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The essentiality of sulfur is closely related to nitrogen metabolism: a clue to hyperhomocysteinaemia

Yves Ingenbleek[1]* and Vernon R. Young[2]

[1]*Laboratory of Nutrition, Faculty of Pharmacy, University Louis Pasteur, 74 Route du Rhin, 67401 Illkirch, France*
[2]*Laboratory of Human Nutrition, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA O2139-4307, USA*

N and S metabolisms are closely interwoven throughout both the plant and animal kingdoms. The essentiality of S relates to its participation in the structure of S-containing amino acids (SAA), to its inclusion in many sulfonated molecules, and to a myriad of metabolic and catalytic reactions of vital importance. Methionine (Met) is the indispensable SAA supplied by food proteins and its plasma homeostasis is achieved via a number of highly efficient regulatory mechanisms. In all conditions characterised by a negative body protein balance such as in dietary restriction or cytokine-induced hypercatabolic losses, N and S endogenous pools manifest parallel tissue depletion rates. Adaptive conservation of N and S body stores is reached by a functional restraint of the trans-sulfuration cascade, through the depression of cystathionine β-synthase activity. As a result, upstream accumulation of homocysteine favours its re-methylation conversion to Met which helps maintain metabolic pathways of survival value. In addition to the measurement of vitamin indices, that of plasma transthyretin, a sensitive marker of protein nutritional status, is proposed to identify the fluctuations of the total body N component accountable for the alterations of homocysteine concentrations in body fluids.

Sulfur biology: Methionine: Homocyst(e)ine: Protein metabolism: Inflammatory disorders

Introduction

Homocysteine (Hcy) is a metabolically important amino acid (AA) generated by the enzymic de-methylation of methionine (Met) and is situated at a crucial crossroad regulating the fate of S-containing compounds. Hcy may be either re-methylated through the activity of Met synthase (*EC* 2.1.1.13) or irreversibly lost as a catabolite of S-containing AA (SAA) metabolism through the action of cystathionine (Cysta) β-synthase (CβS; *EC* 4.2.1.22).

Hyperhomocysteinaemia is an acquired metabolic disorder found in apparently healthy individuals and recognised of increasing importance for health in the general population (Finkelstein, 2000; Refsum *et al*. 2004). The condition has been determined to be an independent and graded risk factor for thrombovascular lesions unrelated to hypercholesterolaemia, arterial hypertension, diabetes and smoking (McCully, 1996; Hankey & Eikelboom, 1999). Considerable efforts have been undertaken in recent years to identify the causal factors responsible for its occurrence and to clarify the aetiopathogenic mechanisms implicated in tissue damage. To date, it has been established that dietary folate (Kang *et al*. 1987) and cobalamin (Stabler *et al*. 1990) shortage are both capable of depressing the activity of Met synthase, favouring the *downstream* accumulation of Hcy in extracellular fluids. Moreover, dietary pyridoxine deprivation may contribute to impair CβS activity, the rate-limiting step initiating the trans-sulfuration cascade (Ubbink *et al*. 1996), thereby promoting the *upstream* sequestration of Hcy.

Preventive and/or therapeutic supplementation trials have highlighted that the three water-soluble vitamins do not operate with the same degree of potency on the enzymic machinery regulating Met homeostasis (Ubbink, 1997; Weir & Scott, 1998). Dietary folate is regarded as a most important factor whereas pyridoxine appears to be less critical. Cobalamin deficiency occupies an intermediary position particularly affecting vegans. Recent studies using stepwise multiple regression analysis have concluded that folate and cobalamin deprivation, working together,

Abbreviations: AA, amino acid; CβS, cystathionine β-synthase; Cys, cysteine; Cysta, cystathionine; Hcy, homocysteine; IAA, indispensable amino acid; Met, methionine; MM, molecular mass; PEM, protein–energy malnutrition; SAA, S-containing amino acid; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; Tau, taurine; TBN, total body N; TTR, transthyretin.

***Corresponding author:** Dr Yves Ingenbleek, fax +33 390 24 43 17, email ingen@pharma.u-strasbg.fr

account for 28 % of the variance in total Hcy concentrations (Pancharuniti *et al*. 1994), whereas the contribution played by folate, cobalamin and pyridoxine taken as a whole ranges from about 35 to 40 % of Hcy variance (Lussier-Cacan *et al*. 1996). In an attempt to explain much of the remaining variation, we postulate that the gap might be identified by largely considering the integrative aspects of total body N (TBN) as a major determinant of Met and Hcy homeostasis (Ingenbleek *et al*. 2002; Ingenbleek & Young, 2002).

Our approach is grounded on the hypothesis that in any morbid condition resulting from either reduced N intake or excessive N losses, causing the downsizing of TBN, leads to depression in the trans-sulfuration pathway, with stimulation of the re-methylation of Hcy to Met. The concept implies that a functional CβS blockade is an N-status-sensitive step, allowing the conservation of Met to maintain adaptive mechanisms of survival importance under nutritional and/or stressful disorders (Ingenbleek *et al*. 2002). Furthermore, the N-dependent component of acquired hyperhomocysteinaemia is best appraised by the serial measurement of plasma transthyretin (TTR; previously named pre-albumin) whose fluctuations are correlated with a shrinking or expanding of TBN (Ingenbleek & Young, 2002). The present review is designed to assemble and evaluate all available information linking S essentiality to N metabolism and to provide substantial support for our hypothesis.

The natural cycle of sulfur-containing compounds

Astrophysical data and the chemical analysis of meteorites point to the participation of S in stellar evolution. There is geological evidence that S was present early amongst primordial compounds, either in free form, combined with other elements (N, O, Si, Fe) or in the reduced form of hydrogen sulfide (H_2S; Holland, 1974; Winnewisser & Herbst, 1987; Wächtershäuser, 2000). For example, the theory of a pressurised Fe-S world suggests an autotrophic metabolism of low-molecular-mass (MM) constituents in an environment of iron sulfide and hot magmatic exhalations (Wächtershäuser, 2000). In the earth's crust, S naturally occurs as a mixture of four isotopes, the most abundant being ^{32}S (95·1 %). The availability of *d*-orbitals for bonding allows S to assume eight different valencies ranging from −2 to +6 (Huxtable, 1986). The most oxidised and most stable state is represented by the physiologically inert SO_4^{2-} oxyanion, whereas sulfide (S^{2-}) constitutes the most reduced and reactive state. From the former to the latter, all intermediate species exhibit increasing instability and reacting capacity (Huxtable, 1986).

In the anaerobic atmosphere of early geological times, the biological cycle of S is thought to have been initiated by micro-organisms transforming SO_4^{2-} to inorganic H_2S. This reducing process is a form of primitive respiration requiring considerable energy from bacteria and fungi. The production and accumulation of H_2S has resulted in profound geochemical consequences favouring the emergence of anaerobic life on earth (Sult & Kulp, 1959). The growth of both oxidising and reducing micro-organisms and the later occurrence of photosynthesis in microscopic plants has created reciprocal exchanges between anaerobic and aerobic ecosystems (Kelly, 1980). It may be worth recalling that S is located directly below O in the periodic table of elements. The progressive enrichment in O_2 of the atmosphere has favoured the evolution of higher plants and aerobic animals. Remnants of primitive ecosystems still remain, largely confined to inhospitable places such as swamps, volcanoes and ocean beds (Mitchell, 1996).

Regulation of sulfur metabolism in the vegetable kingdom

A large number of micro-organisms and plants utilise reduced or oxidised S compounds as donors or acceptors of electrons with the capacity to reduce precursor molecules to the lowest oxidation states. These assimilatory reducing processes are highly regulated and share a close relationship with N metabolism. Their precursor anions found in the environment are usually available to the plant as SO_4^{2-} and NO_3^-, their maximum oxidised forms (Mohr & Schopfer, 1994). The cellular uptake of these oxyanions displays multiphasic characteristics along which K_m and V_{max} values are modified following plant requirements. The permeation systems involve both active accumulation using saturable (carrier-mediated) components and non-saturable passive diffusion (Mohr & Schopfer, 1994). SO_4^{2-} and NO_3^- must undergo reductions to the lowest oxidation states through the stimulation of sulfate and nitrate reductase enzymes to yield SH and NH_4, respectively. SH is then transferred to the O-acetylserine molecule whose decay allows the formation of cysteine (Cys) whereas NH_4 is converted to glutamine through the ATP-dependent activation of glutamine synthetase (Mohr & Schopfer, 1994). These metabolic pathways mainly occur in the leaves during photosynthesis, releasing Cys and glutamate as precursor substrates for the production of most other S- and N-containing organic molecules. Tightly regulated mechanisms operate to prevent the excessive accumulation or shortage of S and N components. The rate of SO_4^{2-} uptake and transport by the plant is rapidly depressed as a result of an expanded intracellular sulfate pool (Smith, 1975) but is also subject to the negative feedback control of Cys and Met concentrations (Hart & Filner, 1969). Overfeeding the plant with Cys involves its breakdown to SO_4^{2-} by degradative enzymes. In contrast, sulfate deficiency in the medium stimulates the ATP-sulfurylase activity of cultured tobacco cells, driving a greater proportion of the available SO_4^{2-} into reducing processes (Reuveny & Filner, 1977) while inhibiting the activity of nitrate reductase, the rate-limiting enzyme governing NO_3^- assimilation (Friedrich & Schrader, 1978). The data indicate that S bioavailability works as a limiting factor for protein synthesis and plant growth. Deprivation of S resources causes failure to thrive and complete exhaustion leads to cessation of growth (Huxtable, 1986). The accumulation of NO_3^- and of dispensable AA in free form (Bergmann, 1981) reflects either the inability of the plant to achieve an adequate rate of protein synthesis and deposition, or the catabolic breakdown of fully mature endproducts. Under normal circumstances, the cellular uptake of S and N in most higher plants and their incorporation into protein molecules operates along narrowly fluctuating S:N

molar ratios ranging from 1:31 to 1:40 (Dijkshoorn & van Wijk, 1967). Likewise, and despite an almost 3000-fold variation in the medium sulfate, there exists a striking constancy in the rate of production of reduced SH and NH_4 groups and in the synthesis of Cys and Met anabolites (Datko *et al*. 1978). These two S-containing AA represent 80 to 90 % of all vegetable protein-bound S molecules (Huxtable, 1986). The concentrations of free Cys and free Met in higher plants is normally low and relatively constant even when the external S supply varies over a wide range of levels (Datko *et al*. 1978).

Regulation of sulfur metabolism in the animal kingdom

The animal kingdom is unable to reduce SO_4^{2-} to sulfide or to produce SAA and must rely on the plant kingdom, bacteria and fungi for these converting processes. Met is one of the eight nutritionally indispensable AA (IAA) whose dietary intake in man is, therefore, obligatory for the synthesis of body proteins, maintenance of normal growth and vital activities. The molecule was discovered by Mueller (1923) and its essentiality was demonstrated by the growth retardation observed in rats given Met-free diets (Womack *et al*. 1937). S is the seventh most abundant element after H, C, O, N, Ca and P in the tissues of higher vertebrates. Thus, the body of a healthy adult man, weighing 70 kg, contains about 140 g S (4400 mmol) or about the same as that of total K (140 g; 3600 mmol) (Forbes, 1996); each element thus represents 0·2 % of body weight. The S:N molar ratio (4400 mmol:64 mol or 1:14·5) is higher than that recorded for plant tissue but is similar to the 1:14·5 ratio characteristic of animal proteins (Peters & Van Slijke, 1931; Kennedy, 1974). Although the tissue distributions of S and N are not strictly aligned and that part of the total S pool is involved in the structure of lipid and carbohydrate components, the higher overall abundance of S leads to the view that it has a relatively greater degree of involvement in the animal economy. As a corollary, most plants used for human consumption reveal a limiting Met content in relation to human requirements. Indeed, Met may become a limiting nutritional factor in some vegetable products, notably in legumes (Young & Pellet, 1994), particularly for the young growing infant and child (Beach *et al*. 1942).

An important biological function of Met is in the initiation of protein synthesis which starts with the attachment of a free Met molecule to initiator transfer RNA to yield formyl-methionyl-tRNA, launching the process of mRNA translation (Rudland & Clark, 1972; Flaim *et al*. 1982). Met is also the major donor of methyl (CH_3) groups (Cantoni, 1951). Through the catalytic activation of Met adenosyltransferase (*EC* 2.5.1.6) Met condenses with ATP, yielding the dephosphorylated S-adenosylmethionine (SAM) molecule whose de-methylation releases, in turn, S-adenosylhomocysteine (SAH; Finkelstein, 1990). SAM serves as a proximal CH_3 donor for more than 100 acceptor substrates, including nucleic acids, myelin, creatine, phospholipids, neurotransmitters, hormones, polyamines and AA (Giulidori *et al*. 1984; Fig. 1). The liver is the main site where most transmethylation reactions take place with the methyl groups supplied from dietary sources or resulting from the *de novo* synthesis of endogenous molecules (Mudd & Poole, 1975). The enzymic hydrolysis of SAH allows the recycling of the adenosyl moiety and the production of Hcy (Finkelstein, 1990). The SAM:SAH ratio is frequently proposed as an indicator of cellular transmethylation potential (Cantoni, 1985). However, and because SAH is a potent inhibitor of SAM-dependent methylation reactions, more recent studies have advocated that an increased SAH intracellular concentration might be more consistently associated with hypomethylating capacities (Caudill *et al*. 2001).

Hcy is not incorporated into proteins via peptide-bond synthesis but rather constitutes a transitory branch-point regulating Met metabolism. On the one hand, Hcy may be re-methylated mainly through the stimulation of Met synthase. This is a rate-limiting enzyme requiring CH_3 from $[N^5]CH_3$-tetrahydrofolate, the reduced product of $[N^{5-10}]$methylenetetrahydrofolate released by the activation of a specific reductase (methylenetetrahydrofolate reductase; Finkelstein, 1990). Several point mutations have been found on the methylenetetrahydrofolate reductase gene, including a thermolabile 677 cytosine-to-thymine mutation causing an alanine-to-valine substitution in the enzyme protein whose activity is normally dependent upon riboflavin (vitamin B_2) coenzyme bioavailability (Hustad *et al*. 2000);

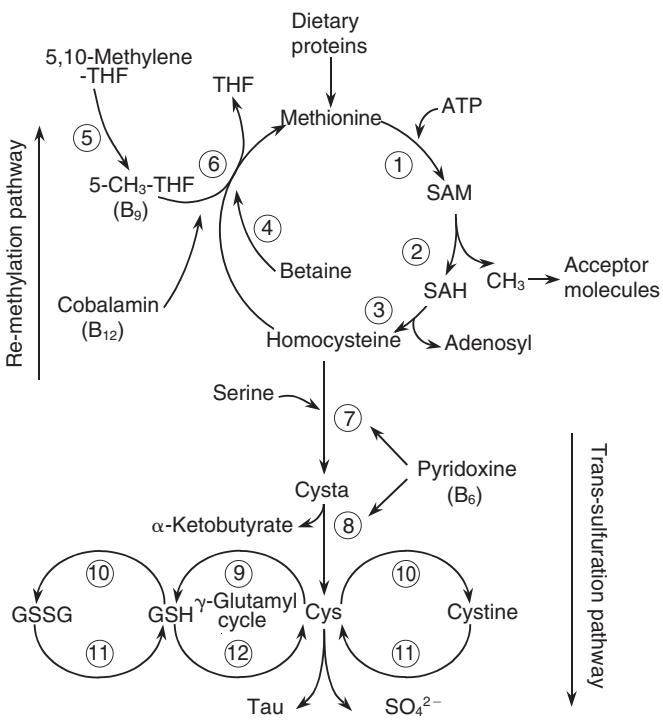

Fig. 1. Schematic outline of methionine (Met) and homocysteine pathways. THF, tetrahydrofolate; SAM, adenosylmethionine; SAH, adenosylhomocysteine; Cysta, cystathionine; Cys, cysteine; Tau, taurine; (1), Met adenosyltransferase; (2), SAM methyltransferases; (3), adenosylhomocysteinase; (4), betaine-homocysteine methyltransferase; (5), methylene-THF reductase; (6), Met synthetase; (7), Cysta β-synthase; (8), γ-cystathioninase; (9), γ-glutamyl synthetase; (10), oxidase; (11), reductase; (12), γ-glutamyl transpeptidase.

the homozygous C677T state affects from 1 to 15 % of investigated populations (Motulsky, 1996). It seems unlikely that the gene mutation, working alone, contributes to an Hcy-induced increased risk for cardiovascular disease (Brattström, 1997; Reinhardt *et al*. 1998) but rather potentiates the effects of a pre-existing folate deficiency. A betaine-Hcy methyltransferase (*EC* 2.1.1.5) pathway may serve as a surrogate supplier of Met molecules in mammals (Ericson *et al*. 1955). The former Met synthase-converting route is cobalamin dependent and widely distributed in animal tissues (Banerjee & Mathews, 1990). The latter pathway is confined to the liver and kidneys, and utilises betaine as the source of the methyl group (Skiba *et al*. 1982). Also, Hcy may undergo irreversible degradation along the trans-sulfuration pathway which is initiated by CβS activity (Finkelstein, 1990). The enzyme condenses Hcy with serine to form Cysta, which, in turn, is converted to Cys through the activity of γ-cystathioninase (*EC* 4.4.1.1; Cooper, 1983). Both of these steps are dependent on pyridoxal 5'-phosphate as a cofactor. Cys is transported to other tissues or it may be oxidised to hypotaurine and taurine (Tau) along the cysteinesulfinate pathway or degraded to SO_4^{2-}, its major catabolite (Cooper, 1983; Fig. 1).

Under physiological circumstances, kinetic studies undertaken on healthy adults consuming well-balanced diets with appropriate Met intake levels indicate that both Hcy trans-sulfuration and re-methylation pathways split into nearly equivalent fractions (Storch *et al*. 1990). These findings are in close concordance with previous clinical investigations (Mudd & Poole, 1975) and with experiments using perfused rat liver (Finkelstein, 1974) or enzyme preparations (Finkelstein & Martin, 1984*a*). In the case of alterations in Met supply, the body exploits its enzymic equipment to modify the respective proportions of Met fluxes driven into re-methylation and trans-sulfuration pathways so as to maintain intra- and extracellular Met homeostasis. The partitioning of Met flow between these two converting routes is reportedly coordinated by the intracellular SAM concentration working as an *inhibitor* of both methylenetetrahydrofolate (Kutzbach & Stokstad, 1967) and betaine–Hcy methyltransferase (Finkelstein & Martin, 1984*b*) and as an *activator* of CβS (Finkelstein *et al*. 1975) enzymes.

Cys undergoes a number of metabolic fates; it may be incorporated into many proteins, keeping its reduced SH group required to achieve functional properties or reversibly converted to GSH via the γ-glutamyl cycle (Beutler, 1989). GSH (γ-glutamyl-cysteine-glycine) is a tripeptide critically involved in the maintenance of the cellular redox state and the scavenging of free radicals. Its production starts from Cys, and involves glutamic acid and glycine through the activation of two ATP-dependent γ-glutamyl and glutathione synthetases working in succession (Meister & Anderson 1983; Beutler, 1989; Fig. 1). In return, GSH may be subsequently cleaved by γ-glutamyl transpeptidase and dipeptidase enzymes, yielding Cys as the endproduct through cysteinylglycine as an intermediate compound (Cooper, 1983; Beutler, 1989). Cys and GSH may be oxidised to cystine and GSSG, respectively, through the binding of two precursor molecules undergoing oxidation of their SH groups to form a disulfide S–S bridge (Cooper, 1983). GSH is the dominant thiol compound synthesised at millimolar level in all cells, including erythrocytes (Mortensen *et al*. 1956). GSH is in constant metabolic turnover with a relatively slow biological $T_{\frac{1}{2}}$ of 65 h in erythrocytes (Mortensen *et al*. 1956) but forming a very much faster labile pool in hepatocytes (Meister & Anderson, 1983; Beutler, 1989). The liver GSH reservoir is significantly increased with generous SAA intake (Leaf & Neuberger, 1947) and depleted in starvation (Tateishi *et al*. 1977). The data are consistent with the concept that GSH constitutes a hepatic storage and readily available transport form of Cys in animal physiology (Tateishi *et al*. 1977). Only trace amounts of Cys, cystine and GSSG in the micromolar range are found in animal cells (Cooper, 1983; Meister & Anderson, 1983).

The essentiality of sulfur in tissue building and metabolic pathways

S fulfils essential roles in all living systems, determining the structure and activity of a number of molecules and modulating a myriad of metabolic and catalytic processes. The formation of S–S bonds is grounded on the oxidation of sulfhydryl groups and they form a cornerstone of molecular structures. Intra- or intercatenary S–S bridges, occurring during the growth of nascent proteins in the endoplasmic reticulum of eukaryocytic cells, determine their tertiary and quaternary conformations, increasing their thermal stability and resistance to physico-chemical denaturing mechanisms (Betz, 1993). Full protein maturation may require the involvement of sulfonation processes through the activation of a sulfonate group (SO_3^-) from the universal donor 3′-phosphoadenosine-5′-phosphosulfate precursor to an appropriate acceptor substrate (Farooqui, 1980). Sulfonation of molecules develops in the cytoplasm of most mammalian cells with the higher activity found in the liver, requiring the activation of two enzyme systems pertaining to a single bifunctional entity (3′-phosphoadenosine-5′-phosphosulfate synthase) and reacting in succession with two ATP molecules (Lyle *et al*. 1994). The highly charged sulfonate groups remain fully ionised at the pH encountered in biological systems, favouring electrostatic interactions (Strott, 2002). All types of acceptor molecules may be involved, ranging from less than 10^3 as MM to greater than 10^6 (Strott, 2002).

Sulfonation is, thus, an important, ubiquitous process taking place along two main pathways in the Golgi network: O-sulfonation mobilises an alcohol group and appears as the dominant reaction in *macromolecules* such as glycosaminoglycans, proteoglycans and galactoglycerolipids. N-sulfonation necessitates an amide group and plays a crucial role in the alteration of carbohydrate moieties of macromolecules such as heparin and heparan sulfate proteoglycans (Strott, 2002). So far, thirty-two different sulfotransferases located within the Golgi organelles have been recognised and they are capable of causing post-translational changes to the sugar residues of lipid and protein macromolecules (Fukuda *et al*. 2001). These sulfotransferases are characterised by having high affinity but low capacity. The converting steps are

reversible as sulfonated products may be subjected to sulfohydrolysis through the activation of specific sulfatases (Parenti *et al*. 1997). Glucoaminoglycans and proteoglycans fulfil structural or functional roles. They participate in the building of cell membranes, transmembrane signalling and extracellular matrix proteins. They are responsible for the hydration and elasticity of fibrous and cartilaginous joints (Kjéllen & Lindahl, 1991; Mitchell, 1996). Sphingolipids and galactoglycerolipids are prevalent in myelin, the kidneys and small intestine and are implicated in cell-receptor adhesiveness, blood coagulation and complement activation (Kjéllen & Lindahl, 1991). Heparin is exclusively produced and stored in mast cells whereas heparan sulfate is more widely distributed (Vos *et al*. 1994).

Sulfonate esterification of relatively *low MM compounds* is a widespread biological process. Such reactions take place in the cytosol and are catalysed by a superfamily of soluble sulfotransferases. At least forty-four cytosolic enzymes have been identified and fall into five different families, determined according to their molecular targets and physiological impact (Nagata & Yamazoe, 2000). O-sulfonation processes of tyrosine residues are prevalent post-translational events occurring in all metazoan species (Niehrs *et al*. 1994) which may lead to either the activation of a biological effect (as reported for thyroid and steroid hormones, and for vitamins) or to its inactivation (as shown for catecholamines and for potentially harmful drugs and xenobiotics) (Huxtable, 1986; Mitchell, 1996). The pituitary–thyroid axis is a major sulfonation target with modulation of the synthesis, metabolism and clearance of the endocrine parameters in several ways. Post-translational sulfonation of thyreostimulin is reportedly capable of enhancing the thyroperoxidase-dependent coupling reaction between iodotyrosine residues (Nlend *et al*. 1999*a*) while down-regulating the sulfonation rate of thyroglobulin (Nlend *et al*. 1999*b*). Both thyroid hormones thyroxine and triiodothyronine may undergo sulfonation of their phenol-hydroxyl group, yielding derivatives characterised by modified biological activity and disposal rates (Visser, 1996). Most hormonal compounds originating from the cortico-adrenal glands and sexual organs (cortisol, testosterone, androstenedione, dehydroepiandrosterone, oestradiol, oestrone and pregnenolone) may be subjected to varying degrees of sulfonation regulated by a set of sulfotransferase isoenzymes displaying substrate specificities (Strott, 2002). Sulfonation of steroid molecules could modulate several biological properties, notably brain maturation (Beaulieu, 1998) and the ageing process (Yen, 2001). Catecholamines (adrenaline, noradrenaline, dopamine) are produced by the adrenomedullary cells in free and physiologically active form characterised by a short $T_{\frac{1}{2}}$ of 1 to 3 min (Strobel *et al*. 1994). The bulk (from 73 to 97 %) of these secretory compounds undergoes sulfoconjugation in phenolsulfotransferase-rich tissues, mainly the gastrointestinal tract (Goldstein *et al*. 1999). Sulfonated catecholamines thus constitute the preponderant hormonal pool found in human blood, endowed with a considerably longer $T_{\frac{1}{2}}$ ranging from 3 to 4 h but devoid of biological potency (Strobel *et al*. 1994; Onasch *et al*. 2000). This large reservoir of inactivated molecules represents a circulating pool that is readily available to meet tissue requirements via transformation to bioactive compounds by sulfohydrolysis (Kuchel, 1994). It has been suggested that the sulfonation of bile acids might influence the removal rate of cholesterol (Radominska *et al*. 1990). Besides the liver, the skin contributes to the bioconversion of steroidogenic molecules. Cholesterol sulfate accumulates in human epidermis and serves as a growth factor, stimulating the differentiation of normal keratinocytes (Jetten *et al*. 1989). Sulfonation processes also modulate the binding affinities, turnover rates and biological properties of a large number of other endogenous molecules. This is notably illustrated by pituitary glycoprotein hormones such as chorionic gonadotrophin (Bielinska, 1987) or luteinising hormone (Parsons & Pierce, 1980), by gastrointestinal peptides such as cholecystokinin (Williams, 1982) or gastrins (Andersen *et al*. 1983) and even by vitamins such as ascorbic acid (Tolbert, 1985) and cholecalciferol (Axelson, 1985).

Finally, S may participate in its *elemental form* to affect the structure and activities of molecules with important roles in normal growth, metabolism, defence, transport and detoxification processes. Sulfhydryl groups are frequently implicated in metal coordination, notably in the conformation of Zn finger motifs involved in protein–DNA recognition and replication (Pabo *et al*. 2001). The coupling of GSH to seleno-dependent peroxidase enzyme comprising four identical selenocysteine subunits allows the protection of body tissues from the deleterious effects of organic and inorganic peroxides (Forstrøm *et al*. 1978; Chance *et al*. 1979). CoA is a complex molecule condensing adenine, ribose 3′-phosphate and pantothenic acid with a cysteamine residue bound to the carboxyterminal group of the vitamin. It is a major esterifying thioester serving as an acyl donor for the synthesis of lipids and as a sensor of the metabolic steps converting carbohydrate substrates into energy (Plesofsky-Vig & Brambi, 1988). The thiamin molecule (vitamin B_1) possesses a thiazole ring and operates in non-redox enzyme reactions, preventing the occurrence of metabolic abnormalities (Gubler, 1991) associated with the beriberi disease affecting rice-eating populations. Biotin is a bicyclic molecule with an S moiety within its tetrahydrothiophene ring and works as a cofactor of enzymes involved in the synthesis of fatty acids and in gluconeogenic processes (Zempleni & Mock, 1999). Metallothioneins are a family of small peptides reaching about 6·5 kDa as MM whose AA sequence reveals up to 30 % Cys residues and fulfilling important storage and transport functions, mainly located in the intestinal mucosa, the liver and kidneys (Bremner & Beattie, 1990). Structural analysis of the six insulin-like growth factor-binding proteins demonstrates the great similarity in their MM ranging from 23 to 31 kDa and in their AA sequence comprising ten to twelve Cys residues (Hwa *et al*. 1999). Many other S-containing molecules are recognised of increasing metabolic and nutritional importance.

It is worth noting that the mean Cys content of intracellular proteins in mammalian tissues is about 2·5-fold lower (1·6 *v*. 4·1 %) than that of proteins extracellularly exported in biological fluids (Fahey *et al*. 1977). This is in keeping with distinct biological properties as Cys prevails in the form of sulfhydrated Cys in the former group but is usually oxidised to disulfide proteins in the latter.

Refractoriness of sulfur-containing molecules to dietary manipulation

The classical experiments by Finkelstein's group (Finkelstein, 1974; Finkelstein *et al*. 1975; Finkelstein & Martin, 1984*a*,*b*) and by others (Kutzbach & Stockstad, 1967) have paved the way for an overall understanding of the enzymic mechanisms regulating Met homeostasis. Dietary Met overload (normally an unlikely condition in human nutrition) occurring under experimental conditions is followed by the intracellular accumulation of SAM which attenuates the activity of re-methylating enzymes and stimulates that of CβS (Finkelstein & Martin, 1986). As a result, the bulk of Met undergoes oxidation along the trans-sulfuration cascade, leading to an increased sulfaturia that is highly correlated with the level of SAA intake (Sabry *et al*. 1965). The kidney clearance of Met excess allows the maintenance of an unmodified methioninaemia and appears as an important adaptive process especially as Met is considered to be one of the most potentially toxic of the AA (Benevenga, 1974).

Preservation of the endogenous Met pools is also achieved through the coordination of intestinal and renal kinetics. Investigations carried out in normal subjects and using oral perfusion of free AA mixtures have shown that Met displays a rapid and efficient absorption rate (90 to 100 % of intake; Horowitz *et al*. 1981) compared with most other IAA (Adibi *et al*. 1967). Moreover, losses of IAA of endogenous origin in the ileostomy fluid of healthy volunteers given a protein-free diet indicate that both Met and Cys together represent 14 % of current daily maintenance requirements, a rate that appears to be below that of all other IAA (Fuller *et al*. 1994). Furthermore, Met loss compared with Cys represents the smaller fraction among the two SAA compounds (Fuller *et al*. 1994). Finally, Met reveals very high renal tubular recovery rates (Werder *et al*. 1966; Stabler *et al*. 1987) resulting in a barely detectable methioninuria. Even after a significant oral loading test (50 mg/kg body weight), Met urinary leakage remains below 1 % of the administered dosage (Horowitz *et al*. 1981). The high degree of Met retention within endogenous pools thus appears as the net result of salvage mechanisms maximising intestinal absorption rates and minimising faecal and urinary losses. The uniqueness of Met is further documented by animal studies assessing the nutritional consequences of the selective withdrawal of single IAA from balanced AA mixtures. Deleting SAA from an otherwise complete diet causes the greatest rate of body N loss, nearly equal to that generated by a protein-free diet (Owens & Bergen, 1983; Fuller *et al*. 1989). The data are in keeping with rat experiments (Aguilar *et al*. 1974) and a series of Massachusetts Institute of Technology clinical and metabolic studies (Storch *et al*. 1990; Hiramatsu *et al*. 1994; Fukagawa *et al*. 1996; Raguso *et al*. 1997) showing that the oxidation rate of Met remains low but constant when its intake level is below requirements (Raguso *et al*. 1997). This oxidative behaviour is qualitatively very similar to that described for other IAA such as leucine (El-Khoury *et al*. 1994) or lysine (Meredith *et al*. 1986) although Met is regarded as the most limiting of the endogenous IAA (Yoshida & Moritoki, 1974). The maintenance of low but measurable post-absorptive (fasting) rates of Met oxidation unrelated to dietary AA supply supports the view that Met fulfils minimal obligatory activities likely to be of survival importance. Dietary Met manipulation studies performed on healthy adults and using multi-labelled Met as a tracer have shown that the fluxes of both methyl and carboxyl end-products remain stable (Raguso *et al*. 1997). This indicates that adaptive changes in the rates of whole-body proteolysis may serve as a mechanism to maintain homeostasis together with the classical re-methylation and trans-sulfuration processes (Kutzbach & Stokstad, 1967; Finkelstein, 1974; Finkelstein *et al*. 1975; Finkelstein & Martin, 1984*a*,*b*).

The first investigation correlating N and S compounds in healthy human subjects was described by Sherman & Hawk (1900). The authors investigated the metabolic consequences of a 7 d period resulting from 'the ingestion of a rather large quantity of protein food when the body is kept in a uniform and perfectly normal condition of nutrition'. In this study, urinary S catabolites were collected in the form of SO_3 compounds and measured by a gravimetric method whereas N output was determined by Kjeldahl analysis (Fig. 2). It is of interest to note that SO_3 values, once converted to elemental S, yielded a molar S:N ratio of 1:13·5, or very close to that characterising the total body chemical composition (Forbes, 1996). More recently, it has been concluded that using sulfate output: (i) depicts a fed-state adaptation to protein restriction in healthy adults (Hamadeh *et al*. 2001); (ii) can provide an accurate method of estimating SAA catabolism (Hamadeh & Hoffer, 2001); (iii) may be a useful method to determine the extent of whole-body protein dysregulation in insulin-dependent diabetes mellitus (Hamadeh & Hoffer, 2003); (iv) allows the N:S balance ratio to serve as a potentially useful indicator of non-protein SAA stores, at least in growing piglets (Hou *et al*. 2003).

The sparing role played by dietary Cys on Met needs in several animal species (Womack & Rose, 1941; Baker, 1976; Burns & Milner, 1981; Finkelstein *et al*. 1988) has been the topic of a recently revived debate with regard to human nutrition. Earlier investigations undertaken on adult human subjects, taking N balance as the criterion of intake adequacy, indicated large variability across individuals with a Cys-sparing role ranging from 16 to 90 % of Met requirements (Reynolds *et al*. 1958; Irwin & Hegsted, 1971). The estimated mean was about 50 % of the requirement for total SAA (Rose, 1957). However, more recent kinetic studies using stable-isotope techniques in healthy volunteers given tracers either orally (Raguso *et al*. 1997) or intravenously (Storch *et al*. 1990) have not confirmed a major capacity of Cys to spare Met unless a very low intake of Met is consumed (Raguso *et al*. 1997). The bioavailability of Cys is met by the dietary intake, by the conversion of Met through the trans-sulfuration cascade, or by the endogenous breakdown of proteins and of GSH reserves. After oral consumption, Cys is almost entirely removed by intestinal tissues (Rérat *et al*. 1992), explaining why Met becomes the predominant SAA found in the portal blood flow (Garcia & Stipanuk, 1992). The large uptake of Cys by the splanchnic (intestine) area thus works as a buffering system so that there is little variation in postprandial Cys values in response to a wide range of Cys

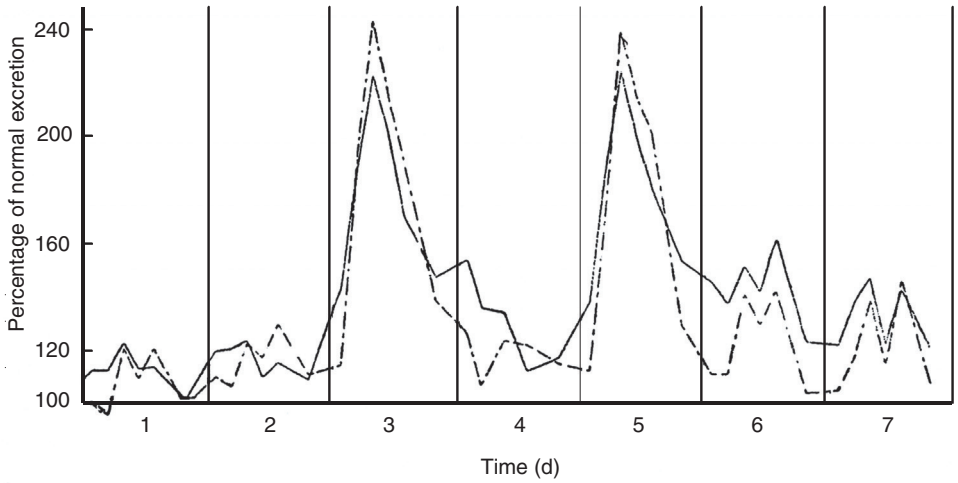

Fig. 2. Evolutionary pattern of a 7 d N and S balance study (Sherman & Hawk, 1900). Baseline values are normalised to 100 % and result from the daily intake by two healthy subjects (the authors of the publication themselves) of a standard regimen providing 10·9 MJ(2600 kcal) and 15 g N. The curves shown here represent the relative fluctuations in the average rates of excretion of N (—) and of S (- - -). An extra supply of 10 g N was ingested in the form of lean beef meat at the start of days 3 and 5, resulting in the near doubling of N and S losses. The study demonstrates that N and S excretory rates remain closely correlated under both basal dietary and supplemented conditions.

intake levels (Hiramatsu *et al*. 1994; Raguso *et al*. 1997). The liver is the major site of synthesis, sequestration and secretion of GSH, utilising either Cys or Met as precursor substrates (Meister & Anderson, 1983; Lauterburg & Smith, 1986; Beutler, 1989). GSH efflux from the liver exceeds 90 % of the total GSH pool found in the bloodstream (Garcia & Stipanuk, 1992), displaying a $T_{\frac{1}{2}}$ of 3 min (Lauterburg *et al*. 1984). The peripheral breakdown of the S-tripeptide yields its Cys moiety in tissue whose membranes are endowed with γ-glutamyl-cycle enzymes. In healthy adults, during the post-absorptive (fasting) period, about 55 % of the plasma Cys flux may result from GSH degradation (Fukagawa *et al*. 1996). An extensive enzymic degradation of GSH occurs in the kidneys which serve as a major supplier of plasma Cys molecules (Abbott *et al*. 1984; Garcia & Stipanuk, 1992). It is worth being reminded that mammals are unable to synthesise Met from Cys. The liver is the major organ of Tau synthesis and the kidney is predominantly involved in Tau excretion (Garcia & Stipanuk, 1992). Part of this last SAA is lost in the biliary flow in the form of taurocholates but may be, to some extent, recovered along the hepatoenteric cycle through the deconjugation of bile salts.

Inorganic SO_4^{2-} is an essential oxyanion required to meet metabolic needs due to its participation in sulfonation processes. The body's requirements are met by SO_4^{2-} via food items, drinking waters and the endogenous breakdown of sulfonated molecules. A minor proportion of dietary SO_4^{2-} may be subjected to bacterial reduction before intestinal uptake (Gibson *et al*. 1993) but the bulk of SO_4^{2-} undergoes very high absorption rates with negligible faecal loss (Krijgsheld *et al*. 1979). Several investigations have documented the close relationship linking the amount of N and/or S intakes and the degree of sulfaturia, confirming the pioneer study performed a century ago (Sherman & Hawk, 1900). Adult volunteers given three different levels of protein intake excrete in their urinary output SO_4^{2-} values highly correlated (r 0·88) with dietary N intake (Wright *et al*. 1960). Similar high correlations are found in healthy adults submitted, during an 8 d period, to SAA-deprived regimens and displaying significantly depressed sulfaturia (Lakshmanan *et al*. 1976). In contrast, stepwise increases in the supply of dietary SAA ranging from normal (0·8 g/d) to high (4 g/d) levels result in a high correlation (r 0·98) with renal SO_4^{2-} output (Sabry *et al*. 1965). Under well-balanced dietary conditions, sulfaturia of healthy individuals usually ranges from 20 to 25 mmol/d (640 to 800 mg/d; Huxtable, 1986), reflecting the amount of ingested S compounds. Taken together, most clinical trials point to complex and narrow interconnections between N and S metabolic pathways which are readily up and down regulated so as to maintain steady state plasma concentrations of Met and Cys in a variety of nutritional circumstances. The conclusion also applies to SO_4^{2-} plasma values which are remarkably stable and likewise manifest strong refractoriness to dietary manipulations (Huxtable, 1986).

Regulation of methionine homeostasis in protein-depleted states

The Massachusetts Institute of Technology clinical and metabolic investigations (Storch *et al*. 1990; Hiramatsu *et al*. 1994; Fukagawa *et al*. 1996; Raguso *et al*. 1997) mentioned earlier were undertaken in healthy adult volunteers who were given their test diet and meals during a limited number of days. These studies have highlighted that Met homeostasis is achieved via the combined results of small changes in tissue protein breakdown and subtle alterations in the partitioning of Met along re-methylation and trans-sulfuration pathways. Under longer-lasting or more severe deprivation conditions, Met homeostasis depends upon other and more drastic regulatory mechanisms. Following

the nature, gravity and duration of dietary deficiencies, several protein–energy malnutrition (PEM) morbidities may develop ranging from overhydrated kwashiorkor cases to emaciated marasmus as extreme clinical forms. Kwashiorkor is a subacute disorder usually taking place during the post-weaning period. It is typically delineated by down-regulated metabolic activities, significant liver steatosis which may serve as an indicator of protein deficiency (Waterlow, 1975), high infectious vulnerability and the depression of protein syntheses, notably those proteins secreted by the liver such as serum albumin and TTR (Ingenbleek *et al*. 1972; Waterlow, 1992). Marasmus is a chronic disease, causing weight and height retardation, exhaustion of both muscular and lipid compartments but with minimal liver fatty infiltration and relatively well-preserved immune and synthetic capacities. The aminoacidaemia of PEM usually reveals unaltered or elevated dispensable AA concentrations whereas IAA exhibit declining trends, affecting more specifically isoleucine, leucine and valine, the so-called branched-chain AA (Arroyave *et al*. 1962; Ittyerah *et al*. 1965). Among the eight IAA, plasma Met concentration remains unmodified in subclinical PEM or marasmus (Polge *et al*. 1997; Ingenbleek *et al*. 2002) or falls within a subnormal range in kwashiorkor disorders (Arroyave *et al*. 1962; Ittyerah *et al*. 1965). The latter anomaly may be partly explained by the fact that Met is relatively low in vegetable products (Young & Pellett, 1994) whose nutritive value may be further reduced by food processing (Friedman, 1992). The departure from normal homeostasis also indicates that the usual regulatory mechanisms described by Finkelstein's and Young's groups no longer operate effectively. Distortion from normal is further documented by the very high plasma Met values recorded in PEM children given an exogenous Met supply (Awwaad *et al*. 1962) or submitted to nutritional rehabilitation (Snyderman *et al*. 1968). The extraordinarily high Met elevation, ranging from two to thirty-five times normal average levels and persisting during many weeks, has led these last authors to postulate 'some failure in its catabolism' (Snyderman *et al*. 1968). The data are reminiscent of liver damage similarly characterised by depressed branched-chain AA plasma concentrations and supranormal Met values (Fisher, 1982; Montanari *et al*. 1988) whose biological $T_{\frac{1}{2}}$ is significantly increased (Kinsell *et al*. 1947; Horowitz *et al*. 1981), again implying an abnormality in the catabolism and removal of Met.

The decline in plasma Met concentration after an oral load follows first-order kinetics and the average clearance rate (170 ml/min) suggests that Met elimination is not a flow-dependent process (Horowitz *et al*. 1981). Hypermethioninaemia is reportedly associated with the genetic absence of the higher K_m I/III isoenzyme of Met adenosyltransferase (Gaull & Tallan, 1974; Finkelstein, 2000). An acquired reduction in Met adenosyltransferase activity to 37·5 % of normal is documented in cirrhotic patients (Cabrero *et al*. 1988), resulting in a depressed rate of Met to SAM conversion. Histopathological lesions found in liver cirrhosis or steatosis could indeed create a portosystemic vascular shunt, mimicking surgical anastomosis (Baldessarini & Fisher, 1967) and forcing Met fluxes to bypass the organ. Intrahepatic SAM depression should be proportionate to the extent of liver damage and more affected in kwashiorkor cases than in marasmic forms of malnutrition. As a result, SAM is no longer capable of properly stimulating CβS activity, as would be expected from healthy individuals submitted to flooding doses of Met. In cirrhotic patients, the lowering of SAM concentration works as a primary defect causing a uniform reduction in derivatives downstream to Met adenosyltransferases, including that of Hcy, unless there is simultaneously a shrinking of their body N reserves. In that case, as in PEM patients affected by diminished TBN pools, there is a gradual elevation in the Hcy concentration in extracellular fluids (Ingenbleek *et al*. 2002). These investigators were the first to postulate the likelihood of an acquired blockade of CβS activity as an adaptive response aimed at preserving endangered N pools. As a general rule in protein metabolism, when the diet is lacking one IAA, the reduced activity of its catabolic enzymes favours a more prolonged conservation of the AA within endogenous pools with increased survival (Carpenter, 1992). Enzymes are protein molecules whose synthesis and/or functional capacities are influenced by changes in N availability (Stephen, 1968). An enzyme-induced sparing of AA whose intake fails to meet optimal requirements is documented for the other IAA namely lysine (Brookes *et al*. 1972), tryptophan (Young & Munro, 1973), threonine (Kang-Lee & Harper, 1978), phenylalanine (Antener *et al*. 1981*b*) and branched-chain AA (Hutson & Harper, 1981).

The concept that CβS activity could remain under the control of N availability, regardless of SAM status, arose from studies undertaken in 1986 on goitrous patients living in the southern region of Senegal (Ingenbleek *et al*. 1986). Despite evenly distributed iodine deficiency, goitrous patients revealed significantly different stages I, II and III of thyroid swelling, according to WHO classification (Pérez *et al*. 1960). Enlargement of the endocrine organ was found to be correlated with declining protein nutritional status as assessed by the measurement of plasma TTR, retinol-binding protein and retinol (Ingenbleek *et al*. 1980); all three components are closely bound within the trimolecular retinol-circulating complex. The aggravating goitrogenic role played by poor nutritional status was attributed to PEM-induced dysmaturation of thyroglobulin (Ingenbleek & De Visscher, 1979; Ingenbleek, 1983). In this preliminary survey, TTR values were found to be negatively correlated with Hcy concentrations, and direct CβS impairment was the proposed explanation (Ingenbleek *et al*. 1986). This first investigation was later extended to another iodine-deprived area in the Republic of Chad where goitrous patients were similarly affected by stepwise PEM states of increasing severity (Ingenbleek *et al*. 2002). Careful attention was given to eliminate from this second study all causal factors which might interfere with the interpretation of TTR and Hcy data. The enlarged battery of biochemical parameters comprised the measurement of the vitamin triad involved in Hcy regulation, that of acute-phase reactants which helped to identify individuals suffering from an underlying inflammatory disorder (Ingenbleek & Young, 1994), and of thyroid hormonal fractions guiding the removal of cases of hypothyroidism

as a potential explanation for high Hcy levels (Nedrebø *et al*. 1998). Values obtained for TTR, for the eight IAA, Hcy and Cysta are summarised in Table 1 showing that all these parameters showed declining trends as protein nutritional status worsened, with the sole exceptions of Met which sustained unmodified levels opposed to the gradual elevation of Hcy values. This latter pattern was unique and the inverse correlation linking TTR and Hcy concentrations is expressed in Fig. 3. The divergent evolution of Hcy and Cysta values point to the progressive inhibition of CβS activity under all conditions threatening endogenous N pools. The data are in agreement with the independent, inverse dose–response relationship existing between the level of protein intake and Hcy values (Stolzenberg-Solomon *et al*. 1999). The causality of deficient N status in the occurrence of acquired hyperhomocysteinaemia must be suspected in all circumstances likely to affect TBN pools such as in weight-reduction programmes (Gallistl *et al*. 2001), anorexia nervosa (Moyano *et al*. 1998) or senile dementia (McCaddon *et al*. 1998) when the vitamin indices remain poorly confirmatory.

Summing up, protein-depleted patients at risk of reduced N body stores are characterised by a defective conversion of Hcy to Cysta, favouring the upstream accumulation and re-methylation of Hcy to Met owing to the higher K_m affinity of Met synthase for Hcy compared with CβS (Finkelstein, 1974, 2000). The acquired metabolic anomaly is accompanied by increased homocystinuria (Antener *et al*. 1981*a*) which contrasts with the fall of most blood and urinary S catabolites downstream to CβS, notably that of Cysta (Ingenbleek *et al*. 2002), Cys (Chawla *et al*. 1985), GSH (Jackson, 1986; Hum *et al*. 1992), Tau (Gray *et al*. 1994) and SO_4^{2-} (Ittyerah, 1969). The exogenous supply of Cys to PEM patients has been shown capable of restoring to some extent the synthesis of GSH in erythrocytes (Baladoo *et al*. 2002). These abnormalities appear to be more pronounced in kwashiorkor than in marasmic cases and result from the combined effects of both inappropriate dietary intake of S-containing molecules and the CβS defect. Many investigators have underscored the role played by PEM in the variance of Hcy concentrations probably because they are using serum albumin, transferrin or creatinine which are meagrely sensitive markers of protein status compared with TTR.

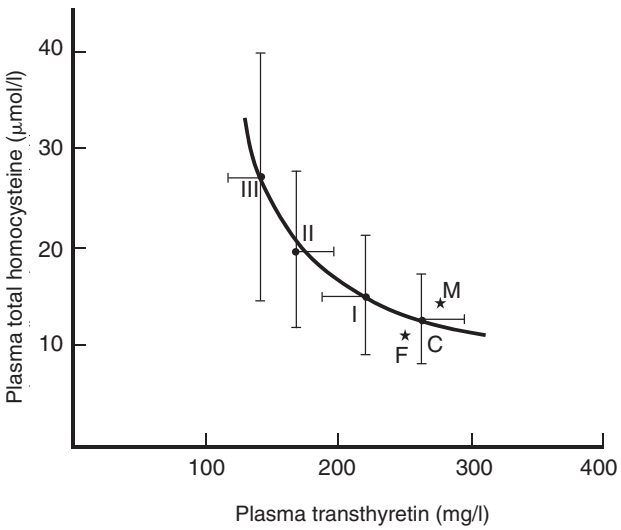

Fig. 3. Measurement of transthyretin (TTR) and homocysteine (Hcy) in control subjects (C) and goitrous patients. WHO recommendations (Pérez *et al*. 1960) served to classify iodine-deprived individuals into three cohorts representing the stages I, II and III of goitrous swelling. Each of the four groups comprised twenty adult subjects (ten males and ten females). TTR and Hcy values were pooled for interpretation and expressed as means, with standard deviations represented by horizontal and vertical bars. In the C group, male (M) and female (F) concentrations are shown separately, indicating that both TTR and Hcy manifest sexual difference. The data reveal that declining protein nutritional status, as assessed by the TTR results, is negatively correlated with rising Hcy concentrations.

Table 1. Plasma concentrations of transthyretin (mg/l) and of amino acids (μmol/l) in control adults and goitrous patients§ (Mean values and standard deviations)

Variables	Control group (*n* 20)		Goitrous patients								
			Stage I (*n* 20)			Stage II (*n* 20)			Stage III (*n* 20)		
	Mean	SD	Mean	SD	% of control	Mean	SD	% of control	Mean	SD	% of control
Transthyretin	268	31	221*	31	83	171†	26	64	146‡	24	55
Lysine	209·3	39	173·1†	46	82	158·8‡	39	76	131·3‡	23	62
Threonine	199·6	51	158·2*	64	79	139·1‡	49	69	125·6‡	29	62
Leucine	179·8	37	129·6‡	41	72	115·1‡	39	64	98·3‡	22	54
Isoleucine	103·6	19	73·7†	18	71	59·4‡	14	57	50·5‡	11	48
Valine	258·2	47	201·0†	42	77	170·9‡	38	66	139·6‡	21	54
Phenylalanine	98·3	14	73·1*	21	74	65·6†	22	66	60·3‡	11	61
Tryptophane	34·5	7·1	21·8†	6·8	63	18·6‡	9·1	53	16·2‡	5·3	46
Methionine	15·2	6·1	17·2	9·4	113	14·8	6·3	97	12·9	4·1	84
Homocysteine	12·7	4·7	15·2†	6·2	119	20·1‡	8·1	158	27·3‡	12·6	214
Cystathionine	4·08	0·81	3·76	0·53	92	3·41†	0·49	84	3·12†	0·41	76

Mean value was significantly different from that for the control group: **P*<0·05, †*P*<0·01, ‡*P*<0·001.
§Measurements were performed in four cohorts of twenty individuals (ten males and ten females in each cohort). The results for male and female subjects were pooled for interpretation. In the control group, transthyretin and homocysteine were the sole variables exhibiting sexual difference (Ingenbleek *et al.* 2002).

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Regulation of methionine homeostasis in stressful disorders

Inflammatory disorders encompass a large cytokine-induced spectrum of metabolic, endocrine and immune alterations developed by the injured body in response to various agents (Wolfe *et al*. 1989). Rising concentrations of the so-called counter-regulatory hormones (glucocorticoids, insulin, glucagon, somatotrophin) work in concert with cytokines to generate an overall insulin resistance and down-regulation of thyroid function aimed at sparing protein and energy resources in healthy tissues (Ingenbleek & Bernstein, 1999). In contrast, diseased tissue areas manifest up-regulated events locally promoting defence mechanisms and tissue repair. Taken together, the adaptive changes point to a dichotomous partitioning of the whole body economy (Ingenbleek & Bernstein, 1999). A salient feature is the strong stimulation of protein turnover as a result of both augmented tissue proteolysis (mainly in the muscle mass) and enhanced specific tissue protein synthesis (mainly in the liver and at the site of injury). The rate of protein degradation generally exceeds the rate at which AA are used for protein syntheses, yielding a net negative N balance whose magnitude is proportionate to the severity and duration of the stress reaction (Ingenbleek & Young, 1994; Ingenbleek & Bernstein, 1999).

Clear evidence that tissue injury of any cause leads to early catabolic losses of both N and S substrates came from studies performed by Cuthbertson (1931) in adult individuals with bone fractures. The rise in N excretion was mainly due to an increased urinary output of urea whereas S was principally excreted in the form of inorganic SO_4^{2-}. The Scottish author stated that the excretory curves of N and of S ran closely parallel, reaching a peak within 3 to 6 d from the initial insult. Moreover, he also predicted, 50 years before the discovery of the roles played by cytokines in stressful disorders, that the increased urinary leakage of urea and SO_4^{2-} as chief products of enhanced muscular catabolism could be due to an 'excessive number of abnormal stimuli (triggering) direct poisoning of the tissue cells such as might be supposed to take place in febrile conditions and in tissue injury'.

The metabolism of Met and S derivatives is complex and not entirely clarified in inflammatory disorders. There is no doubt that GSH is centrally involved in the adaptive responses to stressful stimuli. GSH is indeed implicated in the synthesis of leucotrienes (Anderson *et al*. 1982), contributes to promote lymphocyte (Dröge *et al*. 1991) and anti-inflammatory (Bragt & Bonta, 1980) activities, to neutralise hydrogen organic peroxides (Uhlig & Wendel, 1992) and to scavenge potentially toxic heterologous products (Meister & Anderson, 1983; Beutler, 1989). Assuming that an unaltered level of any metabolite results from the balance between synthesis and consumption rates, the increased demand for molecules mediating the adaptive responses to inflammation implies increased rates of production. This is illustrated by the plasma concentrations of Met, Cys and GSH usually found within physiological ranges despite accelerated turnover rates. Met fluxes are significantly enhanced in burned patients and principally directed into SAM transmethylation and Hcy re-methylation pathways whereas the proportion of Met driven into the Hcy trans-sulfuration cascade is relatively diminished (Yu *et al*. 1993). This suggests that under these conditions of greater turnover rates, Met is oriented towards the feeding of the free AA tissue pools maintaining the S anabolic drive (Yu *et al*. 1993). The mechanism underlying this fate of Met appears to be supported by the inhibition of γ-cystathioninase, an enzyme whose activity seems poorly sensitive to nutritional influences (Viña *et al*. 1992) but selectively depressed by inflammatory stimuli (Viña *et al*. 1992; Malmezat *et al*. 2000*a*,*b*). Due to the relative impairment of the trans-sulfuration pathway, the turnover rates of Cys and GSH cannot be fully or directly explained by an increased metabolic conversion of Met but rather depend on the breakdown of endogenous proteins or on the release of tissue reserves. The concept is consistent with studies showing that the production of Cys is not significantly affected when γ-cystathioninase activity is inhibited (Rao *et al*. 1990) and that the liver uptake of Cys (presumably of GSH or tissue origin) is paradoxically increased when the enzymic conversion of Met is reduced (Viña *et al*. 1992). As expected, the inhibition of γ-cystathioninase activity is accompanied by the upstream accumulation of significant amounts of Cysta in the plasma and the liver of infected rats (Viña *et al*. 1992; Malmezat *et al*. 2000*b*). Well-nourished animals submitted to a sepsis burden manifest increased rates of GSH synthesis seemingly adequate to sustain normal or even high tissue concentrations (Viña *et al*. 1992; Malmezat *et al*. 2000*b*). In contrast, absolute synthesis rate and blood GSH concentrations are found significantly lowered in symptom-free HIV (Buhl *et al*. 1989; Jahoor *et al*. 1999) and in burned patients (Mårtensson *et al*. 1987; Yu *et al*. 2002), suggesting distinct kinetic regulations between animal models and human subjects. In the case of pre-existing PEM in pigs, GSH status is severely compromised in the intestinal mucosa and in erythrocytes but relatively well preserved in the liver, reflecting a functional priority (Jahoor *et al*. 1995). In this inflammatory context, the relative independency of Cys from its Met precursor pools as well as its conditionally limited availability for appropriate GSH synthesis confers a relative degree of essentiality to this dispensable AA (Grimble *et al*. 1992; Viña *et al*. 1992; Jahoor *et al*. 1995). It has been shown that an exogenous supply of Cys has beneficial consequences on the response of rats to stress created by TNFα (Grimble *et al*. 1992). Obviously, the breakdown of endogenous proteins while helping to meet increased tissue Cys requirements simultaneously contributes to a deterioration in N and S balances.

The negative N balance accompanying any hypercatabolic state and typically delineated by N and S urinary losses running in parallel (Cuthbertson, 1931) has been subjected to more recent inquiry in burn injury. During the acute stage of injury, the substantial urinary output of urea and of minor N catabolites, including creatinine and 3-methylhistidine (Young & Munro, 1978), reflects the depletion of both metabolic and structural N compartments (Ingenbleek & Young, 2002) whereas the increased cyst(e)inuria, taurinuria and sulfaturia (Larsson *et al*. 1982) are indicative of the degradation of S pools. In contrast, the anabolic phase of recovery is characterised by a greatly

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depressed elimination of these S compounds whose nadir levels coincide with the period of most intense tissue repair (Mårtensson *et al*. 1987). These urinary patterns are usually associated with an overall shrinking of systemic GSH pools reported in burned patients (Larsson *et al*. 1982) but also in HIV infection (Buhl *et al*. 1989) and in many other chronic inflammatory disorders (White *et al*. 1994). The data are consistent with the greatly reduced bioavailability of these S compounds measured in the leucocytes of burned patients during the most intense phase of anabolic recovery (Mårtensson *et al*. 1987). Low GSH concentrations usually persist as long as defence and healing processes are active and maintain a magnified peroxidative burden. The aminoacidaemia found in stressed or septic patients usually displays a reduction by 10 to 30 % of virtually all AA concentrations but with unmodified methioninaemia (Vente *et al*. 1989), again stressing efficient preservation mechanisms and a relative disconnection of Met from the S metabolites downstream to γ-cystathioninase, at least under mild or moderate stressful conditions. The data collected on N turnover are consistent with the view that both the *metabolic pool* (comprising organs endowed with rapidly turning over proteins) and the *structural pool* (mainly made up of muscular and cutaneous proteins with slower turnover rates) participate in the response of the stressed body. However, the balance between these N compartments is determined by the nature and severity of the injury (Ingenbleek & Young, 2002). Rat experiments based on long-term tissue labelling with [^{35}S]Met have shown that S similarly partitions into two distinct compartments characterised by fast and slow turnover rates (Jackson *et al*. 1968), whose fluctuations seem closely correlated with N fluxes. Under severe morbid circumstances or in the case of major complications, the regulatory mechanisms governing N:S homeostasis may nevertheless be disrupted. A supranormal SAA plasma profile has been described for Cys and Tau as a consequence of renal failure (Vente *et al*. 1989). The concomitant elevation of Met, Cys and Tau plasma values in septic patients points to severe metabolic disturbances, carrying ominous prognosis significance (Freund *et al*. 1979).

Conclusion and recommendations

S found in the biosphere is an essential element and cycles in both vegetable and animal kingdoms in close relationship with N. Body composition analysis shows that S and N accumulate in mammalian tissues at a molar ratio close to 1:15. Animal requirements for S-containing compounds can be entirely or mainly covered by the dietary intake of Met. The endogenous pools of Met and derivatives, chiefly sequestered within the liver, are very well conserved in part due to the combined effects of minimal gut losses, efficient intestinal uptake and renal recovery rates. In addition, the remarkably constant Met and Hcy plasma concentrations appear to be the net result of subtle competitive mechanisms between re-methylation and trans-sulfuration pathways together with adaptive alterations in the overall homeostasis of Met, depending on its bioavailability. Balance studies performed on healthy adult subjects and using labelled material have shown that the oxidative loss of Met reflects the composition of mixed body proteins and occurs in close relationship with the N excretion rate (Young *et al*. 1997; Raguso *et al*. 1999). Protein malnutrition is characterised by TBN depletion affecting primarily the N metabolic pool and by urinary excretion of N and S catabolites proportionate to the level of tissue breakdown. An enhanced re-methylation rate is favoured by the N-depletion-induced acquired blockade of CβS activity, leading to an increased upstream disposal of Hcy. As a consequence, significant homocystinuria may develop, together with decreased urinary losses of S-containing derivatives downstream to CβS. Inflammatory disorders of mild or moderate severity are characterised by the impairment of γ-cystathioninase activity, an upstream accumulation of Cysta and altered Cys and GSH turnover rates. More aggressive hypercatabolic states usually lead to N and S balances becoming even more negative (Cuthbertson, 1931), resulting from the shrinking of both metabolic and structural N and S compartments (Jackson *et al*. 1968; Ingenbleek & Young, 2002).

Although the aetiopathogenic events of stress are basically unrelated to those found in PEM, both conditions may nevertheless similarly endanger N and S compartments and threaten life expectancy. The acquired blockade of CβS activity serves as a salvage mechanism allowing Met to maintain functional properties of survival importance. N-induced hyperhomocysteinaemia thus appears as the dark side of adaptive processes striving to maintain Met homeostasis of the stressed body. The impact of the N status on the trans-sulfuration pathway is an independent variable unrelated to the effects of the water-soluble vitamin status. In both malnourished and injured patients, Hcy plasma values are negatively correlated with the alterations of body N compartments. Reduction in the size of TBN, as seen in most debilitating conditions, is faithfully identified by the serial measurement of TTR which also constitutes a valuable tool for monitoring the efficacy of nutritional support and/or antiphlogistic therapy.

Normal TTR concentrations indeed manifest sex- and age-distribution patterns (Ingenbleek & De Visscher, 1979; Bienvenu *et al*. 1996) superimposed on those of TBN and mainly explained by a larger proportion of muscle protein in male subjects (Ingenbleek & Young, 2002). The higher Hcy values found in men (Pancharuniti *et al*. 1994; Lussier-Cacan *et al*. 1996) are similarly attributed to the larger fat-free mass characterising healthy male individuals (Dierkes *et al*. 2001). These sex- and age-related evolutionary patterns point to regulatory mechanisms maintaining from birth to adulthood tight correlations between the size of the TBN compartment and the plasma concentrations of TTR and Hcy. These biological parameters manifest, on the contrary, diverging profiles in healthy elderly individuals likely to result from the genetically programmed shrinking of their structural TBN pools and affecting more markedly elderly males than female counterparts. While TBN and TTR values outline closely correlated declining trends until the end of life (Bienvenu *et al*. 1996; Ingenbleek & Young, 2002), Hcy molecules display rising tendencies and accumulate in the body fluids (Rosenberg *et al*. 1997; Refsum *et al*. 2004). These data suggest that the regulatory mechanisms set in motion during the pre-senescent period no

longer operate in the course of ageing. Healthy elderly individuals thus represent a target group incurring an increasing risk for Hcy-related thrombovascular disorders, being even more exposed to any additional factor further depleting their protein status.

Epidemiological studies have clearly established the significance of folate, cobalamin and pyridoxine deficiencies in the causation of augmented Hcy concentrations in several vulnerable population groups. These dietary factors, however, do not sufficiently account for the sizeable proportion of individuals with hyperhomocysteinaemia and who are poorly responsive to vitamin therapy. We are proposing the combined measurement of Hcy and TTR as a means to fill the gap between the recorded public health findings and the vitamin causal factors already recognised to date, allowing the assessment of the additional contribution of subclinical and clinical states of protein malnutrition in the occurrence of the metabolic anomaly.

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