 mM; BALF 10 +/- 2 mu M). Similar trends in plasma and BALF TEAC were observed in smoking patients with IPF in comparison with healthy smokers. The decrease in BALF TEAC was concomitant with a decrease in BALF protein thiol levels, but the decrease TEAC levels in plasma in IPF patients was not accompanied by a decrease in protein thiol levels. Reduced glutathione (GSH) was lower in BALF in nonsmoking patients with IPF (1.0 +/- 0.1 mu M) compared with healthy nonsmokers (2.3 +/- 0.2 mu M, p < .001). In contrast, GSH levels were higher in smoking patients with IPF (5.2 +/- 1.1 mu M, p < .001) than in nonsmoking patients. GSSG levels were not different in any of the groups. The levels of products of lipid peroxidation measured as thiobarbituric acid reactive substances (TBARS) in plasma and BALF were significantly increased in both smoking (plasma 2.2 +/- 0.5 mu M, p < .01; BALF 0.18 +/- 0.04 mu M, p < .001), and nonsmoking (plasma 2.1 +/- 0.3 mu M, p < .01; BALF 0.22 +/- 0.05 mu M, p < .001) IPF patients, compared with healthy nonsmokers (plasma 1.4 +/- 0.3 mu M; BALF 0.05 +/- 0.004 mu M). These data show evidence of oxidant/antioxidant imbalance in the lungs of patients with IPF, which is also reflected as systemic oxidant stress. (C) 1999 Elsevier Science Inc.

114. Meyer, A., R. Buhl, S. Kampf and H. Magnussen (1995). "Intravenous N-acetylcysteine and lung glutathione of patients with pulmonary fibrosis and normals." Amer. J. Respir. Crit. Care Med. 152(3): 1055-1060.

Idiopathic pulmonary fibrosis (IPF) is characterized by a huge alveolar oxidant burden and a deficiency of glutathione, a major antioxidant, in the pulmonary epithelial lining fluid (ELF). Therefore, a rational therapeutic strategy is to increase lung glutathione to augment the pulmonary antioxidant protective screen. To evaluate this concept, different doses of N-acetylcysteine (NAC), a glutathione precursor, were administered intravenously to eight patients with pulmonary fibrosis and six control subjects. In patients, bronchoalveolar lavage fluid (BALF) total glutathione increased significantly from 0.99 +/- 0.25 mu M to 1.79 +/- 0.37 mu M within 3 h following 1.8 g NAC, whereas 4.8 g NAC had no additional effect (1.47 +/- 0.34 mu M). In the control subjects, NAC did not significantly alter BALF total glutathione (baseline: 0.79 +/- 0.17 mu M, 600 mg NAC: 0.92 +/- 0.33 mu M, 1.8 g NAC: 1.39 +/- 0.41 mu M, 4.8 g NAC: 1.33 +/- 0.46 mu M). The same was true in ELF, 1.8 g NAC significantly raised ELF total glutathione in patients from 186 +/- 47 mu M to near normal levels (373 +/- 103 mu M), with no further increase following 4.8 g NAC (293 +/- 62 mu M). In the control subjects, ELF total glutathione remained unchanged independent of the NAC dose (baseline: 342 +/- 91 mu M, 600 mg NAG: 385 +/- 135 mu M, 1.8 g NAG: 633 +/- 220 mu M, 4.8 g NAG: 646 +/- 263 mu M) The increases in total glutathione were almost entirely due to increased levels of reduced glutathione, the form functional as an antioxidant. No adverse effects were noted. In conclusion, intravenous NAC augments lung glutathione of patients with pulmonary fibrosis but had no significant effect on lung glutathione in the control subjects.

115. Behr, J., K. Maier, B. Braun, M. Schwaiblmair and C. Vogelmeier (2000). "Evidence for oxidative stress in bronchiolitis obliterans syndrome after lung and heart-lung transplantation." Transplantation 69(9): 1856-1860.

Bronchiolitis obliterans syndrome (BOS) is the most serious long-term sequel of lung or heart-lung transplantation (H/LTX), Neutrophilia in the lower respiratory tract is a prominent feature of BOS, Because polymorphonuclear leukocytes (PMN) are capable of releasing large quantities of reactive oxygen species, we measured indicators of oxidative stress and glutathione levels representing antioxidant defense in H/LTX patients (HLTX, n = 6; double-LTX, n = 7; single-LTX, n = 9), We analyzed 19 bronchoalveolar lavage (BAL) samples from 13 non-BOS patients (nine female, four male: age 39+/-4 years) and 17 BAL samples from nine BOS patients (five female, four male: age 33 +/- 2 years). PMN were the predominant BAL cell population in BOS (61.7 +/- 7.8% vs. 12.3 +/- 3.4%, P < 0,001), Myeloperoxidase activity in the epithelial lining fluid and oxidized methionine residues in BAL-derived proteins were elevated in BOS (8.6 +/- 1.6 U/ml vs. 2.2 +/- 0.6 U/ml, P < 0,01; and 12.6 +/- 1.1% vs. 7.7 +/- 0.8%, P < 0,001, respectively). In addition, the concentration of reduced glutathione in epithelial lining fluid was decreased in BOS (162.6 +/- 20.1 mu M vs, 345,8 +/- 57,1 mu M, P < 0.01) whereas the proportion of oxidized glutathione was increased (13.9 +/- 2.0% vs, 6.7 +/- 1.2%, P < 0.001). PMN, myeloperoxidase, and oxidized methionine residues were inversely correlated, whereas reduced glutathione was positively correlated with forced expiratory volume in 1 sec (P < 0,05 to P < 0.001). We conclude that excessive oxidative stress and a lack of glutathione are associated with BOS after H/LTX and may play relevant roles in the development of this disorder.

116. Engelen, M. P. K. J., A. M. W. J. Schols, J. D. Does, N. E. P. Deutz and E. F. M. Wouters (2000). "Altered glutamate metabolism is associated with reduced muscle glutathione levels in patients with emphysema." Amer. J. Respir. Crit. Care Med. 161(1): 98-103.

Chronic obstructive pulmonary disease (COPD) is often characterized by an impaired skeletal muscle energy metabolism, which is at least partly related to chronic hypoxia and a reduced diffusing capacity. We have found that muscle glutamate (Glu), which is negatively influenced by these conditions, was reduced in patients with severe COPD. The aim of this study was to investigate whether the reduced intracellular Glu level in patients with emphysema is associated with an increased muscle glycolytic metabolism Since Glu is an important substrate in the synthesis of glutamine (Gln) and glutathione (GSH), the influence of Glu status on muscle GSH and Gln was also examined. In 13 patients with emphysema and 25 control patients, arterial blood and biopsies from the vastus lateralis muscle were obtained. Expressed as a percentage of the control values, the patients with emphysema had reduced values for muscle Glu (64 +/- 12%; p < 0.001), GSH (76 +/- 23%; p < 0.01), and Gln (93 +/- 5%; p < 0.01), and higher values for lactate (p < 0.01) and pyruvate (p < 0.05). No differences were found in plasma values. Muscle Glu was highly associated with GSH (R-2 = 0.61; P < 0.001), but not with Gln. This study illustrates that reduced Glu levels in skeletal muscle of patients with emphysema are possibly related to an enhanced glycolytic activity and associated with decreased GSH levels.

117. Dincer, Y., T. Akcay, D. Konukogku and H. Hatemi (1999). "Erythrocyte susceptibility to lipid peroxidation in patients with coronary atherosclerosis." Acta Med. Okayama 53(6): 259-264.

In recent years it has been reported that free oxygen radicals play an important role in the pathogenesis of degenerative diseases and that antioxidant vitamins such as vitamins E or C prevent their harmful effects. In this study, we evaluated the following: Erythrocyte susceptibility to lipid peroxidation; the role of erythrocyte glutathione (GSH) as an antioxidant; plasma lipid fractions; and the relationship between plasma lipid peroxides and antioxidant vitamin levels. Thiobarbituric acid-reactive substance (TBARS) levels were measured to determine the levels of plasma lipid peroxides and the susceptibility to lipid peroxidation when erythrocytes were stressed by hydrogen peroxide for 2 h h vitro. Erythrocyte TBARS production was significantly higher in patients with coronary atherosclerosis than in the controls. On the other hand, the levels of plasma high-density lipoproteins, vitamin C, vitamin E and erythrocyte GSH were significantly lower, and the levels of plasma total cholesterol, triglycerides, low-density lipoproteins and TBARS were significantly higher in the patients with coronary atherosclerosis than in the controls. In conclusion, our results indicate that erythrocytes from patients with coronary atherosclerosis are more susceptible to oxidation than those of controls and that these patients have lowered antioxidant capacity as revealed by decreased plasma levels of vitamins C and E.

118. Yucel, D., S. Aydogdu, S. Cehreli, G. Saydam, H. Canatan, M. Senes, B. C. Topkaya and S. Nebioglu (1998). "Increased oxidative stress in dilated cardiomyopathic heart failure." Clin. Chem. 44(1): 148-154.

In the present study, we assessed oxidative stress in patients with dilated cardiomyopathy of ischemic or idiopathic etiology. For this reason we measured whole blood reduced glutathione, erythrocyte superoxide dismutase, susceptibility of erythrocyte membranes and erythrocytes to peroxidation, and SH content of erythrocyte membranes in 12 patients (8 men and 4 women, ages 31 to 66 years) with idiopathic dilated cardiomyopathy, in 11 patients (8 men and 3 women, ages 32 to 65 years) with ischemic dilated cardiomyopathy, and in 21 healthy volunteers (12 men and 9 women, ages 25 to 67 years). There was no statistically significant difference between the two patient groups for the indicators studied (P >0.05). Blood glutathione, erythrocyte superoxide dismutase, and membrane SH content of both groups of patients was decreased compared with controls (P <0.05), whereas erythrocyte and membrane susceptibility to peroxidation were increased (P <0.05). We conclude that patients with idiopathic or ischemic dilated cardiomyopathy exhibit abnormalities of a range of markers of increased oxidative stress. These abnormalities may contribute to contractile dysfunction, increased incidence of fatal arrhythmias, and sudden death.

119. Usal, A., E. Acarturk, G. T. Yuregir, I. Unlukurt, C. Demirci, H. I. Kurt and A. Birand (1996). "Decreased glutathione levels in acute myocardial infarction." Jpn. Heart J. 37(2): 177-182.

Although experimental studies have demonstrated that reduced glutathione (GSH) is involved in cellular protection from deleterious effects of oxygen free radicals (OFRs) in ischemia and reperfusion, there are controversial data on the correlation between the levels of erythrocyte GSH and the ischemic process. To clarify, we determined the erythrocyte GSH levels in 21 patients with acute myocardial infarction (AMI), aged 39-70, who were not given thrombolytic therapy and 21 age- and sex- matched healthy controls. Samples of blood were taken on days 1, 3, 5 and 7 from AMI patients and on the same days from the controls. The GSH levels of patients with AMI were significantly depressed by 11.5% as compared to the controls on the second day after infarction (7.44 +/- 1.71 vs 8.41 +/- 1.54 U/gHb p < 0.05). Although the total mean of GSH levels for all days was lower (3.8%) in patients than in the controls, this finding did not reach statistical significance (7.41 +/- 1.71 vs 7.71 +/- 1.27 U/gHb, ns). There was no correlation between the erythrocyte GSH levels and cardiac enzyme concentrations, infarct localization, hemodynamic status according to Killip classification and the frequency of ventricular arrhythmias. This preliminary work suggests that depressed GSH levels may be associated with an enhanced protective mechanism to oxidative stress in AMI. Measurements of erythrocyte GSH can be helpful in the estimation of oxidative stress in the course of AMI. However, further research must be done to determine the primary scavenger in AMI by analyzing all the enzymes and substrates involved in the endogeneous system that controls the effects of OFRs.

120. Lou, M. F., J. E. Dickerson Jr, W. H. Tung, J. K. Wolfe and L. T. Chylack Jr (1999). "Correlation of nuclear color and opalescence with protein S-thiolation in human lenses." Exp. Eye Res. 68(5): 547-552.

Human lens nuclei were collected during routine cataract surgery and used to study the role of oxidation in cataract formation and brunescence. This study focused on the comparison of the intensities of nuclear opacity and pigmentation (brunescence) with the changes in free glutathione (GSH) and the three species of protein-thiol mixed disulfides: protein-S-S-glutathione (PSSG), protein-S S-cysteine (PSSC) and protein-S-S-gamma-glutamylcysteine (PSSGC). Eighty-one freshly excised human lens nuclei from a population with a mean age of 77 were used. The nuclear color was graded using the CCRG system, ranging from yellow to dark brown. The nuclear cataract opalescence of these lenses was also graded using the LOGS II system, ranging from LOGS II NO-1 to NO-4. Three normal human lenses (average age of 88 yr) were also included in the study as controls. The nuclear samples were each analyzed for free GSH and protein-thiol mixed disulfides, respectively. It was found that nuclear GSH decreased as the nuclear color increased from yellow to dark, brown (from 0.73+/-0.13 to 0.13+/-0.03 mu mole g wet wt(-1)) and as the nuclear opalescence increased from NO.1 to NO.4 (from 0.80+/-0.19 to 0.20+/-0.01 mu mole g wet wt(-1)). All these values were lower than that of GSH in normal controls (1.43+/-0.59 mu mole g wet wt(-1)). Levels of both PSSG and PSSC progressively increased, however, as the nuclear color intensified. PSSG increased from 0.29+/-0.05 to 0.91+/-0.11 mu mole g wet wt(-1) while PSSC increased from 0.13+/-0.04 to 0.41+/-0.06 mu mole g wet wt(-1). PSSGC concentration progressively increased with increases in both nuclear pigmentation (from 0.05+/-0.01 to 0.23+/-0.05 mu mole g wet wt(-1)) and nuclear opacity (from 0.02+/-0.00 to 0.20+/-0.02 mu mole g wet wt(-1)). In comparison, normal controls had lower levels of all three mixed disulfide species: PSSG, 0.22 +/- 0.06; PSSC, 0.08+/-0.02; PSSGC, 0.02+/-0.06 mu mole g wet wt(-1), respectively. The correlation of lens nuclear color and opalescence intensity with nuclear protein S-thiolation indicates that protein-thiol mixed disulfides may play an important role in cataractogenesis and development of brunescence in human lenses. (C) 1999 Academic Press.

121. Saxena, S., D. Kumar, P. Srivastava, V. K. Khanna and P. K. Seth (1999). "Low levels of platelet glutathione in Eales' disease." Med. Sci. Res. 27(9): 625-626.

Eales' disease is an idiopathic obliterative vasculopathy of the peripheral retina of young adults. The retina, because of its high oxygen requirement and content of polyunsaturated fatty acid (PUFA), is an elective site for oxidative stress. Since platelets also contain a considerable quantity of PUFA, we have undertaken a study based on a tertiary care centre, of 18 Eales' disease cases and 20 healthy controls, to determine the comparative status of platelet glutathione (reduced form, GSH). Levels were significantly lower in the cases as compared to controls ($P < 0.001$). Since GSH is one of the major antioxidant defence systems in the retina, lowered levels may contribute to enhanced oxidative stress in Eales' disease. Med Sci Res 27:625-626 (C) 1999 Lippincott Williams & Wilkins.

122. Seth, R. K., A. S. Saini and S. K. Aggarwal (1985). "Glutathione peroxidase activity and reduced glutathione content in erythrocytes of patients with chronic renal failure." Scand. J. Haematol. 35: 201-204.

Erythrocytes from 18 patients with chronic renal failure (CRF) and 10 healthy subjects were examined with respect to glutathione peroxidase (GSH-Px) activity and reduced glutathione (GSH) contents. The activity of GSH-Px and GSH content were found to be lower in RBC from CRF patients as compared with normal RBC. These reduced levels of GSH and GSH-Px in the red cells of uraemic patients may predispose the cells to oxidative damage.

123. Pasqualotto, F. F., R. K. Sharma, J. M. Potts, D. R. Nelson, A. J. Thomas and A. Agarwal (2000). "Seminal oxidative stress in patients with chronic prostatitis." Urology 55(6): 881-5.

OBJECTIVES: An association between prostatitis and male infertility has been suspected, yet is poorly understood. Prostatitis is often associated with granulocytes in the prostatic fluid that generate reactive oxygen species (ROS), known to impair male fertility. We compared ROS, the total antioxidant capacity (TAC), and a novel index of oxidative stress (ROS-TAC score) in patients with chronic prostatitis and in healthy controls.

METHODS: Semen specimens from 36 men with chronic prostatitis (National Institutes of Health category IIIa), 8 men with prostatodynia (National Institutes of Health category IIIb), and 19 controls attending our urologic clinic were examined according to the World Health Organization criteria. Leukocytospermia was measured by the Endtz test (myeloperoxidase assay). ROS and TAC production was measured by chemiluminescence assay. A composite ROS-TAC score was also calculated in patients and controls.

RESULTS: The sperm concentration, percentage of motility, and morphology among the groups did not differ. The mean +/- standard error log-transformed ROS level was significantly higher in patients with leukocytospermia (3.2 +/- 0.6) than in patients without leukocytospermia (1.8 +/- 0.2; $P = 0.04$) and controls (1.3 +/- 0.3, $P = 0.01$). TAC was significantly lower in patients with or without leukocytospermia (859.69 +/- 193.0 and 914.9 +/- 65.2, respectively) than in controls (1653.98 +/- 93.6, $P = 0.001$). The mean ROS-TAC score of controls (50.0 +/- 4.1) was significantly higher than those of patients with chronic prostatitis and leukocytospermia (8.2 +/- 9.2) and those without leukocytospermia (34.2 +/- 2.9; $P < 0.001$).

CONCLUSIONS: Men with chronic prostatitis or prostatodynia have seminal oxidative stress, irrespective of their leukocytospermia status. These observations may help shed light on the long-standing controversy surrounding prostatitis and infertility.

124. Kokcam, I. and M. Naziroglu (1999). "Antioxidants and lipid peroxidation status in the blood of patients with psoriasis." Clin. Chim. Acta 289(1-2): 23-31.

The aim of this research was to determine levels in blood of vitamin E, beta carotene, lipid peroxidation as malondialdehyde (MDA), reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) activity in patients with psoriasis. Studies were carried out on 34 patients with moderate and severe psoriases and healthy age-matched controls. Red blood cell (RBC) and plasma samples from healthy and patient subjects were taken. Levels of GSH and the activity of GSH-Px in both plasma and RBC samples were significantly ($P < 0.001$) lower in patients with psoriasis than in controls, whereas beta carotene levels in plasma and MDA levels in RBC samples were significantly ($P < 0.01$, $P < 0.001$) higher in patients with psoriasis than in controls. However, vitamin E and MDA levels in plasma did not differ statistically. Although being far from conclusive, these results provide some evidence for a potential role of increased lipid peroxidation and decreased antioxidants in psoriasis. (C) 1999 Published by Elsevier Science BN. All rights reserved.

125. Maurice, M. M., H. Nakamura, E. A. M. van der Voort, A. I. van Vliet, F. J. T. Staal, P.-P. Tak, F. C. Breedveld and C. L. Verweij (1997). "Evidence for the role of an altered redox

state in hyporesponsiveness of synovial T cells in rheumatoid arthritis." J. Immunol. 158(3): 1458-1465.

In rheumatoid arthritis (RA), T cells isolated from the synovial fluid (SF) show impaired responses to mitogenic stimulation compared with T cells from the peripheral blood (PB), Here it is reported that hyporesponsiveness of SF T cells correlated with a significant decrease in the levels of the intracellular redox-regulating agent glutathione (GSH). GSH was decreased in both CD4(+) ($p = 0.0022$) and CD8(+) ($p = 0.0010$) SF T cell subsets compared with PB CD4(+) and CD8(+) T cells in RA patients. Levels of thioredoxin (TRX), another key redox mediator, previously found to be secreted under conditions of oxidative stress, were found to be significantly increased in SF compared with plasma samples of RA patients ($p = 0.005$), Increased levels of TRX in the SF of inflamed joints was found to be associated with RA when compared with Ether arthritides ($p = 0.007$), Restoration of GSH levels in SF T cells with N-acetyl-L-cysteine (NAG), enhanced mitogenic induced proliferative responses and IL-2 production. Collectively, these data impute an important role to an altered redox state in the hyporesponsiveness of joint T cells in patients with RA.

126. **Gringhuis, S. I., A. Leow, E. A. M. Papendrecht-van der Voort, P. H. J. Remans, F. C. Breedveld and C. L. Verweij (2000). "Displacement of linker for activation of T cells from the plasma membrane due to redox balance alterations results in hyporesponsiveness of synovial fluid T lymphocytes in rheumatoid arthritis." J. Immunol. 164(4): 2170-2179.**

The T lymphocytes that reside in the synovium of the inflamed joints in patients with rheumatoid arthritis display severe hyporesponsiveness upon antigenic stimulation, which is probably due to their constant subjection to high levels of oxidative stress. Here we report that the synovial fluid T lymphocytes exert severely impaired phosphorylation of the adaptor protein linker for activation of T cells (LAT), a crucial component of the TCR-mediated signaling pathways. In healthy T lymphocytes, LAT is a membrane-bound protein and becomes phosphorylated by zeta-associated protein of 70 kDa (ZAP-70) upon TCR engagement. The molecular basis underlying the deficient phosphorylation of LAT and consequently the hyporesponsiveness of the synovial fluid T lymphocytes lies in the membrane displacement of LAT, We demonstrate that the subcellular localization of LAT is sensitive to changes in the intracellular levels of the antioxidant glutathione. The membrane anchorage of LAT, and consequently the phosphorylation of LAT and the cellular activation of the synovial fluid T lymphocytes upon TCR engagement, is restored in synovial fluid T lymphocytes after supplementation of the intracellular glutathione levels with N-acetyl-L-cysteine. These data suggest a role for the membrane displacement of LAT in the hyporesponsiveness of the synovial fluid T lymphocytes as a consequence of oxidative stress.

127. **Aukrust, P., A. M. Svardal, F. Muller, B. Lunden, R. K. Berge and S. S. Froland (1995). "Decreased levels of total and reduced glutathione in CD4+ lymphocytes in common variable immunodeficiency are associated with activation of the tumor necrosis factor system: possible immunopathogenic role of oxidative stress." Blood 86(4): 1383-1391.**

We have previously shown chronic immune activation and enhanced generation of reactive oxygen species in common variable immunodeficiency (CVI). In the present study, we examined levels of glutathione, the dominant intracellular thiol, that play an important protective role against oxidative and inflammatory stress in plasma and in monocytes and lymphocyte subsets in 20 CVI patients and in 16 healthy controls. CD4(+) lymphocytes from CVI patients had significantly lower levels of both total and reduced glutathione as well as a lower ratio of reduced to total glutathione compared with healthy controls. This decrease in glutathione levels in CD4(+) lymphocytes was most pronounced in the CD45RA(+) subset. Plasma levels of total glutathione were also significantly decreased in CVI. In contrast, monocytes from CVI patients exhibited increased levels of both total and reduced glutathione compared with blood donor monocytes. CVI patients had significantly raised serum levels of tumor necrosis factor alpha (TNF alpha) and TNF alpha concentration was strongly associated with glutathione depletion in CD4(+) lymphocytes. Furthermore, the lowest levels of both total and reduced glutathione were found in a subgroup of CVI patients characterized by persistent immune activation in vivo, decreased numbers of CD4(+) lymphocytes in peripheral blood, and splenomegaly. Finally, supplementation of cell cultures with glutathione-monoethyl ester did significantly enhance interleukin-2 production from peripheral blood mononuclear cells in CVI patients. These glutathione abnormalities in CVI indicate increased oxidative stress, particularly in CD4(+) lymphocytes, and intracellular depletion of reduced glutathione of the demonstrated magnitude may have profound implications for CD4(+) lymphocyte function and the immunodeficiency in CVI. (C) 1995 by The American Society of Hematology.

128. Hammarqvist, F., J. L. Luo, I. A. Cotgreave, K. Andersson and J. Wernerman (1997). "Skeletal muscle glutathione is depleted in critically ill patients." Crit. Care Med. 25(1): 78-84.

OBJECTIVE: To investigate the concentrations of reduced and total glutathione in relation to the muscle free amino acid pattern in critically ill patients and matched healthy controls.

DESIGN: Prospective case control.

SETTING: University hospital intensive care unit (ICU).

PATIENTS: Eleven critically ill patients in the intensive care unit were studied after a stay of at least 4 days. Eleven age- and gender-matched metabolically healthy patients undergoing elective surgical procedures served as controls.

INTERVENTIONS: None.

MEASUREMENTS AND MAIN RESULTS: Reduced and total glutathione concentrations were determined in skeletal muscle, in plasma, and in whole blood, together with muscle free amino acid concentrations. In the ICU group, reduced and total glutathione values were 57% and 62%, respectively, of the values seen in the control group ($p < .001$). In addition, a decreased ratio between reduced and total glutathione compared with the controls was seen (0.80 as compared with 0.91, $p < .001$). The glutamine concentration in skeletal muscle in the ICU group was 72% lower compared with that value seen in healthy controls ($p < .001$). Correlations were found between the concentrations of glutamine and the total muscle glutathione ($r2 = .46$, $p < .001$), as well as between glutamine and the ratio of reduced and total glutathione ($r2 = .45$, $p < .001$) in skeletal muscle, suggesting that the redox status of glutathione and the glutamine status of the tissue are related.

CONCLUSIONS: Critical illness is associated with alterations in muscle glutathione metabolism. The muscle-reduced glutathione concentrations decrease and, in addition, the ratio between reduced and total glutathione decreases, indicating a situation of oxidative stress in this tissue. This decrease may impair the defense of muscle against oxygen free radicals and influence amino acid transport, thus contributing to the loss of balance between protein synthesis and protein degradation that is characteristic of protein catabolism.

129. Verjee, Z. H. and R. Behal (1976). "Protein-calorie malnutrition: a study of red blood cell and serum enzymes during and after crisis." Clin. Chim. Acta 70(1): 139-147.

This paper reports a study of changes in red blood cell enzymes and some serum parameters during and after treatment of protein-calorie malnutrition. The red cell GSH levels were low during the crisis, together with the levels of GSSG:NADPH reductase, GSH:H2O2 peroxidase, aspartate aminotransferase and alanine aminotransferase. After treatment the levels of all these enzymes increased significantly to normal values. Of the serum parameters investigated, significant reduction in the activity of the enzymes cholinesterase, catecholamine oxidase, total proteins, albumin, urea and electrolytes were obvious, and returned to normal values after treatment. Ceruloplasmin activity remained low even after three weeks' treatment and could not be related to copper levels. The results are discussed in relation to anemia and liver damage that may accompany the syndrome.

130. Reid, M., A. Badaloo, T. Forrester, J. F. Morlese, M. Frazer, W. C. Heird and F. Jahoor (2000). "In vivo rates of erythrocyte glutathione synthesis in children with severe protein-energy malnutrition." Amer. J. Physiol. - Endocrinol. Metab. 278(3): E405-E412.

Although the compromised GSH status of children with edematous protein-energy malnutrition (PEM) has been documented, the in vivo kinetic mechanism(s) responsible for this is not known. To determine if decreased synthesis contributes to the alteration of GSH homeostasis, the fractional and absolute rates of synthesis of erythrocyte GSH were determined shortly after admission (study 1), similar to 9 days postadmission (study 2), and at recovery (study 3) in seven children with edematous PEM and seven children with nonedematous PEM. Children with edematous PEM had significantly lower erythrocyte GSH and slower absolute rates of GSH synthesis than children with nonedematous PEM both shortly after admission, when they were both malnourished and infected, and similar to 9 days later, when the infection had resolved but they were still malnourished. At these times, the edematous group also had significantly lower erythrocyte GSH concentrations and absolute rates of synthesis than at recovery. Plasma and erythrocyte-free cysteine concentrations of the edematous group were significantly lower at studies I and 2 than at recovery. In contrast, erythrocyte GSH concentrations, rates of GSH synthesis, and plasma and erythrocyte free cysteine concentrations of the nonedematous group were similar at all three time points and greater at studies 1 and 2 than in the edematous group. These results confirm that GSH deficiency is characteristic of edematous PEM and suggest that this is due to a reduced rate of synthesis secondary to a shortage in cysteine.

131. **Bernard, G. R. (1991). "N-Acetylcysteine in experimental and clinical acute lung injury." Am. J. Med. 91 (S3C)(S3C): S54-S59.**

Clinically, lung injury is characterized by one or more of the following: altered gas exchange, dyspnea, decreased static compliance, and nonhydrostatic pulmonary edema. Although many antioxidants have been investigated in in vitro systems and in animal models, only some are at the developmental stage, or safe for clinical trials. Considerable evidence has recently accumulated supporting the hypothesis that leukocyte activation involves release of large quantities of highly reactive oxygen radicals, and hydrogen peroxide is partially responsible for diffuse microvascular and tissue injury in septic patients. Granulocyte depletion in animal models reduces the degree of fall in dynamic lung compliance and the increase in airflow resistance, lymph flow, and hypoxemia secondary to endotoxin administration. We hypothesized that the partial benefit derived from granulocyte depletion was due to the effective removal of a major source of oxygen radicals. Among the list of free radical scavengers, N-acetylcysteine stands out, because of its established usefulness in at least one human disease thought to be secondary to free radical organ damage (acetaminophen or paracetamol overdose). It is an extremely safe agent with a wide toxic-therapeutic window. An increasing number of animal studies indicate efficacy for this agent in the prevention and therapy of lung injury involving toxic oxygen species. We developed a randomized, double-blind protocol for the study of intravenous N-acetylcysteine in patients with established adult respiratory distress syndrome (ADRS). Results of this trial are preliminary. Nevertheless, they indicate that plasma and red cell glutathione levels are decreased in ADRS patients, and that N-acetylcysteine increases plasma cysteine as well as plasma and red cell glutathione. There are also indications that cardiopulmonary physiology is favorably affected by such therapy including improvements in chest radiograph edema scores, pulmonary vascular resistance, static compliance, oxygen delivery, and oxygen consumption.

132. **Pacht, E. R., A. P. Timerman, M. G. Lykens and A. J. Merola (1991). "Deficiency of alveolar fluid glutathione in patients with sepsis and the adult respiratory distress syndrome." Chest 100(5): 1397-1403.**

The adult respiratory distress syndrome (ARDS) is a devastating clinical illness characterized by refractory hypoxemia and high-permeability pulmonary edema. Reactive oxygen species such as hydrogen peroxide and hypochlorous acid may play a key role in the pathogenesis of the acute lung injury. Glutathione (GSH) is a tripeptide that is able to react with and effectively neutralize oxidants such as hydrogen peroxide and hypochlorous acid. The present study found that the alveolar epithelial lining fluid of patients with ARDS was deficient in total GSH compared to normal subjects (21.7-mu-mol +/- 7.8-mu-mol vs 91.8-mu-mol +/- 14.5-mu-mol; p = 0.002). In addition, if GSH was measured in unconcentrated bronchoalveolar lavage (BAL) fluid and indexed to total BAL protein, there was also a deficiency in patients with ARDS compared to normal subjects (0.004 +/- 0.003 nmol of GSH per microgram of total protein vs 0.026 +/- 0.005 nmol of GSH per microgram of total protein; p = 0.002). Since patients with ARDS are subjected to an increased burden of oxidants in the alveolar fluid, principally released by recruited neutrophils, this deficiency of GSH may predispose these patients to enhanced lung cell injury.

133. **Eck, H.-P., H. Gmunder, M. Hartmann, D. Petzoldt, V. Daniel and W. Droge (1989). "Low concentrations of acid-soluble thiol (cysteine) in the blood plasma of HIV-1-infected patients." Biol. Chem. Hoppe-Seyler 370: 101-108.**

Blood plasma samples from HIV-1-infected persons contain elevated glutamate concentrations up to 6-fold the normal level and relatively low concentrations of acid-soluble thiol (i.e. decreased cysteine concentrations). The intracellular glutathione concentration in peripheral blood-mononuclear cells (PBMC) and monocytes from HIV antibody-positive persons are also significantly decreased. Therapy with azidothymidine (AZT) causes a substantial recovery of the plasma thiol levels; but glutamate levels remain significantly elevated and intracellular glutathione levels remain low. Cell culture experiments with approximately physiological amino-acid concentrations revealed that variations of the extracellular cysteine concentration have a strong influence on the intracellular glutathione level and the rate of DNA synthesis [(3H]thymidine incorporation) in T cell clones and human and murine lymphocyte preparations even in the presence of several-fold higher cystine and methionine concentrations. Cysteine cannot be replaced by a corresponding increase of the extracellular cystine or methionine concentration. These experiments suggest strongly that the low cysteine concentration in the plasma of HIV-infected persons may play a role in the pathogenetic mechanism of the acquired immunodeficiency syndrome.

134. **Droge, W., H.-P. Eck, H. Naher, U. Pekar and V. Daniel (1988). "Abnormal Amino-Acid Concentrations in the Blood of Patients with Acquired Immunodeficiency Syndrome (AIDS) May Contribute to the Immunological Deficit." Biol. Chem. Hoppe-Seyler 369: 143-148.**

The acquired immunodeficiency syndrome (AIDS) is accompanied by a metabolic disturbance. Serum samples from persons with antibodies against the AIDS associated human immunodeficiency virus (HIV/LAV/HTLV III) including persons without overt symptoms, patients with lymphadenopathy syndrome (LAS) and patients with AIDS or AIDS-related complex (ARC) contain on the average significantly elevated concentrations of arginine and glutamate. The serum from patients with overt AIDS contains also, on the average, significantly reduced concentrations of methionine and cystine. In vitro experiments revealed that the [3H]thymidine incorporation by mitogenically stimulated murine lymphocytes and cloned T cells is inhibited by an elevation of the extracellular glutamate concentration and augmented by the addition of cysteine. This suggests the possibility that the abnormal concentrations of glutamate and cystine in the blood of HIV-infected persons may contribute to the defect in the lymphoid system.

135. **Roederer, M., S. W. Ela, F. J. T. Staal, L. A. Herzenberg and L. A. Herzenberg (1992). "N-Acetylcysteine: a new approach to anti-HIV therapy." AIDS Res. Human Retrov. 8(2): 209-217.**

Several investigators have implicated depletion of glutathione (GSH) and production of reactive oxygen intermediates (ROIs) in the regulation of the human immunodeficiency virus (HIV). We have shown directly that N-acetylcysteine (NAC) blocks HIV expression in chronic and acute infection models, and HIV replication in normal peripheral blood mononuclear cells. NAC is a cysteine prodrug which maintains intracellular thiol levels during oxidative stress and replenishes depleted GSH. The observed antiviral effect of NAC is due to inhibition of viral stimulation by ROIs, which are produced in response to inflammatory cytokines. We have also shown that HIV-infected individuals have decreased intracellular GSH levels in their circulating T cells. Since GSH is the major protection against the production of ROIs, we hypothesize that the observed decrease is due to a chronic oxidative stress induced by continual exposure to elevated levels of inflammatory cytokines. Together, these results provide a rationale for clinical trials testing the efficacy of GSH-replenishing drugs such as NAC in the treatment of AIDS. NAC is different than many other antiviral drugs in that it inhibits host-mediated stimulation of viral replication arising in normal immune responses, and may thereby extend latency. In addition, it inhibits the action of inflammatory cytokines which may mediate cachexia, thereby raising the possibility that it may alleviate the deleterious wasting that accompanies late stage AIDS.

136. **Roederer, M., F. J. T. Staal, S. W. Ela, L. A. Herzenberg and L. A. Herzenberg (1993). "N-Acetylcysteine: potential for AIDS therapy." Pharmacology 46(3): 121-129.**

The observations that people infected with HIV suffer not only from an inflammatory stress but also from depleted glutathione levels have led to a general hypothesis that these two are causally related, and that treatment of AIDS should include thiol-replenishment therapy. In particular, inflammatory stimulations are dependent on intracellular thiol levels, as they are potentiated at low glutathione levels (oxidative stress) and inhibited at high glutathione levels. Inflammatory stress may itself lead to decreased levels of glutathione. HIV has taken advantage of inflammatory signals to regulate its own replication; thus, the HIV infection is exacerbated by low levels of glutathione. We have shown that N-acetylcysteine can inhibit inflammatory stimulations, including that of HIV replication. Since N-acetylcysteine can replenish depleted glutathione levels in vivo, we suggest that it be used as an adjunct in the treatment of AIDS.

137. **Roederer, M., F. J. Staal, M. Anderson, R. Rabin, P. A. Raju and L. A. Herzenberg (1993). "Disregulation of leukocyte glutathione in AIDS." Ann N Y Acad Sci 677: 113-25.**

138. **Staal, F. J. T., M. Roederer, D. M. Israelski, J. Bubp, L. A. Mole, D. McShane, S. C. Deresinski, W. Ross, H. Sussman, P. A. Raju, M. T. Anderson, W. Moore, S. W. Ela, L. A. Herzenberg and L. A. Herzenberg (1992). "Intracellular glutathione levels in T cell subsets decrease in HIV infected individuals." AIDS Res. Human Retrov. 8(2): 305-314.**

The authors have shown previously that intracellular glutathione (GSH) plays an important role in the regulation of human immunodeficiency virus (HIV) transcription and replication in vitro, through modulation of signal transduction by inflammatory cytokines. Moreover, intracellular GSH levels are known to regulate T lymphocyte function. In

multiparameter FACS studies presented here, we show that relative GSH levels in CD4+ and CD8+ T cells from HIV+ individuals are significantly lower than in corresponding subsets from uninfected controls. These studies define the relative intracellular glutathione (GSH) levels in CD4+ T cells, CD8+ T cells, B cells, and monocytes from 134 HIV infected individuals and 31 uninfected controls. The greatest decreases in intracellular GSH occur in subsets of T cells in individuals in the later stages of the HIV infection. In AIDS patients, GSH levels are 63 % of normal in CD4+ T cells ($p < 0.0001$) and are 62 % of normal in CD8+ T cells ($p < 0.0001$). Similarly, in AIDS-related complex (ARC) patients, GSH levels are 66% of normal in CD4+ T cells ($p < 0.003$) and are 69% of normal in CD8+ T cells ($p < 0.003$). These findings suggest that low intracellular GSH levels may be an important factor in HIV infection and in the resulting immunodeficiency.

139. Staal, F. J. T., S. W. Ela, M. Roederer, M. T. Anderson, L. A. Herzenberg and L. A. Herzenberg (1992). "Glutathione deficiency and human immunodeficiency virus infection. {Comment, Letter }." <u>Lancet</u> 339(April 11): 909-912.

140. Droge, W. (1993). "Cysteine and glutathione deficiency in AIDS patients - a rationale for the treatment with N-Acetyl-Cysteine." <u>Pharmacology</u> 46(2): 61-65.

A series of clinical studies and laboratory investigations suggests that the acquired immunodeficiency syndrome (AIDS) may be the consequence of a virus-induced cysteine deficiency. HIV-infected persons at all stages of the disease were found to have decreased plasma cystine and cysteine concentrations and decreased intracellular glutathione levels. In rhesus macaques, cysteine levels decrease already within 1-2 weeks after infection with the closely related virus SIV(mac). HIV-infected persons and SIV-infected rhesus macaques have also, on the average, substantially increased plasma glutamate levels. Increased glutamate levels aggravate the cysteine deficiency by inhibiting the membrane transport of cystine. Even moderately elevated extracellular glutamate levels as they occur in HIV-infected persons cause a substantial decrease of intracellular cysteine levels. Clinical studies revealed that individual cystine and glutamate levels are correlated with the individual lymphocyte reactivity and T4+ cell counts but not T8+ cell counts. This phenomenon was demonstrated not only in HIV-infected persons but also in healthy human individuals. The cellular cysteine supply affects amongst others the intracellular glutathione level and IL-2-dependent proliferation of T cells and (inversely) also the activation of the transcription factor NF-kappaB. The cysteine deficiency of HIV-infected persons is, therefore, possibly responsible not only for the cellular dysfunction but also for the overexpression of tumor necrosis factor-alpha (TNF-alpha), interleukin-2 receptor alpha-chain, and beta2-microglobulin. All the corresponding genes are associated with kappaB-like enhancer sequences. We have suggested that N-acetyl-cysteine may be considered for the replenishment of cysteine and glutathione levels in HIV-infected patients, since N-acetyl-cysteine is a well-established drug in clinical medicine with well-documented pharmacokinetics and safety.

141. Holroyd, K. J., R. Buhl, Z. Borok, J. H. Roum, A. D. Bokser, G. J. Grimes, D. Czerski, A. M. Cantin and R. G. Crystal (1993). "Correction of glutathione deficiency in the lower respiratory tract of HIV seropositive individuals by glutathione aerosol treatment." <u>Thorax</u> 48(10): 985-989.

Background-Concentrations of glutathione, a ubiquitous tripeptide with immune enhancing and antioxidant properties, are decreased in the blood and lung epithelial lining fluid of human immunodeficiency virus (HIV) seropositive individuals. Since the lung is the most common site of infection in those who progress to AIDS it is rational to consider whether it is possible to safely augment glutathione levels in the epithelial lining fluid of HIV seropositive individuals, thus potentially improving local host defence. Methods-Purified reduced glutathione was delivered by aerosol to HIV seropositive individuals ($n = 14$) and the glutathione levels in lung epithelial lining fluid were compared before and at one, two, and three hours after aerosol administration. Results-Before treatment total glutathione concentrations in the epithelial lining fluid were approximately 60% of controls. After three days of twice daily doses each of 600 mg reduced glutathione, total glutathione levels in the epithelial lining fluid increased and remained in the normal range for at least three hours after treatment. Strikingly, even though >95% of the glutathione in the aerosol was in its reduced form, the percentage of oxidised glutathione in epithelial lining fluid increased from 5% before treatment to about 40% three hours after treatment, probably reflecting the use of glutathione as an antioxidant in vivo. No adverse effects were observed. Conclusions-It is feasible and safe to use aerosolised reduced glutathione to augment the deficient glutathione levels of the lower respiratory tract of HIV seropositive individuals. It is rational to evaluate further the efficacy of this tripeptide in improving host defence in HIV seropositive individuals.

142. **Jahoor, F., A. Jackson, B. Gazzard, G. Philips, D. Sharpstone, M. E. Frazer and W. Heird (1999). "Erythrocyte glutathione deficiency in symptom-free HIV infection is associated with decreased synthesis rate." Amer. J. Physiol. - Endocrinol. Met. 39(1): E205-E211.**

Although several studies have documented intra- and extracellular glutathione (GSH) deficiency in asymptomatic human immunodeficiency virus (HIV) infection, the mechanisms responsible for the altered GSH homeostasis remain unknown. To determine whether decreased synthesis contributes to this alteration of GSH homeostasis, a primed-constant infusion of[H-2(2)]glycine was used to measure the fractional and absolute rates of synthesis of GSH in five healthy and five symptom-free HIV-infected subjects before and after supplementation for 1 wk with N-acetylcysteine. The erythrocyte GSH concentration of the HIV-infected group was lower ($P < 0.01$) than that of the control group (1.4 +/- 0.16 vs. 2.4 +/- 0.08 mmol/l). The smaller erythrocyte GSH pool of the HIV-infected group was associated with a significantly slower ($P < 0.01$) absolute synthesis rate of GSH (1.15 +/- 0.14 vs. 1.71 +/- 0.15 mmol.l(-1).day(-1)) compared with controls. Cysteine supplementation elicited significant increases in both the absolute rate of synthesis and the concentration of erythrocyte GSH. These results suggest that the GSH deficiency of HIV infection is due in part to a reduced synthesis rate secondary to a shortage in cysteine availability.

143. **Walmsley, S. L., L. M. Winn, M. L. Harrison, J. P. Uetrecht and P. G. Wells (1997). "Oxidative stress and thiol depletion in plasma and peripheral blood lymphocytes from HIV-infected patients: toxicological and pathological implications." AIDS 11(14): 1689-1697.**

Objectives: To determine, first, whether the plasma and lymphocytes of HIV-positive individuals and AIDS patients have alterations in the major thiols glutathione and cysteine, and/or their oxidative disulphide and mixed disulphide products; and, secondly, whether thiol/disulphide status differs in patients with sulphonamide drug hypersensitivity reactions. Design: Thiols provide critical cellular defence against toxic drug reactive intermediates and endogenous oxidative stress, and may modulate HIV replication. Glutathione is reported to be low in HIV-positive individuals and AIDS patients, but this is controversial and the mechanism responsible is unknown. Also unknown is whether altered thiol/disulphide status determines the predisposition of HIV-positive and AIDS patients to drug reactions. Methods: Thiols and disulphides were measured by high-performance liquid chromatography. Results: Both plasma thiols were decreased by approximately 58% in HIV-positive individuals and AIDS patients compared with uninfected controls ($P < 0.05$), with increases of up to threefold in oxidized products ($P < 0.05$). Similarly, in lymphocytes, thiols were decreased by 30-35% ($P < 0.05$), with apparent increases in oxidized products. For both glutathione and cysteine, the thiol/disulphide ratios also were decreased ($P < 0.05$). The plasma and lymphocyte glutathione thiol/disulphide ratios were highly correlated ($r = 0.7661$; $P = 0.0001$) among all subjects. No parameters differed in patients with drug reactions, or with antiretroviral therapy. Conclusions: The enhanced thiol oxidation in HIV-positive individuals and AIDS patients indicates oxidative stress, which also contributes to thiol depletion, and may enhance damage to macromolecular targets. These mechanisms may contribute to enhanced viral replication and other pathological outcomes. HIV-positive individuals' and AIDS patients' predisposition to drug hypersensitivity reactions appears to be unrelated to thiol/disulphide status.

144. **Pacht, E. R., P. Diaz, T. Clanton, J. Hart and J. E. Gadek (1997). "Alveolar fluid glutathione decreases in asymptomatic HIV-seropositive subjects over time." Chest 112(3): 785-788.**

Background: Initial investigations demonstrated a deficiency of glutathione (GSH) in the epithelial Lining fluid (ELF) of HIV-seropositive patients, In a recent study, our laboratory was unable to document such a deficiency. The current study was performed in an attempt to reconcile those disparate findings. Study objectives: To determine if ELF GSH decreases over time in asymptomatic HIV-seropositive subjects. Design: Prospective, longitudinal study. Setting: Major university medical center. Patients or participants: Thirty-three asymptomatic HIV-seropositive volunteers. Interventions: None. Measurements and results: BAL was performed on 33 asymptomatic HIV-seropositive subjects at baseline, 6 months later, and 12 months later. The volume of ELF and the concentration of GSH and oxidized GSH were determined. The concentration of total GSH in ELF was 689.0 +/- 100.4 mu M. This significantly decreased when measured 6 and 12 months later (355.9 +/- 41.7 mu M, and 397.9 +/- 52.7 mu M, respectively, $p=0.01$, compared with baseline, both comparisons). Significant decreases were also noted in the HIV-seropositive subjects who smoked cigarettes (baseline - 762.6 +/- 142.4 mu M; 6 months - 373.7 +/- 45.9 mu M; 12 months - 459.3 +/- 73.8 mu M, $p<0.03$, for baseline vs 6 months, and baseline vs 12 months). In nonsmoking HIV-seropositive subjects, there was a decrease in ELF GSH over time, but it did not reach statistical significance (baseline - 589.1 +/- 138.2 mu M; 6 months - 335.3 +/- 74.1 mu M; 12 months - 345.8 +/- 74.0 mu M, $p>0.1$, all comparisons), The percentage of total GSH in the oxidized form was similar at all three time points (baseline - 3.8 +/- 0.5%; 6 months - 3.1 +/- 0.5%; 12 months - 3.9 +/- 0.9%, $p>0.1$, all comparisons).

Conclusions: The current study demonstrates that the GSH level in ELF is significantly decreased in HIV-seropositive subjects 6 and 12 months after the initial determination.

145. Repetto, M., C. Reides, M. L. G. Carretero, M. Costa, G. Griemberg and S. Llesuy (1996). "Oxidative stress in blood of HIV infected patients." Clin. Chim. Acta 255(2): 107-117.

The oxidative stress in human erythrocytes was studied in asymptomatic and symptomatic patients infected by the human immunodeficiency virus (HIV), and patients with the acquired immunodeficiency syndrome (AIDS). tert-Butyl hydroperoxide-initiated chemiluminescence, superoxide dismutase and catalase activities, and total glutathione were evaluated in the erythrocytes and the total antioxidant capacity in the plasma of control, patients infected with HIV that have not yet developed acquired immunodeficiency syndrome, and patients in the later stage of AIDS. tert-Butyl hydroperoxide initiated chemiluminescence was increased by 33% in asymptomatic (stage A1) and symptomatic patients (stage B2) infected with HIV and 82% for patients with AIDS (stage B3) ($P < 0.05$). While catalase activity did not show any difference between patients and controls, other indices showed differences that, in some cases, reached statistical significance. Superoxide dismutase activity was increased by 24% in stages A1 and B2 of HIV infection and 65% in patients in stage B3 ($P < 0.05$). Glutathione was decreased by 20% in stages A1 and B2, and by 32% in stage B3 patients ($P < 0.05$). Total plasma antioxidant capacity was increased in 30 and 57% for the asymptomatic and AIDS patients groups, respectively ($P < 0.05$). The data indicate that erythrocyte's oxidative stress is associated with the progressive development of HIV disease. Parameters indicating oxidative stress could be an interesting form to screen the evolution of these patients and their response to anti-oxidant therapies.

146. Skurnick, J. H., J. D. Bogden, H. Baker, F. W. Kemp, A. Sheffet, G. Quattrone and D. B. Louria (1996). "Micronutrient profiles in HIV-1-infected heterosexual adults." J. Acq. Immun. Defic. Synd. Hum. R. 12(1): 75-83.

Then is compelling evidence that micronutrients can profoundly affect immunity. We surveyed vitamin supplement use and circulating concentrations of 22 nutrients and glutathione in 64 HIV-I seropositive men and women and 33 seronegative controls participating in a study of heterosexual HIV-I transmission. We assayed antioxidants (vitamins A, C, and E; total carotenes), vitamins B-6 and B-12, folate, thiamin, niacin, biotin, riboflavin, pantothenic acid, free and total choline and carnitine, biopterin. inositol, copper, zinc, selenium, and magnesium. HIV-infected patients had lower mean circulating concentrations of magnesium ($p < 0.0001$), total carotenes ($p = 0.009$), total choline ($p = 0.002$), and glutathione ($p = 0.045$), and higher concentrations of niacin ($p < 0.0001$) than controls. Fifty-nine percent of HIV + patients had low concentrations of magnesium, compared with 9% of controls ($p < 0.0001$). These abnormal concentrations were unrelated to stage of disease. Participants who took vitamin supplements had consistently fewer low concentrations of antioxidants, across HIV infection status and disease stage strata ($p = 0.0006$). Nevertheless, 29% of the HIV + patients taking supplemental vitamins had subnormal levels of one or more antioxidants. The frequent occurrence of abnormal micronutrient nutriture, as found in these HIV + subjects, may contribute to disease pathogenesis. The low magnesium concentrations may be particularly relevant to HIV-related symptoms of fatigue, lethargy, and impaired mentation.

147. Smith, C. V., L. K. Rogers, R. L. Rabin, Y. A. Maldonado, L. A. Herzenberg, L. A. Herzenberg and A. Petru (1994). "Effects of human immunodeficiency virus (HIV) infection on plasma glutathione status in children." Pediatr. Res. 35: 196A (Abstract #1163).

148. Helbling, B., J. Von Overbeck and B. H. Lauterburg (1996). "Decreased release of glutathione into the systemic circulation of patients with HIV infection." Eur. J. Clin. Invest. 26(1): 38-44.

Low glutathione (GSH) in patients with HIV infection could contribute to their immune deficiency since GSH plays an important role in the function of lymphocytes and sulphydryls decrease the expression of HIV in vitro. In order to gain more insight into the mechanisms responsible for the deranged sulphydryl homeostasis in HIV infection, the release of GSH into the circulation, an estimate of the systemic production of GSH, was determined using a pharmacokinetic approach. The basal plasma concentrations of free GSH (3.3 +/- 1.3 vs. 5.3 +/- 1.9 mu mol L(-1)) and cysteine (7.7 +/- 2.6 vs. 13.4 +/- 4.9 mu mol L(-1)) were significantly lower in eight HIV-infected patients than in eight controls. Upon infusion of GSH at a constant rate of 1 mu mol min(-1) kg(-1), GSH in plasma reached a new plateau. The increment in plasma GSH was significantly larger in the HIV-infected patients than in the controls. The input of GSH into the circulation (12.9 +/- 5.7 vs. 30.1 +/- 11.7 mu mol min(-1); P < 0.01) and the clearance of GSH (25 +/- 7 vs. 35 +/- 7 mL min(-1) kg(-1)) were significantly lower in patients with HIV-infection. During infusion of GSH the concentration of cysteine in peripheral blood mononuclear cells of the HIV-infected patients increased significantly. Nevertheless, intracellular GSH did not increase. Thus. the consumption of GSH is not increased in HIV infection. Rather, the present data suggest that GSH in patients with HIV infection is low because of a decreased systemic synthesis of GSH.

149. **Buhl, R., K. J. Holroyd, A. Mastrangeli, A. M. Cantin, H. A. Jaffe, F. B. Wells, C. Saltini and R. G. Crystal (1989). "Systemic glutathione deficiency in symptom-free HIV-seropositive individuals." Lancet ii(Dec 2): 1294-1298.**

150. **Staal, F. J. T., M. Roederer, D. M. Israelski, J. Bubp, L. A. Mole, D. McShane, S. C. Deresinski, W. Ross, H. Sussman, P. A. Raju, M. T. Anderson, W. Moore, S. W. Ela, L. A. Herzenberg and L. A. Herzenberg (1992). "Intracellular glutathione levels in T cell subsets decrease in HIV-infected individuals." AIDS Res. Human Retrov. 8(2): 305-311.**

The authors have shown previously that intracellular glutathione (GSH) plays an important role in the regulation of human immunodeficiency virus (HIV) transcription and replication in vitro, through modulation of signal transduction by inflammatory cytokines. Moreover, intracellular GSH levels are known to regulate T lymphocyte function. In multiparameter FACS studies presented here, we show that relative GSH levels in CD4+ and CD8+ T cells from HIV+ individuals are significantly lower than in corresponding subsets from uninfected controls. These studies define the relative intracellular glutathione (GSH) levels in CD4+ T cells, CD8+ T cells, B cells, and monocytes from 134 HIV infected individuals and 31 uninfected controls. The greatest decreases in intracellular GSH occur in subsets of T cells in individuals in the later stages of the HIV infection. In AIDS patients, GSH levels are 63 % of normal in CD4+ T cells (p < 0.0001) and are 62 % of normal in CD8+ T cells (p < 0.0001). Similarly, in AIDS-related complex (ARC) patients, GSH levels are 66% of normal in CD4+ T cells (p < 0.003) and are 69% of normal in CD8+ T cells (p < 0.003). These findings suggest that low intracellular GSH levels may be an important factor in HIV infection and in the resulting immunodeficiency.

151. **Eylar, E. H., I. Baez, A. Vazquez and Y. Yamamura (1995). "N-acetylcysteine (NAC) enhances interleukin-2 but suppresses interleukin-4 secretion from normal and HIV+ CD4+ T-cells." Cell. Mol. Biol. 41(Suppl.I): S35-S40.**

We find that purified CD4+ T cells from 30 HIV+ individuals have a suppressed Interleukin-4 (IL-4) production compared to normal controls regardless of activator (anti-CD3 or Con A) or co-activator [phorbol ester (PMA or anti-CD28)], generally by 2-4 fold. In every case, the cells producing IL-4 respond more strongly to anti-CD28 co-activation than to PMA, ie, 1150 pg/ml compared to 2070 pg/ml for controls and 398 pg/ml compared to 1250 pg/ml for HIV+ cells, respectively. In contrast, anti-CD3 with PMA gives a more vigorous IL-2 response than with anti- CD28, ie, 37.3 ng/ml compared to 12.3 ng/ml for controls and 28.5 ng/ml versus 15.1 ng/ml for HIV+ cells, respectively. These data are not compatible with the TH1/TH2 switch hypothesis since IL-4 production is decreased, not increased for CD4+ HIV+ T-cells and while IL-2 production is decreased with PMA, it is not decreased significantly with anti-CD28. Interestingly, 5 mM N-acetylcysteine (NAC) acts as an immunoenhancer; mitogenesis was enhanced 2 fold or more in general for control and HIV+ CD4+ T-cells and IL-2 production was enhanced 2-3 fold for anti-CD3 (with PMA or anti-CD28) for both controls and HIV+ CD4+ cells. However, NAC suppressed IL-4 production induced by anti-CD3 and anti-CD28 in both control and HIV+ CD4+ T cells. In the other cases, it produced in general no significant change.(ABSTRACT TRUNCATED AT 250 WORDS)

152. **Cayota, A., F. Vuillier, G. Gonzalez and G. Dighiero (1996). "In vitro antioxidant treatment recovers proliferative responses of anergic CD4+ lymphocytes from human immunodeficiency virus-infected individuals." Blood 87(11): 4746-53.**

Oxidative stress has been proposed to be involved in the immunologic defeat observed in effector calls of the immune system as well as in lymphocyte cell death and viral replication in human immunodeficiency virus (HIV)-infected patients. Because thiol-containing antioxidants such as N-acetyl-L-cysteine have been shown to have beneficial effects on

CD4+ lymphocyte survival and to inhibit programmed cell death and HIV-1 replication, they may play a role in therapeutic strategies of this disease. In this work we have studied the cellular thiol levels and the affect of in vitro antioxidant treatment of purified CD4+ lymphocytes from HIV-infected patients, and correlated these parameters to proliferative responses and programmed cell death. We show that CD4+ lymphocytes from HIV-infected patients display impaired proliferative responses and a significant decrease in cellular thiol levels, indicating a disturbed redox status. Interestingly, antioxidant treatment succeeded to restore defective proliferative responses to CD3- mediated activation in 8 of 11 patients (high antioxidant responders). In contrast to high responders, patients failing to respond to antioxidant treatment (low antioxidant responders), were characterized by an abnormal ratio of apoptotic cells, which was not affected by N- acetyl-L-cysteine and/or 2-beta-mercaptoethanol preincubation. These results demonstrate for the first time that antioxidant treatment is able to revert the impaired proliferative activity of CD4 cells from HIV-infected patients and could help designing therapeutic strategies with antioxidant drugs. However, this action is not observed in cells undergoing programmed cell death.

153. **Cayota, A., F. Vuillier, G. Gonzalez and G. Dighiero (1996). "CD4+ lymphocytes from HIV-infected patients display impaired CD45- associated tyrosine phosphatase activity which is enhanced by anti- oxidants." <u>Clin Exp Immunol</u> 104(1): 11-7.**

It has been proposed that signal transduction defects may, at least partially, account for the functional impairment of CD4+ lymphocytes during HIV-1 infection. Recently, we have demonstrated that unresponsive CD4+ lymphocytes from these patients had reduced protein tyrosine phosphorylation after CD3 engagement, and that this defect was associated with constitutively altered levels of p56lck and p59fyn kinases. Since CD45 is essential for T cell receptor (TCR) and CD2- mediated activation of protein tyrosine kinases, we study here CD45- associated tyrosine phosphatase activity in resting and activated CD4 T cells from HIV-infected patients. We found a significant decrease in the basal and post-activation phosphatase activity of CD45 which correlated well with impairment of proliferative responses. In addition, decreased levels of cellular thiols observed in resting CD4+ lymphocytes from these patients suggested a disturbed redox status. Although expression levels of CD45 were decreased in most patients, a significant recovery of phosphatase activity and proliferative responses was observed in most patients by preincubating cells with N- acetyl-L-cysteine and beta2-mercaptoethanol. In some patients, anti- oxidant treatment failed to significantly enhance phosphatase activity and proliferative responses. The low responses of purified CD4+ lymphocytes from these patients were associated with a high ratio of apoptotic cell death which did not appear to be influenced by anti- oxidant treatment.

154. **Herzenberg, L. A., W. A. Moore and S. C. De Rosa (1999). "Estimation of missing values [letter]." <u>Lancet</u> 354(9179): 686.**

155. **Barbaro, G., G. Di Lorenzo, M. Soldini, G. Bellomo, G. Belloni, B. Grisorio and G. Barbarini (1997). "Vagal system impairment in human immunodeficiency virus-positive patients with chronic hepatitis C: Does hepatic glutathione deficiency have a pathogenetic role?" <u>Scand. J. Gastroenterol.</u> 32(12): 1261-1266.**

Background: Both an autonomic impairment and a systemic depletion of reduced glutathione (GSH) may be documented in patients with chronic liver diseases and in human immunodeficiency virus (HIV)-positive patients. Methods: The coefficients of electrocardiographic R-R interval variation (CVc) were assessed in 125 patients with chronic hepatitis C (CHC) (65 HIV-positive and 60 HIV-negative) and in 61 healthy controls. The CVc values were correlated with hepatic (H-GSH), plasmatic (P-GSH), lymphocyte (L-GSH), and erythrocyte (E-GSH) concentrations of GSH and with erythrocyte malondialdehyde (MDA) levels. Results: Compared with healthy controls, in CHC patients the concentrations of H-GSH, P-GSH, L-GSH, and E-GSH were reduced, whereas MDA levels were increased with a statistically significant difference ($P < 0.001$). CVc was significantly reduced in patients with CHC (especially in those who were HIV-positive) and correlated significantly with the values of H-GSH, P-GSH, L-GSH, E-GSH, and MDA ($P < 0.001$). Conclusions: A dysfunction of the cardiac vagal system may be detected in patients with CHC (especially in those who are HIV-positive); this abnormality may be related to a reduced response to oxidative stress because of a systemic depletion of GSH.

156. **Barbaro, G., G. Di Lorenzo, M. Soldini, S. Parrotto, G. Bellomo, G. Belloni, B. Grisorio and G. Barbarini (1996). "Hepatic glutathione deficiency in chronic hepatitis C: quantitative evaluation in patients who are HIV positive and HIV negative and correlations with**

plasmatic and lymphocytic concentrations and with the activity of the liver disease." Am. J. Gastroenterol. 91(12): 2569-2573.

OBJECTIVES: Reduced glutathione (GSH) is decreased in patients affected by chronic hepatitis C (CHC) as well as in patients who are HIV positive. Because the liver is the most important source of plasmatic GSH, we measured the concentrations of GSH in the liver (H-GSH) of patients with CHC who were either HIV positive or negative, correlating it to the concentrations of GSH in plasma (P-GSH) and in peripheral blood mononuclear cells (PBMCs) (L-GSH), to the replication activity of hepatitis C virus (HCV) in PBMCs, to the activity of the liver disease, and to the state of immunodeficiency in patients who were HIV positive.

METHODS: One hundred, five patients with serologically and histologically demonstrated CHC (55 HIV positive and 50 HIV negative) entered the trial. Fifty-one healthy individuals made up a control group for P-GSH and L-GSH concentrations. H-GSH concentration was determined by high performance liquid chromatography on liver specimens obtained by ultrasound-guided biopsy according to the method described by Reed et al. The concentrations of P-GSH and L-GSH were determined according to the method described by Suarez et al. The detection of HCV RNA strands in PBMCs was performed according to the method described by Qian et al. Histological findings and degree of fibrosis were scored according to the numerical scoring system proposed by Scheuer and by Knodell et al.

RESULTS: H-GSH, P-GSH, and L-GSH were significantly reduced in patients affected by CHC compared with healthy controls ($p < 0.001$). H-GSH and particularly L-GSH were more significantly reduced in patients who were HIV positive compared with those who were HIV negative ($p < 0.001$), without significant correlation with the values of the T cell subset CD4+. The reductions in H-GSH, P-GSH, and L-GSH were significantly correlated to the replication activity of HCV in PBMCs ($p < 0.001$) and to the grade of activity of the liver disease assessed by the values of ALT ($p < 0.001$) and by histological and fibrosis scores of CHC ($p < 0.001$). In both groups of patients with CHC, H-GSH, P-GSH, and L-GSH were more reduced in patients addicted to drugs than in patients who were not addicted.

CONCLUSIONS: In patients with CHC, particularly those who are HIV positive, a systemic depletion of GSH is present. This depletion may be a factor underlying the resistance to interferon therapy and, in patients who are HIV positive, to antiretroviral drugs, fostering HCV and/or HIV replication. This may represent the biological basis for GSH replacement therapy.

157. **Suarez, M., O. Beloqui, J. V. Ferrer, B. Gil, C. Qian, N. Garcia, P. Civeira and J. Prieto (1993). "Glutathione depletion in chronic hepatitis C." Internatl. Hepatol. Commun. 1: 215-221.**

158. **Verhulst, M.-L., A. H. A. M. Van Oijen, H. M. J. Roelofs, W. H. M. Peters and J. B. M. J. Jansen (2000). "Antral glutathione concentration and glutathione S-transferase activity in patients with and without Helicobacter pylori." Digest. Dis. Sci. 45(3): 629-632.**

Previously we demonstrated an inverse relation between cancer of the gastrointestinal tract and glutathione S-transferase activity of the gastrointestinal mucosa. Chronic infection with H. Pylori has been associated with an increased risk of gastric cancer. The aim of this study was to investigate the levels of glutathione and glutathione S-transferase activity in H. Pylori-infected and noninfected antral mucosa. Glutathione and glutathione S-transferases were measured in antral biopsies of patients with nonulcer dyspepsia without Pi pylori infection (A), with prior H, pylori infection who became H. pylori negative after eradication therapy (B) and with proven ii pylori infection (C), Glutathione concentration and glutathione S-transferase activity in group A were 31.0 (range 6.0-59.6) nmol/mg protein and 810 (range 165-1312) nmol/min/mg protein, in group B 27.0 (range 5.0-53.8) nmol/mg protein and 745 (range 403-1199) nmol/min/mg protein, and in group C 18.5 (range 1.6-55.8) nmol/mg protein and 572 (range 144-1047) nmol/min/mg protein, respectively. The glutathione and glutathione S-transferase values were significantly lower in patients infected with H. Pylori than in patients who were H. Pylori negative.

159. **Knapen, M. F. C. M., T. P. J. Mulder, I. A. L. M. Van Rooij, W. H. M. Peters and E. A. P. Steegers (1998). "Low whole blood glutathione levels in pregnancies complicated by preeclampsia or the hemolysis, elevated liver enzymes, low platelets syndrome." Obstet. Gynecol. 92(6): 1012-1015.**

Objective: To investigate the pathophysiologic involvement of glutathione in pregnancies complicated by preeclampsia or the hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome. Methods: Total whole blood glutathione levels were measured by high performance liquid chromatography in 23 women with pregnancies complicated by preeclampsia or the HELLP syndrome and in 22 normotensive gravidas. Total glutathione levels and the total glutathione/hemoglobin ratios of patients were compared with those of controls by the Mann-Whitney U test. Results: Median total glutathione levels were lower in preeclamptic pregnancies or those complicated by the HELLP syndrome than in normotensive pregnancies (647 [range 268-986] and 750 [range 495-1572] mu mol/L, P = .05). The median total glutathione/hemoglobin ratios were significantly lower in preeclamptic pregnancies or in those complicated by the HELLP syndrome than in normotensive pregnancies (0.079 [range 0.033-0.122] and 0.101 [range 0.073-0.210], P = .02). Conclusion: Decreased total glutathione levels in maternal whole blood might indicate decreased detoxificating or free radical scavenging capacity in pregnancies complicated by preeclampsia or the HELLP syndrome. (Obstet Gynecol 1998;92:1012-15. (C) 1998 by The American College of Obstetricians and Gynecologists.).

160. Smith, C. V., T. N. Hansen, N. E. Martin, H. W. McMicken and S. J. Elliott (1993). "Oxidant stress responses in premature infants during exposure to hyperoxia." Pediatr. Res. 34(3): 360-365.

To assess oxidant stress responses in newborn infants treated with elevated concentrations of oxygen, we measured plasma concentrations of glutathione (GSH) and glutathione disulfide (GSSG) in newborn infants ranging from 23 to 42 wk gestational age. All infants recruited into the study were mechanically ventilated and had catheters placed in their umbilical arteries as part of their normal clinical management. Blood samples were obtained on d 1, 3, and 5 and weekly thereafter or until the catheters were removed. We observed plasma concentrations of GSSG in these infants that were frequently an order of magnitude higher than the 0.1 to 0.3 muM we find in adults. Interestingly, plasma GSSG concentrations were inversely correlated to the inspired oxygen tensions. This effect appeared to arise from the patient selection criteria whereby, of the infants studied, those breathing the lowest partial pressures of oxygen were the smallest and gestationally youngest. A second observation was that plasma concentrations of GSH in the premature infants were substantially, indeed often dramatically, lower than we have observed in adult humans (6 to 10 muM). Finally, we found that in patients with both umbilical arterial and umbilical venous catheters arterial GSSG concentrations were consistently higher than venous concentrations; conversely, arterial GSH concentrations were lower than venous concentrations. The elevated GSSG concentrations we observed in these infants indicate marked oxidant stress responses in prematurely born infants, even in those infants exposed only to room air. The positive arteriovenous gradients of GSSG concentrations across the lungs of these infants suggest that at least some of the increased plasma GSSG originates in the lung. The low plasma GSH concentrations we observed in these same infants suggest deficiencies in an antioxidant that has been shown in numerous animal studies to be critical for prevention of hyperoxia-induced lung injury. Finally, the negative arteriovenous gradients of GSH concentrations across the lung provide the first evidence in humans for pulmonary uptake of GSH.

161. Lang, C. A., S. Naryshkin, D. L. Schnieder, B. J. Mills and R. D. Lindeman (1992). "Low blood glutathione in healthy aging adults." J. Lab. Clin. Med. 120(5): 720-725.

The objective of this investigation was to test the hypothesis that blood glutathione levels are lower in aging human subjects as previously found in blood and tissues of standard rodent models of aging. Thus a study was conducted with 39 men and 130 women, 20 to 94 years old, who were selected by the criteria of being ambulatory, healthy, and free from diabetes mellitus, thyroid disease, anemias, and cancer. The reference group was comprised of the 20- to 39-year-old subjects, whose blood glutathione levels were 547 +/- 53.5 micrograms/10(10) erythrocytes (mean +/- SD) for 40 individuals and defined the reference range (95% confidence limits) of 440 to 654. Based on the 440 micrograms/10(10) erythrocyte cutoff, the incidence of low blood glutathione content in the older subjects increased significantly, particularly in the 60- to 79-year-old group. Their glutathione levels were 452 +/- 86.8 micrograms/10(10) erythrocytes, 17% lower than the reference group ($p < 0.001$). These findings demonstrate an increased incidence of low glutathione levels in apparently healthy elderly subjects, who thus may be at risk because of a decreased capacity to maintain many metabolic and detoxification reactions mediated by glutathione.

162. Julius, M., C. A. Lang, L. Gleiberman, E. Harburg, W. DiFranceisco and A. Schork (1994). "Glutathione and morbidity in a community-based sample of elderly." J. Clin. Epidemiol. 47(9): 1021-1026.

This study examined the association of blood glutathione level, a potential marker of physiological/functional aging, with a number of biomedical/psychological traits in a subgroup (N = 33) of a representative sample of community-based elderly. Higher glutathione levels were associated with fewer number of illnesses ($p < 0.05$), higher levels of self-rated health ($p < 0.01$), lower cholesterol ($p < 0.05$), lower body mass index, and lower blood pressures. Subjects with diagnoses of arthritis, diabetes, or heart disease (as assessed by physicians) had at least marginally significant lower glutathione levels than those who were disease free. Glutathione, together with age and a measure of suppressed anger, accounted for 39% of the variance of an index of morbidity. Glutathione, by itself, accounted for 24% of the variance. To our knowledge, this is the first evidence of an association of higher glutathione levels with higher levels of physical health in a sample of community-based elderly. Further studies in large samples are needed to investigate glutathione as a potential overall health risk factor for morbidity among the elderly.

163. Samiec, P. S., C. Drews-Botsch, E. W. Flagg, J. C. Kurtz, P. Sternberg, R. L. Reed and D. P. Jones (1998). "Glutathione in human plasma: Decline in association with aging, age-related macular degeneration, and diabetes." Free Radical Biol. Med. 24(5): 699-704.

Blood samples were analyzed for GSH and GSH redox state in 40 age-related macular degeneration (ARMD) patients (> 60 y), 33 non-ARMD diabetic patients (> 60 years), 27 similarly aged non-ARMD and nondiabetic individuals (> 60 years), and 19 younger individuals (< 60 years) without ARMD or diabetes. Results showed a significantly lower plasma GSH in older individuals (ARMD, diabetes, and controls) than in younger individuals ($p < .01$). Total GSH (GSHt) obtained following treatment with dithiothreitol was significantly lower only in diabetic cases ($p < .05$) but also approached significance for ARMD cases ($p = .089$). Estimation of redox potential indicated that the plasma GSH pool is considerably more oxidized in all of the older groups. Analyses of whole blood GSH showed that GSH was significantly lower in diabetic cases compared to the other groups, but did not reveal any difference associated with age or ARMD. In contrast, GSSG in whole blood was significantly higher in the older groups compared to the younger controls. The results suggest that in studies of age-related pathologies, oxidation of GSH may be a more important parameter than a decline in pool size, while in specific pathologies such as diabetes, both oxidation and a decline in pool size may be important. (C) 1998 Elsevier Science Inc.

164. Nuttall, S. L., F. Dunne, M. J. Kendall and U. Martin (1999). "Age-independent oxidative stress in elderly patients with non-insulin-dependent diabetes mellitus." QJM - Monthly J. Assoc. Physicians 92(1): 33-38.

Impaired antioxidant defence is implicated in the development of cardiovascular complications in non-insulin-dependent diabetes (NIDDM). However, as many of these patients are elderly, observed changes in antioxidant status may be due to the patient's age rather than their disease. We sampled blood from 47 elderly NIDDM patients (21 male and 26 female; mean age +/- SD 75.62+/-7.97 years), 66 young (30 male and 36 female; 24.52 +/-4.72 years) and 58 healthy elderly volunteers (17 male and 41 female; 70.74+/-4.85 years), and measured the antioxidant glutathione, the marker for free-radical-damage lipid hydroperoxide products (LHP), vitamin E and total antioxidant capacity) (TAC). There was a significant increase in LHP in the healthy elderly group compared with the young volunteers (3.14 +/-1.5 vs. 2.14+/-1.38 mu mol/l, $p<0.01$). The values were much higher in NIDDM patients (7.02 +/- 2.29 mu mol/l, $p<0.0001$ vs. Healthy elderly). There was a reduction in TAC in healthy elderly compared with the young (359.99 +/-154.82 vs. 471.47 +/- 94.29 mu mol/l trolox equivalents, $p< 0.0001$), but there was no further reduction in NIDDM patients. Similarly, glutathione was reduced to the same degree in healthy elderly and NIDDM patients (0.29+/-0.09, 0.30+/-0.11 vs. 0.54 0.19 mu mol/l in young volunteers, $p<0.0001$). Vitamin E concentrations were comparable in all groups (26.34+/-5.39 young volunteers, 31.50 +/-8.23 healthy elderly and 30.98+/-9.03 mu mol/l NIDDM patients), but after correction for serum cholesterol there was a significant reduction in the diabetic group compared with the young, but not with the elderly (5.54 +/-1.55 vs. 6.67 +/-1.86 vs. 6.31 +/-1.85 (mu mol/l)/(mmol/l), $p<0.01$). We have demonstrated an age-dependent reduction in total antioxidant capacity and glutathione defence and an age-independent increase in LHP in elderly patients with NIDDM. Reduced concentrations of vitamin E were demonstrated in NIDDM patients compared with young, but not elderly, volunteers. Increased oxidative damage occurs independently of age in NIDDM patients despite comparable antioxidant defences in this age group.

165. van Lieshout, E. M. and W. H. Peters (1998). "Age and gender dependent levels of glutathione and glutathione S- transferases in human lymphocytes." Carcinogenesis 19(10): 1873-5.

Glutathione S-transferases (GSTs) are a family of enzymes involved in the detoxification of a wide range of chemicals including chemical carcinogens. Human cytosolic GSTs are divided into four major classes; alpha, mu, pi and theta. This study was performed to evaluate the influence of age and gender on the GST isoenzyme expression and glutathione (GSH) content in lymphocytes. Blood was collected from 124 healthy controls, which were divided into age groups of 20-

40 years (21 females, 20 males), 40-60 years (20 females, 21 males) and 60-80 years (20 females, 22 males). Lymphocytes were isolated by density centrifugation on Histopaque-1077. After homogenization, cytosolic fractions were isolated. Herein, GST isoenzyme levels were determined by densitometrical analysis of western blots after immunodetection with monoclonal antibodies. Total GSH content was determined by high performance liquid chromatography after conjugation with monobromobimane. Spearman rank correlation and Wilcoxon rank sum tests were used for statistical evaluation. Lymphocytic GSTmu and pi levels were not correlated with age or gender. GSTalpha was not detectable in lymphocytes. GSH contents were not different in males and females, but decreased with age in both males and females. In age group 60-80, GSH content was significantly lower as compared with age groups 20-40 and 40-60 in both sexes. Since high GSH is an essential factor in the detoxification of many compounds, these data indicate that the detoxification potential of the GSH/GST system in lymphocytes may decrease with age in man.

166. van Bakel, M. M., G. Printzen, B. Wermuth and U. N. Wiesmann (2000). "Antioxidant and thyroid hormone status in selenium-deficient phenylketonuric and hyperphenylalaninemic patients." Am J Clin Nutr 72(4): 976-81.

BACKGROUND: Subjects consuming protein-restricted diets, such as patients with phenylketonuria (PKU) or milder hyperphenylalaninemias (HPAs) are at risk of selenium deficiency. Selenium is a cofactor of the antioxidant enzyme glutathione peroxidase and of the thyroid hormone converting enzyme thyroxine deiodinase. OBJECTIVE: Our goal was to investigate the effects of low plasma selenium on antioxidant and thyroid hormone status. DESIGN: We assessed plasma selenium, plasma total antioxidant status and the individual components thereof, erythrocyte antioxidant status, and plasma thyroid hormones in 24 PKU and 10 HPA patients and in 42 age-matched control subjects. RESULTS: Selenium was significantly lower in both PKU and HPA patients than in control subjects and the PKU patients had lower values than did the HPA patients. Total antioxidant status was lower in both patient groups than in the control group, whereas alpha-tocopherol, albumin, and uric acid were not significantly different among groups. Plasma selenium correlated well ($r = 0.76$) with erythrocyte glutathione peroxidase. PKU patients had lower glutathione peroxidase activity than did HPA patients and control subjects and lower glutathione concentrations than did control subjects. Both patient groups had lower superoxide dismutase activity than did control subjects. Free triiodothyronine was higher in both patient groups than in control subjects, whereas free thyroxine was higher in the PKU patients only. Free thyroxine and reverse triiodothyronine were inversely correlated with selenium. CONCLUSION: Supplementation with selenium seems to be advisable for patients consuming diets low in natural protein.

167. Miners, J. O., R. Drew and D. J. Birkett (1984). "Mechanism of action of paracetamol protective agents in mice in vivo." Biochem. Pharmacol. 33: 2995-3000.

The mechanism of action of cysteine, methionine, N-acetylcysteine (NAC) and cysteamine in protecting against paracetamol (APAP) induced hepatotoxicity in male C3H mice in vivo has been investigated by, characterising the effect of the individual protective agents on the metabolism of an hepatotoxic dose of APAP, and determining the efficacy of the protective agents in animals treated with buthionine sulphoximine (BSO), a specific inhibitor of glutathione (GSH) synthesis. Co-administration of cysteine, methionine or NAC increased, while co-administration of cysteamine decreased, the proportion of GSH- derived conjugates of APAP excreted in the urine of mice administered APAP, 300 mg/kg. Pretreatment of animals with BSO abolished the protective effect of cysteine, methionine and NAC, whereas cysteamine still afforded protection against APAP after BSO treatment. In conjunction with other data, these results suggest the most likely mechanism for the protective effect of cysteine, methionine and NAC is by facilitating GSH synthesis, while the most likely mechanism for the protective effect of cysteamine is inhibition of cytochrome P-450 mediated formation of the reactive metabolite of APAP.

168. Slattery, J. T., J. M. Wilson, T. F. Kalhorn and S. D. Nelson (1987). "Dose-dependent pharmacokinetics of acetaminophen: evidence of glutathione depletion in humans." Clin. Pharmacol. Ther. 41: 413-418.

The time course of excretion of acetaminophen and its metabolites in urine was determined in eight healthy adults (seven men and one woman) who ingested 1 gm of the drug and collected timed urine samples for 24 hours. The mean time of peak excretion rate was 1.3 to 3.7 hours for acetaminophen, its glucuronide, sulfate, cysteine, mercapturate, and methoxy metabolites but 13.5 hours for methylthioacetaminophen. The mean half-life of acetaminophen was 3.1 hours and the mean half-life of the metabolites other than methylthioacetaminophen ranged from 4.1 to 5.7 hours. The half-life of methylthiometabolite could not be determined because of its very late peak time. In a second study the effect of dose on the clearance of acetaminophen was determined in nine healthy adult subjects (eight men and one woman) who received doses of 0.5 and 3 gm acetaminophen on separate occasions, separated by 4 to 10 days. The renal clearance of acetaminophen and the formation clearances of the sulfate, glutathione, and catechol metabolites were lower (by 38%, 41%, 35%, and 46%, respectively) at the higher dose. The renal clearance of acetaminophen sulfate and glucuronide conjugates were not different between doses. In a third study (10 men), 10 gm N- acetylcysteine was found to increase the formation clearance of the sulfate conjugate by 27% and that of the glutathione conjugate by 10%. The data suggest that the hepatic supply of reduced glutathione and 3'- phosphoadenosine 5'-phosphosulfate begins to be depleted over the range of 0.5 to 3 gm acetaminophen and that the depletion is overcome by the administration of N-acetylcysteine.

169. Lauterburg, B. H. and M. E. Velez (1988). "Glutathione deficiency in alcoholics: risk factor for paracetamol hepatotoxicity." Gut 29: 1153-1157.

Patients chronically abusing ethanol are more susceptible to the hepatotoxic effects of paracetamol. This could be due to an increased activation of the drug to a toxic metabolite or to a decreased capacity to detoxify the toxic metabolite by conjugation with glutathione (GSH). To test these hypotheses paracetamol 2 g was administered to five chronic alcoholics without clinical evidence of alcoholic liver disease and five control subjects. The urinary excretion of cysteine- plus N-acetyl-cysteine-paracetamol, the two major products of detoxification of the reactive metabolite of paracetamol, was not significantly higher in chronic alcoholics arguing against a substantially increased metabolic activation of paracetamol. Chronic alcoholics had significantly lower plasma concentrations of GSH than healthy volunteers, however (4.35 (1.89) microM v 8.48 (2.68) microM, p less than 0.05) before the administration of paracetamol, and plasma GSH reached lower concentrations in the alcoholics after paracetamol (2.40 (1.36) v 6.26 (2.96) microM). In a group of patients with alcoholic hepatitis intrahepatic GSH was significantly lower than in patients with chronic persistent hepatitis and patients with non-alcoholic cirrhosis, suggesting that low plasma GSH in alcoholics reflects low hepatic concentrations of GSH. The data indicate that low GSH may be a risk factor for paracetamol hepatotoxicity in alcoholics because a lower dose of paracetamol will be necessary to deplete GSH below the critical threshold concentration where hepatocellular necrosis starts to occur.

170. Moss, M., D. M. Guidot, M. Wong-Lambertina, T. Ten Hoor, R. L. Perez and L. A. S. Brown (2000). "The effects of chronic alcohol abuse on pulmonary glutathione homeostasis." Amer. J. Respir. Crit. Care Med. 161(2): 414-419.

The incidence and severity of the acute respiratory distress syndrome (ARDS) is increased in critically ill patients with a prior history of chronic alcohol abuse; however, the specific mechanisms responsible for this association are unknown. Recently, we determined that chronic ethanol ingestion in rats decreased the alveolar epithelial lining fluid (ELF) concentration of the antioxidant glutathione (GSH), which is a characteristic finding in patients with ARDS, However, the effects of chronic alcohol abuse on the human alveolar epithelium are essentially unknown. Therefore, as a first step we asked if chronic alcohol abuse, independent of other comorbid conditions, decreases the concentration of GSH in the human lung. We determined that otherwise healthy chronic alcoholics had significantly decreased ELF concentrations of GSH compared with nonalcoholic control subjects (79 mu mol [48 to 118 mu mol] versus 576 mu mol [493 to 728 mmol], $p < 0.001$). Furthermore, the percentage of GSH in the oxidized form was higher in the chronic alcoholics (9.8% [2.2 to 14.8%] versus 2.8% [0.4 to 4.0%] $p = 0.05$), indicative of increased utilization of GSH. This is the first report that chronic alcohol abuse alters GSH homeostasis in the human lung, and suggests a potential mechanism by which chronic alcohol abuse predisposes susceptible patients to develop ARDS.

171. Grattagliano, I., G. Vendemiale, C. Sabba, P. Buonamico and E. Altomare (1996). "Oxidation of circulating proteins in alcoholics: role of acetaldehyde and xanthine oxidase." J. Hepatol. 25(1): 28-36.

Background/Aims: This study aimed to evaluate the protein and lipid redox status in plasma, erythrocytes and erythrocyte ghosts of alcoholics and of patients with non-alcoholic liver disease; we also investigated the relation to glutathione levels and the role of acetaldehyde and xanthine oxidase activity in plasma. Methods: Carbonyl and sulfhydryl proteins, glutathione and malondialdehyde levels and the activity of the circulating xanthine oxidase were determined in: active and abstinent alcoholics, patients with chronic viral hepatitis and healthy controls. Results: Active alcoholics showed a decrease of sulfhydryl protein and glutathione concentrations in plasma, erythrocytes and ghosts compared to the other groups. Also, an increase of the carbonyl protein and malondialdehyde levels and of the activity of circulating xanthine oxidase (9.2+/-1.8 nmol min ml, $p<0.001$) were observed. Significant correlations between carbonyl protein and malondialdehyde concentrations in plasma ($r=0.775$, $p<0.001$), as well as between daily alcohol intake and carbonyl protein content in plasma ($r=0.879$, $p<0.001$) and erythrocytes ($r=0.605$, $p<0.01$) were observed. However, carbonyl protein levels did not correlate with the degree of liver injury. Incubation of plasma with acetaldehyde, but not with ethanol, significantly increased the carbonyl protein formation. Administration of N-Ethylmaleimide, a thiol depletor, or glutathione significantly increased or delayed, respectively, the carbonyl protein formation. Conclusions: Proteins are oxidatively modified in plasma and erythrocytes of active alcoholics, whereas no such alterations are detectable in patients with non-alcoholic liver disease. Protein oxidation in alcoholics does not seem to result directly from ethanol; circulating xanthine oxidase, delivered from injured cells, may play a contributory role and glutathione appears to be directly involved in the protection of plasma proteins against acetaldehyde toxicity.

172. Banerjee, B. D., V. Seth, A. Bhattacharya, S. T. Pasha and A. K. Chakraborty (1999). "Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers." Toxicol. Lett. 107(1-3): 33-47.

Oxidative stress was studied in blood samples obtained from lindane, malathion and propoxur poisoning cases admitted to the Guru Teg Bahadur Hospital, Delhi and evaluated for lipid peroxidation, oxygen free radical (OFR) scavenging enzymes, and glutathione (GSH) and related enzymes. Acetylcholine esterase (AChE), gamma glutamyl transpeptidase (GGT) and GSH level were also assayed in lymphocytes. The level of thiobarbituric acid reacting substances and activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and GGT were increased and GSH level was decreased in pesticide poisoning. Apparently lindane (at the concentration examined) was more potent than malathion and propoxur in producing alteration in lipid peroxidation, GSH related parameters and OFR scavenging enzymes. However, AChE activity and GSH level in lymphocytes of malathion poisoning cases were reduced and GGT activity was enhanced in comparison to control subjects. The present results suggest that OFR scavenging enzymes were induced while combating oxidative stress in a differential manner in organochlorine, organophosphate and carbamate poisoning. Increased lipid peroxidation, coupled with altered levels of GSH and OFR scavenging enzymes in the blood are discussed in the light of oxidative stress.

173. Jones, A. L. (1998). "Mechanism of action and value of N-acetylcysteine in the treatment of early and late acetaminophen poisoning: A critical review." J. Toxicol. - Clin. Toxicol. 36(4): 277-285.

Introduction: The mechanism of action of N-acetylcysteine in early acetaminophen poisoning is well understood, but much remains to be learned of the mechanism of its possible benefit in acetaminophen poisoning presenting beyond 15 hours. Methods: Selective review of medical literature. N-acetylcysteine should be used in all cases of early acetaminophen poisoning where the plasma acetaminophen concentration lies "above the line;" which line is chosen depends on individual preference and whether enzyme induction is suspected. Particular care should be taken with the use of the nomogram for patients with chronic excess ingestion of acetaminophen or for those who have taken slow-release formulations. Conclusions: While there is a trend suggesting a beneficial effect of N-acetylcysteine in some patients presenting beyond 15 hours, further research is necessary to establish just how effective N-acetylcysteine is, particularly in patients presenting with fulminant hepatic failure. Candidate mechanisms for a beneficial effect include improvement of liver blood flow, glutathione replenishment, modification of cytokine production, and free radical or oxygen scavenging. Hemodynamic and oxygen delivery and utilization parameters must be monitored carefully during delayed N-acetylcysteine treatment of patients with fulminant hepatic failure, as unwanted vasodilation may be deleterious to the maintenance of mean arterial blood pressure.

174. Pela, R., A. M. Calcagni, S. Subiaco, P. Isidori, A. Tubaldi and C. M. Sanguinetti (1999). "N-acetylcysteine reduces the exacerbation rate in patients with moderate to severe COPD." Respiration 66(6): 495-500.

Objective: This study was performed to confirm the efficacy of a 6-month therapy with a formulation of N-acetylcysteine (NAC; 600 mg/day p.o.) on frequency and severity of exacerbations in patients suffering from chronic obstructive pulmonary disease (COPD).

Methods: One hundred sixty-nine patients attending five Italian centres were recruited in an open, randomized, controlled study. The patients were randomly allocated to standard therapy plus NAC 600 mg once a day or standard therapy alone over a 6-month period. At baseline, medical history was evaluated, and physical examination was performed; occurrence and severity of exacerbations and side effects of NAC were analyzed after 3 and 6 months.

Results: The results showed a decreased number of exacerbations (by 41%) in the group of patients treated with NAC and standard treatment: 46 patients had at least one exacerbation as compared with 63 patients of the group treated with standard therapy alone. Also the number of the patients with two or more exacerbations was lower in the NAC group (26%) than in the standard-therapy group (49%). The number of sick days was less (82) in the NAC group as compared with the standard-therapy group (155). There was a small but significant improvement in FEV1 and MEF50 in the NAC group. NAC once a day was well tolerated. There were no differences in the number of side effects reported in both groups.

Conclusions: These data confirm results of previous studies which reported a reduction in the number of exacerbations in patients having moderate to severe COPD treated with the antioxidant NAC. Further, the once-daily formulation is well tolerated and is likely to improve patient compliance with the prescribed regimen. Copyright (C) 1999 S. Karger AG, Basel.

175. Riise, G. C., S. Larsson, P. Larsson, S. Jeansson and B. A. Andersson (1994). "The intrabronchial microbial flora in chronic bronchitis patients: a target for N-acetylcysteine therapy?" Eur. Respir. J. 7(1): 94-101.

Chronic bronchitis is common among smokers, often together with recurrent infectious exacerbations. Streptococcus pneumoniae and Haemophilus influenzae are the pathogens traditionally considered most important. N-acetylcysteine (NAC) treatment has been shown to reduce the number of infectious exacerbations in patients with chronic bronchitis. The mechanism behind this is unknown. We attempted to characterize the intrabronchial bacterial flora in patients with chronic bronchitis in an infection-free interval, and to determine whether pharmacological and immunological factors effected the bacterial occurrence. Twenty two smokers with non-obstructive chronic bronchitis, 19 smokers with chronic bronchitis and chronic obstructive pulmonary disease (COPD) and 14 healthy nonsmokers underwent bronchoscopy. To obtain uncontaminated intrabronchial samples, a protected specimen brush was used. Quantitative bacterial cultures and virus isolations were performed. Significantly positive bacterial cultures (> 1,000 colony-forming units (cfu).ml-1) were found only in the patients. S. pneumoniae and H. influenzae were found in five patients, and only in the patients without NAC treatment. The most common bacterium was alpha-haemolytic streptococcus. Negative cultures were more common in the healthy controls. Of the various factors examined, only NAC medication had an influence on bacterial numbers. Significantly fewer patients with NAC medication had positive cultures (3 out of 16) than in the group of patients without NAC therapy (15 out of 21). Our results confirm that chronic bronchitis in smokers leads to increased intrabronchial bacterial colonization. We could also confirm that 1,000 cfu.ml-1 is an adequate cut-off level for significant bacterial growth when using the protected specimen brush. NAC medication was associated with low bacterial numbers.

176. Zimmerman, R. J., B. J. Marafino Jr., A. Chan, P. Landre and J. L. Winkelhake (1989). "The role of oxidant injury in tumor cell sensitivity to recombinant human tumor necrosis factor in vivo. Implications for mechanism of action." J. Immunol. 142: 1405-1409.

The intracellular glutathione levels of two human tumor lines and seven murine tumor lines were determined in order to investigate the role of oxidant injury in tumor cell sensitivity to human rTNF (rhTNF). Correlations were found between high intracellular glutathione levels and in vivo tumor resistance to rhTNF, and on the other hand, low glutathione levels and rhTNF sensitivity. The transplantable murine fibrosarcoma, Meth A, a TNF-sensitive line in vivo, was less sensitive to rhTNF and host toxicity was reduced when the hosts were pretreated with uric acid, a major reactive oxygen scavenger in humans and certain other primates. Conversely, pretreatment of the tumor-bearing hosts with DL-buthionine-(S,R)-sulfoximine, an inhibitor of GSH biosynthesis, resulted in an increased sensitivity of Meth A to rhTNF. This effect was not limited to tumor-bearing mice, as rats pretreated with diethyl maleate, a compound which irreversibly binds glutathione, were more sensitive to rhTNF toxicity than control rats. On the other hand, pretreatment with N-acetyl cysteine, an oxidant scavenger, reduced the toxicity of rhTNF treatment in rats. The data are consistent with the hypothesis that tumor cell sensitivity to rhTNF in vivo is dependent on its capacity to buffer oxidative attack. In addition, host toxicity is also related to the production of reactive oxygen species. Activated effector cells such as granulocytes and macrophages are hypothesized to produce most of this damage by their respiratory burst and oxidant release, although the direct action of rhTNF may also contribute to oxidative injury in vivo.

177. Altomare, E., P. Colonna, C. Dagostino, G. Castellaneta, G. Vendemiale, I. Grattagliano, F. Cirelli, F. Bovenzi and L. Colonna (1996). "High-dose antioxidant therapy during thrombolysis in patients with acute myocardial infarction." Curr. Ther. Res. 57(2): 131-141.

This study investigated the effects of 24-hour intravenous infusion of glutathione (GSH) on plasma malondialdehyde (MDA) levels and on clinical recovery after myocardial infarction in 67 patients treated with recombinant tissue-type plasminogen activator (rt-PA) and 29 patients not given rt-PA, Baseline MDA levels were also measured in 20 healthy control subjects matched for sex, age, and smoking habits, Administration of rt-PA resulted in an earlier recovery of ST elevation. A higher number of patients with fast ST recovery, a lower creatine phosphokinase (CPK) maximum peak, earlier CPK peak, and a lower incidence of arrhythmic episodes were among those who received GSH in addition to rt-PA, Patients who did not receive rt-PA showed a rather steady trend of plasma MDA levels, Patients receiving thrombolytic therapy showed increased MDA concentrations beginning 6 hours after starting therapy, However, while the patients treated only with rt-PA showed a continuous increase in MDA levels, those patients who also received GSH had a significant decrease in MDA levels, Plasma MDA levels significantly increased after thrombolysis; administration of GSH appeared to limit some adverse effects associated with reperfusion-induced oxidative stress.

178. Smilkstein, M. J., A. C. Bronstein, C. Linden, W. L. Augenstein, K. W. Kulig and B. H. Rumack (1991). "Acetaminophen overdose: a 48-hour intravenous N-acetylcysteine treatment protocol." Ann Emerg Med 20(10): 1058-63.

STUDY OBJECTIVE: To determine the safety and efficacy of a 48-hour IV N- acetylcysteine (IV NAC) treatment protocol for acute acetaminophen overdose.

DESIGN: Nonrandomized trial open to all eligible patients.

SETTING: Multicenter; hospitals included moderate- and high-volume private, university, and municipal hospitals in urban and suburban settings.

TYPE OF PARTICIPANTS: Two hundred twenty-three patients were entered. Of these, 179 met inclusion criteria: acute acetaminophen overdose, plasma acetaminophen concentration above the treatment nomogram line, treatment with IV NAC according to the protocol, and sufficient data to determine outcome.

INTERVENTIONS: IV NAC treatment consisted of a loading dose of 140 mg/kg followed by 12 doses of 70 mg/kg every four hours.

MEASUREMENTS AND MAIN RESULTS: Patients were grouped for analysis according to risk group based on the initial plasma acetaminophen concentration. Hepatotoxicity (aspartate aminotransferase or alanine aminotransferase of more than 1,000 IU/L) developed in 10% (five of 50) of patients at "probable risk" when IV NAC was started within ten hours of acetaminophen ingestion and in 27.1% (23 of 85) when therapy was begun after ten to 24 hours. Among "high-risk" patients first treated 16 to 24 hours after overdose, hepatotoxicity occurred in 57.9% (11 of 19). There were two deaths (two of 179, 1.1%). Adverse reactions resulting from NAC occurred in 32 of 223 cases (14.3%), consisting in 29 of 32 patients (91% of reactions) of transient, patchy, skin erythema or mild urticaria during the loading dose that did not require discontinuation of therapy.

CONCLUSION: This 48-hour IV NAC protocol is safe and effective antidotal therapy for acetaminophen overdose. Based on available data, it is equal to 72-hour oral and 20-hour IV treatment protocols when started early and superior to the 20-hour IV regimen when treatment is delayed. Further study will be required to determine its relative efficacy in the high-risk patient treated very late.

179. Jones, A. L., D. R. Jarvie, D. Simpson, P. C. Hayes and L. F. Prescott (1997). "Pharmacokinetics of N-acetylcysteine are altered in patients with chronic liver disease." Aliment Pharmacol Ther 11(4): 787-91.

BACKGROUND: The threshold plasma paracetamol concentration at which N- acetylcysteine (NAC) treatment is recommended to treat paracetamol poisoning in a patient with induced liver enzymes (for example, with chronic liver disease or taking anticonvulsant drugs) is 50% lower than in a patient without induced liver enzymes. More patients with chronic liver disease might therefore be expected to be exposed to NAC treatment than previously. In addition, there is increasing use of NAC in patients with chronic liver disease for multiorgan failure or hepatorenal syndrome. Little is known of NAC's pharmacokinetic properties in patients with cirrhosis. AIM: The aim was to determine if the pharmacokinetics of NAC are altered by chronic liver disease. SUBJECTS AND METHODS: NAC was given intravenously in a dose of 600 mg over 3 min to nine patients with biopsy-proven cirrhosis (Child's grade; 1 A, 4 B, 4 C: aetiology: 7 alcohol-related, 1 primary biliary cirrhosis, 1 secondary biliary stenosis) and six healthy matched controls. Venous blood was taken at 20, 40, 60 and 90 min then at 2, 3, 4, 6, 8 and 10 h after NAC administration. Serum NAC was estimated by HPLC. The data were normalized to a standard body weight of 70 kg. RESULTS: The area under the serum concentration-time curve was increased (152.34 mg/L.h +/- 50.38 s.d.) in cirrhotics compared with normal controls (93.86 mg/L.h +/- 9.60 s.d.) ($P < 0.05$). The clearance of NAC was reduced in patients with chronic liver disease (4.52 L/h +/- 1.87 s.d.) compared with controls (6.47 L/h +/- 0.78: $P < 0.01$). CONCLUSIONS: Increased vigilance for untoward anaphylactoid reactions is necessary in cirrhotics as they may have higher plasma NAC concentrations. Further studies to determine the optimum dosage regimen in such patients are required.

180. Ballatori, N., M. W. Lieberman and W. Wang (1998). "N-acetylcysteine as an antidote in methylmercury poisoning." Environ Health Perspect 106(5): 267-71.

Methylmercury is a ubiquitous environmental pollutant and potent neurotoxin. Treatment of methylmercury poisoning relies almost exclusively on the use of chelating agents to accelerate excretion of the metal. The present study demonstrates that oral administration of N- acetylcysteine (NAC), a widely available and largely nontoxic amino acid derivative, produces a profound acceleration of urinary methylmercury excretion in mice. Mice that received NAC in the drinking water (10 mg/ml) starting at 48 hr after methylmercury administration excreted from 47 to 54% of the 203Hg in urine over the subsequent 48 hr, as compared to 4-10% excretion in control animals. When NAC- containing water was given from the time of methylmercury administration, it was even more effective at enhancing urinary methylmercury excretion and at lowering tissue mercury levels. In contrast, excretion of inorganic mercury was not affected by oral NAC administration. The ability of NAC to enhance methylmercury excretion when given orally, its relatively low toxicity, and is wide availability in the clinical setting indicate that it may be an ideal therapeutic agent for use in methylmercury poisoning.

181. Anderson, M. E., A. Naganuma and A. Meister (1990). "Protection against cisplatin toxicity by administration of glutathione ester." FASEB J. 4: 3251-3255.

The role of cellular glutathione in the prevention of toxicity due to the anti-cancer drug cisplatin (cis-diamminedichloroplatinum) was explored in mice treated with buthionine sulfoximine (BSO), a selective inhibitor of gamma-glutamylcysteine synthetase (and therefore of glutathione synthesis), and with glutathione and glutathione monoisopropyl ester. Pretreatment of mice with BSO enhanced the lethal toxicity of cisplatin by about twofold. Administration of glutathione ester (dose, 2.5-7.5 mmol/kg) protected against lethal cisplatin toxicity; glutathione was also effective, but much less so. Glutathione ester, in contrast to glutathione, is effectively transported into cells and split to glutathione intracellularly. The previous findings that administered glutathione does not protect against lethal toxicity due to cadmium ions and mercuric ions, whereas glutathione ester does, suggest that intracellular glutathione is required for protection against these heavy metal ions. That administration of glutathione has a protective effect on cisplatin toxicity suggests that the toxic effects of cisplatin may be exerted both intracellularly and extracellularly, and that extracellular glutathione (or its degradation products) may form a complex with cisplatin extracellularly. The finding that glutathione ester is more effective than glutathione in protecting against the toxicity of cisplatin suggests that use of glutathione ester may be therapeutically advantageous.

182. Ercal, N., P. Treeratphan, T. C. Hammond, R. H. Matthews, N. H. Grannemann and D. R. Spitz (1996). "In vivo indices of oxidative stress in lead-exposed C57BL/6 mice are reduced by treatment with meso-2,3-dimercaptosuccinic acid or N-acetylcysteine." Free Radical Biol. Med. 21(2): 157-161.

Knowledge of lead's capacity to disrupt the prooxidant/antioxidant balance within mammalian tissues suggests that definitive therapy for chronic lead poisoning should encompass both chelating and antioxidant actions. The dithiol meso-2,3-Dimercaptosuccinic Acid (DMSA) is the first orally administered metal chelating agent to receive U.S. Food and Drug Administration (FDA) approval for the treatment of childhood plumbism and possesses the potential to function as an antioxidant by removing lead from the site of deleterious oxidation reactions. Five weeks of lead exposure was found to deplete glutathione (GSH) levels, increase oxidized glutathione (GSSG), and promote malondialdehyde (MDA) production in both liver and brain samples taken from C57BL/6 mice. GSH levels increased and GSSG and MDA levels decreased in groups of lead-exposed mice that received 1 mmol/kg DMSA or 5.5 mmol/kg N-acetylcysteine (NAC) for 7 d prior to sacrifice. Treatment with DMSA caused a reduction in blood, liver, and brain lead levels consistent with its function as a chelating agent, while treatment with NAC did not reduce these lead levels. However, NAC did cause a reduction in indices of oxidative stress in both brain and liver samples, which implies that this synthetic thiol-containing antioxidant is capable of abrogating lead-induced oxidative stress in vivo. Overall, these results suggest that lead-induced oxidative stress in vivo can be mitigated by pharmacologic interventions, which encompass both chelating as well as thiol-mediated antioxidant functions.

183. **Gurer, H., H. Ozgunes, R. Neal, D. R. Spitz and N. Ercal (1998). "Antioxidant effects of N-acetylcysteine and succimer in red blood cells from lead-exposed rats." Toxicology 128(3): 181-189.**

This study examined whether lead-induced alterations in selected parameters that are indicative of oxidative stress accompany the toxic effects of lead in red blood cells (RBCs) in vivo. It also explored the possibility that treatment with N-acetylcysteine (NAC) or succimer (meso-2,3-dimercaptosuccinic acid) was capable of reversing parameters indicative of lead-induced oxidative stress. Fisher 344 rats were given 2000 ppm lead acetate in their drinking water for 5 weeks. The lead was then removed and the animals were given NAC (800 mg/kg/day) or succimer (90 mg/kg/day) in their drinking water for 1 week, after which the RBCs were harvested. Animals not given lead and those given lead, but not NAC or succimer, served as negative and positive controls, respectively. At the end of the experiment, blood-lead levels were 35 +/- 4 mu g/dl in lead-treated animals, which were reduced to 2.5 +/- 1 mu g/dl by treatment with succimer and to 25 +/- 3 mu g/dl by treatment with NAG. Lead-exposed animals demonstrated signs of anemia as evidenced by anisocytosis, poikilocytosis, and alterations in hemoglobin, hematocrit, and mean corpuscular volume. Lipid peroxidation, as evidenced by increased malondialdehyde (MDA) content; as well as decreases in reduced glutathione (GSH) and increases in catalase and glucose 6-phosphate dehydrogenase (G6PD) activity were noted in RBCs from lead-treated rats, suggesting that the lead induced oxidative stress. In addition, a significant reduction in blood delta-aminolevulinic acid dehydratase (ALAD) activity suggested that accumulation and autooxidation of delta-aminolevulinic acid might contribute to lead-induced oxidative stress. Treatment with either NAC or succimer reversed lead-induced alterations in MDA and GSH content, but only succimer appeared to partially restore ALAD activity. These results provide in vivo evidence supporting the hypothesis that lead induces oxidative stress in RBCs, which is reversible by treatment with a thiol antioxidant (NAC), as well as a chelating agent (succimer).

184. **Neal, R., K. Cooper, H. Gurer and N. Ercal (1998). "Effects of N-acetylcysteine and 2,3-dimercaptosuccinic acid on lead induced oxidative stress in rat lenses." Toxicology 130(2-3): 167-174.**

Lead (Pb) is known to disrupt the pro-oxidant/anti-oxidant balance of tissues which leads to biochemical and physiological dysfunction. The present study investigated the effects of exposure on the redox status of the lenses of Fisher 344 rats and examined whether antioxidant or chelator administration reversed these changes. Animals were given 5 weeks of 2000 ppm Pb exposure followed by 1 week of either antioxidant, chelator or distilled water administration. Glutathione (GSH) and cysteine (CYS) levels decreased in the Pb-exposed group. N-acetylcysteine or 2,3-dimercaptopsuccinic acid (Succimer) supplementation following Pb intoxication resulted in increases in the GSH and CYS levels. Protein bound glutathione (PSSG) and cysteine (PSSC) increased following Pb exposure. In the Succimer-treated animals, the PSSG decreased significantly. The glutathione disulfide (GSSG) levels remained unchanged. Malondialdehyde (MDA) levels, a major lipid peroxidation byproduct, increased following Pb exposure and decreased following Succimer treatment. Our results suggest that antioxidant supplementation, as well as chelation, following Pb exposure may enhance the reductive status of lenses.

185. **Sieg, K. J. and R. E. Billings (1997). "Lead/cytokine-mediated oxidative DNA damage in cultured mouse hepatocytes." Toxicol. Appl. Pharmacol. 142(1): 106-115.**

Hepatocytes exposed to the cytokines tumor necrosis factor alpha (TNF alpha) and interferon gamma (IFN gamma), in combination with a nontoxic dose of lead (Pb), exhibited significantly greater cytotoxicity compared to cytokine-treated hepatocytes after 24 hr treatment. Concentrations of Pb which interact with the cytokines to cause cytotoxicity stimulated hepatocytes cultured at this high cell density (63,830 cell/cm(2)) to undergo active DNA synthesis. DNA synthesis was also observed in hepatocytes plated at a low cell densities (21,276 cells/cm(2)) and these cells were sensitive to the toxic effects of the cytokines in the absence of Pb. Under conditions of active DNA synthesis, induced by either Pb or cell density, cytokine-induced toxicity appeared to be the result of extensive DNA fragmentation. Both DNA fragmentation and cytotoxicity were inhibited by treatment with an antioxidant mixture. Pb potentiated cytokine-induced oxidative stress within hepatocytes as indicated by decreased intracellular glutathione (GSH) and increased efflux of oxidized glutathione (GSSG) prior to cytotoxicity. The combination of cytokines and Pb also caused a significant decline in intracellular ATP concentrations prior to the onset of cytotoxicity. ATP concentrations were not altered by treatment with Pb or cytokines alone. Pb treatment did not alter total protein synthesis within hepatocytes. These results indicate that the cytotoxic interaction between Pb and the cytokines TNF alpha and IFN gamma may be mediated by oxidative DNA damage resulting from cytokine-induced oxidative stress and stimulation of mitogenic signals.

186. **Yusof, M., D. Yildiz and N. Ercal (1999). "N-acetyl-L-cysteine protects against delta-aminolevulinic acid-induced 8-hydroxydeoxyguanosine formation." Toxicol. Lett. 106(1): 41-47.**

5-Aminolevulinic acid (ALA) is a heme precursor that accumulates in acute intermittent porphyria and lead poisoning. It has been shown that ALA induces free radical generation and may cause damage to proteins and DNA. In the present study, the effects of ALA on DNA damage and its prevention by N-acetyl-L-cysteine (NAC) and the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) are investigated. Oxidative damage to DNA was quantitated by measuring the increase in 8-hydroxy-2'-deoxyguanosine (oh(8)dG) formation. The time-course study demonstrated that ALA causes a linear increase in oh(8)dG levels in Chinese hamster ovary (CHO) cells. However, direct lead exposure did not cause any measurable increase in oh(8)dG levels. In the presence of either NAC (1 mM) or antioxidant enzymes (10 u/ml SOD and 10 u/ml CAT), oh(8)dG levels returned to the corresponding control levels. This suggests a protective role for NAC and the antioxidant enzymes. To determine the effect of ALA on cell proliferation, cell numbers were counted at the end of 24 h of incubation in the presence and absence of ALA at different concentrations. Results showed that levels of ALA up to 5 mM do not inhibit cell proliferation.

187. **Lamson, D. W. and M. S. Brignall (2000). "The use of nebulized glutathione in the treatment of emphysema: a case report [In Process Citation]." Altern Med Rev 5(5): 429-31.**

We present the case of a 95-year-old man with an acute respiratory crisis secondary to emphysema and apparent bronchial infection. Treatment with nebulized glutathione led to a rapid resolution of the crisis, as well as a marked improvement in the chronic course of the disease. This treatment has been used since for a number of patients with emphysema. The safety and bioavailability of this method of delivery have been established in human studies. Preliminary results suggest efficacy for nebulized administration of glutathione in this patient population. We suggest this treatment can be considered an option for acute respiratory crises due to COPD.

188. **Prasad, A., N. P. Andrews, F. A. Padder, M. Husain and A. A. Quyyumi (1999). "Glutathione reverses endothelial dysfunction and improves nitric oxide bioavailability." J. Amer. Coll. Cardiol. 34(2): 507-514.**

OBJECTIVES We investigated whether glutathione (GSH), a reduced thiol that modulates redox state and forms adducts of nitric oxide (NO), improves endothelium-dependent vasomotion and NO activity in atherosclerosis.

BACKGROUND Endothelial dysfunction and reduced NO activity are associated with atherosclerosis and its clinical manifestations such as unstable angina.

METHODS In the femoral circulation of 17 patients with atherosclerosis or its risk factors, endothelium-dependent vasodilation with acetylcholine (ACH), and endothelium-independent vasodilation with nitroglycerin and sodium nitroprusside were studied before and after GSH. In 10 patients, femoral vein plasma cyclic guanylate monophosphate (cGMP) levels were measured during an infusion of ACH before and after GSH. Femoral artery flow velocity was measured using a Doppler flow wire and the resistance index (FVRI) calculated as mean arterial pressure divided by flow velocity.

RESULTS Glutathione strongly potentiated AGH-mediated vasodilation; at the two doses, FVRI decreased by 47% and 56% before, and by 61% and 67% after GSH ($p = 0.003$). Glutathione also elevated cGMP levels in the femoral vein during ACH infusion from 17.6 +/- 3 to 23.3 rt 3 pmol/ml ($p = 0.006$). Augmentation of ACH responses was only observed in patients with depressed endothelial function. Glutathione did not influence endothelium-independent vasodilation with either NO donor.

CONCLUSIONS Thiol supplementation with GSH selectively improves human endothelial dysfunction by enhancing NO activity.

189. **Boesgaard, S., J. Aldershvile, H. E. Poulsen, S. Christensen, H. Dige-Petersen and J. Giese (1993). "N-acetylcysteine inhibits angiotensin converting enzyme in vivo." J. Pharmacol. Exp. Ther. 265(3): 1239-44.**

Nitrate tolerance has been explained by 1) a direct loss of pharmacological effect due to reduced bioconversion and 2) an indirect effect due to activation of the renin/angiotensin system and counter-regulatory vasoconstriction. The sulfhydryl compound N-acetylcysteine (NAC) has been shown to attenuate and partly counteract tolerance to nitrates, and this effect has been attributed to a nitrate/sulfhydryl interaction and increased production of vasoactive intermediates. The effect of NAC on counter-regulatory mechanisms is, however, unknown. This study examined whether NAC modulates the function of the renin/angiotensin system in normal rats and in nitrate-tolerant healthy volunteers. Animal study: Conscious rats received NAC (5 mmol/kg/hr i.v., n = 8) or placebo (N-acetylserine, n = 8). Two hours of NAC infusion significantly reduced the pressor effect of angiotensin I (ANG I) by 39 +/- 14% (mean +/- SEM) and reduced angiotensin converting enzyme activity by 31% in plasma (N-acetylserine: 74 +/- 9 nmol/min/mg, NAC: 51 +/- 7) and 43% in kidney (N-acetylserine: 0.9 +/- 0.3, NAC: 0.5 +/- 0.1 nmol/min/mg protein) (P < .05). Clinical study: Isosorbide dinitrate (5 mg/hr) was infused into six male volunteers for 48 hr. NAC (2 g i.v. followed by 5 mg/kg/hr) was co-infused from 24 to 48 hr. Plasma angiotensin II (ANG II) increased during the first 24 hr of isosorbide dinitrate infusion and decreased from 28 +/- 4 to 14 +/- 2 ng/l after 2 hr of NAC infusion (P < .05). The results suggest that sulfhydryl supplementation modifies the function of the renin/angiotensin system in vivo, an effect probably mediated by inhibition of angiotensin converting enzyme activity.(ABSTRACT TRUNCATED AT 250 WORDS)

190. Ventura, P., R. Panini, M. C. Pasini, G. Scarpetta and G. Salvioli (1999). "N-acetyl-cysteine reduces homocysteine plasma levels after single intravenous administration by increasing thiols urinary excretion." Pharmacol. Res. 40(4): 345-350.

A decrease of plasma homocysteine (Hcy) may represent a therapeutic promise for reducing the impact of atherosclerosis. N-Acetyl-cysteine (NAC) is a thiol-containing compound interfering with endogenous thiols, cysteine (Cys) and Hcy, by forming with them mixed disulphides with a possibly more efficient renal clearance. The aim of this work was to assess the effect of NAC intravenous infusion on plasma levels of different forms of Hcy and particularly to verify the effect on Hcy renal excretion. We collected basal blood samples at 0.5, 1, 2, 5, 8 and 24 h after the beginning of NAC infusion (50 mg kg-l body wt.) and also 24-h urine samples of the day of NAC infusion and of the day before and of the day after the infusion in ten healthy subjects (mean age 73 +/- 15). Urinary and plasma thiols (Hcy, Cys and NAC) were assayed by HPLC. Both total plasma Hey (approx. 69% vs basal values) and Cys (approx. 40% vs basal values) fell progressively, reaching a minimum 5 h after infusion start; total free (i.e. Not bound to proteins) Hcy (2.2 +/- 1.8 down from 4.4 +/- 4.2 nmol ml(-1)) and Cys (70.4 +/- 39.8 down from 113.3 +/- 61.2 nmol ml(-1)) decreased as well. Reduced (thiolic-free form) Hey and Cys decreased during infusion, though not as pronounced as for the other forms. Percentagewise, out of the total plasma levels, Hey and Cys total free form and reduced form tended to increase over infusion as well as their difference (i.e. The plasma mixed disulphide moiety), thus supporting the idea that excess NAC displaces thiols from their plasma binding sites forming mixed disulphides. Urinary total Cys and Hey excretion significantly increased at the end of the day of NAC infusion (tenfold for Cys and fivefold for Hey) and reduced appreciably on the following day. Also urinary excretion of the free form of Cys and Hey increased at the end of the day of NAC infusion, although in a lower amount with respect of total amounts, meaning a reduction of percentage Cys and Hey excreted as the free form; for none of the patients had proteinuria, the 'free' form of urine thiols has to be identified in the 'reduced' form, the difference between the total and free form reflecting the 'mixed disulphide' moiety. NAC intravenous administration induces an efficient and rapid reduction of plasma thiols, particularly of Hey; our data support the hypothesis that NAC displaces thiols from their binding protein sites and forms, in excess of plasma NAG, mixed disulphides (NAC-Hcy) with an high renal clearance. This effect may represent the start of an alternative approach in the treatment of hyperhomocysteinaemic conditions. (C) 1999 Academic Press.

191. Hultberg, B., A. Andersson, P. Masson, M. Larson and A. Tunek (1994). "Plasma homocysteine and thiol compound fractions after oral administration of N-acetylcysteine." Scand. J. Clin. Lab. Invest. 54(6): 417-422.

The total concentration of the atherogenic aminothiol acid homocysteine in plasma of healthy volunteers was decreased after oral administration of N-acetylcysteine (NAC), whereas the reduced and free (non-protein bound) fractions of homocysteine were increased. The decrease of the total fraction varied between 20 and 50% and was dose-related. Cysteinylglycine was also decreased after the administration of NAC, whereas cysteine did not change. Administration of high amounts of NAC probably displaces homocysteine and cysteinylglycine from their protein binding sites by disulfide interchange reactions. This leads to the formation of mixed low molecular-weight cystein and NAC disulfides with high renal clearance and possibly also increased metabolic bio-availability, thereby eliminating homocysteine and cysteinylglycine from plasma. Since only a small amount of additional urinary homocysteine was recovered it is likely that this aminothiol acid is taken up by the tubular cells and further metabolized.

192. **Redondo, P. and A. Bauza (1999). "Topical N-acetylcysteine for lamellar ichthyosis." Lancet 354(9193 (27 Nov)): 1880.**

The antioxidant N-acetylcysteine has an antiproliferative effect on a culture of human keratinocytes. We report a patient with lamellar ichthyosis satisfactorily treated with topical N-acetylcysteine.

193. **Tripi, S., G. Di Gaetano, M. Soresi, A. Carroccio, G. Bonfissuto, A. Savi, O. Vuturo and G. Montalto (1998). "Acetylcysteine therapy for chronic hepatitis C: Are its effects synergistic with interferon alpha? A pilot study." Clin. Drug Invest. 16(4): 297-302.**

Objective: This trial reports the 6-month results of a pilot study using lymphoblastoid interferon alpha (IFN alpha) and acetylcysteine (N-acetylcysteine) separately and in combination in patients with chronic hepatitis C, genotype 1b, who were nonresponders to previous treatment with recombinant IFN alpha alone. Patients and Methods: 21 patients were randomly divided into three groups of seven each. Group A was treated with lymphoblastoid IFN alpha 6MU three times a week for 6 months; group B received the same schedule of lymphoblastoid IFNa as group A plus acetylcysteine 1200 mg/day per os in two administrations, and group C received only acetylcysteine 1200 mg/day per os in two administrations. Results: Mean serum alanine aminotransferase (ALT) levels at 6 months in groups A and B, but not in group C, were significantly lower than baseline values ($p < 0.05$ and $p < 0.03$, respectively). Two patients in group A (28.6%) and three in group B (42.9%), but none in group C, had normalised ALT levels at 6 months. During follow-up, levels flared in one group A and in one group B patient. Thus, at the end of follow-up one group A and two group B patients were sustained responders. At the end of therapy and follow-up, hepatitis C virus (HCV)-RNA was negative in one patient in group A and two patients, in group B. As no serious adverse effects were observed, therapy was never interrupted or suspended. Conclusion: Acetylcysteine alone had no effect on hepatic cytolysis and viral replication; lymphoblastoid IFN alpha showed a modest, but better, response than recombinant IFN alpha, and the combination therapy, although in a limited number of patients, appeared to be more efficient than lymphoblastoid IFN alpha alone.

194. **Pendyala, L. and P. J. Creaven (1995). "Pharmacokinetic and pharmacodynamic studies of N-acetylcysteine, a potential chemopreventive agent during a phase I trial." Cancer Epidemiol Biomarkers Prev 4(3): 245-51.**

A Phase I, pharmacokinetic and pharmacodynamic study of N- acetylcysteine (NAC), a potential chemopreventive agent, given daily p.o. for 6 months was carried out in 26 volunteers at higher than normal risk of malignancy. The goals of the study were to define the highest nontoxic dose, the toxicity profile, and the pharmacokinetics and pharmacodynamics of NAC. The pharmacodynamic end points studied included glutathione (GSH) in plasma, RBC and peripheral blood lymphocytes (PBL), cysteine in plasma, and two GSH-metabolizing enzymes glutathione S-transferase and oxidized glutathione reductase in PBL. The study was carried out in 2 stages. The first stage consisted of an inter- and intrasubject dose escalation; the second, an assessment of a single daily dose. Starting doses for the first 4 cohorts of 3 subjects were 400, 800, 1600, and 3200 mg/m2/day in divided doses doubled at the end of each month in the absence of toxicity to a final dose of 6400 mg/m2/day. The total planned period on NAC for each subject was 6 months. Pharmacokinetic and pharmacodynamic measurements were carried out at the beginning of the study and at the end of each month. The second stage of the study consisted of a daily dose of 800 mg/m2/day. During this part of the study, NAC in plasma and GSH and oxidized glutathione reductase (GRD) in PBL were measured on day 1 and again at the end of first, second, and sixth month on NAC. Major toxicities were bad taste and gastrointestinal disturbances. The highest nontoxic dose was 800 mg/m2/day in most of the subjects.(ABSTRACT TRUNCATED AT 250 WORDS)

195. **Woo, O. F., P. D. Mueller, K. R. Olson, I. B. Anderson and S. Y. Kim (2000). "Shorter duration of oral N-acetylcysteine therapy for acute acetaminophen overdose." Ann Emerg Med 35(4): 363-8.**

STUDY OBJECTIVE: We sought to evaluate the safety and efficacy of a shorter N-acetylcysteine (NAC) regimen in the treatment of acute acetaminophen overdose. METHODS: We performed a retrospective case series in a large urban county hospital. Of 305 patients identified through the emergency department, 75 patients met the criteria inclusion: an acute overdose ingestion, serum acetaminophen concentration in toxic range according to the Rumack-Matthew nomogram, and oral NAC treatment initiated within 24 hours of the ingestion. The regional poison control center recommended oral treatment with NAC 140 mg/kg, followed by maintenance doses of 70 mg/kg every 4 hours until the serum acetaminophen level was no longer detectable, rather than the standard 72-hour treatment regimen. RESULTS: The primary outcome measure was the development of hepatotoxicity. Twenty-five (33.3%) patients were treated for a period

of less than 24 hours, 25 (33.3%) were treated for 24 to 36 hours, and 25 (33.3%) were treated for 37 to 64 hours; the mean and median duration of treatment was 31 hours. None of the patients treated for less than 24 hours had evidence of hepatotoxicity (aspartate aminotransferase [AST] or alanine aminotransferase [ALT] level >1,000 IU/L); hepatotoxicity developed in 2 (8%) patients treated for 24 to 36 hours and 4 (16%) patients treated for 37 to 64 hours. There were no deaths or patients who received liver transplantation. The overall incidence of hepatotoxicity in our patients was similar to that found in other protocols with administration of oral NAC for 72 hours or intravenous NAC for 20 or 48 hours. CONCLUSION: This observational study suggests that a shorter course of oral NAC therapy in patients who do not show evidence of hepatotoxicity within 36 hours of an acute acetaminophen overdose is safe and effective.

196. Wu, M. L., W. J. Tsai, J. F. Deng and C. C. Yang (1999). "Hemodialysis as adjunctive therapy for severe acetaminophen poisoning: a case report." Chung Hua I Hsueh Tsa Chih (Taipei) 62(12): 907-13.

Acetaminophen overdose is a common intoxication in daily practice the standard treatment is N-acetylcysteine (NAC) antidotal therapy for possible poisoning. However, dialysis procedures can remove the drug from the body effectively. We describe a case of acetaminophen overdose that was treated with both hemodialysis (HD) and NAC due to severe intoxication and slow drug clearance. A 37-year-old woman attempted suicide by ingestion of 100 tablets (500 mg each) of acetaminophen, and presented with vomiting, hematemesis and abdominal pain. The patient had elevated liver enzymes, coagulation defects, thrombocytopenia a high serum acetaminophen level (201 mg/l at 12 hours post-ingestion) with a prolonged half-life. Oral NAC was given; however, it was ineffective due to severe vomiting and hematemesis. HD as adjunctive therapy was initiated at 19 hours post-ingestion. HD reduced the serum acetaminophen level from 102.77 to 35.77 mg/l. Severe hepatic injury, bacteremia and pancytopenia were noted in the following days. The patient later recovered after treatment with NAC, HD and intensive supportive care. HD removed 66% of the total acetaminophen body burden during a single four-hour session, increased the clearance by 2.75-fold and shortened the half-life from 7.2 hours to 2.6 hours during HD. Through NAC therapy is the standard regimen for acetaminophen poisoning, in the severely poisoned patient who cannot tolerate NAC therapy, HD may be used as adjunctive therapy to enhance the elimination of acetaminophen.

197. Bond, G. R. and L. K. Hite (1999). "Population-based incidence and outcome of acetaminophen poisoning by type of ingestion [see comments]." Acad Emerg Med 6(11): 1115-20.

OBJECTIVES: 1) To determine, in a population-based sample, the observed frequency of acetaminophen overdose-related ED evaluation and hospitalization. 2) To examine the relative frequency of hospitalization by pattern of ingestion, the outcome of each group, and the presence or absence of postulated risk factors. METHODS: This study was a 46-month, retrospective chart review of all acetaminophen-related visits, by patients at least 10 years of age, to either of the two hospitals that serve a four-county region of central Virginia. RESULTS: Of 636 charts identified for review, only 137 involved acute or chronic acetaminophen overdose. One hundred twenty-six patients presented after an acute ingestion; 122 of these patients gave a history of a single, supratherapeutic ingestion of acetaminophen. Twenty-five patients were hospitalized for treatment. Eighteen of these were treated with N- acetylcysteine (NAC) based on the Rumack-Matthew nomogram; one suffered significant hepatic injury. The other seven presented at least 18 hours after ingestion, with no measurable serum acetaminophen. Two of these suffered significant hepatic injury. Four additional patients presented after multiple ingestions within 24 hours. Three were hospitalized, but none experienced significant injury. Only 11 patients were evaluated for chronic acetaminophen overmedication for pain (more than 6 g/day over a period of more than 24 hours). Four were admitted for treatment; three suffered significant hepatic injury. Thus, the observed incidence of acute acetaminophen ingestion in this region was 21.4/100,000/yr (95% CI = 17.7 to 25.2). The observed incidence of hospitalization for acute acetaminophen toxicity was 4.8/100,000/yr (95% CI = 3.0 to 6.5). The observed incidence of hospitalization for all acetaminophen poisoning was 5.5/100,000/yr (95% CI = 4.1 to 7.0). High ethanol consumption was present more frequently in those who suffered hepatic injury. CONCLUSIONS: Most patients evaluated for acetaminophen ingestion present early following acute single overdose. Relatively few of these patients require hospitalization and, for those hospitalized, the outcome is good. More significantly, acetaminophen overdose patients whose risk cannot be estimated using the Rumack-Matthew nomogram represented 44% of those hospitalized and 83% of those who suffered significant hepatic injury. Emergency physicians need to determine how they can impact the outcome of these patients. Efforts should be directed at further characterizing historical, physical, and biochemical markers of risk and at determining in which circumstances hospitalization for NAC or other therapies is justified.

198. Tucker, J. R. (1998). "Late-presenting acute acetaminophen toxicity and the role of N-acetylcysteine." Pediatr Emerg Care 14(6): 424-6.

NAC is an effective antidote for APAP toxicity. NAC has been shown to be effective for early toxicity and is gaining acceptance for late toxicity. As the knowledge of APAP toxicity advances, the duration and route of NAC administration may be clarified.

199. Perry, H. E. and M. W. Shannon (1998). "Efficacy of oral versus intravenous N-acetylcysteine in acetaminophen overdose: results of an open-label, clinical trial [see comments]." J Pediatr 132(1): 149-52.

We compared the clinical course of pediatric patients (n = 25) with acetaminophen poisoning treated with an investigational intravenous preparation of N-acetylcysteine (IV-NAC) with that of historical control subjects (n = 29) treated with conventional oral NAC (O-NAC) therapy. Patients received IV-NAC for 52 hours; historical control subjects received O-NAC (72 hours). There were no significant intergroup differences between treatment groups in age (15.5 vs 15.9 years), gender (88% vs 90% female) or distribution of risk categories (probable risk, 12 vs 15; high risk; 13 vs 14). The peak prothrombin time was significantly higher in the IV-NAC group (14.2 vs 13.6 seconds; $p = 0.048$). Mean treatment delay was significantly longer in the IV-NAC group (14.4 vs 10.4 hours; $p = 0.001$). Hepatoxicity was noted in two (8.0%) patients in the IV-NAC treatment group and two (6.9%) patients in the O-NAC group. All patients recovered. Our results indicate that 52 hours of intravenous NAC is as effective as 72 hours of oral NAC.

200. Kelly, G. S. (1998). "Clinical applications of N-acetylcysteine." Altern Med Rev 3(2): 114-27.

N-acetylcysteine (NAC), the acetylated variant of the amino acid L- cysteine, is an excellent source of sulfhydryl (SH) groups, and is converted in the body into metabolites capable of stimulating glutathione (GSH) synthesis, promoting detoxification, and acting directly as free radical scavengers. Administration of NAC has historically been as a mucolytic agent in a variety of respiratory illnesses; however, it appears to also have beneficial effects in conditions characterized by decreased GSH or oxidative stress, such as HIV infection, cancer, heart disease, and cigarette smoking. An 18-dose oral course of NAC is currently the mainstay of treatment for acetaminophen-induced hepatotoxicity. N-acetylcysteine also appears to have some clinical usefulness as a chelating agent in the treatment of acute heavy metal poisoning, both as an agent capable of protecting the liver and kidney from damage and as an intervention to enhance elimination of the metals.

201. Kirk, M. and S. Pace (1997). "Pearls, pitfalls, and updates in toxicology." Emerg Med Clin North Am 15(2): 427-49.

Pearls and pitfalls learned from our practical experiences caring for poisoned patients are presented. Clinical pearls include the following: using diagnostic tests to detect end-organ toxicity, applying physiologic principles to the management of hemodynamically unstable poisoned patients, and dealing with psychologic injuries from hazardous materials incidents. Recognizing serious complications from poisoning and adverse drug effects, including the serotonin syndrome, are offered as pitfalls. Pharmaceutical companies are rapidly developing and marketing new therapies. Therefore, updates on the evolving role of NAC as an antidote for acetaminophen poisoning, new psychotropic medications, and new antidotes were included in this article. These pearls, pitfalls, and updates are intended to provide practical information that is readily applicable to the clinical practice of emergency medicine.

202. Cotgreave, I. A. (1997). N-acetylcysteine: Pharmacological considerations and experimental and clinical applications. Advances in Pharmacology. Volume 38. Antioxidants in Disease Mechanisms and Therapy. H. Sies. 525 B Street, Suite 1900, San Diego, CA 92101-4495, Academic Press Inc. 38: 205-227.

The diversity of application of the thiol drug NAC in both the experimental setting, as a tool for the study of the mechanisms and consequences of oxidative stress, and the clinical setting, as a therapeutic agent, clearly reflects the central role played by the redox chemistries of the group XVI elements, oxygen and sulfur, in biology. As our understanding of such redox processes increases, particularly their roles in specific pathophysiological processes, new avenues will open for the use of NAC in the clinical setting. As a drug, NAC represents perhaps the ideal xenobiotic, capable of directly entering endogenous biochemical processes as a result of its own metabolism. Thus, it is hoped that the experience gained with this unique agent will help in future efforts to design antioxidants and chemoprotective principles which are able to more accurately utilize endogenous biochemical processes for cell- or tissue-specific therapy.

203. Cetaruk, E. W., R. C. Dart, K. M. Hurlbut, R. S. Horowitz and R. Shih (1997). "Tylenol Extended Relief overdose." Ann Emerg Med 30(1): 104-8.

In this report we describe the toxicokinetics of the Tylenol Extended Relief (TER) preparation of acetaminophen in human overdose. We collected 41 cases of TER overdose from five regional poison centers. Patients who met the following criteria were studied: a single ingestion of TER alone; confirmed time of ingestion; at least four acetaminophen determinations; and normal concentrations of liver function enzymes. With the exception of standard decontamination measures, treatment with N-acetylcysteine (NAC) if any acetaminophen level was above the treatment line of the Rumack-Matthew nomogram, and additional acetaminophen determinations, no interventions were recommended. Our study group comprised 13 patients, 12 female and 1 male, with single overdoses of 10.4 to 65 g TER. The acetaminophen elimination half-life was 3.1 +/- .8 hours (mean +/- SD; range, 1.3 to 4.0 hours; n = 12). The elimination phase for patients 2, 3, 4, 6, 8, 9, 11, 13 was delayed until 8.0 +/- 2.8 hours (range, 5 to 14 hours) after ingestion. Patients 3, 8, and 11--who had initial acetaminophen levels below the "possible toxicity" line of the Rumack-Matthew nomogram--later had acetaminophen levels above this line. No patient demonstrated a late or second acetaminophen peak. We conclude that the elimination half-life of TER acetaminophen is similar to that reported in overdose of immediate-release acetaminophen overdose. In a subgroup of patients, drug absorption continued beyond the 2 to 4 hours previously reported in immediate-release acetaminophen overdose. On the basis of our data, the use of a single 4-hour acetaminophen determination may lead to failure to recognize patients with potentially toxic TER ingestion. Until more toxicokinetic data are available, a reasonable approach would be to obtain at least one additional acetaminophen determination at least 4 to 6 hours after the first, if the first is obtained 4 to 8 hours after ingestion. NAC treatment should be initiated if either level is above the nomogram line but not if both levels fall below the nomogram line.

204. Dean, B. S., J. D. Bricker and E. P. Krenzelok (1996). "Outpatient N-acetylcysteine treatment for acetaminophen poisoning: an ethical dilemma or a new financial mandate?" Vet Hum Toxicol 38(3): 222-4.

The mainstay of treatment for acetaminophen-induced hepatotoxicity, produced by the accumulation of the toxic metabolite N- acetylbenzoquinoneimine, is an enteral 18-dose course of N- acetylcysteine (NAC). However, absence of characteristic symptomatology is a frequent reason for premature cessation of NAC and early discharge of the toxic acetaminophen poisoned patient. We report a series of confirmed acetaminophen poisonings who were discharged early with NAC and instructions to self-administer. All cases of acute acetaminophen poisoning without concomitant drugs, reported to a certified Regional Poison Information Center for a 3-mo period of time, were reviewed. Inclusion criteria included patients who were discharged with orders to complete the course of NAC outside of a hospital, despite toxic serum acetaminophen concentrations. Data parameters evaluated included age, amount taken, symptoms, laboratory results, treatment, and medical outcome. 131 cases of confirmed toxic acetaminophen poisoning yielded 6 patients who received 4 to 6 doses of NAC during hospitalization, but were discharged to home with the remaining 11-13 doses. Patients' ages ranged from 16-28 y (mean 20.0 y). Serum acetaminophen concentrations measured at 4 h post-ingestion ranged from 171-198 mcg/ml (mean 182 mcg/ml). Follow-up by the certified Regional Poison Information Center at 1-3 w post-discharge determined dosing compliance to be 83%. All 6 patients remained asymptomatic with normal liver function testing. Since health care reform encourages practitioners to reconsider established approaches to the delivery of health care, perhaps home delivery of NAC would not only be clinically preferred to premature cessation of the antidote, but also offer cost savings. Self- administration of NAC in the home setting may be representative of a new era in America's health care delivery system.

205. Clemmesen, J. O., P. Ott, K. P. Dalhoff, L. B. Astrup, U. Tage-Jensen and H. E. Poulsen (1996). "[Recommendations for treatment of paracetamol poisoning. Danish Medical Society, Study of the Liver]." Ugeskr Laeger 158(48): 6892-5.

Based on recent reports concerning the efficacy of N-acetylcysteine (NAC) in paracetamol (acetaminophen) poisoning, guidelines for treatment and control of these patients are reviewed by a study group under the Danish Association for the Study of the Liver. It is recommended that NAC-treatment is initiated immediately after referral and continued for 36 hours in all cases. Further NAC-treatment should not be discontinued before a decrease in INR has been observed.

206. **Brandwene, E. L., S. R. Williams, C. Tunget-Johnson, S. G. Turchen, A. S. Manoguerra and R. F. Clark (1996). "Refining the level for anticipated hepatotoxicity in acetaminophen poisoning." J Emerg Med 14(6): 691-5.**

Treatment of an acetaminophen overdose with N-acetyl cysteine usually is based on the position of the 4-h acetaminophen (APAP) level on the Rumack-Matthew nomogram; however, there is disagreement on the level at which clinically relevant hepatotoxicity occurs. A retrospective review of all acute adult formulation APAP exposures reported to our poison center between 1986 and 1993 was performed and cases corresponding to the "possible risk or toxicity" range on the nomogram were identified. Our current poison center protocol for APAP poisoning does not recommend treatment with N-acetylcysteine (NAC) in low-risk patients if the 4-h serum APAP level or the extrapolated equivalent falls within the possible toxicity range on the nomogram. Seventeen cases met the inclusion criteria for the study and received no NAC; six additional patients met inclusion criteria but received one or two doses of NAC before therapy was discontinued. No patients in either group demonstrated clinical evidence of hepatotoxicity. This pilot study suggests that patients with no risk factors and APAP levels in the "possible risk" range may not require NAC therapy.

207. **Chan, T. Y., A. Y. Chan and J. A. Critchley (1993). "Paracetamol poisoning and hepatotoxicity in Chinese--the Prince of Wales Hospital (Hong Kong) experience [see comments]." Singapore Med J 34(4): 299-302.**

From 1989 to 1991, 104 Chinese patients were admitted to the Prince of Wales Hospital with paracetamol poisoning. Only 11 subjects had a plasma paracetamol concentration above the published treatment line. Intravenous N-acetylcysteine (NAC) was completely effective when given within 8 hours (3 patients), while late treatment with NAC at 16 and 26 hours after overdose (2 patients) was ineffective in preventing liver damage as evidenced by elevations in plasma alanine transaminase concentrations. Of the 6 patients receiving NAC between 10 to 15 hours, two had liver damage. Two other subjects who presented late or in whom a plasma paracetamol concentration was not measured also developed liver damage. Fortunately, none of these 6 subjects developed hepatic encephalopathy. We recommend that a standard protocol be readily available for junior hospital staff to use when treating patients with paracetamol overdosage.

208. **Mrvos, R., S. M. Schneider, B. S. Dean and E. P. Krenzelok (1992). "Orthotopic liver transplants necessitated by acetaminophen-induced hepatotoxicity." Vet Hum Toxicol 34(5): 425-7.**

BACKGROUND: Acetaminophen-induced hepatotoxicity has been recognized since 1966. Patients experiencing a massive hepatic insult due to acetaminophen (APAP) may recover with minimal residual complications or develop fulminant hepatic necrosis. We report 3 patients with hepatic failure due to an APAP overdose who received orthotopic liver transplants and survived. CASE REPORTS: An 18-y-o female ingested 60 500 mg APAP tablets (30 g). She presented with tachycardia and lethargy stating that she had taken amoxipine, carbamazepine, and lorazepam. She began to recover but on day 2 experienced an upper gastrointestinal bleed and became hypotensive and hyperpyrexic. She developed hepatic encephalopathy and it was then determined she had ingested APAP. Her APAP level was 13 micrograms/ml 96 h post-ingestion. She was successfully transplanted 19 d post-ingestion with recovery. A 40-y-o female was admitted for flu-like symptoms persisting for 7 d. She was jaundiced, hyperventilating and hypotensive. She admitted ingesting approximately 17 g APAP over 36 h. Her APAP level was 12.2 micrograms/ml. Her condition worsened and on day 3 she was in grade IV coma. She was successfully transplanted 4 d post-arrival with recovery. A 16-y-o female ingested an unknown amount of APAP. She presented approximately 24 h post-ingestion with a serum APAP level of 130 micrograms/ml. Her condition deteriorated and she became encephalopathic with grade IV coma. She was successfully transplanted on day 7 post-arrival. DISCUSSION: Hepatotoxicity can occur as a result of either acute or chronic APAP overdose. Although n-acetylcysteine (NAC) is effective antidotal therapy, it must be used within 8-12 h post-ingestion to be optimally effective. Inaccurate patient histories may prevent NAC administration resulting in hepatotoxicity. CONCLUSION: Liver transplantation is a viable option to be considered in those APAP overdose patients who experience rapidly progressing encephalopathy, hemolysis, and hepato-renal failure.

209. **Brotodihardjo, A. E., R. G. Batey, G. C. Farrell and K. Byth (1992). "Hepatotoxicity from paracetamol self-poisoning in western Sydney: a continuing challenge." Med J Aust 157(6): 382-5.**

OBJECTIVE: To determine the annual incidence of admissions for paracetamol overdosage in the years 1985 to 1990, morbidity and mortality rates, predictors of poor prognosis and the most appropriate use of N-acetylcysteine (NAC).

DESIGN: A retrospective review of case records of all patients with a discharge diagnosis of paracetamol overdosage. SETTING: A 900-bed tertiary referral teaching hospital in western Sydney with a busy accident and emergency department. PATIENTS: 306 patient records were reviewed and details of the overdose and admission were recorded. INTERVENTIONS: NAC infusion in patients with possible paracetamol hepatotoxicity. MAIN OUTCOME MEASURES: Blood paracetamol levels; elevated alanine aminotransferase levels; prolonged prothrombin time; severe liver injury; and NAC side effects. RESULTS: Annual admission rate was constant at circa 55 per annum. Female to male ratio was 2:1. Predictors of liver injury included paracetamol dose over 10 g, presentation more than 10 hours after the overdose and chronic ingestion of more than 80 g alcohol per day. There were no deaths. Fifty-five patients (18%) had toxic paracetamol levels, 51% received treatment with NAC, including 40% of those with non-toxic levels, and 11% of those treated with NAC experienced side effects. CONCLUSION: Paracetamol overdosage continues to be a significant cause of hospital admissions in western Sydney. Severe hepatic damage occurs infrequently and the prognosis for liver injury, when it occurs, is good. Treatment with NAC should be reserved for patients with definite indications for the drug.

210. Beckett, G. J., B. J. Chapman, E. H. Dyson and J. D. Hayes (1985). "Plasma glutathione S-transferase measurements after paracetamol overdose: evidence for early hepatocellular damage." Gut 26(1): 26-31.

Plasma glutathione S-transferase (GST) measurements have been used to study early changes in hepatocellular integrity after paracetamol overdose and treatment with N-acetylcysteine (NAC). Patients admitted within seven hours and successfully treated had raised or equivocal GST on admission and each showed a transient peak in GST approximately 12 hours after the overdose. Similar, though smaller changes in GST, were seen in untreated patients whose paracetamol level fell below the treatment line. The plasma GST concentrations in successfully treated patients were small compared with values found in patients who subsequently developed severe liver damage. The changes in GST concentration observed in patients who developed severe liver damage indicated that distinct early and late phases of paracetamol-induced hepatotoxicity occurred. Although the mechanism by which paracetamol exerts its early toxic effect is unclear, our data suggest that prompt treatment with NAC can successfully prevent both clinical and subclinical hepatotoxicity in this early period.

211. **Linden, C. H. and B. H. Rumack (1984). "Acetaminophen overdose." Emerg Med Clin North Am 2(1): 103-19.**

N-acetylcysteine (NAC) is the treatment of choice for acetaminophen overdose. With this therapy, morbidity from overdose can be held to a minimum. Mortality is rare in any case and virtually nonexistent in treated patients. Unless a high index of suspicion is maintained, the diagnosis may be missed until it is too late for effective antidotal treatment.

212. **Bailey, B. O. (1980). "Acetaminophen hepatotoxicity and overdose." Am Fam Physician 22(1): 83-7.**

Acetaminophen is a widely available and frequently recommended over-the- counter analgesic and antipyretic. Chronic doses in excess of 5 Gm. per day and acute doses of as little as 7 Gm. have caused hepatic damage in adults. Larger doses may be fatal. The hepatotoxicity, which is due to metabolic transformation of the acetaminophen to an alkylating agent, can be palliated or avoided by prompt treatment. Blood levels over 200 micrrograms per mL. four hours after ingestion correlate with severe hepatotoxicity. Clinical trials have shown N-acetylcysteine (NAC) to be a specific antidote when administered within eight hours of an acute ingestion.

213. **Girardi, G. and M. M. Elias (1991). "Effectiveness of N-acetylcysteine in protecting against mercuric chloride-induced nephrotoxicity." Toxicology 67(2): 155-64.**

Mercuric chloride (HgCl2)-induced nephrotoxicity, as measured by functional and biochemical parameters was evaluated in rats at different kidney non-protein sulfhydryls (NPS) levels. Diethylmaleate (DEM) induced a 75% of NPS diminution 1 h after the administration. Renal function (clearance) and biochemical measurements (gamma- glutamyltranspeptidase activity in urine, and lipoperoxides in kidney tissue) were impaired when the animals were HgCl2-treated. Values were highly impaired when the kidneys were NPS-depleted and were improved when NPS pools were previously increased although they were not similar to control values. DEM treatment promoted a higher accumulation of HgCl2 in both kidney and liver while NAC-treatment reduced significantly the metal content in these organs. These data are in favour of a positive relationship among mercury content and organ injury. On the other hand, mercury content increased while NPS levels diminished. NPS might play a role in the HgCl2 detoxification and thus avoids mercury accumulation and mercury effects.

214. **Lorber, A., W. A. Baumgartner, R. A. Bovy, C. C. Chang and R. Hollcraft (1973). "Clinical application for heavy metal-complexing potential of N- acetylcysteine." J Clin Pharmacol 13(8): 332-6.**

215. **Lund, M. E., W. Banner, Jr., T. W. Clarkson and M. Berlin (1984). "Treatment of acute methylmercury ingestion by hemodialysis with N- acetylcysteine (Mucomyst) infusion and 2,3-dimercaptopropane sulfonate." J Toxicol Clin Toxicol 22(1): 31-49.**

A case of acute methylmercury ingestion was treated sequentially with oral D-penicillamine, hemodialysis during N-acetylcysteine (NAC) infusion, and 2,3-dimercaptopropane sulfonate (DMPS) an experimental oral agent. Urinary organic mercury elimination rate increased almost 40-fold during and 84-fold after hemodialysis with NAC infusion, compared with elimination during initial D-penicillamine therapy. Mean clearance during hemodialysis was only 13 ml/min with an extraction rate of 3.7 mcg/min. Although whole blood mercury concentrations decreased from 568 to 265 ng/ml during dialysis, a rebound to 525 ng/ml occurred. A total of 1.6 mg mercury was renally eliminated during hemodialysis and in the following 24 hours. A total of 3.3 mg of predominantly organic mercury was renally eliminated during 18 days of combined therapies. Since renal elimination of inorganic mercury is seen with chronic methylmercury poisoning, the high ratio of organic to inorganic mercury in urine supports the acute nature of this exposure. DMPS was begun on day 4 and during the two weeks of administration whole blood concentrations fell by 15% to 355 ng/ml. An expected decrease in elimination half-life to 10 days was not observed during DMPS therapy, possibly due to concurrent administration of vitamins containing zinc and copper. The amount of methylmercury ingested was estimated as 45 mg, based on a post-distribution blood concentration of approximately 450 ng/ml. The patient developed no symptoms of methylmercury poisoning during the one year after the episode. We conclude that NAC may be useful to enhance renal elimination of methylmercury and merits further investigation as a potential binding agent to reduce the body burden of methylmercury.

216. **Zalups, R. K. and D. W. Barfuss (1998). "Participation of mercuric conjugates of cysteine, homocysteine, and N- acetylcysteine in mechanisms involved in the renal tubular uptake of inorganic mercury." J Am Soc Nephrol 9(4): 551-61.**

Mechanisms involved in the renal uptake of inorganic mercury were studied in rats administered a nontoxic 0.5 mumol/kg intravenous dose of inorganic mercury with or without 2.0 mumol/kg cysteine, homocysteine, or N-acetylcysteine. The renal disposition of mercury was studied 1 h after treatment in normal rats and rats that had undergone bilateral ureteral ligation. In addition, the disposition of mercury (including the urinary and fecal excretion of mercury) was evaluated 24 h after treatment. In normal rats, coadministering inorganic mercury plus cysteine or homocysteine caused a significant increase in the renal uptake of mercury 1 h after treatment. The enhanced renal uptake of mercury was due to increased uptake of mercury in the renal outer stripe of the outer medulla and/or renal cortex. Ureteral ligation caused reductions in the renal uptake of mercury in all groups except for the one treated with inorganic mercury plus N-acetylcysteine. Thus, it appears that virtually all of the mercury taken up by the kidneys of the normal rats treated with inorganic mercury plus N-acetylcysteine occurred at the basolateral membrane. Urinary excretory data also support this notion, in that the rate of excretion of inorganic mercury was greatest in the rats treated with inorganic mercury plus N- acetylcysteine. Our data also indicate that uptake of inorganic mercury in the kidneys of rats treated with inorganic mercury plus cysteine occurred equally at both luminal and basolateral membranes. In addition, the renal uptake of mercury in rats treated with inorganic mercury plus homocysteine occurred predominantly at the basolateral membrane with some component of luminal uptake. The findings of the present study confirm that there are at least two distinct mechanisms involved in the renal uptake of inorganic mercury, with one mechanism located on the luminal membrane and the other located on the basolateral membrane. Our findings also show that cysteine and homologs of cysteine, when coadministered with inorganic mercury, greatly influence the magnitude and/or site of uptake of mercuric ions in the kidney.

217. **Montanini, S., D. Sinardi, C. Pratico, A. U. Sinardi and G. Trimarchi (1999). "Use of acetylcysteine as the life-saving antidote in Amanita phalloides (Death cap) poisoning - Case report on 11 patients." Arzneim. Forsch. - Drug Res. 49(12): 1044-1047.**

alpha-Amanitin is an amatoxin known to produce deleterious effects on the liver and the kidneys, when circulating in the blood. It is produced by a particular kind of mushroom called amanita phalloides. Therapeutic options employed to treat mushroom intoxication, such as haemodiaperfusion on activated charcoal, high dosages of penicillin G, oral charcoal, etc., very often failed to act properly and liver transplantation (when a graft is available) appeared to be the only solution. In recent years, as suggest by some authors, it has been postulated that the oxidant effects of alpha-amanitin could be counteracted by the use of antioxidants such as silibinin. High dosages of N-acelyl-cysteine (CAS 616-91-1, NAC), already used as antioxidant in paracetamol poisoning: were successfully used in our Intensive Care Unit (ICU) in the treatment of Amanita phalloides poisoning. In the last two years, 11 patients (mean age of 5-72 = 38.5) were treated for Amanita phalloides poisoning of various degrees, with a protocol (haemodiaperfusion on activated charcoal, high dosages of penicillin G. Etc.) further comprehending NAC (fluimucil(R)). All the patients recovered successfully but one (bearing precedent liver disease) needed liver transplantation. Daily monitoring of liver enzymes, creatinine, coagulation, LDH, blood and urinary alpha-amanitin were used to screen the progresses of the patients.

218. **Beloqui, O., J. Prieto, M. Suarez, B. Gil, C. H. Qian, N. Garcia and M. P. Civeira (1993). "N-acetyl cysteine enhances the response to interferon-alpha in chronic hepatitis-C: a pilot study." J. Interferon Res 13: 279-282.**

Hepatitis C virus (HCV) is an RNA virus that replicates in both the liver and lymphoid cells. Interferon-alpha (IFN-alpha) is a useful treatment of chronic hepatitis C (CHC) although resistance to this drug occurs frequently. The mechanisms underlying resistance to IFN remain unknown. In this work, we have measured the levels of glutathione in plasma and peripheral lymphoid cells from 15 healthy controls and 24 CHC patients, 10 of whom were without treatment and 14 showed high serum alanine aminotransferase (ALT) values despite therapy with lymphoblastoid IFN for more than 4 months. In all patients, glutathione levels in plasma and in mononuclear cells were depressed in comparison to controls. In IFN-unresponsive patients, the addition of 600 mg tid of oral N-acetyl cysteine (NAC), a glutathione precursor, resulted in a steady decrease of ALT values in all patients, with complete normalization in 41% of cases after 5-6 months of combined therapy. Administration of NAC alone for 1 month was without effect in the 10 patients that were not receiving IFN. Supplementation of IFN with NAC induced a near normalization of intralymphocytic glutathione, but plasma levels were only moderately increased. HCV replication was markedly inhibited in lymphocytes and viremia was cleared in one of the 8 patients tested. In conclusion, NAC enhances the response to IFN in CHC. Controlled studies are needed to ascertain whether antioxidant therapy might act in synergy with IFN in chronic viral hepatitis.

219. **Levy, E. M., J. M. Wu, M. Salibian and P. H. Black (1992). "The effect of changes in thiol subcompartments on T cell colony formation and cell cycle progression: relevance to AIDS." Cell. Immunol. 140(2): 370-380.**

220. **Marmor, M., P. Alcabes, S. Titus, K. Frenkel, K. Krasinski, A. Penn and R. W. Pero (1997). "Low serum thiol levels predict shorter times-to-death among HIV-infected injecting drug users." AIDS 11(11): 1389-1393.**

Objectives: To investigate whether serum thiol levels are altered by HIV disease, and whether low serum thiols predict time to death among HIV-infected injecting drug users (IDU). Design: A cross-sectional study of serum thiol levels among 13 HIV-seronegative IDU, 116 HIV-seropositive IDU, and 17 HIV-seropositive IDU with a history of AIDS, and a cohort study of the 133 HIV-infected IDU who took part in the cross-sectional study.

Methods: Subjects were recruited from a methadone-maintenance treatment program during 1990-1991. Total serum thiols were determined spectrophotometrically at enrolment; low serum thiols were defined as those with an absorbance at 412 nm less than or equal to 0.46. Deaths through 31 December 1993 were determined from the National Death Index (NDI). Twenty-six HIV-seropositive subjects died during follow up; death certificates, which were obtained for 23 subjects, indicated AIDS or HIV infection for 20. Product-limit estimation was used to calculate survival. Multivariate analyses employed Cox proportional-hazards regression.

Results: Analysis of cross-sectional data showed that serum thiols did not differ significantly among HIV-free subjects, HIV-infected subjects, and HIV-infected subjects with a history of AIDS. Cohort analysis, adjusted for age, revealed that persons with low serum thiols had a significantly increased hazard of death compared with those with high serum thiols (relative hazard = 2.83; 95% confidence interval (CI), 1.15, 6.97); a significant interaction between low serum thiols and a history of AIDS was associated with a relative hazard of 5.65 (95% CI, 1.22, 26.1).

Conclusions: Among HIV-infected persons, low serum thiols, especially in concert with a history of AIDS, predict mortality risk. These findings support the hypothesis that oxidative stress is critical to the pathogenesis of HIV infection.

221. **Westendorp, M. O., V. A. Shatrov, K. Schulze-Osthoff, R. Frank, M. Kraft, M. Los, P. H. Krammer, W. Droge and V. Lehmann (1995). "HIV-1 Tat potentiates TNF-induced NF-kappaB activation and cytotoxicity by altering the cellular redox state." EMBO J. 14(3): 546-554.**

This study demonstrates that human immunodeficiency virus type 1 (HIV-1) Tat protein amplifies the activity of tumor necrosis factor (TNF), a cytokine that stimulates HIV-1 replication through activation of NF-kappaB. In HeLa cells stably transfected with the HIV-1 tat gene (HeLa-tat cells), expression of the Tat protein enhanced both TNF-induced activation of NF-kappaB and TNF-mediated cytotoxicity. A similar potentiation of TNF effects was observed in Jurkat T cells and HeLa cells treated with soluble Tat protein, TNF-mediated activation of NF-kappaB and cytotoxicity involves the intracellular formation of reactive oxygen intermediates. Therefore, Tat-mediated effects on the cellular redox state were analyzed. In both T cells and HeLa cells HIV-1 Tat suppressed the expression of Mn-dependent superoxide dismutase (Mn-SOD), a mitochondrial enzyme that is part of the cellular defense system against oxidative stress. Thus, Mn-SOD RNA protein levels and activity were markedly reduced in the presence of Tat. Decreased Mn-SOD expression was associated with decreased levels of glutathione and a lower ratio of reduced:oxidized glutathione. A truncated Tat protein (Tat(1-72)), known to transactivate the HIV-1 long terminal repeat (LTR), no longer affected Mn-SOD expression, the cellular redox state or TNF-mediated cytotoxicity. Thus, our experiments demonstrate that the C-terminal region of HIV-1 Tat is required to suppress Mn-SOD expression and to induce pro-oxidative conditions reflected by a drop in reduced glutathione (GSH) and the GSH:oxidized GSH (GSSG) ratio. They further imply a distinct mechanism of Mn-SOD suppression as compared with HIV-1 LTR transactivation by Tat. Taken together, our data suggest that Tat expressed in HIV-1-infected cells and Tat taken up by non-infected cells modulates TNF activity by altering the cellular redox state. These findings may be relevant for HIV-1 replication and for T cell depletion in acquired immune deficiency syndrome.

222. **Opalenik, S. R., Q. Ding, S. R. Mallery and J. A. Thompson (1998). "Glutathione depletion associated with the HIV-1 TAT protein mediates the extracellular appearance of acidic fibroblast growth factor." Arch Biochem Biophys 351(1): 17-26.**

Primary murine embryonic fibroblasts transfected with HIV-1 TAT demonstrated decreased levels of high energy phosphates (ATP, GTP, UTP/CTP), adenine nucleotides (ATP, ADP, AMP), and both NAD+/NADH redox pairs, resulting in a substantial loss of redox poise. A greater than 50% decrease in intracellular reduced glutathione (GSH) concentration was accompanied by the extracellular appearance of acidic fibroblast growth factor (FGF-1). Addition of either N-acetyl-L-cysteine or glutathione ester (GSE), but not L-2-oxothiazolidine 4-carboxylate, partially restored intracellular GSH levels and resulted in loss of extracellular FGF-1. Treatment of FGF-1-transduced cells with buthionine sulfoximine (BSO) resulted in a time- and dose-dependent decrease in total cellular GSH concentration that was accompanied by the extracellular appearance of FGF-1. Inclusion of GSE during BSO treatment eliminated the extracellular appearance of FGF-1. BSO treatment of cells transfected with a mutant form of FGF-1, in which all three cysteine residues were replaced with serines, also decreased total cellular GSH concentration but failed to induce the extracellular appearance of FGF-1. Collectively, these results suggest that HIV-1 TAT induces a condition of oxidative stress, which mediates cellular secretion of FGF-1, an observation relevant to the pathophysiologic development and progression of AIDS-associated Kaposi's sarcoma.

223. **Choi, J., R. M. Liu, R. K. Kundu, F. Sangiorgi, W. Wu, R. Maxson and H. J. Forman (2000). "Molecular mechanism of decreased glutathione content in human immunodeficiency virus type 1 Tat-transgenic mice." J Biol Chem 275(5): 3693-8.**

Human immunodeficiency virus (HIV) progressively depletes GSH content in humans. Although the accumulated evidence suggests a role of decreased GSH in the pathogenesis of HIV, significant controversy remains concerning the mechanism of GSH depletion, especially in regard to envisioning appropriate therapeutic strategies to help compensate for such decreased antioxidant capacity. Tat, a transactivator encoded by HIV, is sufficient to cause GSH depletion in vitro and is implicated in AIDS-associated Kaposi's sarcoma and B cell lymphoma. In this study, we report a decrease in GSH biosynthesis with Tat, using HIV-1 Tat transgenic (Tat+) mice. A significant decline in the total intracellular GSH content in liver and erythrocytes of Tat+ mice was accompanied by decreased gamma-glutamylcysteine synthetase regulatory subunit mRNA and protein content, which resulted in an increased sensitivity of gamma-glutamylcysteine synthetase to feedback inhibition by GSH. Further study revealed a significant reduction in the activity of GSH synthetase in liver of Tat+ mice, which was linearly associated with their GSH content. Therefore, Tat appears to decrease GSH in vivo, at least partially, through modulation of GSH biosynthetic enzymes.

224. **Ehret, A., M. O. Westendorp, I. Herr, K.-M. Debatin, J. L. Heeney, R. Frank and P. H. Krammer (1996). "Resistance of chimpanzee T cells to human immunodeficiency virus type 1 Tat-enhanced oxidative stress and apoptosis." J. Virol. 70(9): 6502-6507.**

CD4(+) T-cell depletion in AIDS patients involves induction of apoptosis in human immunodeficiency virus (HIV)-infected and noninfected T cells. The HIV type 1 (HIV-1)-transactivating protein Tat enhances apoptosis and activation-induced cell death (AICD) of human T cells, This effect is mediated by the CD95 (APO-1/Fas) receptor-CD95 ligand (CD95L) system and may be linked to the induction of oxidative stress by Tat. Here we show that HIV-1 Tat-induced oxidative stress is necessary for sensitized AICD in T cells caused by CD95L expression. Tat-enhanced apoptosis and CD95L expression in T cells are inhibited by neutralizing anti-Tat antibodies, antioxidants, and the Tat inhibitor Ro24-7429. Chimpanzees infected with HIV-1 show viral replication resembling early infection in humans but do not show T-cell depletion or progression towards AIDS. The cause for this discrepancy is unknown. Here we show that unlike Tat-treated T cells in humans, Tat-treated chimpanzee T cells do not show downregulation of manganese superoxide dismutase or signs of oxidative stress. Chimpanzee T cells are also resistant to Tat-enhanced apoptosis, AICD, and CD95L upregulation.

225. **Aukrust, P., F. Muller, E. Lien, I. Nordoy, N. B. Liabakk, D. Kvale, T. Espevik and S. S. Froland (1999). "Tumor necrosis factor (TNF) system levels in human immunodeficiency virus-infected patients during highly active antiretroviral therapy: Persistent TNF activation is associated with virologic and immunologic treatment failure." J. Infec. Dis. 179(1): 74-82.**

Because persistent tumor necrosis factor (TNF)-alpha activation may play a pathogenic role in human immunodeficiency virus infection, TNF component levels were assessed over 78 weeks in plasma and peripheral blood mononuclear cells (PBMC) during highly active antiretroviral therapy (HAART) in 40 HIV-infected patients. HAART induced a significant decline in plasma levels of TNF-alpha and soluble TNF receptors and was associated with a fall in the abnormally increased unstimulated and a rise in the abnormally low Mycobacterium avium complex-purified-protein derivative-

stimulated TNF-alpha released from PBMC, However, concentrations of these TNF components were not normalized. Patients with virologic and immunologic treatment failure after 52 weeks had higher levels of several TNF components than other patients early after initiation of therapy, also during periods with adequate virologic response. Although TNF components significantly decreased during HAART, these results support data indicating that full immunologic normalization is not achieved during such therapy. The persistent activation of the TNF system in a subgroup of persons may be involved in treatment failure.

226. **Walker, R. E., H. C. Lane, C. M. Boenning, M. A. Polis, J. A. Kovacs, J. Falloon, R. T. Davey, H. Sussman, L. Gabel, R. Correa-Coronas, H. Masur and A. S. Fauci (1992). "The safety, pharmacokinetics, and antiviral activity of N-acetylcysteine in HIV-infected individuals."** Abstract (MoB 022). VIII International AIDS Conference, Amsterdam.

227. **Walker, R. E., H. C. Lane, C. M. Boenning and A. S. Fauci (1992). "The safety, pharmacokinetics, and antiviral activity of N-acetylcysteine in HIV-infected individuals." Keystone Symposium- "Prevention and Treatment of AIDS": Abstract #Q 560.**

228. **Walker, R. E., C. M. Boenning, G. Murphy, M. A. Polis, J. A. Kovacs, J. Falloon, R. T. Davey, H. H. Sussman, L. Gabel, R. L. Correa-Coronas, H. Masur, H. C. Lane and A. S. Fauci (1993). "The safety, pharmacokinetics and antiviral activity of N-acetylcysteine in HIV-infected individuals." Am. Rev. Respir. Dis. 147: A1004.**

229. **Witschi, A., E. Junker, C. Schranz, R. F. Speck and B. H. Lauterburg (1995). "Supplementation of N-acetylcysteine fails to increase glutathione in lymphocytes and plasma of patients with AIDS." AIDS Res. Human Retrov. 11(1): 141-143.**

Because glutathione (GSH) in plasma and lymphocytes of HIV-infected patients is low, adjunct therapy with N-acetylcysteine (NAC) to restore GSH homeostasis has been proposed. To investigate the effect of NAC on the GSH status we treated six patients with AIDS with 1.8 g/day of NAC for 2 weeks. During treatment the plasma concentration of cysteine, a precursor for GSH synthesis, increased significantly. Nevertheless, there was no significant increase in GSH in plasma and peripheral blood mononuclear cells. The failure of sulfhydryl supplementation to increase GSH suggests that the low concentrations of the tripeptide are not the result of an increased consumption secondary to an oxidant stress, but rather the consequence of a decreased rate of synthesis of GSH in HIV infection.

230. **Jones, D. P., J. L. Carlson, P. S. Samiec, P. Sternberg Jr, V. C. Mody Jr, R. L. Reed and L. A. S. Brown (1998). "Glutathione measurement in human plasma Evaluation of sample collection, storage and derivatization conditions for analysis of dansyl derivatives by HPLC." Clin. Chim. Acta 275(2): 175-184.**

Literature values for human plasma GSH vary over 10-fold despite the use of apparently valid analytical procedures for GSH measurement. The purpose of this study was to develop a procedure to minimize error in sample collection, processing and storage that could contribute to such differences. HPLC with fluorescence detection of dansyl derivatives was used for quantification. The results show that collection of blood with a butterfly needle and syringe reduces overestimation due to limited hemolysis and that use of a preservation solution designed to inhibit autooxidation and enzymatic degradation allows quantitative recovery of both GSH and GSSG. Stability tests showed that non-derivatized samples were stable for at least 2 months at - 80 degrees while dansyl derivatives were stable in the dark at 0-4 degrees for 12 months. Results from 59 healthy individuals (20-43 years) provided a mean (+/-1 SD) GSH value of 2.09 +/- 1.14 micromolar. (C) 1998 Elsevier Science B.V. All rights reserved.

231. **Huengsberg, M., R. Waring, D. Moffitt, R. Round, J. Winer, M. Gompels and M. Shahmanesh (1998). "Serum cysteine levels in HIV infection. {LETTER, COMMENT}." AIDS 12(10): 1245.**

232. **Rapezzi, D., E. M. Porqueddu, L. Fenu, O. Racchi, A. M. Ferraris, G. F. Gaetani and A. Aceti (1998). "Survival of people who are HIV-1-positive and G6PD-deficient is unaffected by virus-induced oxidative stress [LETTER]." Lancet 351(9098): 264-5.**

233. **Malorni, W., R. Rivabene, B. M. Lucia, R. Ferrara, A. M. Mazzone, R. Cauda and R. Paganelli (1998). "The role of oxidative imbalance in progression to AIDS: Effect of the thiol supplier N-acetylcysteine." AIDS Res. Hum. Retroviruses 14(17): 1589-1596.**

In this study we investigate the redox profile of HIV+ patients at different stages of disease with regard to immunological parameters, i.e., the number of circulating CD4(+) and CD8(+) lymphocytes, For this purpose, peripheral blood mononuclear cells (PBMCs) obtained from healthy donors, HIV+ patients in the asymptomatic phase, long-term nonProgressors (LTNPs), and AIDS patients have been considered. Cells have been exposed in vitro to the prooxidizing agent menadione, which is able to induce superoxide anion formation, and the susceptibility of the cells to the induced oxidative stress was estimated. Moreover, the possibility that the susceptibility of the cells to oxidative stress might be reduced by preexposing them to the antioxidizing agent N-acetylcysteine (NAC) has also been analyzed. The results obtained can be summarized as follows: (1) treatment with the prooxidant agent is capable of inducing massive morphological alterations in PBMCs, In particular, a significant correlation was found between the decrease in number of CD4(+) lymphocytes in patients at different stages of disease and the susceptibility of their PBMCs to oxidative stress; (2) preincubation with NAC was able to preserve partially the ultrastructural characteristics of PBMCs isolated from HIV+ patients. In particular, a direct relationship was found between the efficacy of NAC protection and CD4 counts; (3) evaluation of the plasma index of peroxidation and the number of circulating CD4 lymphocytes indicates the existence of a positive correlation between "systemic" oxidative imbalance and stage of the disease; and (4) cells from LTNPs display either oxidative susceptibility or oxidative markers similar to those of healthy donor cells. Our study suggests that the redox profile of patients may be considered a predictive marker of AIDS progression and that the acute infection and the asymptomatic phase of the disease may represent a useful period in which the combined use of antiretroviral and antioxidant drugs may be beneficial.

234. **Roberts, R. L., V. R. Aroda and B. J. Ank (1995). "N-acetylcysteine enhances antibody-dependent cellular cytotoxicity in neutrophils and mononuclear cells from healthy adults and human immunodeficiency virus-infected patients." J. Infect. Dis. 172(6): 1492-1502.**

Patients with AIDS have decreased levels of the intracellular antioxidant, glutathione, in their circulating lymphocytes and plasma. N-acetylcysteine (NAC) increases intracellular stores of glutathione and has direct antioxidant properties. In this study, the effects of glutathione and NAC on the cytotoxicity of neutrophils and mononuclear cells were tested using cells from healthy controls and human immunodeficiency virus (HIV)-infected patients, NAC (1 and 5 mM) enhanced the antibody-dependent cellular cytotoxicity (ADCC) of neutrophils from healthy adult controls and HIV-infected adults and children, The antineoplastic drug, 1,3 bis(2-chloroethyl)-1-nitrosourea (BCNU), which depletes intracellular glutathione, inhibited the ADCC of neutrophils; the addition of NAC partially reversed this inhibition. Similar effects of BCNU and NAC were seen when the cytotoxicity of mononuclear cells was tested using CEM tumor cells bearing the HIV gp120 antigen as targets. Thus, NAC enhances various forms of cytotoxicity and may be beneficial to AIDS patients whose defects in leukocyte cytotoxicity may be due to glutathione depletion.

235. **Droge, W., H.-P. Eck, H. Gmunder and S. Mihm (1991). "Modulation of lymphocyte functions and immune responses by cysteine and cysteine derivatives." Am. J. Med. 91 (Suppl. 3C): 140S-144S.**

AB - Mitogenically stimulated human peripheral blood lymphocytes and T cell clones were found to have weak membrane transport activity for the disulfide cystine but strong membrane transport activity for the thiol amino acid cysteine. Cysteine, however, is represented at the lowest concentration among all protein-forming amino acids in the blood plasma. Complementary laboratory experiments have shown that the cysteine supply is indeed limiting for important lymphocyte functions. Proliferative responses of mitogenically stimulated lymphocytes and T-cell clones and the activation of cytotoxic T cells in allogeneic mixed lymphocyte cultures are strongly influenced by small variations in the extracellular cysteine concentration even in the presence of relatively high andapproximately physiologic concentrations of cystine. Cysteine can besubstituted by N-acetylcysteine but not by cystine. The more detailedanalysis revealed that the extracellular supply of cysteine influencesstrongly the intracellular level of glutathione (GSH) and also the activity of the transcription factor NF kappa B that regulates theexpression of several immunologically relevant genes. In vitroexperiments including double-chamber experiments with macrophages and lymphocytes revealed, moreover, that cysteine plays an important role asa regulatory mediator between these cell types. The cysteine supply is impaired directly or indirectly in several pathologic conditions that are associated with immunodeficiencies, including the acquired immune deficiency syndrome (AIDS). Cysteine or cysteine derivatives may therefore be considered for the treatment of patients with HIV-1 infection.

236. Eylar, E., C. Rivera-Quinones, C. Molina, I. Baez, F. Molina and C. M. Mercado (1993). "N-acetylcysteine enhances T cell functions and T cell growth in culture." <u>Int Immunol</u> 5(1): 97-101.

N-Acetylcysteine (NAC) is highly nontoxic for peripheral blood T cells and immunostimulatory enhancing T cell functions such as mitogenesis, interleukin-2 (IL-2) production, and growth in culture. NAC has been proposed for the treatment of AIDS based on its inhibition of human immunodeficiency virus (HIV) replication in cultured cells. Therefore its effect on normal T cells from 10 young donors and one elderly donor has been investigated as a prelude to clinical consideration. T cell function was evaluated in the presence and absence of accessory cells. With concanavalin A and anti-CD3 activation, NAC enhanced mitogenesis by approximately 2- to 2.5-fold at 5-10 mM. Mitogenesis of purified T cells with anti-CD2 was not affected by NAC; in the presence of accessory cells, NAC enhanced mitogenesis by approximately 2-fold at 1- 10 mM. Importantly, NAC levels above 10 mM completely inhibited activation of peripheral blood mononuclear cells by anti-CD2. IL-2 secreted by T cells was also enhanced by NAC, approximately 1.5-fold, but IL-2 secreted by cells from old donors was enhanced by 3-fold. In cultures of peripheral blood T cells, NAC (10 mM) stimulated growth by at least 4- to 6-fold after two passages. These results show that NAC, nontoxic even at 20 mM, is an effective enhancer of T cell function and a remarkable enhancer of growth. Results from other laboratories show that NAC, which increases glutathione levels, suppresses HIV replication presumably via suppression of the activation of transcriptional factor NF-kappa B.(ABSTRACT TRUNCATED AT 250 WORDS)

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