For many years there has been a focus on indispensable (essential) amino acids in both enteral and parenteral nutrition. These are considered to be those the body cannot synthesize because it lacks the necessary enzymes. However, over the last 20 years evidence has accumulated that the requirement of some non-indispensable amino acids may be increased under certain conditions – either physiological (pregnancy and lactation) or pathological (for example, burns, cancer). In these situations, demand for the amino acid may exceed the capacity of the organism to synthesize it. Amino acids that behave in this way may be considered ‘conditionally indispensable’.

Among the amino acids that may be conditionally indispensable the most studied have been arginine and glutamine. There is evidence that glutamine supplementation of parenteral nutrition improves nitrogen balance and has beneficial effects on gut function, as glutamine is a major fuel for the intestine. With respect to arginine, there are data suggesting a potential role in the full development of the immune defense mechanisms. More recently, new data have been produced [1, 2] strongly suggesting that cysteine could also be a conditionally indispensable amino acid under various pathological conditions. This increased cysteine requirement is related to the inflammatory component of certain diseases, which generates specific needs for cysteine that are linked to the acute phase response and perhaps even more to oxidative stress.

The aim of this chapter is to give an overview of the metabolism of cysteine and its related metabolites (in particular glutathione) under physiological conditions, and of the modifications to cysteine metabolism that occur with injury and
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oxidative stress, and to try to understand the nutritional implications of these modifications.

**Roles of Cysteine and its Metabolites**

**Cysteine**
There are different pathways of cysteine utilization. Quantitatively the most important is protein synthesis. Cysteine residues are major components of the catalytic sites of many enzymes. The thiol group on the cysteine molecule allows the formation of disulfide bonds which are necessary for protein structure. Through its reversible biotransformation into cystine, cysteine is also an antioxidant and participates in the regulation of the redox status of cells. More recently, it has been suggested that cysteine could play a role in the regulation of immune function, mainly through modulating the translocation of NF-κB, a major transcriptional factor \[3\]. Finally, cysteine is the precursor of taurine and glutathione, both of which have important functions described later.

There are few data on the potential functions of this amino acid in catabolic states. Grimble \[4\] was probably the first to suggest that the high content of cysteine in some acute phase proteins could reflect a specific role of this amino acid in inflammatory syndromes. More recent data from other investigators have revealed the importance of cysteine in catabolic situations, either as the precursor of glutathione \[5, 6\], or through regulation of the transcriptional factor NF-κB \[3\].

**Taurine**
For many years it was thought that taurine was simply a catabolite of cysteine that was used to conjugate bile acids and that its main role was in the solubilization of fat. However, it is now known that taurine is ubiquitous; indeed it is present in mammal cells at the highest concentration of all the free amino acids, and particularly in nervous tissues and lymphocytes, where it accounts for 60% of the total free amino acids. For this reason, it has been suggested that taurine has an important function in the regulation of the immune system, and that efforts should be made to understand its function better \[7\]. In lymphocytes, taurine plays a role as an antioxidant and could participate in the control of peroxide ion production. It is also thought that taurine helps to stabilize cell membranes and to regulate ionic (Ca\(^{2+}\)) flux across membranes \[8\]. This last function seems to be important in the control of cardiac muscle contraction. It has been suggested that taurine participates in the development and maintenance of the brain, in thermoregulation, and in the control of sleep and food intake. It is also known to be important for retinal function.

Specific functions of taurine under catabolic conditions are poorly documented. However, on the basis of its role as an antioxidant, it could be important in protecting cells against oxidative stress.
Glutathione

Glutathione is ubiquitous in eukaryotic cells and is the most abundant non-protein thiol compound in mammalian cells. It is involved in many cellular functions, and particularly in lymphocyte function [9].

Glutathione is one of the most important antioxidant and reducing agents of the body, through its conversion from the reduced form (GSH) to the oxidized form (GSSG). It is active in the neutralization of free radicals and reactive oxygen intermediates, and also in protection against exposure to radiation and ultraviolet light. It maintains the thiol groups of proteins in their reduced (–SH) form, which is essential in many cases for the maintenance of normal protein function. Glutathione plays an important role in maintaining the intracellular redox potential and in the metabolism of various compounds such as prostaglandins, leukotrienes, and oestrogens. It serves as a reservoir for cysteine and is of central importance in the detoxification of xenobiotics. It also participates in amino acid transport between cells [9].

As an antioxidant, glutathione is of primary importance in the defense against injury, particularly after oxidative stress.

Cysteine Metabolism under Healthy Conditions

Cysteine is not an indispensable amino acid, as all mammalian species can synthesize it from methionine and serine. It has several different functions (protein synthesis, glutathione synthesis, taurine synthesis, and so on). It is therefore in equilibrium in the body when the various pathways of utilization are balanced by dietary intake and de novo synthesis.

Cysteine Synthesis

The major pathway of methionine degradation in mammals is the transmethylation-transulfuration pathway, which leads to cysteine synthesis [10] (Fig. 1). This pathway can occur in different tissues, but the liver is quantitatively the most important site. Transmethylation induces homocysteine synthesis in three steps, but is reversible through the remethylation pathway. In mammals, this pathway is the major source of methyl groups that are used in the synthesis of compounds such as choline and creatinine (step 2 from S-adenosyl-L-methionine to S-adenosyl-L-homocysteine). Through the action of cystathionine β-synthetase, homocysteine can condense with serine in an irreversible reaction producing cystathionine, then cleaving into cysteine and α-cetobutyrate.

Cysteine Utilization

Among the different pathways of cysteine utilization, only two are significant from a quantitative point of view: those for protein synthesis and glutathione synthesis. Glutathione is a tripeptide composed of glutamate, glycine, and cysteine. It plays a pivotal role in cysteine utilization as about half the cysteine flux
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measured in healthy postabsorptive adults can be accounted for by the turnover of GSH [11]. The structure of this peptide is unique because the N-terminal glutamate of GSH is linked to cysteine through a \(\gamma\)-glutamyl bond, instead of an \(\alpha\)-peptidyl bond as usual in peptides. This makes glutathione resistant to peptidases and thus increases its stability.

Glutathione is present in tissues at millimolar concentrations but in plasma at micromolar concentrations. It is present in two forms. The reduced form (GSH) corresponds to the tripeptide with a free thiol group. The oxidized form (GSSG) is obtained by condensation of two GSH molecules through a disulfide bond between the two thiol groups. The ratio of GSH to GSSG varies from one tissue to another, but always favors the GSH form (ratio from 10 to 100).

A summary of glutathione metabolism is given in Figure 2. Glutathione metabolism occurs through the \(\gamma\)-glutamyl cycle which accounts for the synthesis and breakdown of GSH [12]. Most of glutathione synthesis occurs in the liver and kidneys in a two-step enzymatic reaction that takes place in the cytosol. The first step, catalyzed by \(\gamma\)-glutamylcysteine synthetase, induces the formation of \(\gamma\)-glutamylcysteine. This is the rate-limiting step in GSH synthesis and the reaction is subjected to feedback inhibition by GSH. In a second step, the action of

Fig. 1. Metabolism of methionine.
the glutathione synthetase allows formation of GSH. Substrates for glutathione synthesis are provided either by the transport of amino acids into the cells or by the action of the γ-glutamyl transpeptidase, which is the key enzyme responsible for interorgan flow and breakdown of GSH.

The degradation of glutathione occurs extracellularly in an export-import process involving GSH and its constitutive amino acids. Glutathione metabolism is linked to interorgan flows of glutathione and therefore must be considered at the level of the whole organism. The liver is a net producer and exporter of GSH, as GSH efflux accounts for about 90% of the hepatic turnover of GSH [13]. The kidneys, lungs, and gut are the major consumers of glutathione. GSH uptake by the kidneys accounts for up to 70% of the hepatic production of glutathione. γ-Glutamyl transpeptidase is bound to the external surfaces of cell membrane. This enzyme is present in high concentrations in the kidneys and intestine. Circulating GSH is bound to the γ-glutamyl transpeptidase and transpeptidation transfers the γ-glutamyl moiety to an amino acid acceptor, which is often cystine. This reaction leads to the formation of cysteinylglycine and γ-glutamylcysteine which are often internalized. The former is split into glycine and cysteine and the latter is reduced in cysteine and γ-glutamylcysteine. These metabolites can be
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used for new glutathione synthesis. If transpeptidation induces the formation of another γ-glutamyl amino acid instead of γ-glutamyl cystine, it is converted into 5-oxoproline, which is metabolized to glutamate and the corresponding amino acid. In the intestine, the transpeptidation reaction allows the reabsorption of any GSH present in the lumen because of exportation from the liver in bile. In the kidneys, intestine, and lung, GSH can also enter cells as the whole peptide, without any degradation. The quantitative importance of this is unknown.

GSH is also the substrate of various GSH S-transferases, enzymes that catalyze the conjugation of xenobiotic compounds. The GSH S-conjugates follow the mercapturic pathway with formation of S-conjugates of N-acetylcysteine and elimination in the urine. This pathway therefore induces a net loss of cysteine and leads to decreased tissue GSH concentrations.

Finally, GSH serves as an antioxidant by reacting directly with reactive oxygen species through the action of GSH peroxidase. In this reaction, GSH is converted to the oxidized form (GSSG), which in turn can be recycled back to the reduced form in a reaction catalyzed by GSH reductase. Under normal conditions, the cell maintains GSH primarily in the reduced form, GSSG being present only to a limited extent.

Cysteine Catabolism

Cysteine catabolism pathways are usually described as two groups of pathways: the cysteine sulfinate pathway, which is considered to be the major one, and the cysteine sulfinate-independent pathway (Fig. 3). Both pathways ultimately allow the production of sulfate, but only the cysteine sulfinate pathway induces production of taurine.

In the first step, the cysteine sulfinate pathway leads to the production of cysteine sulfinate, which can be either decarboxylated into hypotaurine and then further oxidized to taurine, or deaminated and oxidized finally into CO₂ and sulfate. The cysteine sulfinate-independent pathway is in fact a grouping of different metabolic pathways, but all finally cause liberation of pyruvate and sulfur, the latter being oxidized and eliminated as sulfate.

Nutritional Regulation of Cysteine Metabolism

Nutritional Regulation of Cysteine Synthesis

The nutritional regulation of methionine catabolism (and thus of cysteine synthesis) has been much studied and was reviewed by Finkelstein [10]. Methionine degradation increases with the protein content (and the methionine content) of the diet. It is also generally considered that cyst(e)ine in the diet has a sparing effect on methionine (ranging from 20 to 80% of the total methionine requirement) by reducing methionine oxidation. This regulation is documented by measurement of the hepatic enzyme activities involved in methionine catabolism [14] and the rate of [¹⁴C]methionine oxidation [15]. The sparing effect is not due to methionine synthesis from cysteine as the cystathionine synthase reaction is irreversible. Dietary cystine seems to exert a methionine-sparing effect at two levels: the major
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Fig. 3. Metabolism of cysteine.

regulatory site would be the distribution of homocysteine between remethylation and cystathionine synthesis. The second regulatory level is linked to competition between protein synthesis and the formation of S-adenosylmethionine. However, using tracer techniques which permit a quantitative estimation of the partitioning of methionine through transmethylation, remethylation, and transulfuration, recent results seem to indicate that the efficacy of cystine in sparing methionine depends on the relative levels of methionine and cystine in the diet [16, 17]. Some of the data are therefore still controversial, but it appears that de novo cysteine synthesis accounts for less than 15% of the total cysteine flux. Therefore the biggest proportion of the cysteine flux comes from the diet and from protein and glutathione breakdown.

Nutritional Regulation of Cysteine Catabolism

In hepatocytes, basal metabolism seems to be equilibrated between cysteine sulfinate and cysteine sulfinate-independent pathways. The taurine pathway represents about 10% of total catabolism [18]. Production of each of the major
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metabolites of cysteine increases as protein, methionine, or cysteine concentration increases in the culture cell medium, but the partitioning is modified depending on the concentration. Low cysteine availability (0.2 mmol/l) favors its utilization for glutathione and high availability increases catabolism through the sulfate and taurine pathways [19]. Furthermore, high protein diets favor the sulfate pathway against the taurine pathway. By contrast, if the sulfur amino acids are specifically increased in the diet, the percentage of cysteine catabolized as taurine is increased [20]. Modulation of other macronutrients of the diet does not influence cysteine.

Nutritional Regulation of GSH Status

There is a complex regulation of GSH homeostasis. It is difficult to increase tissue GSH concentration above physiological levels because of the feedback inhibition of γ-glutamylcysteine synthetase. This feedback regulation is an important regulatory mechanism for limiting the maximum tissue concentration [9].

The availability of substrates is the major determinant of hepatic GSH concentration within the physiological range. Glutamate and cysteine are the two substrates of the rate-limiting step in the glutathione synthesis pathway, and therefore seem to be the main candidates for such regulation. Glutamate and glutamine are generally present in large excess in the cell, and the cysteine concentration is low in tissues and plasma. For instance, in the liver, cysteine is maintained at a concentration approximately 50 times lower than GSH. For this reason, we would expect cysteine to be the rate-limiting amino acid, even when dietary sulfur amino acid intake is increased [9, 21]. Starvation induces GSH depletion in liver, intestine, and muscle of rats [22]. Similar GSH depletion has been reported in animals receiving a protein-deficient diet, either in rat liver [21, 23] or in pig erythrocytes [24], but also in the liver of chicks receiving a cysteine-deficient diet [25]. In contrast, it seems that the erythrocyte GSH concentration is independent of diet in rats and chicks [22, 25]. Furthermore, supplementation of a diet low in either protein or sulfur amino acids with cysteine or 2-oxothiazolidine-4-carboxylate (a precursor of cysteine) leads to normalization of the glutathione concentration in various species [21, 22]. These results suggest that only cysteine is the limiting factor in maintaining glutathione status, and not glutamate. However, it cannot be ruled out that under catabolic conditions, where glutamine or glutamate are depleted, glutamate might also be rate limiting for glutathione synthesis [26].

The efficiency of different sulfur amino acids for glutathione synthesis has been compared. In isolated hepatocytes, cysteine was incorporated in glutathione at a higher rate than 2-oxothiazolidine-4-carboxylate, cystine, or methionine, but at a similar rate as N-acetylcysteine [19, 27]. On the other hand, cysteine is more rapidly metabolized into taurine and sulfate than N-acetylcysteine. Therefore cysteine appears to be the more available amino acid for cells. These results suggest that cystine, cysteine, GSH, and methionine are all equivalent.
as sources of cysteine for growth, but that cysteine is more efficiently utilized for glutathione synthesis.

**Modification of Cysteine Metabolism in Catabolic States**

The regulation of cysteine metabolism is thus partially understood in health. There are few data examining the effect of catabolic states or oxidative stress on these metabolic pathways. However, diseases such as renal or liver failure, sepsis, or other major stresses may compromise the body’s normal capacity to synthesize non-indispensable nutrients in such a way as to alter nutritional requirements. For this reason, it is now accepted that the sick individual, with either a chronic or an acute illness, may have amino acid requirements that are quite different from those of age-matched healthy control individuals [28].

Sulfur amino acids, and especially cysteine and glutathione, are unstable because of their susceptibility to oxidative processes. Furthermore, amino acid concentrations are sensitive to nutritional status, and it is well known that catabolic states can modify this. For instance, plasma amino acid concentrations are higher in septic rats than in pair-fed control animals [6]. It is therefore very important to take into account the nutritional status of patients or animals.

For these reasons, cysteine and glutathione concentrations are somewhat difficult to interpret and even more so when comparisons are attempted between different studies.

**Taurine**

Data on taurine concentrations under pathological conditions are controversial. Plasma concentrations have been found to be increased in sepsis [29] but decreased in severe trauma [30]; another study found concentrations to be decreased during sepsis [31]. The significance of these discrepancies is still unclear.

**Cyst(e)ine and Methionine**

Effect of Catabolic States on Circulating Concentrations

In most studies, only cystine has been measured and has been assumed to be representative of total cysteine + cystine, because most of the cysteine is oxidized to cystine during the technical process. Moreover, cysteine can bind to proteins but the bound form is generally not measured. However, the amount of free cystine can vary according to the speed of the sampling processing. Most studies from the 1980s considered that sulfur amino acid concentrations are either maintained or increased in catabolic states. The increase is generally more pronounced for methionine than for cyst(e)ine [29, 30]. More recent studies, however, now show that there are low plasma cysteine concentration in HIV patients [32]. In Ghanaian volunteers, decreased serum cysteine and blood glutathione have been found in individuals with evidence of liver inflammation,
Cysteine and Glutathione in Catabolic States

and it was suggested that deficits in cysteine and glutathione may increase the risk of liver toxicity [33]. In fact, a combination of abnormally low cyst(e)ine and glutathione levels, low natural killer cell activity, and skeletal muscle wasting or muscle fatigue is found in patients with different pathologies such as HIV infection, cancer, major injuries (sepsis), and Crohn’s disease. The hypothesis of a role of cysteine and glutathione in muscle wasting and immunological dysfunction has been discussed but not demonstrated [34].

Hypotheses concerning the effects of catabolic states on sulfur amino acid concentrations and the reasons for these modifications are thus controversial. However, most studies have described decreased concentrations of these amino acids in patients with chronic diseases such as AIDS, or moderately severe injury. In contrast, increased concentrations are generally described in acute severe disorders such as septic shock [29]. Decreased concentrations could reflect the increased utilization of sulfur amino acids in response to catabolic stress and the associated oxidative stress (as this would result in increased glutathione synthesis). Very acute stress could cause liver dysfunction, and this would compromise the body’s capacity to synthesize glutathione and the acute phase proteins. This may cause accumulation of the sulfur amino acids and could explain the observations of Freund et al. [29] of a dramatic increase in cystine concentration in fatal cases of sepsis, but concentrations within the normal range in survivors.

Disturbances of Cysteine Metabolism in Catabolic States

In premature infants, cysteine has been considered to be an indispensable amino acid because synthesis from methionine through the transulfuration pathway is very low. The reason for this is that cystathionase activity is not sufficient to support adequate cysteine synthesis.

Only two studies have described the effect of injury on cysteine synthesis from methionine and the results were conflicting. In rats exposed to surgical stress, Vina et al. [35] found an increased plasma methionine to cysteine ratio, impaired cystathionase activity, and a decreased rate of cysteine synthesis from methionine. They concluded that cysteine might become indispensable during surgical stress not because of an increased metabolic demand but because of a decreased cysteine supply from the transulfuration pathway. However, Malmezat [6] found that cystathionase activity in septic rats was similar in infected and pair-fed animals and that the fraction of cysteine flux coming from methionine was increased in infected animals. He concluded that the cysteine requirement was probably increased during infection owing to increased metabolic demand, and that the transulfuration pathway was not stimulated sufficiently to cover the demand. This hypothesis seems to be in agreement with the fact that cysteine coming from the transulfuration pathway accounts for 5–15% of the cysteine flux, depending on the species (human and rat) and on the nutritional status (fed or postabsorptive) [6, 11].
Cysteine and Glutathione in Catabolic States

Burn-injury patients do not have an impaired ability to oxidize methionine and cysteine [36]. Patients with mild forms of liver dysfunction also show normal metabolism of sulfur amino acids [37]. As observed in healthy individuals, the sulfate pathway is the major elimination pathway for sulfur in burn patients, but the relative importance of taurine elimination is increased [36]. Furthermore, incorporation of a complete amino acid mixture in the parenteral diet induces better improvement in sulfur balance than in nitrogen balance, suggesting good utilization of sulfur amino acids in these patients. In contrast, when liver dysfunction increases in severity (as with different degrees of cirrhosis), there is a decreased ability to metabolize sulfur amino acids, initially at the level of the transulfuration pathway, and later at the level of cysteine oxidation into sulfate and taurine. Thus sulfur amino acid metabolism needs to be carefully monitored in patients with advanced forms of liver disease [37].

Decreased catabolism of sulfur amino acids in association with acute stress does not always imply decreased liver function. Different studies have shown that a decrease in sulfur amino acid catabolism was associated with an increase in utilization, suggesting active regulation. Thus in septic rats both a dramatic increase in the whole body content of cyst(e)ine and a concomitant decrease in the catabolism of this amino acid have been observed simultaneously [2]. Hunter and Grimble [38] found a reduction in the excretion of urinary inorganic sulfate following injection of tumor necrosis factor (TNF) in rats. After injection of $^{35}$S-cysteine, septic rats showed decreased $^{35}$S-sulfate production in all tissues (reflecting an overall decrease in cysteine catabolism) and decreased taurine production in the kidneys, spleen, and gut. At the same time, incorporation of the tracer in hepatic taurine and in proteins and glutathione in most splanchnic tissues was increased [2]. These results all suggest that cysteine was spared during sepsis in order to synthesize the GSH necessary for protection against the oxidative stress associated with sepsis. The increased synthesis of taurine and acute phase proteins in the liver could also contribute to an increased requirement for sulfur amino acids.

Glutathione

Glutathione is the major intracellular antioxidant and its function in the protection of cells against free radical reactive oxygen species and toxic compounds is essential in the mechanism of defense against injury. Thus catabolic states are generally associated with increased utilization of glutathione, which can lead to depletion of the glutathione pools. Unfortunately, depletion of glutathione in the tissues is not always associated with a decrease in blood. This discrepancy between blood and tissue measurements has been observed in rats [22] and chicks [25], and it appears that the blood concentration of glutathione is maintained within a physiological range in different states of nutrition and under pathological conditions. Nevertheless, blood glutathione may be a more sensitive indicator of tissue levels in the human. In chronic hepatitis C, for example, the glutathione level in the plasma and in peripheral blood mononuclear cells is
Cysteine and Glutathione in Catabolic States

positively correlated with liver GSH [39]. However, the link between blood and tissue concentrations is not clearly established in the human, and in mild injury it has been shown that glutathione may be depleted in muscle but be unchanged in blood [40].

Glutathione loss can be induced in different ways. Oxidative stress induces oxidation of GSH in GSSG, and a low ratio of GSH to GSSG has been observed in certain clinical conditions such as HIV infection [41] and peritonitis [42]. However, the concentration of GSSG is tightly regulated, so GSSG is rapidly reduced to GSH by GSH reductase. GSSG is also actively transported from the cells into the extracellular space for elimination, so a decrease in intracellular glutathione level sometimes accounts for the efflux of GSSG from the cells [43]. In this case, there is a net loss of GSH, but GSSG can be maintained in the physiological range, as observed after zymozan inflammation in rats [42]. This implies that a catabolic state or an oxidative stress does not induce an obligatory increase in GSSG.

Various pathologies (either chronic or acute) have been associated with low GSH status. GSH is depleted in plasma, erythrocytes, and peripheral blood mononuclear cells in HIV patients [44, 45] or after viral infections [39, 46], in the gut of patients with chronic inflammatory disorders of the colon [47], and in muscle in patients undergoing elective abdominal surgery [40]. Similar data were found in clinical situations associated with oxidative damage [48]. In animal models, GSH was low in erythrocytes and the jejunal mucosa of protein-deficient pigs after the induction of inflammation with turpentine [24], and in the liver of rats exposed to a long-term model of sepsis (Fig. 4). However, in this latter case, the response was biphasic and account needs to be taken of the nutritional status of animals: pair-fed control rats showed a depleted GSH level after 2 days of food restriction, and then a progressive recovery on days 6 and 10, when food intake was normalized; on the other hand, infected animals had a normal liver glutathione level 2 days after infection (and therefore an increased level compared with pair-fed controls), but an acutely depleted level on days 6 and 10 after infection. Several investigators have reported early depletion of glutathione status in the first hours following stress, and a nearly normal or sometimes increased level 24 h later [5, 24, 42, 49]. Thus glutathione status may follow a rebound kinetic: the increased consumption of glutathione owing to catabolism would rapidly induce depletion of glutathione stores; between 12 and 24 h after the initial stress, a rebound effect occurs, resulting in a net increase in organ levels of glutathione [48]. This rebound could reflect a stimulation of glutathione synthesis, which would be a mechanism whereby cellular adjustment to stress occurs. Such an adaptation is possible if substrate availability is sufficient. After prolonged stress, the low availability of nutrients linked to anorexia could induce depletion of GSH status, as shown in Figure 4. This might occur in various clinical situations characterized by low glutathione.

These data suggest that monitoring the glutathione concentration is sometimes a poor indicator of disturbances of glutathione metabolism. For this reason,
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Fig. 4. Effect of infection on liver glutathione concentration in rats. Infection was induced in rats by an intravenous injection of live bacteria. The liver glutathione concentration was measured in well-fed animals (●, day 0), in infected animals (■) and pair-fed animals (□) 2, 6 and 10 days after infection [Breuillé D, Malmzat T, Rosé F, Pouyet C, Obled C. Assessment of tissue glutathione status during experimental sepsis. Clin Nutr 1994; 13: 5]. *p < 0.05 vs day 0. †p < 0.05 vs pair-fed rats.

Attempts have recently been made to measure the GSH synthesis rate or the activity of the enzymes implicated in GSH synthesis. Lung epithelial cells from rats exposed to oxidative stress or glutathione depletion show activation of transcription of the regulatory subunit of γ-glutamylcysteine synthetase. In vivo, this enzyme seems unaffected by the inflammatory response, showing that the GSH-synthesizing capacity should not be compromised [5, 6]. These observations suggest that substrate availability may be the major determinant of glutathione synthesis in vivo, though in one study, the decreased glutathione concentration observed in muscle after surgical trauma was correlated with low glutathione synthetase activity [40]. However, this correlation has not been reported by other investigators, and glutathione synthetase is not generally considered to be the rate-limiting enzyme. Following indirect estimation of the glutathione synthesis rate in rats injected with TNFα, a positive correlation was found between increased glutathione concentrations and the synthesis rate observed in the liver [5]. Using a direct method for measuring glutathione synthesis, concomitant increases in the concentration and synthesis rate were found in liver, spleen, muscle, lung, heart, and large intestine of infected rats [6] (Table 1, 2). These variables were unchanged in the small intestine and decreased in the blood. These results are in agreement with those found in erythrocytes of HIV patients [45] and in the intestine of pigs fed an adequate protein level in the diet [24]. Thus the data indicate that the glutathione synthesis rate is increased in nearly all tissues after infection or inflammation, with the exception of erythrocytes and the small
Cysteine and Glutathione in Catabolic States

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Pair-fed rats</th>
<th>Infected rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/g tissue</td>
<td>µmol/g tissue</td>
</tr>
<tr>
<td>Liver</td>
<td>3.52 ± 0.21</td>
<td>8.18 ± 1.40*</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.45 ± 0.09</td>
<td>3.22 ± 0.22</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>0.56 ± 0.13</td>
<td>0.78 ± 0.11*</td>
</tr>
<tr>
<td>Lung</td>
<td>1.81 ± 0.09</td>
<td>2.01 ± 0.11*</td>
</tr>
<tr>
<td>Heart</td>
<td>1.53 ± 0.11</td>
<td>2.02 ± 0.14*</td>
</tr>
<tr>
<td>Small intestine</td>
<td>2.45 ± 0.21</td>
<td>2.61 ± 0.21</td>
</tr>
<tr>
<td>Large intestine</td>
<td>1.65 ± 0.14</td>
<td>1.94 ± 0.18*</td>
</tr>
<tr>
<td>Blood</td>
<td>1.60 ± 0.09</td>
<td>0.79 ± 0.21</td>
</tr>
</tbody>
</table>

Infection was induced in rats by an intravenous injection of live bacteria. Control animals were pair-fed to infected animals, since infection induced a large decrease in food intake. Meal values ± SD are given for 8 animals in pair-fed and infected rats. Significantly different from pair-fed rats, p < 0.05.

intestine. Furthermore, increased or decreased synthesis rates were associated with parallel changes in concentration.

A critical point is the demonstration of a link between GSH depletion and clinical disturbances. GSH deficiency could be one of several factors responsible for immune deficiency in HIV infection. Immune disorders linked to glutathione deficiency are associated with lymphocyte proliferation and activation, natural killer cell activation, and cytotoxic T-cell activity [44]. The clinical relevance of GSH depletion is strongly suggested by the association between GSH deficiency and impaired survival in AIDS [50]. In chronic hepatitis C, the level of glutathione, as measured in plasma, peripheral blood mononuclear cells, and liver, correlated significantly with the replication of hepatitis virus C and the activity of the liver disease [39]. GSH depletion in lymphoid cells may interfere with the immunological mechanisms implicated in viral clearance. In patients with common variable immunodeficiency, the depletion of total and reduced glutathione observed in CD4+ lymphocytes was strongly associated with increased TNFα level in the serum [46]. Furthermore, interleukin (IL)-2 production from peripheral blood mononuclear cells was increased in these patients after supplementation of cell cultures with GSH-monoethyl ester. GSH depletion, or a decreased ratio of GSH to GSSG, is therefore associated with immunological disorders by mechanisms which need further clarification.

**Nutritional Modulation of Catabolic States by Sulfur Amino Acids**

The data reported above suggest a possible increase in cyst(e)ine requirement in catabolic states. A few trials modulating the level of sulfur amino acids in the diet have been performed in animals and humans. We have discussed
Table 2. Glutathione synthesis rates in tissues of infected and pair-fed control rats

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Fractional synthesis rate</th>
<th>Absolute synthesis rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%/day</td>
<td>µmol/tissue-day</td>
</tr>
<tr>
<td></td>
<td>pair-fed rats</td>
<td>infected rats</td>
</tr>
<tr>
<td></td>
<td>pair-fed rats</td>
<td>infected rats</td>
</tr>
<tr>
<td>Liver</td>
<td>385 ± 33</td>
<td>534 ± 117†</td>
</tr>
<tr>
<td>Spleen</td>
<td>249 ± 46</td>
<td>342 ± 36†</td>
</tr>
<tr>
<td>Muscle†</td>
<td>124 ± 44</td>
<td>209 ± 83†</td>
</tr>
<tr>
<td>Lung</td>
<td>188 ± 46</td>
<td>278 ± 68†</td>
</tr>
<tr>
<td>Heart</td>
<td>68.8 ± 16.2</td>
<td>86.6 ± 21.5</td>
</tr>
<tr>
<td>Small intestine</td>
<td>401 ± 52</td>
<td>394 ± 65</td>
</tr>
<tr>
<td>Large intestine</td>
<td>252 ± 80</td>
<td>491 ± 119†</td>
</tr>
<tr>
<td>Blood‡</td>
<td>40.1 ± 13.8</td>
<td>41.7 ± 0.1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Infection was induced in rats by an intravenous injection of live bacteria. Control animals were pair-fed to infected animals, since infection induced a large decrease in food intake. Animals were infused for 6 h with L-[15N]cysteine and glutathione synthesis rate was calculated from free and glutathione-bound cysteine enrichments in tissues at the end of the infusion.

1 For absolute synthesis rate calculation in whole skeletal muscle, skeletal muscle was estimated to be 45% of body weight for the pair-fed rats [Miller SA. In Munro HN, ed. Mammalian protein metabolism. New York: Academic Press, 1969: 3, 183–233] and as 40% of body weight for the infected rats [2].

2 For absolute synthesis rate calculation in whole blood, blood was estimated as 5.5% of body weight in the two groups [Miller SA. In Munro HN, ed. Mammalian protein metabolism. New York: Academic Press, 1969: 3, 183–233].

The total amount of glutathione synthesized in the whole body was estimated by summing the total glutathione synthesis of the tissues examined and extrapolating the value to the body weight of the rat.

Means values ± SD are given for 8 rats in each group (except for values obtained in liver where n = 5 for infected rats).

* Significantly different from pair-fed rats, p < 0.05.

how, in vitro, the sulfur amino acid concentration regulates cysteine metabolism to glutathione, sulfate, and taurine [19, 27], and how cysteine availability is probably the limiting factor in glutathione synthesis. It is therefore clinically relevant to determine whether manipulation of the diet can modulate glutathione status and other clinical variables.

In vitro, it has been shown that cystine uptake into endothelial cells is competitively inhibited by glutamate, so that incubation of cells with high concentrations of glutamate induce intracellular GSH depletion [43]. This observation needs to be borne in mind, but we do not know whether such competition has any clinical relevance.

Among the various possible precursors of cysteine, it is interesting to note that in healthy rats, GSH given in the diet is directly absorbed as intact GSH,
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and that oral gavage with GSH causes an increase in plasma glutathione for several hours [51]. However, this effect is transient and the effects on tissue GSH status are unknown. Furthermore we do not know how GSH is absorbed in man, especially in catabolic states or after an oxidative stress.

After intravenous administration of TNFα in rats fed a protein-deficient diet, supplementation with methionine or cysteine (but not alanine) induces a trophic reaction in the liver (increased weight and protein content), and an increase in glutathione content of liver and lungs but does not modulate liver protein synthesis [52]. After endotoxin administration, this effect has been shown to be associated with an increased glutathione synthesis rate in liver [5]. The effect was even more pronounced with methionine supplementation than with cysteine. These results are in agreement with those of Breuillé et al. [54], who demonstrated that cysteine supplementation of the diet of septic rats was followed by an increase in hepatic glutathione content back to normal values. However, their results indicate that this did not occur when methionine rather than cysteine was supplemented [54].

In AIDS patients, depletion of cysteine and glutathione was the rationale for treatment with N-acetylcysteine [34]. However, treatment of patients with 1.8 g/day of N-acetylcysteine for 2 weeks failed to increase glutathione in lymphocytes or plasma [55]. The authors suggest that the low GSH concentration might not be the result of oxidant stress, but rather was the consequence of a decreased rate of GSH synthesis. On the other hand, treatment of AIDS patients for up to 8 months with an average of 4.4 g of N-acetylcysteine/day caused replenishment of blood glutathione and improved survival [50]. In a recent study it was found that N-acetylcysteine supplementation for only 1 week at a level representing a 33% increase in the dietary intake of cysteine caused a marked increase in plasma and erythrocyte GSH levels, associated in most patients with an increase in GSH synthesis rate [45]. This study suggests that the glutathione deficiency in HIV infection is partly the result of reduced synthesis secondary to a shortage of cysteine. Results obtained in infected rats (see above) suggest that this clinical problem could be relevant in other clinical situations characterized by disturbances of cysteine and glutathione status, such as trauma or sepsis. Indeed, a recent paper [56] showed that infusion of N-acetylcysteine for 24 h (150 mg/kg) in patients with early septic shock improved respiratory function and shortened the length of stay of survivors in the intensive care unit. This effect was associated with attenuated production of IL-8, a potential mediator of septic lung injury.

References

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Discussion

Dr. Furst: I was a little surprised to see the glutathione levels following stress. Dr. Wernerman has shown that trauma is associated with a 40% decline in glutathione in muscle [1], and severe injury or critical illness is associated with a reduction to around 60% of the initial glutathione level [2], but you have shown here that there is an increase in synthesis.

Dr. Breuillé: I think the data from Wernerman’s team were obtained at a time when there was a depression of glutathione because of a lack of substrate for synthesis. I think they also showed an initial increase in the glutathione concentration but later on, when substrate deficiency occurs – cysteine and possibly glycine – there is a depression of glutathione concentrations. In our data, we observed during the acute phase an increase in synthesis which correlates with an increase in concentration. Later on, we observed a decrease in concentration but we have no measurement of synthesis at this time.

Dr. Furst: The question is of course the limiting amino acid. We just did a study measuring glutathione synthesis and catabolism in patients suffering from inflammatory bowel disease and in patients with tumors. We found that cysteine was not limiting in any of these patients, but glutamate and glycine were.

Dr. Breuillé: I saw the data but intracellular concentrations of glutamate or glycine are 20–40 times higher than the concentration of cysteine. This low cysteine concentration suggests that this amino acid could be limiting for glutathione synthesis. In our experiments, we gave supplements of either glutamine or cysteine, and we only found improvement with cysteine. The diets were isonitrogenous, which means that we had the same quantity of nitrogen as glutamine or cysteine. Perhaps we didn’t add enough glutamine to reveal a beneficial effect since studies demonstrating a beneficial effect of glutamine supplementation have all been performed with levels much higher than we used.

Dr. Déchelotte: In studies that we did in animals some years ago we found that during experimental infection liver glutathione decreased initially but later exceeded the initial values, while muscle glutathione was decreased [3]. Our impression was that there was recirculation of glutathione within organs. In relation to Crohn’s disease, we have recently performed a study, which is to appear in Clinical Nutrition, where we were surprised to find that neither the plasma concentrations of cysteine, glutamine or glycine, nor the intracellular (intramucosal) concentrations of these three amino acids, were reduced in these patients, so there was no evidence of limiting availability of substrate for synthesis [4]. Recent data indicate that the activity of the glutathione synthetase is depressed [5], so the problem may not be of substrate but rather of enzyme activity. Finally, about the influence of glutamine on glutathione, we also were unable to show any effect on liver glutathione during inflammatory challenge in rats, but in the jejunum the glutathione...
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concentration tended to be higher in glutamine-supplemented rats, and significantly so after 2 or 3 days.

Dr. Breuillé: It seems to me that the cysteine concentration is very well maintained but I don’t know why. Cysteine is probably involved in the regulation of redox potential and perhaps it is of primary importance to maintain cysteine levels. Even if we provide a very large quantity of cysteine in the diets of animals, there is little or no modification of cysteine concentration in the blood. I want to underscore what Dr. Déchelotte says, that there is an exchange between different organs and between glutathione and cysteine, so that we have a system which is very well maintained. Concerning the idea that the problem of glutathione depletion may be linked to a depression of glutathione synthetase, I do not share this opinion for two reasons:

- Firstly, it is generally considered that the rate-limiting enzyme is the γ-glutamylcysteine synthetase and not the glutathione synthetase. Thus, a decrease in the latter is perhaps not physiologically relevant.
- Secondly, a small number of measurements of the glutathione synthesis rate are available but they all report an increased synthesis rate in catabolic states. Furthermore, the recent paper of Jahoor shows that supplementation of the diet with N-acetylcysteine induces a concomitant increase in the glutathione concentration and synthesis rate in erythrocytes of HIV patients.

Dr. Millward: I’d like to ask about the relation between methionine and cysteine. I have a problem in understanding the concept of the conditional essentiality of cysteine, given that it is formed on the catabolic pathway from methionine, and given that the metabolic scheme that you showed us for methionine metabolism only had one catabolic pathway for methionine, the transulfuration pathway. In that scheme, therefore, in order for cysteine to be conditionally essential, there is either not enough methionine catabolism going on or there is an alternative pathway for methionine catabolism which is the major one. You showed us that in your rats that were supplementing with methionine, glutathione levels were not restored by the same amount as with cysteine, so clearly it was not methionine that was limiting. The consequence of that must be that methionine is being catabolized by a different route from the one that we think it normally goes through after being deaminated. In other words, the conditional essentiality of cysteine requires that you can’t make enough of it from methionine or that methionine actually becomes limiting under those circumstances, and clearly you’ve shown that it’s not limitation of methionine.

Dr. Breuillé: I think that there are two solutions for the possible conditional essentiality of cysteine. The first is that cysteine synthesis is depressed and so cysteine production is insufficient to provide substrate for glutathione synthesis. The second is that utilization of cysteine, either for protein synthesis or for glutathione synthesis, is so greatly increased that its formation from methionine is insufficient, even with maximally increased synthesis. The initial hypothesis of our team was that cysteine synthesis was depressed because of low cystathionase activity, but this seems not to be the case in our model, as we showed there was increased formation of cysteine from methionine. We believe there is a regulatory mechanism that induces increased cysteine synthesis, but this increase is not sufficient to provide enough substrate.

Dr. Jackson: Although the sulphydryl group of cysteine is derived from methionine, the carbon skeleton is derived from serine. Therefore any limitation in availability or in the ability to generate sufficient serine/glycine would constrain the flow through the transulfuration pathway; therefore a limitation of cysteine formation has to be seen in the context of the ability to form glycine, serine, and cysteine as a carbon triplet. The other obviously important consideration is that, in terms of the formation of nonessential amino acids, the limiting consideration may be cofactors for the pathway rather than substrate,
and it’s not at all clear to what extent cofactor limitations play an important role in these states, and whether or not there is an unusual requirement for cofactors over and above what we would consider to be the normal requirement. The other difficulty we have is in interpreting what concentrations mean if concentrations are actually controlled, and it is the flux through the pathway which is being modulated in relation to those concentrations.

Dr. Millward: I accept those are important issues, but it still leaves us with the question of where does the methionine go under these circumstances? If there’s a limitation of serine or of vitamin B6, both of which would limit the transulfuration pathway, then methionine would accumulate, and do we observe that under these circumstances?

Dr. Jackson: Well, I think one of the interesting considerations is the extent to which there is hyperhomocystinemia under some of the conditions that are being talked about and whether there is a constraint in the flow either through the remethylation pathway or down the transulfuration pathway indicated by hyperhomocystinemia.

Dr. Breuillé: I am not sure that we have a problem with the catabolism of methionine. Firstly, we have effectively observed a greater methionine concentration in infected animals as compared to pair-fed controls but not when compared to ad libitum control rats. So, we cannot consider that we have an accumulation in infected rats but we do not observe the decrease linked to food restriction. Secondly, I showed in my presentation that the transulfuration pathway was activated in infected animals. That means increased cysteine production but also increase methionine catabolism. The third point is that for methionine, we have a competition between two major pathways: protein synthesis and catabolism through the transulfuration pathway, and both of them seem to be activated after injury.

Dr. Young: I wonder if Dr. Millward’s conundrum, which I think is understandable one, might be explained by metabolic or anatomic compartmentation of metabolism and that the catabolism of methionine is going on at a different site from where cysteine utilization is enhanced and required? This would mean that you could have increased rates of methionine catabolism through the transulfuration pathway simultaneously with limitation of cysteine for the purposes of glutathione synthesis if that’s occurring somewhere else in the cell, or somewhere else in another anatomic site in the liver.

Dr. Breuillé: I’m confused that in one of your very early slides you indicated that for most amino acids there was a reduction in body content and an increase in catabolism, but for cysteine there was an increase in whole body content and a reduced rate of catabolism. Yet you showed in a later slide that there was an increased rate of taurine synthesis, which is derived from the catabolism of cysteine. So is there an increased rate of cysteine catabolism in sepsis, or a reduction? You can’t have it both ways!

Dr. Breuillé: I think there is increased utilization of most amino acids for energy purposes during catabolic states, but not for cysteine; indeed total cysteine catabolism appears to be depressed under these circumstances but we observed at the same time an increased rate of taurine synthesis from cysteine. The concomitant increased rate of taurine synthesis and decreased catabolism of cysteine is possible because taurine is only one of the multiple pathways of cysteine metabolism. However, I am not sure that we have to consider taurine as a catabolite of cysteine since taurine also exhibits important functions. We can suspect that increased taurine production could be an active regulation participating in the defense of the organism. For me, the real catabolite of cysteine is sulfate, not taurine. In anyway, we observed a sparing effect of injury on cysteine catabolism and this one is because cysteine is necessary for synthesis of acute phase proteins or glutathione, functions that have primary importance in the defense of the organism. These functions are associated with an increased substrate requirement, probably mainly cysteine but also glycine and glutamate.

Dr. Dechelotte: I think we should be cautious to separate acute inflammatory stress from chronic disease. The situation is quite different in the early days after an inflammatory challenge when there is no deficiency of circulating compounds but simply a need
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for interorgan exchange – reutilization of muscular cysteine in the liver, for instance, or biliary exchange between the liver and the gut. In chronic diseases, such as HIV or Crohn’s disease, there’s probably an irreversible loss of oxidized circulatory compounds, in the gastrointestinal lumen for instance, because oxidized glutathione diffuses much more readily than reduced glutathione within the lumen and may be irreversibly lost.

Dr. Reeds: We have good evidence of extremely tight compartmentation of cysteine metabolism. One of my colleagues has been studying cysteine synthesis in low birth weight infants, where glutathione is a very big issue. If you give the appropriate precursor, you cannot find labeled cysteine in plasma, but you can find it in apo-B 100 at quite a reasonable enrichment. Furthermore, some years ago Bill Heird tried to demonstrate cysteine synthesis by giving an industrial scale dose of U13C serine. Again he didn’t find label in cysteine but found an increase in plasma taurine [personal communication]. So we need to be very careful in examining extracellular cysteine and drawing conclusions about biosynthesis. I think, moreover, that this sort of compartmentation applies to all the conditionally indispensable amino acids and to a number of the essential ones as well.

Dr. Breuillé: As you described yesterday, when you perfuse a tracer you find the same incorporation of the tracer in the lumen as in the enterocyte, so that shows that there is large scale exportation of intestinal glutathione. We don’t know whether there is recycling or not. Do we have global losses of cysteine or glutathione in the stools or is there recycling to spare glutathione? We don’t know.

Dr. Reeds: We looked at that in our earlier glutathione experiment. We had more than enough label in mucosal and luminal glutathione for it to have been readily measurable in plasma if it was recirculating as glutathione, but plasma glutathione was essentially unlabeled. Yet there’s good evidence that if you give glutathione by the stomach, you can materially affect glutathione status in the body, so compartmentation again rears its ugly head.

Dr. Wernerman: I don’t think there is an important conflict between our human data and the experimental data presented here. After elective surgery we’ve seen a decrease in glutathione which is restored after 48–72 h; there may be an overshoot later on, but we don’t know because we haven’t looked. It’s reasonable that the time course in rats should be much shorter than in humans. In intensive care patients there is a decrease in glutathione during the first three days [6]. In a recent poster presentation we showed a long-term effect where total glutathione was restored [7] after 6 or 9 days in the ICU. We can’t explain that and we were very astonished, but those results are in keeping with what Dr. Breuillé has shown here. The difference that we see between postoperative patients and ICU patients is the redox state of glutathione. We see a much larger fraction in the oxidized form in the ICU patients, for example those with generalized inflammation, than we do in patients with clean trauma, as represented by the postoperative group.

Dr. Fürst: Were the changes in glutathione paralleled by similar changes in glutamine?

Dr. Wernerman: Not glutamine but glutamate. If you look at concentrations, you see that the decrease in glutamate seen after trauma or during sepsis comes before the changes in glutamine, and glutathione appears to mirror the glutamate.

Dr. Reeds: The balance between GSH and GSSG is absolutely crucial to the interpretation of all our tracer experiments. I have to continually remind myself that redox cycling is not going to lead to glutathione turnover in the way we measure it. If glutathione is cycling between GSSG and GSH – and I agree with you that GSSG is readily moved out of the cell, for no other reason than it’s a ribosomal poison – what happens to that GSSG? That’s the crucial question in all of this.

Dr. Breuillé: I think that is a crucial question, but we have no clear evidence of a net loss of GSSG.

Dr. Jackson: We find that when we shift normal people from one level of dietary protein to another, there are changes that only become evident after 5 or 6 days. So
without any unusual stress or trauma there are metabolic changes relating to glutathione metabolism that become manifest after 5 days on a changed intake. It's going to be very interesting to find out about the processes within and between tissues that allow these adjustments to occur. This suggests to me that there are certain functions that operate as buffering functions. In terms of nonessential amino acids, one can envisage what these might be – for example, the extent to which you do or do not synthesize creatine in large
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amounts, the extent to which you do or do not synthesize heme in large amounts, and how long you can sustain that reduction in their synthesis. But the measurement of those metabolic pathways that consume relatively large amounts of essential amino acids can’t be sacrificed indefinitely.

Dr. Wernerman: Do you want to comment?

Dr. Fürst: After this sophisticated discussion I would like to take up a practical point concerning N-acetylated amino acids. Actually, humans do not possess N-acetylases. This has been confirmed in several Swedish studies in which utilization of N-acetylcysteine by humans [8, 9] was extremely poor, in contrast to animals [10–12]. The Swedish data show that, though much of the acylated amino acids are excreted, there is accumulation after intravenous infusion in both the extracellular and intracellular compartment (Fig. 5). We have calculated from the available data that not more than 2% of the available N-acetylcysteine is utilized by various organs, except for the kidney [Dröge W, personal communication]. All pharmaceutical companies seem to use it in their products, I believe more for cosmetic than for nutritional purposes. It is possible that in various situations N-acetylcysteine may act as an antioxidant, but it does not act as a cysteine precursor.

Dr. Breuilé: The recent human study from Jahoor’s team showed that N-acetylcysteine at a relatively moderate level induced a concomitant increase in glutathione concentration and synthesis rate in AIDS patients [13]. So, it appears that at least in AIDS patients, N-acetylcysteine can be metabolized and utilized as glutathione precursor!

Dr. Reeds: That was an oral supplementation study, I believe. We don’t know what the gut flora or the enterocytes do to N-acetylcysteine. It may be deacetylated in first pass for all we know.

References

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