SULFUR AMINO ACID REQUIREMENTS AND THE BIOAVAILABILITY OF OXIDIZED SULFUR AMINO ACIDS IN THE GROWING RAT FED EIGHT PERCENT DIETARY PROTEIN

by

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ABSTRACT

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The requirement for sulfur amino acids (SAA) was found to be 0.34% of diet (0.32% L-methionine + 0.02% L-cystine) as measured by optimal feed/gain ratio, RNPR, RPER and Liver Protein Utilization of growing rats fed diets providing 4% protein from ANRC casein and amino acids (except methionine and cystine) equivalent to 4% casein protein and supplemented with increments of L-methionine. Replacement of portions of L-methionine with L-cystine in similar diets indicated that L-cystine could replace up to 60% of the methionine requirement. Plasma amino acid (PAA) data supported rat growth results.

L-methionine sulfoxide was shown to be as available and L-methionine sulfone and L-cysteic acid less available when used to partially replace respective unoxidized forms as measured by Relative Methionine Availability, rat growth and PAA data.

Two commercial infant formulas in liquid concentrate and powdered forms were tested relative to their fresh condensed skim milk (CSM) source, as supplements to low SAA rat diets balanced in terms of amino acid, lipid and lactose content. Liquid concentrate from one manufacturer provided less available methionine than the CSM supplement and rat growth and PAA data suggested liquid concentrates provide less available methionine than respective powered forms likely reflecting the greater heat processing involved in preparation of liquid concentrates.

RÉSUMÉ

R.W. Peace M.Sc. Zootechnie

Besoins en acides aminés sulfurés et biodisponibilité des acides aminés sulfurés oxydés chez le jeune rat sous régime à huit pourcent de protéine

Le besoin en acides aminés sulfurés (AAS) a été de 0.34% du régime (0.32% L-méthionine + 0.02% L-cystine) selon le rapport nourriture/gain optimal, le RNPR, le RPER et l'Utilisation Protéique Hépatique chez le rat en croissance sous régime à 4% de protéine de caséine et d'acides aminés ANRC (méthionine et cystine exceptées) équivalent à 4% de protéine de caséine et additionné de doses croissantes de L-méthionine. En la substituant à la L-méthionine, la L-cystine a remplacé jusqu'à 60% des besoins en méthionine dans un régme semblable. Les valeurs des acides aminés plasmatiques (AAP) concordaient avec les résultats de croissances.

Lorsque substituée partiellement à la forme non oxydée, la L-méthionine sulfoxide avait une biodisponibilité comparable mais celle de la L-méthionine sulfone et de l'acide L-cystéique était inférieure d'après la Biodisponibilité Relative de la Méthionine, la croissance chez le rat et les valeurs d'AAP.

Deux mélanges lactés commercials pour biberons, sous formes de concentré liquide et de poudre, ont été testés chez le rat par rapport à leur source de lait écrémé condensé (LEC) frais comme suppléments à des régimes pauvres en AAS et équilibrés pour leur teneur en acides aminés, lipide et lactose. Un concentré liquide de l'un des fabriquants a manifesté une biodisponibilité méthionine moindre que le supplément LEC; les données de

croissance et d'AAP ont suggéré que les concentrés liquides avaient une biodisponibilité méthionine moindre que les poudres correspondantes, reflétant vraisemblablement le traitement (chaleur) plus poussé subit durant le préparation des concentrés liquides.

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ABBREVIATIONS AND DEFINITIONS

Amino Acids

Arg	- arginine	α-Aba	- α-amurobutyric acid
His	- histidine	Ala	- alanine
Ile	- isoleucine	Asp	- aspartic acid
Leu	- leucine	Glu	- glutamic acid
Met	- methionine	Pro	- proline
Cys	- cystine	Ser	- serine
Phe	- phenylalanine	Tau	- taurine
Tyr	- tyrosine	Cit	- citrulline
Thr	- threonine	Orn	- ornithine
Trp	- tryptophane	Val	- valine

CSM - condensed skim milk

LC-I, II - infant formula, liquid concentrate form - Manufacturer I, II

LPU - Liver Protein Utilization expresses the amount of food nitrogen 'retained' in the liver as a percentage of food nitrogen intake.

NPR - Net Protein Ratio is the weight gain of a test diet-fed animal plus the weight loss of a control animal fed a non-protein diet per gram of protein consumed under standard conditions.

RNPR - Relative Net Protein Radio compares NPR of test diet fed animals (or test protein) to NPR obtained using animals fed a high quality reference protein under standard conditions at the same time.

NPU - Net Protein Utilization is the proportion of nitrogen intake that is retained.

PSPS - 'Active sulfate' 3'-phosphate-5'-phosphosulfate

PER - Protein Efficiency Ratio is the weight gain of an animal per weight of protein consumed under standard conditions.

RPER - Relative Protein Efficiency Ratio compares PER of test diet fed animals (or test protein) to PER of animals fed a high quality reference protein under standard conditions at the same time.

PF-I, II - Infant formula, powdered form - Manufacturer I, II

Relative Methionine Availability compares NPR obtained using a test diet containing a potential supplementary source of available methionine to NPR obtained using a reference diet.

For more complete definitions, references cited in text of thesis are recommended.

I. INTRODUCTION

The sulfur amino acid requirements of the rat for growth and maintenance have been subjects of considerable study (Rao et al. 1959, Sowers et al. 1972, Byington et al. 1972, Stockland et al. 1973). The weanling rat is widely used in bioassays to estimate adequacy and quality of food proteins and such estimates are generally considered to have predictive significance in terms of human nutrition (Campbell and McLaughlan 1971, Young and Scrimshaw 1974).

The requirement of the essential amino acid methionine has particular importance due not only to its structural role in proteins but also to its unique biological function in the initiation of protein synthesis (Bhagavan 1978) and in transmethylation reactions (Aguilar et al. 1974). Methionine is frequently the first limiting amino acid in important human food sources such as legumes. It may also be rendered unavailable as a result of oxidation reactions that occur in food processing (Slump and Schreuder 1973, Kunachowicz et al. 1976, Strange et al. 1980).

Determination of the requirement for methionine is complicated to some extent by the partial sparing effect of the sulfur-containing amino acid cysteine. Usually cystine (comprised of 2 molecules of cysteine), is considered in terms of methionine replacement. The National Research Council (1978) lists an L-methionine requirement for growth, lactation or gestation in rats of 0.6 percent of diet (5.0 g/100 g protein) when a high quality, 100% digestible, whole egg protein-based diet is fed at the twelve percent protein level. L-cystine is reported to be able to replace one third to one half of this methionine requirement.

Sulfur amino acid requirements of children, ranging from 3.5 g/100 g protein to 2.6 g/100 g protein (FAO/WHO 1973, Torun et al. 1981, NRC 1980) are lower than reported rat growth requirements. In addition it is generally agreed that any rat growth assay underestimates protein quality for humans of food sources deficient in sulfur amino acids (Steinke 1979, Sarwar et al. 1983, Codex Alimentarius Commission 1984). Current regulatory and research practice in terms of protein quality assessment involves in part, rat bioassays with diets supplying eight to ten percent dietary protein (McLaughlan 1974, Sarwar and McLaughlan 1981). At these lower levels of protein intake, rat growth responses to deficits in component amino acids are readily shown and differences in protein quality of individual protein sources can be determined. Since the efficiency of protein and amino acid utilization is linearly related to the amount of protein consumed (Edozien and Switzer 1978), it was decided to determine if rat sulfur amino acid requirements at the eight percent protein level are less than those set by NRC (1978) and hence more closely related to human requirements.

There are conflicting reports in the literature as to the bioavailability of oxidized sulfur amino acids (Anderson et al. 1976, Sjöberg and Böstrum 1977, Cuq et al. 1978). Any reduction in availability could be important in terms of human nutrition, in foods limiting in sulfur amino acids and fed as sole sources of dietary protein to groups such as vegans and formula fed infants. As a result it was also decided to test the bioavailability of the main oxidized forms of sulfur amino acids - methionine sulfoxide, methionine sulfone and cysteic acid, and to test the relative methionine availability of potentially oxidized commercially-prepared infant formulas.

SAM has been shown to serve as a methyl group donor for a variety of enzymatically catalyzed reactions. Guanidoacetic acid methyl transferase for example catalyzes the reaction of guanidoacetate with SAM to produce creatine, H^+ ion and S-adenosylhomocysteine (du Vigneaud et al. 1940). Specific methyl transferases are involved in other reactions of SAM resulting in compounds such as N-methylnicotinamide, methylhistamine, phosphatidylcholine, melatonin, anserine, epinephrine, metanephrine, ergosterol and certain purines and alkaloids (Meister 1965, Cooper 1983).

Schlenk and DePalma (1957) isolated SAM from cultures of the yeast Torula utilis grown in methionine containing media. SAM has also been isolated in adrenal, liver, heart, spleen, kidney, lung and brain tissues of the rat (Baldessarini and Kopin 1963).

S-adenosylhomocysteine (SAH) derived from the various methyl transfer reactions is a potent inhibitor of most transmethylation reactions (Finkelstein et al. 1982). It is subsequently acted upon by cleavage enzyme (a specific hydrolase EC 3.3.1.1) with addition of water and loss of adenosine moiety to form homocysteine. Homocysteine has been shown to support rat growth with methionine-deficient diets, but only if folate and Vitamin B_{12} or choline or other methyl source were present in the diet.

At least two potential salvage pathways for remethylation of homocysteine and formation of methionine exist. Betaine-homocysteine methyl transferase (EC 2.1.1.5) catalyzes the demethylation of betaine to dimethylglycine with formation of methionine by transfer of methyl group to homocysteine. In the absence of dietary choline from which betaine is derived, a depletion of methyl group can occur. Three methionine molecules are required to synthesize a single betaine or choline molecule (Finkelstein 1974). Both

dimethylglycine and methionine act as inhibitors of the reaction and this inhibition may be important in terms of control of the reaction (Finkelstein et al. 1972).

N^5-methyltetrahydrofolate acts as the methyl donor in the second remethylation reaction of homocysteine. Vitamin B_{12} is required as a co-enzyme and trace amounts of SAM act as cofactor in the reaction. This remethylation is important not only in terms of formation of methionine but also as a means of releasing folate from its N^5-methyl derivative. Release of folate thus prevents it from becoming 'trapped' or unavailable which could result in folate deficiency (Krebs et al. 1976). It has been demonstrated that both B_{12} and folate can spare choline, preventing rat renal damage and chick perosis in cases of folate deficiency. The net result of the transmethylation cycle is thus transfer of methyl group and conservation of both the four carbon unit and sulfur atom of methionine.

Transsulfuration - the transfer of sulfur atom via homocysteine to the carbon chain of serine and beyond is the third major metabolic route of methionine. Because of the common source and common intermediate, this pathway is closely related to the transmethylation pathway described previously. It is at the level of homocysteine that the metabolic fate of methionine is regulated. Cystathionine synthase (EC 4.2.1.22) catalyzes the irreversible condensation of homocysteine with serine to form cystathionine. Finkelstein and Mudd (1967) have shown significantly lower levels of cystathionine synthase and of methionine activating enzyme in livers of rats fed low methionine diets relative to hepatic levels in rats fed diets adequate in methionine with or without cysteine supplementation. Tissue saturation with methionine has been shown to favour accelerated cystathionine synthesis due to

decreased methyltetrahydrofolate formation and continued unmethylation of homocysteine (Krebs et al. 1976).

Cystathionine is cleaved to cysteine and α-ketobutyrate in the presence of the pyridoxal phosphate dependent enzyme cystathionase (EC 4.4.1.1). The sulfur atom originally present in methionine is thus present in cysteine - the second major sulfur amino acid to be considered.

An additional route for the catabolism of methionine, independent of S-adenosylmethionine activation has been demonstrated in rat liver tissue. Formaldehyde and formate have been proposed as intermediates in the oxidation of the methyl group to CO_2 by this pathway (Case and Benevenga 1976).

b) Cysteine/Cystine - FUNCTIONS AND METABOLISM

Cysteine is involved in a complex series of metabolic reactions. It can be incorporated as a structural component of proteins and of the tripeptide glutathione (Meister et al. 1980). Coenzyme A and adenosine 3'-phosphate-5'-phosphosulfate (PAPS or 'active sulfate') are also important derivatives of cysteine. It has been suggested that disulfides of cysteine and its derivatives may be involved in regulation of metabolic reactions in vertebrates (Ziegler 1985). Inorganic sulfate, the major urinary catabolite of cysteine and taurine, the major tissue catabolite have key structural and metabolic roles in the normal animal (Mudd 1980, Cooper 1983).

Cystine, the oxidized dimer of cysteine, is the major free form of cysteine found in the body and is readily converted to cysteine by enzymatic activity. Both cystine and cysteine can be formed in the body or supplied in the diet. Cysteine has been considered a non-essential amino acid for mammals and can be synthesized from methionine via cystathionine and serine. The absence of cystathionase enzyme in human fetal liver has been used to suggest that

cysteine is an essential amino acid for the human fetus (Sturman et al. 1970, Sturman 1980).

Two major pathways for the catabolism of cysteine have been determined in mammals. The direct oxidation of cysteine to cysteine sulfinic acid is catalyzed by cysteine dioxygenase (EC 1.13.11.20). Taurine is subsequently derived either via oxidation to cysteic acid followed by decarboxylation or via decarboxylation to hypotaurine and oxidation to taurine. Cysteine sulfinic acid can also be converted to pyruvate, SO_4^{2-} and NH_4^+. Transamination via 3-mercaptopyruvate to pyruvate and inorganic sulfate is the second major pathway though the oxidative route is considered to predominate (Stipanuk 1979). Martin et al. (1974) have described subsequent reactions of inorganic sulfate involving formation of PAPS. This latter compound can contribute to formation of cysteic acid and subsequent taurine formation or to sulfate conjugates that are important in detoxification reactions.

c) Taurine - FUNCTIONS AND METABOLISM

Taurine is the most abundant amino acid of animals and is the major free amino acid of the mammalian central nervous system. Its concentration varies with the species, sex, stage of development and tissue or fluid considered. Despite its ubiquitous occurrence, the only established roles for taurine involve the conjugation of bile acids and the maintenance of structural and functional integrity of the retina of cats. In this latter species, taurine has been shown to be an essential amino acid that must be supplied in the diet to prevent retinal degradation and blindness (Hayes and Sturman 1981). In other species, high tissue and urinary concentrations of taurine under normal nutritional regimens and the potential for taurine synthesis from methionine or cysteine involving cysteine sulfuric acid decarboxylase (CSAD) enzyme (EC 4.1.1.29) have supported the long standing opinion that taurine is a non-essential amino acid (Hayes 1976).

Evidence is accumulating that supports additional roles for taurine in animal metabolism. Cellular deletion studies and spike discharge inhibition experiments involving the cerebellum of the rat suggests taurine has a role in neurotransmission (McBride and Frederickson 1980, Bernardi et al. 1978). Taurine distribution studies of rat spinal chord preparations (Yoneda et al. 1978) and dietary and in vitro electrical stimulation studies of optic nerve and cerebral cortex preparations (Yoneda and Kuriyama 1978, Kuriyama 1980, Schaffer et al. 1980) support a neuromodulator function for taurine.

Higher levels of taurine in fetal and neonatal tissues (relative to the same tissues in older animals) and lower levels of CSAD enzyme as well as higher maternal milk taurine levels immediately post partum (relative to milk later in lactation) suggest a potential dietary requirement for taurine in premature and neonatal animals of species other than the cat (Sturman and Gaull 1975, Sturman and Hayes 1980, Rassin et al. 1978). Hayes et al. (1980) have suggested that taurine is essential for maximal growth in monkeys fed soy-based commercial infant formula devoid of taurine. Rassin et al. (1978) found lowered plasma and urinary taurine concentrations in formula fed human infants reflecting the lower taurine content of cow's milk relative to human milk. Lowered I.Q. measurements of low birth weight infants maintained by total parenteral nutrition (using intravenous solutions lacking in taurine) relative to controls not maintained parenterally (Tejani et al. 1979) and a higher taurine requirement to support immature bile acid metabolism in such infants (Dorvil et al. 1983) further support an essential role for taurine in the diet.

d) Inorganic Sulfur - SOURCES, FUNCTIONS, REQUIREMENTS

Inorganic sulfur is an integral part of the body sulfur pool. In non ruminants, sulfate is derived endogenously from the terminal oxidation step of

sulfur amino acid (SAA) metabolism. Sulfate oxidase (EC 1.8.2.1) has been detected in most animal tissues with highest levels occurring in the liver. Minor amounts of sulfite can also be supplied by exposure to atmospheric sulfur dioxide and by ingestion of foods containing bisulfite preservative (Johnson and Rajagopalan 1980). Inorganic sulfate is also a dietary constituent which normally contributes as much to the sulfur pool as does neutral or organic sulfur (Michels and Smith 1965).

Cellular metabolism of sulfate initiates with a series of activation steps with ATP to form adenosine 5'-phosphosulfate (APS) and adenosine 3'-phosphate 5'-phosphosulfate (PAPS). In higher animals specific esterases cause formation of sulfate esters from PAPS with steroids, phenols and polysaccharides (Schiff 1980). Michels and Smith (1965) using ^{35}S-labelled organic and inorganic sulfur sources have shown that little reduction of inorganic sulfur to neutral sulfur occurs in the intestinal mucosa or by intestinal bacteria of rats.

Inorganic sulfur has been shown to be important in sulfur incorporation into rib cartilage mucopolysaccharides and in formation of normal skin collagen (Michels and Smith 1965, Brown et al. 1965). Lack of dietary inorganic sulfur for rats has been reported to result in enhancement of avitaminosis E, impaired collagen metabolism and reduced feed efficiency (Whittle and Smith 1974) and reduced weight gain and Protein Efficiency Ratio (PER) (James and Hove 1978).

Brown and Gametero (1970) found that inclusion of dietary sulfate improved growth performance of young rats fed peanut meal-based diets low in both SAA and inorganic sulfate. Byington et al. (1972), however, found that inorganic sulfate had no significant effect on food intake, weight gain, carcass nitrogen, liver weight, liver lipid or liver choline content in rats fed varying levels of methionine and cysteine. The methionine sparing effect of inorganic sulfate for rats suggested by various authors occurs only in diets intrinsically

low in inorganic sulfur (Brown et al. 1965, Michels and Smith 1965, James and Hove 1978).

Requirements of the rat for inorganic sulfur have been set at 0.03% of diet as sulfate (NRC 1978). Generally intrinsic inorganic sulfur levels of most diets are adequate (Smith 1973, James and Hove 1978).

e) Methionine/Cysteine - INTERACTIONS AND REQUIREMENTS

The concept of dietary requirement or essentiality (indispensability) of an amino acid for any animal species, in general, hinges upon the inability of the animal to synthesize any (or adequate) amounts of the amino acid in question for the purposes of protein synthesis. Growth in the young animal and nitrogen balance in the adult remain the primary criteria for assessment of adequacy of intake of the essential amino acids. Age (stage of life), gestation, lactation and wound healing as well as sources and adequacy of energy and other nutrient factors and unique metabolic functions for particular amino acids can further influence dietary requirement (Bunce and King 1969, NRC 1978, Visek 1984).

The aims of defining amino acid requirements include the need to formulate diets which will maximize the use of essential amino acids for protein synthesis (Kim et al. 1983). This could be in conjunction with optimizing animal growth performance or production or to provide standard reference diets for use in assessment of protein quality of various food sources for human nutrition (Bressani et al. 1973).

The requirement for the essential amino acid methionine has been subject of considerable study. Methionine is frequently the first limiting amino acid in important human food protein such as legumes and dietary inadequacy of methionine may be a particular problem in third world countries or in groups

such as vegans or formula-fed infants where intakes of single classes or sources of dietary protein can be the practice. Methionine is also frequently subjected to oxidation in food processing which may further limit its availability in certain foods (Anderson et al. 1976, Cuq et al. 1978).

The requirement for methionine is complicated to some degree by the sparing effect of cysteine/cystine. In practical nutritional situations methionine and cystine are frequently regarded as equivalent and proteins are often evaluated on the basis of their methionine plus cystine content (James and Hove 1980). Byington et al. (1972) demonstrated the complex relationship existing between methionine, cystine and choline that must be considered in formulation of experimental diets and in examining the amino acid content of foods. The growth promoting capability of combinations of methionine and cystine in rat diets was shown to be dependent not only upon the amount of total organic sulfur but also on the relative dietary proportions of the two SAA and the presence of choline. Choline as earlier outlined can serve as a source of methyl groups which via betaine can be involved in remethylation of homocysteine to methionine permitting recycling of the methionine molecule's methyl donation capacity and conserving potentially limited methionine supply for protein synthesis (Finkelstein 1974).

The ability of cystine to spare methionine derives from the fact that cysteine can be synthesized from methionine via the transsulfuration pathway. Any requirement for cysteine in terms of contribution to protein synthesis, inorganic sulfate or taurine requirements, that is normally supplied via methionine transsulfuration can be met by dietary cysteine/cystine, conserving methionine for protein synthesis.

The metabolic basis of this sparing effect remains under study. Significant decreases in rat hepatic methionine adenosyl transferase (EC 2.5.1.6) and

cystathionine synthase (EC 4.2.1.22) activities were reported by Finkelstein and Mudd (1967) when cystine was added to a low methionine diet. Shannon et al. (1972) similarly reported inhibition of cystathionine synthase activity when rats were fed diets with low proportions of methionine relative to cystine. Increased cystathionase activity following injection of cysteine into chick liver has been shown by Goswammi et al. (1959), while injection or dietary excess of methionine increased rat hepatic cystathionase activity (Daniel and Waismann 1969). It has been suggested that depression of cystathionine synthase activity spares methionine and that regulation of the enzyme is influenced by intakes of both methionine and cystine in diets limiting in methionine (Shannon et al. 1972).

Stipanuk and Benevenga (1977), however, suggested that the sparing effect of cystine was due primarily to stimulation of protein synthesis in diets initially limiting in cysteine. The significant reduction in rat liver extract cystathionase activity they were also able to demonstrate was a secondary effect. Significantly reduced recovery of $^{14}CO_2$ resulting from catabolism of ^{14}C-labelled methionine when rats on low protein diets were supplemented with cystine, was accompanied by a trend towards increased specific activity in muscle tissue suggesting decreased methionine catabolism and increased protein synthesis.

Womack and Rose (1941) found a total DL-methionine requirement for the growing rat to be 0.6% of diet in the absence of dietary cystine and 0.5% of diet if 0.4% cystine was present. Cystine was thus estimated to be able to replace 1/6 of dietary methionine. Williams et al. (1954) using ratios of amino acids to lysine in whole carcasses of animals, determined a total SAA requirement of 0.43% of diet. Methionine requirements were estimated to be 0.22% of diet with the balance to be supplied by L-cystine. It was suggested

that these were the true SAA requirements for tissue synthesis without consideration of additional needs for other functions such as methyl group donation.

It would appear that SAA requirements for growth can vary somewhat depending upon the type and protein level of diets used in testing. Leveille et al. (1961) found a greater requirement for SAA in mice fed diet containing 2.5% nitrogen compared to diet containing 1.5% nitrogen. Kroening et al. (1965) similarly reported increased methionine + cystine requirements for maximal gain in 2-7 week old pigs as dietary protein level was increased from 12 to 24% of diet.

When weanling male Sprague Dawley rats were fed equivalent to 10% dietary protein (5% casein plus equivalent to 5% protein (% N x 6.25) as crystalline amino acids), maximum PER and daily weight gains were obtained with 0.49% diet (4.9% of protein) as total SAA (Rao et al. 1959). Using 10% dietary protein (% N x 6.25) supplied by crystalline amino acids alone, 0.16% methionine and 0.34% cystine were found to be minimum requirement levels for maximal weight gain and PER. L-cystine according to these studies could thus replace up to 68% of the total L-methionine content (Rao et al. 1959, 1961). Sowers et al. (1972) found closely similar requirements (0.47% total SAA, 0.17% absolute methionine requirement and 0.30% or 64% of total SAA as L-cystine) using similar crystalline amino acid based diets.

The importance of consideration of both the quantity of total SAA and the relative proportions of methionine:cystine were emphasized by Byington et al. (1972) and Shannon et al. (1972). These factors were shown to influence rat body composition and growth and it was suggested could be important factors to consider in terms of contribution of various protein sources towards rat growth. Animal proteins usually contain a higher proportion of methionine than cystine

while cereals contain higher proportions of cystine and legumes equal amounts of each (Orr and Watt 1957). Optimal growth of young rats occurred when methionine:cystine ratio was 70:30 or 50:50 (Byington et al. 1972). James and Hove (1980) found that supplemental cystine was ineffective in improving PER and weight gain of young rats fed low SAA, legume protein-based diets when the legumes (lentils, soybean, broadbean, sweet lupin seed) had high endogenous cystine:methionine ratios. Cystine supplementation of similar diets based on casein, yeast, leaf protein concentrate or lupin tops with lower endogenous cystine:methionine ratios was effective. It was suggested that the practice of treating methionine and cystine as equivalent in terms of nutritional requirements could be misleading.

As earlier stated, rat bioassays remain an integral part of testing for adequacy and quality of food proteins for human nutrition. The use of the rat model, however, does have limitations despite obvious biological similarities in terms of digestion and metabolism of proteins and amino acids (Bodwell 1978). The significance of rat requirements and capacity to handle amino acids from various food sources, in terms of human implications is thus coming under increasing scrutiny.

Human infants and children with higher needs for proteins and amino acids for growth, relative to other age groups are the groups most likely at risk of methionine deficit and hence are prime subjects of comparison with rat bioassays. Sulfur amino acid requirements of human children, however, are considerably lower than the current NRC (1978) rat requirements of 0.6% of diet. Sulfur amino acid requirements for human children range from 2.6 g/16 g N to 3.5 g/16 g N (2.6-3.5% of protein) (FAO/WHO 1973, Torun et al. 1981, NRC 1980). It is also generally accepted that rat growth assays tend to

under estimate the protein quality for humans of food sources deficient in sulfur amino acids (Codex Alimentarius 1984).

Because current NRC (1978) rat growth requirements for SAA of 0.6% of diet (5% of protein) assume a 12% protein diet and because it has been shown that the efficiency of protein and amino acid utilization decrease and the sulfur amino acid requirements of animals increase at higher levels of protein intake (Edozien and Switzer 1978, Leveille et al. 1961, Kroening et al. 1965) there is a need to determine if SAA requirements of rats fed 8% dietary protein are perhaps lower than NRC (1978) requirements and more closely related to human requirements - especially requirements of human infants and children. As earlier stated, standard rat bioassays for protein quality involve 8-10% protein levels in test diets (AOAC 1976, McLaughlan and Keith 1977).

f) Oxidized Sulfur Amino Acids - OCCURRENCE AND AVAILABILITY

The quantity and availability of the essential amino acids in a food protein are major determinants of the nutritional quality of the protein. Methionine or lysine are frequently the first limiting amino acids in foods and along with cystine may also often be present in forms unavailable for use by the animal. Unavailability may result from food processing and from factors present in the food itself.

Thermal processing, the major method used in food manufacturing, can result in increased palatability, increased protein digestibility and amino acid availability, and destruction of antinutrient factors such as trypsin inhibitors (Tannenbaum 1974). Hydrogen peroxide treatments have important applications in milk sterilization (Fox and Kosikowski 1967), in bleaching and improvement of baking quality of breads employing yeast and skim milk (Guy et al. 1968), and potentially in the destruction of glucosinolates of rapeseed flour (Anderson et

al. 1975). Both these processing methods can also have detrimental effects on protein digestibility and amino acid availability - especially in terms of the sulfur amino acids.

Protein fragments resistant to enzyme proteolysis can occur with food processing and these fragments may contain sulfur amino acids. Anglemeir and Montgomery (1976) reported that Maillard compound formation, initially involving reaction of epsilon amino groups of lysine molecules with reducing sugars, not only reduces lysine availability but can also block specificity requirements of certain enzymes, preventing hydrolysis and release of amino acids from food proteins. Protein-protein interactions and the formation of new cross linkages in the absence of reducing substances may also occur when proteins are exposed to high temperatures (> 110°C) for prolonged periods (> 24 hrs) (Bjarnason and Carpenter 1970).

Miller et al. (1965) using chick, rat and microbiological assays, and amino acid analysis, showed that heating of cod protein (27 hrs at 116°C) resulted in loss of availability but not destruction of lysine, methionine and tryptophan. Heating at lower temperatures (20 hrs at 85°C) in the presence of glucose resulted in destruction of lysine and cystine and almost complete loss of availability but no destruction of methionine and tryptophan. It would thus appear that bioassays are more relevant to determination of available amino acids than are chemical assays following hydrolysis of proteins.

Cystine is the most heat labile amino acid and its destruction may be especially important in terms of nutritional quality of diets heavily based on milk, cottonseed or fish meals (Miller et al. 1965, Bender 1972).

The effects of hydrogen peroxide treatments on free and protein bound methionine and cystine have been subjects of several studies. Yang (1970) and Inoue and Hayatsu (1971) reported the aerobic oxidation of methionine to

methionine sulfoxide in the presence of sodium sulfite and Mn^{2+} ions. Njaa (1962) showed that pH influenced the extent of free methionine oxidation. Photoxidation and lipid hydroperoxidation of methionine have also been described (Cuq et al. 1978, Tannenbaum et al. 1969).

Protein bound methionine and cysteine also undergo oxidation to varying degrees. Increased methionine sulfoxide levels were found in casein and milk solutions exposed 30 minutes at alkaline pH and 50°C to mild H_2O_2 concentrations. Methionine sulfoxide increased with increasing H_2O_2 molarity but amino acid analysis failed to reveal further oxidation to stable methionine sulfone (Cuq et al. 1973). Slump and Schreuder (1973), however, showed that both fish meal and casein treated with H_2O_2 in the presence of perchloric acid readily underwent oxidation of cysteine and methionine residues with both methionine sulfone and cysteic acid residues being produced. Oxidation to methionine sulfone has also been reported in sunflower protein isolate, possibly due to presence of trace metal catalysts in the isolate (Cuq et al. 1978). Anderson et al. (1975) found that reduction in glucosinolate level in rapeseed flour by 3 and 7% H_2O_2 was accompanied by oxidation of methionine to both methionine sulfoxide and methionine sulfone and oxidation of cysteine to cysteic acid.

Chang et al. (1982) similarly described the oxidation of methionine and cysteine residues in solutions of casein, soy protein isolate and egg white solids. Mild H_2O_2 exposure at 40°C readily resulted in conversion of methionine to its partially oxidized sulfoxide form with only slight formation of methionine sulfone and cysteic acid. H_2O_2 treatment at 90°C resulted in rapid conversion of cysteine to cysteic acid and of methionine to methionine sulfone. The proteins varied in stability from oxidation of their sulfur amino acid residues with egg white solid being least stable. It was suggested that differences in protein structure influenced this stability.

Both lipid hydroperoxides and hydrogen peroxide have been shown to cause varying degrees of oxidation of cysteine residues in intact proteins. More complete oxidation to cysteine sulfuric acid or cysteic acid in soy, safflower, alfalfa leaf protein and lactalbumin gave greater stability to alkali treatments and less formation of potentially toxic lysinoalanine. Partial oxidation of cysteine residues to disulfide or monoxide forms increased their susceptibility to dehydroalanine and lysinoalanine formation (Finley et al. 1982).

The availability of the oxidized forms of sulfur amino acids has been subject of considerable study and controversy. Several authors have reported on the bioavailability of various oxidized forms when used in their free form as supplements or sole sources of sulfur amino acids. Njaa (1962) used various methionine sulfoxides as 0.15% supplements to soya bean meal diets fed to young rats. An index of protein utilization, based upon urinary nitrogen excretion as a percentage of nitrogen intake, showed L-methionine sulfoxide, DL-methionine sulfoxide and D-methionine sulfoxide were respectively 100, 75 and 50% as effective as L-methionine supplementation. Miller and Samuel (1970) using 10 day Net Protein Utilization (NPU) measurements of rats fed 10% casein protein diets with 0.1% supplements of various sulfur compounds, found that DL-methionine sulfoxide was 25% as active as a DL-methionine supplement. Methionine sulfone and cysteic acid supplements caused 11% and 4% decreases in NPU relative to the sulfur amino acid-deficient diet.

Miller et al. (1970) reported, however, that complete replacement of methionine with L-methionine sulfoxide in crystalline amino acid based diets fed to young rats for a 17-day period, resulted in lower weight gains (66%) and higher levels of microbiologically-assayed blood methionine activity. Replacement of only one half the methionine with L-methionine sulfoxide produced no differences in weight gain (methionine utilization).

Longer term feeding (132 days) of the initial diets revealed no significant difference in weight gains with L-methionine sulfoxide or L-methionine supplementation, suggesting an adaptation mechanism for utilization of the sulfoxide or decreased requirement for methionine in older animals.

Anderson et al. (1976) supplemented an 18% crystalline amino acid diet lacking sulfur amino acids with graded amounts of L-methionine, L-methionine sulfoxide, L-methionine sulfone, L-cysteine or L-cysteic acid. A series of two week rat feeding trials revealed that L-methionine sulfoxide used as sole methionine source was only 60% as efficient as methionine for rat growth. Methionine sulfone and cysteic acid used as sole sulfur amino acid sources were unable to support rat growth. Plasma amino acids reflected dietary intakes of sulfur amino acids. Plasma methionine concentrations increased with dietary increments of methionine. Methionine sulfoxide, detected in all rat groups, increased with increased dietary methionine sulfoxide and methionine sulfone was detected only in plasma of rats fed dietary methionine sulfone. Half cystine levels in plasma increased significantly with highest levels of methionine or methionine sulfoxide and cysteine or cysteic acid supplementation most notably caused increased plasma taurine levels.

Gjøen and Njaa (1977) similarly reported poor utilization by young rats of methionine sulfoxide used as sole methionine source in crystalline amino acid-based diets. Substitution of 1/3 of the sulfoxide with cystine increased utilization to that of methionine. A linear relationship was observed between plasma methionine activity measured microbiologically and methionine sulfoxide levels in the diets. L-methionine-DL-sulfoxide, however, has been shown to be as available as L-methionine when used to supplement untreated and hydrogen peroxide-treated fish protein fed to young rats. L-methionine sulfone and cysteic acid had no supplementary effects in terms of improvement in

Protein Efficiency Ratio (PER) or biological value (BV) (Sjöberg and Boström 1977). Njaa and Aksnes (1982) have also found that free L-methionine sulfoxide as well as DL-methionine and L-cystine are as active and D-methionine sulfoxide slightly less active than L-methionine in terms of nitrogen sparing effect (measured as decrease in urinary nitrogen excretion when the various compounds were supplied as supplements to protein-free diets fed to rats).

Recently it has been reported that several oxidized forms of cystine similarly show a variable bioavailability relative to cystine when measured by chick growth assay. Cystine bioavailability was determined using the slope-ratio technique, with L-cystine assigned a value of 100% bioavailability. Cystine monoxide and symmetrical cystine disulfoxide were nearly 100% as available as L-cystine while unsymmetrical cystine dioxide was only 50% as available and L-cystine sulfonate was completely unavailable (Crawford et al. 1984).

Reports concerning the bioavailability of protein- and peptide-bound oxidized SAA also vary. Morrison and Sabry (1963) found reduced methionine availability in heat-treated fish flours as measured by PER and NPR values obtained with rats fed methionine-deficient diets supplemented with the flours. Variable susceptibility to in vitro enzymatic digestion and release of protein-bound methionine from different flours suggested potentially different mechanisms or factors affecting methionine availability. Rao and McLaughlan (1967) reported that increased autoclaving of casein-glucose mixtures led to progressively decreased in vitro enzymatic availability of methionine. Decreases in growth and plasma methionine activity of rats fed methionine-deficient diets supplemented with mixtures subjected to prolonged heating similarly suggested differential methionine availability in the casein-glucose mixtures.

Ellinger and Palmer (1969) ascribed the lower utilization by rats of hydrogen peroxide-treated casein relative to untreated casein, as due to conversion of peptide-bound methionine to methionine sulfoxide. Slump and Schreuder (1973), however, chemically determined levels of methionine, methionine sulfoxide, methionine sulfone, cystine and cysteic acid in H_2O_2-perchloric acid-treated casein and fish meal. They demonstrated that the protein-bound sulfoxide was completely available and that reductions in NPU and true digestibility obtained with rats, were due to the presence of methionine sulfone residues.

A 30% loss of available methionine as measured by in vitro Pronase digestibility was, however, reported in milk in which 51% of methionine residues had been oxidized to methionine sulfoxide by mild (0.018 M) H_2O_2 treatment. No methionine sulfone was detected. Similar Pronase release of methionine in H_2O_2-treated casein was found to be inversely related to methionine sulfoxide present. Enzymes in the Pronase appeared unable to split methionyl peptide bonds involving methionine sulfoxide residues (Cuq et al. 1973). Decreased levels of available SAA measured by various in vitro chemical procedures following enzyme hydrolysis have also been shown in processed foods ranging from condensed and roller dried milks and whey powders to fish and vegetable protein concentrates (Pieniążek et al. 1975, Kunachowicz et al. 1976). Bioassays nevertheless remain the ultimate reference methods for evaluation of amino acid availability.

Rapeseed flour treated with H_2O_2 (0.9 M) to reduce toxic levels of sulfur-containing glucosinolates underwent oxidation of tryptophan and SAA residues. Methioine sulfoxide, methionine sulfone and cysteic acid production in the flour resulted in reduced bioavailability as measured by decreases in weight gain, feed intake and PER and increases in plasma methionine sulfoxide

and methionine sulfone in rats fed diets based on the treated flour. Methionine supplementation of the diet improved weight gain (Anderson et al. 1975).

Methionine sulfoxide readily produced to varying degrees from methionine residues in casein, milk and sunflower protein isolate by a variety of oxidative processes, resulted in very slight reduction in nutritional availability of the proteins for the growing rat. Methionine sulfoxide residues in casein were shown to be mainly released, absorbed, converted to methionine and used for protein synthesis by one month old rats. Accumulation of the sulfoxide in plasma and muscle and changes in plasma amino acid (PAA) patterns, however, suggested a relatively slow conversion of the sulfoxide in the younger rat (Cuq et al. 1978).

Similar increases in plasma methionine sulfoxide and in both methionine sulfoxide and methionine sulfone in rats fed oxidized versus untreated fish meal or protein were reported by Gjøen and Njaa (1977) and Sjöberg and Boström (1977) respectively. The latter authors also detected both forms in liver, kidney and muscle and plasma methionine sulfoxide levels were shown to be dependent upon levels of protein intake. Significant decreases in growth, PER and/or biological value were also reported. It was suggested that low levels of cystine in the fish meals and in casein used in other experiments, contributed to the low utilization of methionine sulfoxide. Reduction of the sulfoxide occurred too slowly in the absence of cystine to contribute to cysteine/cystine synthesis and requirements (Gjøen and Njaa 1977).

The availability of oxidized SAA for animals is likely a complex function of gastrointestinal and metabolic abilities to digest peptide linkages containing the oxidized forms, to absorb the resultant free or peptide-bound forms from the lumen and to reduce them in liver and kidney to useable compounds (Higuchi et al. 1982).

III. EXPERIMENT 1 - METHIONINE REQUIREMENTS

INTRODUCTION

Methionine and cystine are the main sources of organic sulfur for the non ruminant. Methionine as an essential amino acid must be supplied in the diet to meet initiation and structural requirements for protein synthesis and to act as a source of methyl groups for a variety of metabolically important compounds. In addition methionine can serve as a source of cystine (Finkelstein 1974).

Legumes are frequently limiting in sulfur amino acids (SAA) (Bossani and Silano 1978), and often serve as key sources of dietary protein in Third World countries and increasingly in more affluent countries for specific groups such as vegans and formula fed infants (Graham et al. 1976). SAA are also frequently rendered unavailable in foods as a result of processing (Cuq et al. 1978). In order to properly assess nutritional adequacy of various food proteins, determination of methionine requirement is of major importance.

Bioassays involving the laboratory rat remain a major part of determinations of protein quality and adequacy for humans in both Canada and the United States (AOAC 1976, Sarwar and McLaughlan 1981). A general consensus, however, has been held that rat requirements for SAA exceed those of humans, thus making any rat growth assay underestimate protein quality for humans of food sources deficient in SAA (Bodwell 1978, Codex Alimentarius 1984).

Human children constitute an age class with relatively high growth demands for amino acids. SAA requirements for children are lower than reported rat requirements ranging from 3.5 g/16 g N to 2.6 g/16 g N (FAO/WHO 1973, Torun et al. 1981, NRC 1980). The rat growth requirement for

methionine is set by NRC (1978) at 0.6% of diet when an ideal, fully digestible protein is fed at the 12% level of intake (5 g/16 g N). These latter requirements derive from a variety of older reports in which 'protein' sources tested varied from intact casein or other products, to individual crystalline amino acids.

Current regulatory and research practices in North America for protein quality assessment, however, involve rat growth assays using 8 to 10% dietary protein (McLaughlan 1974, Sarwar and McLaughlan 1981). Moreover it has been demonstrated that the efficiency of protein utilization (and hence amino acid utilization) is linearly related to the amount of protein consumed, declining as protein level increases (Edozien and Switzer 1978; Kroening et al. 1965).

Because the methionine requirement for rat growth has not been firmly established at the 8% dietary protein level, it was decided to determine if it was similar to NRC (1978) requirements or perhaps more closely related to human growth requirements. Rat growth performance and plasma free amino acid (PAA) data were used in these studies.

MATERIALS AND METHODS

A non-protein diet as described in Table 1a, and a reference diet providing 8% protein from ANRC casein[1] plus 0.2% L-methionine[2] at the expense of the corn starch of the basal diet were formulated. Basal diets were also formulated as described for the non-protein diet with 4% protein from ANRC casein and equivalent to 4% casein protein from crystalline L-amino acids (except methionine and cystine) (Table 1b) added at the expense of corn

[1] Humko Sheffield Chemical, Division of Kraft Inc. P.O. Box 630, Norwich, N.Y., 13815.

[2] Sigma Chemical Co., P.O. Box 14508, St. Louis, MO. 63172.

Table 1a. Composition of non-protein diet

Ingredient	% Composition (Air dry basis)
AIN Mineral Mix[1]	3.0
AIN Vitamin Mix[1]	1.0
Choline bitartrate[2]	0.2
Nonnutritive cellulose (Alphacel)[3]	5.0
Corn Oil[4]	10.0
Corn Starch[5]	80.8
Total	100.0

1 American Institute of Nutrition (1977), purchased from ICN Nutritional Biochemical Co., Division of ICN Biomedicals Inc., Cleveland, Ohio 4418.

2 Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63172.

3 Teklad Test Diets, P.O. Box 4220, Madison, WI 53711.

4 Mazola, Canada Starch Co., Toronto, Ontario.

5 Canada Starch Co., Toronto, Ontario.

Table 1b. Amino acid composition (%) of basal diets

L-amino acid	Source		Total**
	ANRC Casein*	Supplement	
Arginine	0.16	0.16	0.32
Aspartic Acid	0.31	0.31	0.62
Glutamic Acid	0.97	0.97	1.94
Histidine	0.12	0.12	0.24
Isoleucine	0.22	0.22	0.44
Leucine	0.42	0.42	0.84
Lysine	0.35	0.35	0.70
Methionine	0.12	--	0.12
Cystine	0.02	--	0.02
Phenylalanine	0.23	0.23	0.46
Tyrosine	0.25	0.25	0.50
Proline	0.47	0.47	0.94
Threonine	0.19	0.19	0.38
Tryptophan	0.05	0.05	0.10
Valine	0.28	0.28	0.56
Non-essential			
Glycine	0.08	0.08	0.16
Alanine	0.13	0.13	0.26
Serine	0.25	0.25	0.50

* Based upon amino acid analyses of acid and base hydrolysates of ANRC casein.

** Total levels of individual essential amino acids (except methionine) and total non-essential amino acids met or exceeded rat growth requirements at 8% dietary protein as extrapolated from NRC (1978) requirements.

starch to maintain equivalent energy levels. Supplements of L-methionine were added to the basal diets to make test diets providing 0.14, 0.24, 0.34, 0.44, 0.54, 0.64 and 0.74% total dietary SAA mainly as methionine. In each diet the casein component contributed 0.02% dietary L-cystine. Isonitrogenicity was maintained by suitable increments of a (1:1:1) mixture of the non-essential amino acids glycine, alanine and serine. The reference diet contained 0.48% total SAA (0.44% L-methionine, 0.04% L-cystine).

Weanling, male, Sprague Dawley rats[3], 21-23 days old, were kept in stock cages and fed the reference diet for 2 days. They were then assigned to individual, stainless steel, mesh-bottomed cages, in a randomized complete block design involving 10 replicates, in which each diet (non-protein, casein + 0.2% methionine reference, and 7 test diets) was represented once per block. Blocking was on the basis of matched initial body weight. Diets and water were provided ad libitum for a 2-week period and cages were maintained at 21 $\pm$ 1°C and 50 to 60% relative humidity with 12 hour light/dark cycles, for the entire test period.

Weekly feed intakes and weight gains for each animal were recorded. At the end of the 2-week feeding trial, diets were removed early in the morning and after 2 hours, rats were sacrificed by means of ether anesthesia and decapitation. Blood was collected in heparinized test tubes and individual whole livers weighed, frozen and freeze dried as previously described (Sarwar et al. 1981). Plasma from pairs of rats within each diet group was pooled and deproteinized with 10% trichloroacetic acid (1:1 v/v). The resulting 5 pooled, deproteinized, plasma samples per diet were stored at -20°C until analysed in

[3] Charles River Canada Inc., St. Constant, Québec

duplicate for free amino acids (PAA) by ion exchange chromatography using a Beckman 121 MB Amino Acid Analyzer. Analysis involved a 6 buffer (Na^+) single column system using Beckman AA-10 resin[4] (Appendix A).

Diets and freeze-dried livers were analysed in duplicate for protein content (%N x 6.25) using Macro Kjeldahl techniques (AOAC 1975). Relative Net Protein Ratio (RNPR) (Casein + 0.2% methionine diet = 100) for test and reference diets were determined as previously described (Sarwar and McLaughlan 1981) as were 2-week weight gains, feed intakes, feed/gain ratios and 2 week Relative Protein Efficiency Ratios (RPER) (Casein + 0.2% methionine diet = 100). Liver Protein Utilization (LPU) was also determined as described by Mokady et al. (1969).

The data for food intake, weight gain, feed/gain ratio, RNPR, LPU and plasma amino acids were reported as means $\pm$ SE and subjected to one way analyses of variance and Duncan's Multiple Range Test (Snedecor and Cochran 1967, Duncan 1955).

RESULTS

As shown in Tables 2 and 3, increasing dietary L-methionine above the 0.12% of the basal diet, resulted in significant improvement ($P < 0.05$) in all growth parameters measured. Feed intake and weight gain reached optimal values at 0.42% dietary L-methionine (0.44% total SAA) while optimal feed/gain ratio, RNPR, RPER and LPU values were achieved at 0.32% dietary L-methionine (0.34% total SAA).

4 Beckman Instruments, Spinco Division, 1117 California Ave., Palo Alto CA, 94304

Essential plasma free amino acid and tyrosine concentrations obtained in rats fed the different diets are listed in Table 4. There was a trend towards increased plasma methionine as dietary L-methionine increased. With 0.72% total dietary L-methionine, plasma methionine was significantly higher than for all other diets. Certain other essential amino acids (arginine, histidine, isoleucine, leucine, phenylalanine, threonine, tryptophan), showed a trend towards decreased levels in the plasma as dietary methionine content increased. L-threonine showed the greatest response.

Plasma L-taurine and α-aminobutyric acid (α-Aba) increased significantly with increased dietary L-methionine (Table 5). With 0.42% dietary L-methionine, plasma taurine and α-Aba levels were equivalent to those of the ANRC casein + 0.2% L-methionine reference diet which provided a similar amount of dietary SAA. Other non-essential amino acids (alanine, glycine, proline, serine, ornithine) showed a trend toward decreased plasma concentrations as dietary methionine was increased above basal concentrations. At 0.62 to 0.72% dietary methionine this decrease was often finished or reversed.

Table 6 indicates a trend towards decreased levels of total essential PAA and essential PAA/total PAA ratios as dietary methionine levels increased above 0.12%. Minimum total PAA and total essential PAA were obtained with 0.62% dietary L-methionine although mean values did not differ significantly from those of the diets containing 0.42 or 0.52% dietary L-methionine. Minimum essential PAA/total PAA ratios occurred with the 0.32% L-methionine reference diet although no significant difference in ratios were seen from 0.22 to 0.72% levels of dietary L-methionine.

Table 2. Effects of dietary L-methionine level on rat performance*

% Dietary L-methionine	% Total SAA	Feed Intake g	Weight Gain g	Feed/Gain Ratio
0.12	0.14	101 ± 5^{e}	11 ± 1^{d}	12.2 ± 2.9^{a}
0.22	0.24	139 ± 9^{d}	36 ± 3^{c}	4.1 ± 0.34^{b}
0.32	0.34	192 ± 5^{c}	68 ± 3^{b}	2.8 ± 0.04^{b}
0.42	0.44	217 ± 6^{a}	85 ± 3^{a}	2.6 ± 0.04^{b}
0.52	0.54	214 ± 6^{a}	83 ± 3^{a}	2.6 ± 0.05^{b}
0.62	0.64	221 ± 5^{a}	84 ± 3^{a}	2.6 ± 0.03^{b}
0.72	0.74	195 ± 3^{bc}	74 ± 2^{a}	2.6 ± 0.03^{b}
Reference	0.48	211 ± 7^{ab}	84 ± 4^{a}	2.5 ± 0.05^{b}

* Means $\pm$ SE for 10 animals

Means with different letters in the same column differ significantly ($P < 0.05$).

Table 3. Effects of dietary L-methionine on rat growth parameters*

% Dietary L-methionine	% Total SAA	RNPR %	RPER %	LPU
0.12	0.14	48 ± 2^{c}	28 ± 3^{c}	1.37 ± 0.17^{c}
0.22	0.24	79 ± 3^{c}	68 ± 4^{b}	2.56 ± 0.23^{b}
0.32	0.34	102 ± 2^{a}	99 ± 3^{a}	3.90 ± 0.15^{a}
0.42	0.44	105 ± 2^{a}	104 ± 2^{a}	3.83 ± 0.27^{a}
0.52	0.54	101 ± 2^{a}	101 ± 2^{a}	3.73 ± 0.05^{a}
0.62	0.64	104 ± 2^{a}	104 ± 3^{a}	3.95 ± 0.15^{a}
0.72	0.74	101 ± 2^{a}	101 ± 2^{a}	3.98 ± 0.42^{a}
Reference	0.48	100^{a}	100^{a}	4.16 ± 0.23^{a}

* Means ± SE for 10 animals

Means with different letters in same column differ significantly ($P < 0.05$).

Table 4. Effects of dietary L-methionine on essential plasma amino acid concentrations* in rats

% Dietary L-methionine	Arg	His	Ile	Leu	Met	Phe	Tyr	Thr	Trp	Val	Lys
0.12	16.0^{a}	15.1^{a}	8.2^{ac}	13.9^{ab}	2.8^{a}	5.2^{ab}	4.8^{a}	139.6^{a}	4.8^{ab}	18.7^{ab}	68.5
0.22	14.2^{abc}	14.7^{a}	10.5^{ab}	15.8^{ab}	4.1^{a}	5.3^{b}	9.8^{b}	74.5^{b}	5.1^{b}	21.1^{a}	84.1
0.32	13.7^{abc}	14.3^{a}	10.3^{ab}	15.9^{a}	5.3^{ab}	4.9^{abc}	12.7^{c}	33.2^{c}	4.4^{abc}	21.3^{a}	83.8
0.42	12.6^{bc}	13.5^{abc}	9.3^{abc}	13.4^{abc}	6.2^{ab}	4.7^{abc}	11.7^{bc}	22.2^{cde}	3.6^{cd}	17.6^{bc}	74.7
0.52	13.4^{bc}	12.1^{bcd}	9.1^{abc}	12.4^{bc}	6.6^{ab}	4.8^{abc}	9.4^{b}	23.4^{cd}	4.1^{bcd}	15.5^{c}	74.1
0.62	13.0^{bc}	11.8^{bd}	8.9^{abc}	11.9^{bc}	9.8^{b}	4.6^{c}	9.4^{b}	21.2^{de}	3.8^{cd}	15.2^{c}	70.2
0.72	11.7^{b}	13.9^{ac}	7.7^{c}	11.3^{c}	34.8^{c}	4.6^{c}	9.7^{b}	24.1^{cd}	4.0^{cd}	15.1^{c}	81.3
Reference	14.3^{abc}	10.9^{d}	11.2^{b}	14.7^{ab}	7.2^{ab}	4.5^{c}	10.3^{bc}	11.6^{e}	3.4^{d}	20.7^{ab}	82.2
SEM**	0.8	0.6	0.7	0.8	1.5	0.2	0.9	3.7	0.3	1.0	5.9

* µM/100 mL plasma

** SEM calculated from analysis of variance

Means with different letters in the same column differ significantly ($P < 0.05$).

Table 5. Effects of dietary L-methionine on plasma non-essential amino acids* in rats

% Dietary L-methionine	Ala	Asp	Glu	Gly	Pro	Ser	Tau	Cit	Orn	α-Aba
0.12	71.4^{c}	2.1^{b}	20.4^{b}	36.5^{b}	24.8^{b}	87.7^{a}	6.6^{d}	11.0^{bc}	13.3^{a}	0.4^{e}
0.22	103.3^{ab}	2.4^{ab}	23.5^{b}	41.4^{a}	43.2^{a}	65.1^{b}	4.5^{d}	11.9^{ab}	12.3^{ab}	0.6^{e}
0.32	114.5^{a}	2.9^{ab}	27.7^{ab}	31.0^{c}	46.4^{a}	49.0^{c}	5.2^{d}	12.9^{a}	13.8^{a}	1.1^{e}
0.42	95.5^{ab}	2.6^{ab}	26.2^{ab}	20.2^{d}	42.2^{a}	33.2^{d}	13.0^{c}	10.9^{bc}	10.3^{c}	1.9^{d}
0.52	88.0^{bc}	2.8^{ab}	21.8^{b}	21.5^{d}	34.4^{ab}	34.3^{d}	22.1^{b}	10.0^{c}	10.5^{bc}	3.0^{bc}
0.62	72.4^{c}	2.7^{ab}	21.0^{b}	17.9^{de}	29.0^{b}	30.9^{d}	24.4^{ab}	9.1^{c}	9.1^{c}	3.4^{b}
0.72	98.5^{ab}	2.4^{ab}	26.7^{ab}	15.0^{e}	42.1^{a}	29.2^{d}	27.2^{a}	10.3^{bc}	9.6^{c}	5.9^{a}
Reference	92.8^{abc}	3.1^{a}	33.2^{a}	22.3^{d}	42.6^{a}	35.2^{d}	13.0^{c}	11.9^{ab}	12.9^{a}	2.3^{cd}
SEM**	7.2	0.3	2.8	1.6	3.7	2.1	1.1	0.5	0.6	0.3

* μM/100 ml plasma

** SEM calculated from analysis of variance

Means with different letters in the same column differ significantly ($P < 0.05$).

Table 6. Effects of dietary L-methionine level on plasma free amino acids (PAA) in rats

% Dietary L-methionine	% Dietary SAA	Total PAA*	Essential* PAA	Non Essential* PAA	Essential PAA / Total PAA
0.12	0.14	571^{a}	298^{a}	274^{ac}	0.52^{a}
0.22	0.24	567^{a}	259^{b}	308^{a}	0.46^{b}
0.32	0.34	523^{ab}	219^{c}	303^{ab}	0.42^{b}
0.42	0.44	444^{cd}	189^{cde}	254^{bc}	0.43^{b}
0.52	0.54	432^{cd}	185^{de}	247^{cd}	0.43^{b}
0.62	0.64	396^{d}	180^{e}	217^{d}	0.45^{b}
0.72	0.74	479^{bc}	218^{cd}	261^{c}	0.46^{b}
Reference	0.48	458^{cd}	191^{cde}	266^{bc}	0.42^{b}
SEM**		20	11	12	0.01

* µM/100 mL plasma

** SEM calculated from analysis of variance

Means with different letters in the same column differ significantly ($P < 0.05$).

DISCUSSION

The combination of low sulfur amino acid (SAA) ANRC casein and crystalline amino acids as dietary amino acid - nitrogen sources, permitted manipulation of dietary SAA over a considerable range. It also negated potential differences in feed intakes and growth, possibly due to osmotic effects, reported to occur when intact protein-based and equivalent amino acid-based diets were fed (Itoh et al. 1973, 1974, 1974a, Forsum and Hambraeus 1978). Equivalent feed intakes and weight gains of animals fed the intact protein-based reference diet and the 0.42% methionine test diet, with similar SAA levels, support the use of the combined 'protein' source.

The ANRC casein + 0.2% L-methionine diet has long standing use in protein quality assessment as a high quality reference diet adequate in SAA, to which diets based on other protein sources can be compared (McLaughlan et al. 1980, Sarwar and McLaughlan 1981). Weight gains and feed intakes shown in Table 2, suggest that test diets containing 0.42%-0.62% L-methionine were adequate in dietary SAA.

Feed/gain ratio, the amount of diet consumed for a given unit of gain, permits correction for any differences in food intakes and indicates the efficiency of food conversion for weight gain of the various diets. The lower the value for feed/gain ratio, the better the efficiency. While highest levels of feed intake and weight gain were obtained with 0.42% dietary methionine and above, lowest feed/gain ratios were obtained at 0.32% dietary methionine and above. In both cases, the values did not differ significantly ($P > 0.05$) from those obtained with the reference diet.

NPR determinations involve a 2 week growth assay which is cheaper than the standard 28 day PER assay for protein quality. Unlike the PER test, NPR testing also takes into account maintenance requirements by adding weight loss of non-protein dietary group to weight gains of the various test groups. Due to expense, problems of precision, reproducibility and variation as well as not crediting maintenance, the official PER assay is undergoing replacement by NPR methods (McLaughlan et al. 1980, Sarwar and McLaughlan 1981). As shown in Table 3, diets containing 0.32%-0.62% dietary L-methionine, gave RNPR values equivalent to RNPR of the reference diet. Ranking of the test diets was similar when a non-standard 2 week RPER (Reference diet = 100) was employed.

Measures of Liver Protein Utilization (LPU) correlate well with standard measures of Net Protein Utilization (Mokady et al. 1969, Sotelo-Lopez and Lucas-Florentino 1978). Measurement of total liver nitrogen is also considerably easier than total carcass nitrogen analysis. LPU values obtained with the various test diets further support 0.32% dietary L-methionine as the lowest test level producing optimal protein synthesis.

Plasma amino acid levels have long been used to determine animal requirements for amino acids and to identify limiting amino acids in different protein sources (Young et al. 1972, Graham and Placko 1973, Sarwar et al. 1983). It has been demonstrated that addition of an essential amino acid in graded amounts to a diet deficient in the amino acid, results in a constant and low plasma concentration of that amino acid until requirement levels are reached. Beyond requirement, there is a rapid increase in plasma levels of the amino acid. In addition with the limiting amino acid in deficit, a higher concentration of other amino acid occurs in the plasma and decreases as the

dietary deficit is overcome. With the dietary level of the initially limiting amino acid greatly in excess of requirements, PAA levels of other amino acids increase (Zimmerman and Scott 1965, Mitchell et al. 1968, Almquist 1972). This is due to the high metabolic demand for the amino acid in deficit relative to dietary supply and a low efficiency of utilization of other amino acids. As the requirement level for the deficient amino acid is approached, increasing amounts of the other amino acids can be used for protein synthesis and PAA levels should tend to decrease. Excessive supply of the initially limiting amino acid should result in increased PAA concentrations due to amino acid imbalance problems.

Table 4 shows that plasma levels of individual essential amino acids support rat growth findings. Girard-Globa et al. (1972) reported that methionine is rapidly cleared from plasma of rats fed 18% casein-based diets. In the current experiment, the relatively constant plasma methionine levels obtained once basal methionine intakes were exceeded, were accompanied by a trend towards decreased concentrations of other essential amino acids (Arg, His, Ile, Leu, Phe, Thr, Trp). L-threonine showed the greatest response, suggesting it was the second limiting amino acid in the test diets. Girard-Globa et al. (1972) similarly found that plasma threonine decreased by increasing the dietary methionine intakes of rats.

At 0.72% dietary L-methionine, plasma methionine was higher than for all other diets, suggesting it was in excess of requirements and accumulating in the plasma.

The concentrations of non-essential amino acids in plasma of rats fed the different diets are shown in Table 5. While these by definition can be synthesized endogenously, many of them are involved in or derived from

methionine metabolism. The significant increases in concentrations of methionine catabolites, taurine and α-aminobutyric acid, despite relatively constant plasma methionine concentrations, suggest increasing catabolism of methionine as its dietary intake increased. Significantly higher levels for taurine and α-aminobutyrate at 0.72% dietary methionine, relative to the reference diet and most other test diets suggest methionine was in excess of requirements. Elevated plasma and urine levels of taurine in rats fed excess methionine have also been reported by Girard-Globa et al. (1972) and Daniel and Waisman (1969) respectively. Significant increases in food intakes may also have induced increased taurine synthesis for bile acid metabolism (Anderson et al. 1976).

Decreases in plasma serine and the closely related amino acids glycine and alanine with increased dietary methionine suggest contributions to catabolism of methionine via the transsulfuration pathway (Finkelstein and Mudd 1967). Dietary serine has been shown to facilitate elimination of excess methionine via taurine synthesis in rats (Girard-Globa et al. 1972). Significant decreases in plasma ornithine with increased dietary methionine support methionine/ornithine interrelationships reported in chicks (Smith 1981).

Summaries of total essential, total non-essential and total PAA concentrations obtained with the various diets (Table 6) further support the adequacy of 0.32% dietary methionine for optimal growth and protein synthesis of rats fed the 8% protein diets. Total essential, non-essential and total PAA concentrations decreased with increasing dietary methionine suggesting increased protein synthesis. Minimal values for total essential amino acids were achieved at 0.62% dietary methionine, though values at 0.32% dietary methionine did not differ significantly from those obtained with the high quality casein-based reference diet which supplied 0.48 total SAA.

IV. EXPERIMENT 2 - METHIONINE/CYSTINE REQUIREMENTS

INTRODUCTION

The sulfur amino acid (SAA) requirement of the growing animal is influenced by level of dietary protein (Kroening et al. 1965) and energy and by specific dietary components such as levels of cysteine/cystine, choline and inorganic sulfur. In addition, sulfur amino acid availabilities of dietary protein and unique developmental requirements (hair or feather production) may also be important (Byington, et al. 1972, Stockland et al. 1973).

Generally, inorganic sulfur levels of most diets are adequate (Smith 1973, James and Howe 1978) and it is the practice to supply adequate amounts of sources of both sulfate and choline in diets used in protein quality assessment and other research applications using the laboratory rat (Bernhart and Tomarelli 1966, American Institute of Nutrition 1977, Beare-Rogers et al. 1972, Yew et al. 1979).

The partial sparing effect of the non-essential amino acid cysteine/cystine on dietary methionine requirement is of considerable importance. Many foods have equal total SAA contents but vary in relative proportion of methionine and cystine (Orr and Watt 1957). Frequently methionine and cystine are regarded as nutritionally equivalent. James and Hove (1980), however, have demonstrated that supplementary cystine is ineffective in improving weight gain and PER of rats fed low SAA, legume protein-based diets with intrinsically high cystine/methionine ratios.

In order to increase information on SAA requirements for rat growth at

the 8% dietary protein level, it was decided to test effects of various ratios of methionine/cystine.

MATERIALS AND METHODS

A non-protein diet, 8% ANRC casein + 0.2% L-methionine reference diet and '8% protein' basal diets were formulated as described in Experiment 1. Seven test diets providing 0.44% total SAA but varying in proportion of methionine:cystine (100:0, 80:20, 70:30, 60:40, 50:50, 40:60, 70:30) on a weight basis, were made from the casein + amino acid basal diets by suitable increments of crystalline L-methionine[5] and/or L-cystine[5]. Other dietary components were as described for Experiment 1.

Weanling, male Sprague Dawley rats[3], 21-23 days old, were kept in stock cages and fed the reference diet and water ad libitum for 2 days. The rats were then assigned to stainless steel, mesh-bottomed cages, in a randomized complete block design involving 10 replicates in which each of the 9 diets was represented once. Blocking was on the basis of matched initial body weights. Diets and water were provided *ad libitum* for 14 days and cages were maintained at 21 $\pm$ 1°C and 50 to 60% relative humidity with 12 hour light/dark periods for the entire test.

Similar to Experiment 1, weekly food intakes and weight gains were recorded and animals sacrificed 2 hours after removal of food at the end of the 14 day feeding trial. Livers and diets were analysed for total protein (%N x 6.25) and plasma for plasma free amino acids (PAA) also as described for Experiment 1. The data for food intake, weight gain, feed/gain ratio, LPU,

[5] Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63172

RNPR, 2 week RPER and PAA were subjected to one way analyses of variance and Duncan's Multiple Range Test (Snedecor and Cochran 1967, Duncan 1955).

RESULTS

As L-cystine provided from 0 to 33% of total 0.44% dietary SAA at the expense of L-methionine there was a trend towards increased feed intake and weight gain of the rats (Table 7). With 33% of total SAA supplied by L-cystine, maximum feed intakes and weight gains were obtained. These latter values, however, did not differ significantly ($P > 0.05$) from those obtained with the ANRC casein + 0.2% methionine reference diets, nor from diets in which 20 to 60% of total SAA were supplied by L-cystine. Feed/gain ratios were significantly higher only when L-cystine supplied 70% of the total 0.44% dietary SAA and at this level feed intakes and weight gains were significantly lower than for all other diets.

RNPR, RPER and LPU underwent gradual significant increases as L-cystine increased from 0 to 33% of total dietary SAA supplied (Table 8). Similar to results for weight gain and feed intake, maximal RNPR, RPER and LPU were obtained with 33% of dietary SAA supplied by L-cystine. At this ratio of dietary methionine/cystine, values for all three parameters did not differ significantly from those obtained with the 8% casein protein + 0.2% methionine reference diet. With 30/70 ratio of methionine/cystine, values for RNPR and RPER were significantly lower than for those of the reference diet or for all other ratios of methionine/cystine tested.

Table 9 indicates that a significant drop in plasma free methionine occurred as the cystine proportion of dietary SAA increased at the expense of L-methionine. Plasma arginine, threonine and tryptophan concentrations were

significantly higher with the 30/70 ratio of dietary methionine/cystine, threonine levels being more than twice as high as for all other diets. A non-significant decrease in plasma levels of histidine, isoleucine, leucine, tyrosine, threonine, tryptophan and valine also occurred as cystine replaced up to 40% of total SAA. Plasma arginine, cystine, phenylalanine and lysine concentrations remained relatively constant over this range of cystine replacement of dietary methionine (0 to 40%). Feeding the casein + 0.2% methionine reference diet resulted in significantly higher plasma arginine, isoleucine, leucine, methionine and valine concentrations relative to all the casein + crystalline amino acid-based test diets.

Concentrations of non-essential PAA of rats fed the various diets are listed in Table 10. Alanine, glutamic acid, glycine, serine, taurine, citrulline and ornithine levels were significantly higher in the diet supplying 30/70 ratio of methionine/cystine relative to the other test diets supplying equivalent amounts of dietary SAA but lower proportions of dietary cystine. Alanine, glutamic acid, proline, citrulline and ornithine values of rats fed the reference diet did not differ significantly from those obtained with the test diet supplying 30/70 ratio of methionine to cystine, while reference diet values for glycine, serine and taurine were significantly lower, and equivalent to values for test diets in which cystine supplied 0 to 40% of total dietary SAA.

Total PAA and total essential PAA concentrations decreased relative to basal diet values with all levels of SAA supplementation used. Significantly higher levels of total PAA and total essential PAA were achieved with the 30/70 ratio of methionine/cystine relative to other test diet ratios supplying the same amount of total SAA (Table 11). A non-significant trend towards decreased levels of total PAA, total essential PAA and total non-essential PAA occurred as L-cystine replaced up to 40% of dietary methionine, however,

Table 7. Effects of L-methionine/L-cystine ratios on rat performance*

L-Met:L-Cys Ratio	Feed Intake g	Weight Gain g	Feed/Gain Ratio
100:0	170 ± 4^{b}	68 ± 2^{b}	2.5 ± 0.04^{b}
80:20	177 ± 5^{ab}	72 ± 3^{ab}	2.5 ± 0.04^{b}
67:33	191 ± 4^{a}	81 ± 3^{a}	2.4 ± 0.03^{b}
60:40	178 ± 7^{ab}	74 ± 4^{ab}	2.4 ± 0.04^{b}
50:50	181 ± 6^{ab}	75 ± 3^{ab}	2.4 ± 0.03^{b}
40:60	186 ± 7^{a}	77 ± 4^{a}	2.4 ± 0.05^{b}
30:70	116 ± 9^{c}	39 ± 4^{c}	3.0 ± 0.11^{a}
Reference	178 ± 3^{ab}	76 ± 2^{ab}	2.4 ± 0.02^{b}

* Means $\pm$ SE for 10 rats

Means with different letters in the same column differ significantly ($P < 0.05$).

Table 8. Effects of L-methionine/L-cystine ratios on rat growth parameters*

L-Met:L-Cys Ratio	RNPR %	RPER %	LPU
100:0	93 ± 0.8^{c}	93 ± 0.8^{c}	2.82 ± 0.27^{c}
80:20	95 ± 1.0^{bc}	95 ± 1.4^{bc}	3.15 ± 0.43^{bc}
67:33	100 ± 1.5^{a}	100 ± 2.0^{ab}	3.57 ± 0.26^{abc}
60:40	98 ± 1.5^{ab}	98 ± 1.9^{ab}	3.32 ± 0.31^{bc}
50:50	100 ± 1.4^{a}	101 ± 1.7^{a}	2.96 ± 0.18^{bc}
40:60	99 ± 2.0^{ab}	98 ± 2.4^{ab}	3.33 ± 0.23^{bc}
30:70	88 ± 2.7^{d}	80 ± 3.4^{d}	4.25 ± 0.28^{a}
Reference	100^{a}	100^{ab}	3.69 ± 0.24^{ab}

* Means $\pm$ SE for 10 rats

Means with different letters in same column differ significantly ($P < 0.05$).

Table 9. Effects of dietary L-methionine/L-cystine ratios on essential amino acid concentrations* in rat plasma

L-Met:L-Cys Ratio	Arg	His	Ile	Leu	Met	Cys	Phe	Tyr	Thr	Trp	Val	Lys
100:0	13.8^{c}	13.8^{ab}	8.0^{b}	11.0^{b}	6.3^{b}	1.4^{ab}	4.7^{bc}	12.9^{a}	20.7^{b}	5.6^{b}	16.5^{b}	66.2
80:20	13.7^{c}	14.4^{ab}	7.6^{b}	11.3^{b}	4.8^{bc}	1.4^{ab}	4.5^{bc}	12.5^{a}	17.5^{b}	5.2^{b}	19.0^{b}	65.5
67:33	15.0^{bc}	13.4^{ab}	7.9^{b}	10.4^{b}	4.2^{c}	1.6^{ab}	4.5^{bc}	12.6^{a}	19.1^{b}	5.0^{b}	16.9^{b}	67.1
60:40	13.9^{c}	12.4^{b}	7.1^{b}	9.8^{b}	4.1^{c}	1.7^{ab}	4.4^{bc}	11.0^{a}	18.3^{b}	4.6^{b}	15.9^{b}	65.9
50:50	13.5^{c}	14.1^{a}	8.5^{b}	11.1^{b}	3.9^{c}	1.1^{b}	4.6^{bc}	13.4^{a}	19.4^{b}	5.7^{b}	17.9^{b}	66.7
40:60	13.1^{c}	14.7^{ab}	8.3^{b}	10.6^{b}	3.3^{cd}	1.4^{ab}	4.4^{c}	12.1^{a}	20.2^{b}	4.8^{b}	16.9^{b}	65.9
30:70	17.9^{a}	13.5^{ab}	8.6^{b}	11.1^{b}	2.0^{d}	1.7^{a}	5.4^{ab}	15.0^{a}	47.1^{a}	9.7^{a}	17.9^{b}	69.3
Reference	16.3^{ab}	15.4^{a}	10.6^{a}	14.1^{a}	8.7^{a}	1.6^{ab}	5.7^{a}	14.6^{a}	14.5^{b}	5.3^{b}	25.0^{a}	77.9
SEM**	0.8	0.8	0.6	0.8	0.6	0.2	0.3	1.4	2.7	0.5	1.3	5.8

* μM/100 mL plasma

** SEM calculated from analysis of variance data

Means with different letters in the same column differ significantly ($P < 0.05$).

Table 10. Effects of L-methionine/L-cystine ratios on non-essential amino acids* in rat plasma

L-Met:L-Cys Ratio	Ala	Asp	Glu	Gly	Pro	Ser	Tau	Cit	Orn	α-Aba
100:0	89^{bc}	4.1	32.2^{b}	24.3^{b}	37.7^{b}	39.5^{b}	23.5^{b}	11.9^{b}	8.1^{c}	2.7^{b}
80:20	93^{bc}	4.6	32.9^{b}	26.2^{b}	37.3^{b}	39.7^{b}	24.8^{b}	11.5^{b}	8.6^{c}	2.2^{b}
67:33	77^{c}	4.5	32.6^{b}	25.2^{b}	29.5^{b}	40.4^{b}	24.9^{b}	11.9^{b}	9.6^{bc}	1.5^{c}
60:40	76^{c}	4.4	34.3^{b}	26.8^{b}	28.7^{b}	40.3^{b}	23.4^{b}	11.0^{b}	8.7^{c}	1.1^{c}
50:50	89^{bc}	4.1	32.6^{b}	24.9^{b}	39.0^{b}	41.9^{b}	22.4^{b}	11.7^{b}	8.9^{c}	1.0^{c}
40:60	83^{c}	4.7	33.6^{b}	23.2^{b}	34.6^{b}	41.4^{b}	24.1^{b}	11.3^{b}	8.7^{c}	0.1^{d}
30:70	117^{a}	4.9	39.9^{a}	31.7^{a}	42.1^{ab}	66.4^{a}	35.5^{a}	14.2^{a}	11.9^{a}	0.1^{d}
Reference	110^{ab}	5.1	39.7^{a}	23.9^{b}	52.7^{a}	42.4^{b}	26.2^{b}	14.9^{a}	10.9^{ab}	3.6^{a}
SEM**	8	0.4	1.8	1.1	4.3	2.0	1.8	0.6	0.6	0.2

* μM/100 ml plasma

** SEM calculated from analysis of variance data

Means with different letters in the same column differ significantly ($P < 0.05$).

Table 11. Effect of dietary L-methionine/L-cystine ratios on plasma free amino acid (PAA) parameters of the growing rat

L-Met:L-Cys	Total PAA*	Essential PAA*	Non Essential PAA*	Essential PAA / Total PAA
100:0	454^{b}	181^{bc}	274^{b}	0.40
80:20	459^{b}	177^{bc}	281^{b}	0.39
67:33	435^{b}	178^{bc}	257^{b}	0.41
60:40	424^{b}	169^{c}	255^{b}	0.40
50:50	455^{b}	180^{bc}	275^{b}	0.39
40:60	441^{b}	176^{bc}	265^{b}	0.40
30:70	583^{a}	219^{a}	363^{a}	0.38
Reference	539^{a}	210^{ab}	330^{a}	0.39
SEM**	25	11	16	0.01

* µM/100 mL plasma

** SEM calculated from analysis of variance data

Means with different letters in the same column differ significantly ($P < 0.05$).

levels for these parameters did not differ significantly from those obtained with up to 60% of dietary SAA as L-cystine. No significant differences in essential PAA/total PAA ratios were seen to result from any of the test diets fed.

DISCUSSION

Based upon results of Experiment 1, 0.32% dietary L-methionine (0.34% total SAA), was the minimum level of SAA to produce maximum rat growth response by most variables measured and optimal PAA parameters. In order to ensure that SAA requirements were fully satisfied in this current experiment dealing with the potential for replacement of dietary methionine by cystine, it was decided to use 0.42% L-methionine (0.44% total SAA) as the initial test level. This meant that it was not until the 67/33 ratio of dietary methionine/cystine that dietary methionine became limiting in terms of rat growth requirement at the 8% level of protein intake.

The trend towards increased feed intake and weight gain as L-cystine replaced 0 to 33% of dietary methionine (Table 7) suggests a requirement for dietary cystine at the 8% dietary protein level. The improvements in RNPR, RPER and LPU (Table 8) over the same range of cystine replacement further support a requirement for cystine in order to maximize rat growth response. It is proposed that inclusion of dietary cystine made unnecessary the metabolically expensive conversion of methionine to cysteine (Bhagavan 1978) thereby increasing the efficiency of utilization of dietary methionine for protein synthesis.

Total SAA requirements for growth of chicks and rats have been shown to be less, expressed on a weight basis, when a combination of methionine and cystine was used compared to when methionine alone was used. Expressed on a

molar basis, however, the requirement did not appear to change (Graber and Baker 1971, Sowers et al. 1972, Stockland et al. 1973).

The casein + 0.2% L-methionine reference diet has widespread use in collaborative and other studies of protein quality (McLaughlan et al. 1980, Sarwar et al. 1981, 1985a). It is also completely adequate in terms of rat growth SAA requirements. The fact that feed intakes, weight gains, feed/gain ratios, RNPR and RPER results did not differ significantly from the reference diet with up to 60% replacement of dietary methionine by cystine, confirms that there is a range of acceptable replacement of methionine by cystine. The 60% maximum replacement value at 8% dietary protein (values from 60-70% not tested) slightly exceeds that set by NRC (1978) (50%) but is in accordance with values of Rao et al. (1959) and Sowers et al. (1972) (60-70%).

The significantly lower food intake, weight gain and decreased efficiency of food conversion (higher feed/gain ratio) resulting from 70% of total SAA supplied as L-cystine, suggests that the methionine requirement was not met at this level. This is supported by minimum RNPR and RPER also obtained at this 30/70 ratio of methionine/cystine. Sowers et al. (1972) similarly found that at suboptimal levels of methionine intake, depression in weight gains of rats resulted from increases in dietary cystine level. Cystine has also been reported to have an antagonistic effect on methionine utilization in methionine deficient chickens and pigs (Featherston and Rogler 1978, Baker et al. 1969). It has been suggested that high endogenous cystine/methionine ratios are the reason for ineffectiveness of supplementary cystine in improving PER and weight gain of young rats fed certain low SAA-containing legume based diets (James and Hove 1980). It is proposed that a similar high cystine/methionine ratio in the 70/30 methionine/cystine test diet caused the potential sparing effect of cystine on methionine to be exceeded resulting in poor animal performance.

Liver protein utilization which is an expression of the amount of food nitrogen retained in the liver as a percentage of food nitrogen intake, has been shown to be an effective and easy measure of Net Protein Utilization and to correlate well with PER measures of protein quality (Mokady et al. 1969, Sotelo-Lopez and Lucas-Florentino 1978). Increased LPU values as cystine replaced 0 to 33% of dietary methionine reflect increased efficiency of utilization of food protein for protein synthesis and further support the suggestion that there is a dietary requirement for L-cystine to support maximal growth of the rat at the 8% dietary protein level. Allison (1964) showed that the liver is second only to the blood in terms of susceptibility of different organs to changes in nutritional quality of food proteins.

Mean plasma concentrations of individual essential amino acids (plus cystine and tyrosine) in Table 9, and of non-essential amino acids in Table 10, reflect a combination of change in dietary methionine and cystine intakes and the concurrent effects of these changes in terms of methionine metabolism. The significant drop in plasma methionine with decreased methionine intake is to be expected. Continuing demands for growth or protein synthesis in the young rats would use up first limiting methionine as its dietary supply decreased. The low and relatively constant plasma cystine levels, despite increased dietary cystine in the test diets, were with one exception not significantly different from the level in rats fed the ANRC casein + 0.2% L-methionine reference diet. This latter diet contained minimal levels of dietary cystine. Zlotkin and Anderson (1982) in studies of sulfur balance of intravenously fed newborn infants suggested that low plasma response to supplementary cysteine was likely due to increased catabolism (oxidation) of cysteine as measured by increased sulfur excretion. Relatively constant plasma taurine concentrations in the present study (Table 10) up to the 30/70 ratio of dietary

methionine/cystine would suggest that the taurine catabolic route was relatively stable up to this level and that increased bile acid metabolism (Brueton et al. 1978) was not involved. The reported equilibrium between protein-bound and free plasma cysteine (Malloy et al. 1981) suggests that protein binding of increased dietary cysteine was not a factor involved in the stable plasma cysteine levels though the peptide glutathione has been implicated as a major tissue reservoir for cysteine in rodents (Seligson and Rotruck 1983).

Significantly decreased levels of α-aminobutyric acid with increased cystine contribution to total dietary SAA (Table 10) reflect decreased methionine catabolism (Byington and Howe 1972) as methionine requirements became increasingly in deficit.

The non-significant trend in decreased plasma levels of several essential amino acids (histidine, isoleucine, leucine, tryptophan) and of non-essential amino acids (alanine, proline, tyrosine) as cystine replaced up to 40% of dietary methionine suggest increased protein synthesis with partial replacement of dietary methionine by cystine. This is supported by significant increases in weight gain, RNPR and RPER occurring at the same time with up to 30% replacement of dietary methionine.

At 30/70 ratio of dietary methionine/cystine, several essential amino acids (arginine, phenylalanine, threonine and tryptophan) and non-essential amino acids (tyrosine, alanine, glutamic acid, glycine, proline, serine, taurine, citrulline and ornithine) were significantly higher than for all other test ratios. Most other essential and non-essential amino acids showed a similar, though non-significant increase. It is proposed that this is a reflection of methionine deficiency due to excess replacement of methionine with cystine resulting in failure of protein synthesis and accumulation of plasma amino acids.

The lower plasma concentrations of threonine, tryptophan, glycine, serine and taurine and higher α-aminobutyric acid obtained with the reference diet relative to the 30/70 methionine/cystine test diet indicate adequacy of methionine intake and protein synthesis. This conclusion is also supported by the growth and rat performance data (Table 7, Table 8). As discussed in Experiment 1, many of these amino acids have key roles in the catabolism of methionine and cystine.

In general, summaries of total, total essential and total non-essential amino acid concentrations in rats fed the various diets (Table 11) parallel findings discussed for the individual amino acids. The non-significant decreases in these parameters up to the 40/60 methionine/cystine ratio, as cystine replaces initially more than adequate and then limiting methionine, suggest increased utilization or catabolism of amino acids. Weight gain, RNPR and RPER improvements over this range of cystine replacement of dietary methionine as well as individual PAA findings, support protein synthesis rather than catabolism. At the 30/70 dietary ratio, higher values for the above PAA parameters and minimum weight gain, RNPR, RPER and maximum feed/gain ratio suggest accumulation of amino acids in the plasma due to methionine deficit and resulting decreased protein synthesis.

A depression in cystathionine synthase activity and depressed methionine oxidation in rats fed low methionine diets supplemented with cystine as compared to methionine has been proposed to account for the methionine-sparing effect of cystine (Finkelstein and Mudd 1967, Shannon et al. 1972). Stipanuk and Benevenga (1977), however, found that cystine supplementation resulted not only in decreased cystathionine synthase activity but also in increased protein synthesis and decreased methionine catabolism as determined by significantly decreased recovery of labelled CO_2 arising from metabolism of

L-1-^{14}C-methionine and increased specific activity in muscle tissue. Cystine supplementation of threonine-deficient, methionine-deficient diet had no effect on labelled CO_2 recovery. They concluded that changes in the oxidation of amino acids were secondary to the increased utilization of amino acids for protein synthesis.

Based upon the results of the current experiment, the sparing effect of L-cystine on rat growth requirement for L-methionine at the 8% level of protein intake is confirmed. It would appear that there is an optimal range of cystine replacement (up to 60%). Improvements in rat growth with replacement of up to 33% dietary methionine with cystine suggest a requirement for L-cystine at this low level of protein intake. Further studies are required to confirm this.

Bioavailabilities of amino acids in a protein source are key factors governing whether or not the protein meets animal requirements for those amino acids. Evans et al. (1974) using 10% protein diets based upon optimally heated dry bean (Phaseolus vulgaris) seeds or soybean meal with and without added methionine found higher rat requirements for methionine plus cystine, to obtain maximum growth and PER, in the case of the dry bean-based diet. Methionine and cystine balance studies using the growing rats indicated twice the level of methionine and cystine in the undigested fecal protein of the dry bean-fed rats relative to the soybean-fed animals.

Measurements of methionine and cystine present in the soybean meal and dry bean seeds and correction for availabilities of the two amino acids in the two protein sources contributed to determination of methionine plus cystine requirements for optimal rat growth. Assuming complete availability of supplemental methionine, 0.26% of diet as available methionine was determined to be the requirement in the case of the soybean-meal based diet and at least

0.27% methionine plus cystine requirement in the case of the Phaseolus seed-fed rats.

Sarwar (1984) has reported that the true digestibilities for methionine and cystine of ANRC casein are 96% and 85% respectively. Correction for these digestibilities in the four percent casein protein (0.12% L-methionine, 0.02% L-cystine contribution to total diet) used in these experiments, and assuming supplemented crystalline amino acids are completely available, suggests that the true sulfur amino acid requirement for the growing rat at the 8% protein level is 0.33% of the diet. 4.1% of dietary protein must therefore be supplied as SAA for optimal growth of rats fed 8% protein diets.

Pick and Meade (1971) using corn + amino acid-based diets supplying 10% dietary protein, and Williams et al. (1954) based upon analysis of carcass amino acids of rats, reported total SAA requirements for rat growth of 0.40% and 0.43% of diet. However, Rao et al. (1959), using casein + amino acid-based diets and Sowers et al. (1972), using crystalline amino acid-based diets, reported requirements of 0.49% and 0.47% of diet respectively. These values are closer to the 0.6% dietary SAA requirement set by NRC (1978) for rats fed 12% dietary protein.

The lower requirement determined in the present study is likely related to more efficient utilization of protein and amino acids at lower levels of dietary protein as suggested by Edozien and Switzer (1978). The inclusion of adequate dietary choline in the present study may partially spare methionine requirements for transmethylation reactions. NRC (1978) includes a portion of SAA for potential transmethylation needs.

V. EXPERIMENT 3

AVAILABILITY OF OXIDIZED SULFUR AMINO ACIDS

INTRODUCTION

There are many reports in the literature indicating that food processing or factors inherent in foods themselves, give rise to oxidation of sulfur amino acids (SAA) present in food proteins (Miller et al. 1965, Cuq et al. 1978, Chang et al. 1982). There is considerable controversy concerning the bioavailability of oxidized SAA, whether present as free forms (Miller and Samuel 1970, Miller et al. 1970, Anderson et al. 1976, Gjøen and Njaa 1977, Crawford et al. 1984) or in intact peptides and proteins (Slump and Schreuder 1973, Cuq et al. 1973, Anderson et al. 1975). Gjøen and Njaa (1977) suggested that poor utilization by animals of methionine sulfoxide present in many proteins was due to inherently low levels of cystine. In the absense of dietary cystine, methionine sulfoxide reduction occurred too slowly in the animals to meet cysteine/cystine requirements.

The availability of an amino acid in a food is a key factor contributing to whether or not animal requirements for the amino acid are met. This can be especially important in terms of the SAA which are frequently first limiting amino acids in many food sources that may also be subjected to necessary heating or other processes to destroy antinutrient factors or to render the food more acceptable or digestible.

Methionine sulfoxide and methionine sulfone are the major oxidized forms of methionine and cysteic acid is the major oxidized form of cysteine. Methionine sulfoxide with one additional oxygen per molecule relative to

methionine is only partly oxidized, while methionine sulfone and cysteic acid with two and three oxygen molecules respectively are completely oxidized. The University of Nebraska Food Protein Research Group (1982) has demonstrated rise in methionine sulfoxide and methionine sulfone and of cysteic acid and a concurrent drop in methionine and cystine in samples of soy protein isolate, egg white solids and casein when the samples were subjected to varying times, temperatures and concentrations of hydrogen peroxide. The loss in bioavailability of methionine due to partial oxidation of soy protein and casein as measured by rat PER performance was also demonstrated.

Because of the limiting nature of SAA in many foods and their propensity for oxidation, it was decided to determine the bioavailability of the major oxidized forms of SAA (methionine sulfoxide, methionine sulfone and cysteic acid) in terms of their potential for meeting rat growth requirements when 8% protein diets were fed. This could prove useful in terms of testing the availability of oxidized SAA in important human food protein sources subjected to oxidizing conditions. Heat sterilized infant formulas based upon cow's milk proteins or soy protein would be prime candidates for such testing.

MATERIALS AND METHODS

As described in Experiment 1, a non-protein diet (Table 1a) and an 8% ANRC casein protein + 0.20% L-methionine reference diet were formulated. Four test diets were also made up from basal diets deficient in sulfur amino acids (SAA) and providing at the expense of the corn starch of the non-protein diet, 4.0% protein from ANRC casein and crystalline L-amino acids (except methionine and cystine) equivalent to 4% casein protein. Supplementary SAA (0.20%) were provided in the test diets by the L-forms of either methionine[5] +

cystine[5], methionine sulfoxide[5] + cystine, methionine sulfone[5] + cystine or methionine + cysteic[5] acid. Supplemental methionine or its oxidized forms provided 50% of the total 2/3 methionine portion of dietary SAA and supplemental cystine or cysteic acid approximately 85% of the total cystine portion of dietary SAA.

Weanling male Sprague Dawley rats[3], 21-23 days old, were kept in stock cages and fed the reference diet for 2 days. They were then assigned on the basis of matched body weight, to individual stainless steel, mesh-bottomed cages, in a randomized complete block design involving 8 replicates in which each diet (non-protein, reference, basal and 4 test diets) was represented once per block. Diets and water were provided *ad libitum* for 14 days and cages maintained at 21 $\pm$ 1°C and 50 to 60% relative humidity, with 12 hour light/dark cycles for the entire test period. Weekly food consumption and weight gain of each animal were recorded.

Animal sacrifice, blood sampling and analyses were as described for Experiment 1. Immediate plasma deproteinization after sampling permitted analysis of cystine and elevation of column temperature during amino acid analysis permitted detection of methionine sulfone and methionine sulfoxide in the plasma samples. In addition to rat growth parameters, RNPR, 2 week RPER and PAA data, relative methionine availabilities of the test diets (methionine + cystine-supplemented diet = 100) were determined by using the formula:

$$\text{Relative Methionine Availability} = \frac{\text{NPR of test diet - NPR of basal diet}}{\text{NPR of reference diet - NPR of basal diet}} \times 100$$

[5] ICN Nutritional Biochemicals, Cleveland, Ohio.

as described by Paquet and Sarwar (1980).

Data obtained were listed as means ± standard errors and subjected to one way analyses of variance and Duncan's Multiple Range Test at the 5% level of significance (Snedecor and Cochran 1967, Duncan 1955).

RESULTS

All forms of SAA supplementation used, resulted in significant improvements in rat performance relative to the basal diet as measured by feed intake, weight gain and feed/gain ratio (Table 12). Diets containing methionine sulfone and cysteic acid supplements caused significantly lower food intakes and weight gains and higher feed/gain ratios than the methionine + cystine-supplemented diet. The diet containing methionine + cysteic acid resulted in poorest rat performance. Results for the methionine + cystine and methionine sulfoxide + cystine-supplemented diet did not differ significantly.

RNPR and RPER values (Table 13) showed similarly ranking of the various SAA supplements and paralleled rat performance data. Diets containing methionine or methionine sulfoxide supplements appeared equivalent. Both the methionine sulfone + cystine-supplemented diet and the methionine + cysteic acid-supplemented diet resulted in significantly lower RNPR and RPER values relative to the former two diets, the methionine + cysteic acid-supplemented diet causing lowest values.

Relative methionine availability estimates for methionine + cystine and methionine sulfoxide + cystine supplements were similar. Diets containing methionine sulfone + cystine and methionine + cysteic acid produced lower methionine availability compared to the methionine + cystine-supplemented diet. Relative methionine availability of the diet containing methionine + cysteic acid was lowest of all test diets, however, values did not differ

significantly from those obtained when supplemental methionine sulfone + cystine was used.

Table 14 lists individual SAA levels in plasma of rats fed the different diets. Plasma methionine was significantly higher than all other diets with methionine sulfoxide + cystine supplementation. Methionine + cystine and methionine + cysteic acid supplements resulted in similar methionine values, intermediate to those of the basal and sulfoxide-supplemented diets. Methionine sulfone + cystine supplement caused lowest plasma methionine values and these differed significantly from those of the other three test diets. Methionine sulfoxide was present in the plasma of rats fed both the basal and test diets, however, no significant differences between diets was observed. Methionine sulfone was detected only in the plasma of rats fed methionine sulfone as part of the SAA supplement.

Plasma cystine, undetected in the basal diet-fed rats, was present in low concentrations in rats fed each test diet. It was significantly lower relative to other SAA supplements only in the case of methionine + cysteic acid-supplemented animals. Taurine and cysteic acid catabolites of cystine were detected in all rats, with highest levels for both measured in plasma of rats fed methionine + cysteic acid-supplemented diet. Only taurine level was significantly higher compared to rats fed all the other diets. Relative to the basal diet-fed rats, plasma taurine of all but the methionine + cysteic acid-supplemented rats underwent a significant decrease, although this decrease was much less than increase in plasma taurine that occurred with the latter diet.

Remaining free essential amino acid and tyrosine levels in plasma of rats fed the different diets are listed in Table 15. The methionine sulfone + cystine supplement showed least change relative to the basal diet. In general when supplementation did result in changes, these involved decreases in plasma

concentrations. Thus plasma arginine was significantly lower than the level in the basal diet with methionine + cystine, methionine sulfoxide + cystine and methionine + cysteic acid supplementation. Histidine showed a significant decrease with methionine + cystine and methionine sulfoxide + cystine supplements. Plasma threonine showed the greatest decrease in all supplemented diets relative to the basal diet and the decreases with methionine sulfone and cysteic acid-containing supplements were one half those seen with the methionine + cystine and methionine sulfoxide + cystine supplements. In contrast, free tyrosine underwent significant increase with both methionine + cystine and methionine sulfoxide + cystine supplemented diets. Tyrosine values for the other two diets were comparable to each other and to tyrosine level of the basal diet.

Non-essential amino acids not yet listed but also detected in the plasma samples are shown in Table 16. Free L-glycine showed a significant decrease relative to the basal diet for all test diets except the one containing methionine + cysteic acid supplement. Serine underwent a significant decrease with all test diets compared to the basal diet. α-Aminobutyric acid was higher with all test diets as was glutamic acid. In the case of α-aminobutyric acid the increase was significant only in terms of the methionine + cystine-supplemented diet. In terms of plasma free glutamic acid the increase was significant only in terms of the methionine + cysteic acid-supplemented diet. Summaries of changes in total essential, total non-essential and total PAA as well as essential PAA/total PAA ratios (Table 17) reflect changes already described on an individual basis for PAA. Total essential PAA underwent significant decreases relative to the basal diet with all SAA supplements. The decrease for the methionine sulfone + cystine supplement was significantly less than those of the other three forms of supplementation. Total non-essential PAA underwent a decrease compared to

basal diet levels with all but the cysteic acid-containing supplement. Total PAA were significantly lower with all SAA supplements used, the decrease being smaller (but not significantly smaller) in the case of methionine sulfone + cystine and methionine + cysteic acid supplements. Essential PAA/total PAA ratios decreased significantly relative to the basal diet with all but the methionine sulfone + cystine forms of SAA supplementation. The lowest essential PAA/total PAA ratio was attained with L-methionine + L-cysteic acid containing test diet and this ratio was significantly lower than the ratio measured in all other diets.

DISCUSSION

The use of free forms of oxidized and unoxidized sulfur amino acids in this experiment serves as a model for studies on the bioavailability of oxidized SAA and permits precise control of levels present in the test diets. It must be emphasized, however, that the nutritional bioavailability of an amino acid or its derivative may be influenced by its presence in intact protein or in free form in the diet. This may influence time of exposure to digestive or other enzymes and even stimulation of enzyme production. Utilization of a modified amino acid would also be influenced by its relative conversion rate to the amino acid which could depend upon rate of transport, action of intestinal enzymes and bacteria, rate of absorption, post absorption enzyme activity, renal clearance and possible toxic effects (Friedman and Gumbmann 1981). While it has been demonstrated that oxidation of SAA in intact casein protein reduced in vitro enzymatic release of methionine (Cuq et al. 1973), it has also been shown by everted intestinal sacs in vitro and by in vivo circulated gut loops that efficiency of rat intestinal transport of methionine sulfoxide (the major

Table 12. Effects of source of supplemental SAA on rat performance*

Supplemental SAA source	Feed Intake g	Weight Gain g	Feed/Gain Ratio
Basal	71 ± 3[d]	5 ± 1[d]	16.1 ± 2.0[a]
L-Met + L-Cys	207 ± 5[a]	83 ± 3[a]	2.5 ± 0.04[b]
L-Met sulfoxide + L-Cys	204 ± 8[a]	84 ± 4[a]	2.4 ± 0.05[b]
L-Met sulfone + L-Cys	164 ± 10[b]	51 ± 5[b]	3.3 ± 0.10[b]
L-Met + L-Cysteic Acid	107 ± 8[c]	27 ± 5[c]	5.1 ± 1.14[b]

*Means ± SE for 8 rats

Means with different letters in the same column differ significantly ($P < 0.05$).

Table 13. Effects of source of supplemental SAA on rat growth parameters*

Supplemental SAA source	RNPR %	RPER %	Relative Methionine Availability
Basal	43 ± 2.3[d]	17 ± 2.0[d]	-
L-Met + L-Cys	100[a]	100[a]	100[a]
L-Met sulfoxide + L-Cys	101 ± 3.6[a]	104 ± 2.9[a]	103 ± 5.8[a]
L-Met sulfone + L-Cys	81 ± 2.9[b]	77 ± 2.9[b]	68 ± 4.2[b]
L-Met + L-Cysteic Acid	71 ± 6.8[c]	55 ± 8.4[c]	55 ± 9.9[b]

* Means ± SE for 8 rats

Means with different letters in same column differ significantly ($P < 0.05$).

Table 14. Effects of source of supplemental SAA on plasma sulfur amino acid concentrations* in growing rats

Supplemental SAA Source	Met	Cys	Met Sulfoxide	Met Sulfone	Cysteic Acid	Tau
Basal	2.6^{bc}	-	2.1	-	1.3^{ab}	6.9^{b}
L-Met + L-Cys	3.3^{b}	0.7^{a}	2.1	-	1.2^{ab}	3.3^{c}
L-Met sulfoxide + L-Cys	4.7^{a}	0.7^{a}	2.1	-	1.1^{b}	4.0^{c}
L-Met sulfone + L-Cys	2.1^{c}	0.7^{a}	2.3	12.7	1.1^{b}	3.4^{c}
L-Met + L-Cysteic Acid	3.4^{b}	0.3^{b}	2.3	-	1.5^{a}	24.6^{a}
SEM**	0.3	0.1	0.1		0.1	0.7

* µM/100 mL plasma

** SEM calculated from analysis of variance data

Means with different letters in the same column differ significantly ($P < 0.05$).

Table 15. Effects of source of supplemental SAA on plasma essential amino acid concentrations* in growing rats

Supplemental SAA Source	Arg	His	Ile	Leu	Phe	Tyr	Thr	Trp	Val	Lys
Basal	18.2^{a}	17.0^{a}	7.7	13.2	4.8	5.4^{c}	155.2^{a}	3.3	15.6	75.9^{ab}
L-Met + L-Cys	12.9^{b}	12.8^{b}	7.8	12.2	4.5	12.2^{a}	37.8^{c}	3.8	16.9	74.0^{ab}
L-Met sulfoxide + L-Cys	12.9^{b}	12.4^{b}	7.6	10.3	3.8	11.0^{ab}	27.8^{c}	3.0	17.1	74.9^{ab}
L-Met sulfone + L-Cys	16.1^{ab}	17.4^{a}	7.4	12.6	5.0	6.0^{c}	78.7^{b}	3.8	19.3	90.3^{a}
L-Met + L-Cysteic Acid	12.9^{b}	14.4^{ab}	7.7	12.5	4.6	6.9^{bc}	65.5^{b}	2.9	20.2	69.6^{b}
SEM**	1.2	1.0	0.8	1.3	0.5	1.3	8.5	0.3	2.7	5.0

* μM/100 mL plasma

** SEM calculated from analysis of variance data

Means with different letters in the same column differ significantly ($P < 0.05$).

Table 16. Effects of source of supplemental SAA on plasma non-essential amino acids* in growing rats

Supplemental SAA Source	Ala	Asp	Glu	Gly	Pro	Ser	Cit	Orn	α-Aba
Basal	81.1	2.1	14.8^{b}	44.4^{a}	33.8	102.8^{a}	11.8	10.9	0.3^{b}
L-Met + L-Cys	77.7	1.9	16.2^{ab}	28.2^{b}	31.8	60.3^{b}	11.0	9.3	0.5^{a}
L-Met sulfoxide + L-Cys	74.5	2.1	16.9^{ab}	26.2^{b}	31.9	43.7^{b}	12.2	9.4	0.4^{ab}
L-Met sulfone + L-Cys	65.6	1.9	15.2^{b}	23.3^{b}	31.6	63.5^{b}	12.8	11.8	-
L-Met + L-Cysteic Acid	94.1	2.3	17.8^{a}	40.3^{a}	38.2	69.8^{b}	11.8	11.0	0.4^{ab}
SEM**	8.8	0.2	0.7	3.2	5.2	8.0	0.7	0.8	0.06

* µM/100 ml plasma

** SEM calculated from analysis of variance data

Means with different letters in same column differ significantly ($P < 0.05$).

Table 17. Effects of source of supplemental SAA on plasma free amino acid (PAA)* parameters in growing rats

Supplemental SAA Source	Essential PAA	Non Essential PAA	Total PAA	Essential PAA / Total PAA
Basal	330[a]	312[a]	642[a]	0.52[a]
L-Met + L-Cys	199[c]	244[ab]	443[b]	0.45[b]
L-Met sulfoxide + L-Cys	185[c]	225[b]	409[b]	0.45[b]
L-Met sulfone + L-Cys	260[b]	246[ab]	506[b]	0.52[a]
L-Met + L-Cysteic Acid	218[c]	314[a]	531[b]	0.41[c]
SEM**	18	23	40	0.01

* μM/100 mL plasma

** SEM calculated from analysis of variance data

Means with different letters in the same column differ significantly ($P < 0.05$).

oxidized form of methionine) was similar to that of methionine (Higuchi et al. 1982). The existence of enzymatic, thermal labile, NADH enhanced methionine sulfoxide reducing activity has been demonstrated in rat liver and kidney (Aymard et al. 1979).

The combination of casein plus amino acids used as 'protein' source while limiting the extent of which oxidized SAA can replace unoxidized forms, should also tend to limit potential digestive and absorptive differences caused by use of intact protein versus amino acid based diets. In addition, the 50% replacement of methionine and almost complete replacement of cystine in the test diets by their respective oxidized forms may be more reflective of actual results of food processing than the complete replacement often used in availability studies (Miller and Samuel 1970, Miller et al. 1970, Gjøen and Njaa 1977). Cystine has been shown to be the most heat labile of amino acids (Miller et al. 1965, Bender 1972) and the extent to which methionine undergoes oxidation is dependent upon harshness of processing conditions (Marshall et al. 1982). Conditions sufficiently harsh to cause complete oxidation of methionine would also likely cause loss of available lysine due to Maillard compound formation (Cheftel 1979, Labuza and Saltmarch 1981) which would also contribute to decreased growth performance.

The improvement in rat growth relative to the basal diet obtained with all test diets indicates that all the supplements used were at least partially effective as sources of available SAA. It must be noted, however, that each diet contained unoxidized methionine and/or cystine as part of the SAA supplement. Based upon weight gain, feed/gain ratio, RNPR and RPER (Table 12, Table 13), the diet containing methionine sulfoxide + cystine was as effective at improving rat growth performance as the diet containing equivalent levels of supplemental methionine + cystine. The diets in which

methionine sulfone and cysteic acid contributed to the SAA supplement resulted in significantly poorer rat performance relative to the methionine + cystine - and methionine sulfoxide + cystine-supplemented diets measured by the same criteria. The diet in which almost the entire cystine component was replaced by cysteic acid gave poorest results. It is proposed that free L-methionine sulfoxide is as available and that L-methionine sulfone is unavailable as methionine when used to replace up to 50% of the rat growth requirement for methionine when 8% protein diets are fed. L-cysteic acid is similarly proposed to be unavailable as an SAA source for the growing rat. Improvements in rat performance occurring relative to the basal diet, are proposed to be due to the unoxidized methionine also included in the SAA supplement.

Equivalent bioavailability of methionine and methionine sulfoxide has also been reported as measured by nitrogen retention of rats fed 8% soybean meal protein-based diets supplemented with 0.12% of either SAA (Njaa 1962); as measured by weight gain when methionine sulfoxide replaced 50% of total methionine in crystalline amino acid-based diets (Miller et al. 1970); and as measured by growth and N balance when used as partial SAA supplements to amino acid, soybean meal and casein-based diets fed to rats (Gjøen and Njaa 1977). Complete replacement of methionine by L-methionine sulfoxide resulted in poorer rat performance as measured by decreased 17 day weight gain and microbiologically-measured blood methionine activity (Miller et al. 1970) and poorer rat growth (Anderson et al. 1976, Gjøen and Njaa 1977). An improvement in utilization of methionine sulfoxide with increased age of rats has also been demonstrated (Njaa 1962, Miller et al. 1970). Yanagita et al. (1984) despite similar weight gain, feed intake and liver weight results have, however, reported that L-methionine sulfoxide was less effective than methionine in inducing fatty liver in rats fed low casein plus 0.1% SAA-supplemented diets.

Methionine sulfone and cysteic acid have been reported to decrease 10 day NPU measurements by 11% and 4% respectively, in growing rats fed 10% casein protein diets supplemented with 0.1% of either SAA, relative to unsupplemented casein protein diets (Miller and Samuel 1970). Anderson et al. (1976) found that cysteic acid and methionine sulfone when used as sole SAA sources in crystalline-amino acid based diets, did not support rat growth. Both completely oxidized SAA were found to have no supplementary effects in terms of improvement of PER and biological value in H_2O_2-treated fish protein (Sjøberg and Boström 1977).

Relative Methionine Availability measurement has been used to show the relative availability of methionine in various methione derivatives having potential replacement value for dietary methionine (Paquet and Sarwar 1980) and in methionine-containing tripeptides that can arise during food processing (Sarwar et al. 1985b). Because this measure involves a bioassay of actual animal performance it should give a more realistic measure of bioavailability of SAA in diets than chemical assays of the diets. As shown in Table 13, methionine sulfoxide + cystine supplementation gave equivalent Relative Methionine Availability to methionine + cystine supplementation further supporting the equivalence of methionine sulfoxide and methionine. The cysteic acid-containing diet gave lowest measure of Relative Methionine Availability although values did not differ significantly from that of the methionine sulfone-supplemented diet suggesting both completely oxidized SAA were unavailable as SAA sources for rat growth requirements.

Plasma amino acid findings generally supported rat growth parameters already discussed and reflected the relative bioavailabilities of the various SAA forms for protein synthesis. Anderson et al. (1976) found that amino acid data

permitted detailed interpretation of growth and feed intake data of rats fed diets containing oxidized SAA.

As shown in Table 14, levels of SAA in rat plasma remained relatively low with the equivalent to 8% protein diets employed. Anderson et al. (1975) in studies of protein quality of hydrogen peroxide-treated rapeseed, found that rat plasma SAA generally reflected the amino acid content of the diets fed and that levels of plasma SAA increased with increases from 5 to 20% dietary protein. Lowest levels of rat plasma methionine were obtained in the current experiment with methionine sulfone + cystine supplementation and these levels did not differ significantly from those of rats fed the basal diet with no supplemental SAA. It is suggested that the low levels reflect an inability of the rat to reduce the fully oxidized methionine sulfone supplied in the diet with resultant depletion of plasma methionine due to high protein synthesis requirements. Significantly highest plasma methionine levels occurring with the methionine sulfoxide + cystine-supplemented diet may reflect problems in the accurate determination of the various oxidized forms of SAA (Anderson et al. 1975, 1976). Iwami et al. (1983) in studies of intraperitoneal injection of labelled methionine sulfoxide and its contribution to glutathione or urinary sulfate metabolites found that methionine sulfoxide was obviously reduced in vivo as measured by resultant incorporation of cysteine into the glutathione peptide. Higher plasma methionine levels with methionine sulfoxide containing supplement may also reflect this in vivo reduction and slower utilization of dietary methionine sulfoxide versus methionine. Equivalent plasma methionine levels with methionine + cystine and methionine + cysteic acid supplements might be expected due to equal levels of methionine supplementation.

Plasma cystine was not detected in rats fed the basal diet reflecting the low dietary supply of cystine relative to the test diets. The three diets

containing methionine or either of its two oxidized forms had equivalent and highest levels of plasma cystine reflecting equivalent dietary cystine supply. Significantly lower plasma cystine in rats fed the cysteic acid-supplemented diet relative to the other three test diets similarly reflect dietary cystine supply. Presumably supplemental L-methionine was contributing to plasma cystine via the transsulfuration pathway resulting in slightly higher plasma cystine levels in the cysteic acid-supplemented diet relative to the basal diet.

The detection of methionine sulfoxide at similar levels in plasma of all rats, including those fed the unsupplemented basal diet was in contrast to the findings of Anderson et al. (1976) who reported elevated plasma methionine sulfoxide when methionine sulfoxide was used as a dietary supplement. These authors suggested that the rate limiting step in the utilization of methionine sulfoxide, was its reduction to methionine. The absence of elevated levels of plasma methionine sulfoxide demonstrated in the present study may reflect the ready reduction of the sulfoxide to methionine (Aymard et al. 1979, Iwami et al. 1983) and its contribution to methionine requirement for protein synthesis, for transmethylation reactions or methionine catabolism to CO_2. The formation of N-acetylmethionine sulfoxide in rat kidney and its urinary excretion has been proposed as an additional pathway for removal of excess methionine sulfoxide (Smith 1972).

The presence of methionine sulfone only in plasma of rats fed the methionine sulfone + cystine-supplemented diet, and its high concentration compared to other SAA reflects its unavailability as a methionine source and supports the same conclusion arrived at using rat growth data. High plasma levels of methionine sulfone and poor growth have also been reported for rats fed hydrogen peroxide-treated rapeseed flour containing methionine sulfone (Anderson et al. 1975) or crystalline amino acid-based diets in which methionine

sulfone served as a source of SAA (Anderson et al. 1976). Smith (1972) has stated that methionine sulfone is relatively inert in rats compared to methionine sulfoxide and has shown that a considerable portion of intraperitoneally injected (Me-^{14}C)-methionine sulfone was excreted via the urine, either as methionine sulfone or N-acetylmethionine sulfone.

Higher plasma cysteic acid levels with cysteic acid-containing SAA supplement were not significantly different from those of the basal or methionine + cystine-supplemented diet, although elevated levels might be expected due to higher dietary supply. Anderson et al. (1975, 1976) also found that cysteic acid did not respond to dietary changes. Highest plasma taurine levels obtained with the cysteic acid-containing diet likely reflect the rapid conversion of cysteic acid to taurine (Krebs 1964) which would also explain the lack of plasma cysteic acid response.

Levels of essential and non-essential amino acids not yet mentioned (Table 15, Table 16) generally also support conclusions based upon growth data, concerning the bioavailability of the different oxidized SAA. Significant decreases in levels of plasma free arginine, histidine and threonine relative to the basal diet when methionine + cystine and methionine sulfoxide + cystine supplements were fed suggest adequacy of dietary SAA and removal of essential amino acids for protein synthesis. The decrease in plasma threonine with cysteic acid- and methionine-sulfone-containing supplements compared to the basal diet reflect the contribution of the unoxidized SAA components also provided. The fact that decreases were significantly less than those seen with the other two test diets, reflect the unavailability of the completely oxidized cysteic acid and methionine sulfone. Increased plasma tyrosine levels in the case of methionine + cystine and methionine sulfoxide + cystine supplementation may reflect decreased tyrosine requirements for catecholamine synthesis

with adequacy of SAA supply, increased food intake and decreased level of dietary stress. The stress of the method of sacrifice, operating on rats fed each of the diets, however, might be expected to have more influence in the short term on demands for catecholamine synthesis.

The significant decrease in plasma free serine and glycine and the non-significant decrease in plasma free alanine obtained with all but the methionine + cysteic acid-containing test diet (Table 16), support increased adequacy of available methionine supply and/or cystine sparing effect on methionine requirements for protein synthesis. As discussed in Experiment 1, serine is involved in metabolism of methionine via the transsulfuration pathway (Finkelstein and Mudd 1967) and has been shown to facilitate elimination of excess methionine via taurine synthesis in rats (Girard-Globa et al. 1972). The increase in plasma α-aminobutyric acid relative to the basal diet, seen with methionine + cystine, methionine sulfoxide + cystine and methionine + cysteic acid supplements, while significant only in the case of the first test diet, similarly indicates increased adequacy of available methionine and decreased levels of methionine catabolites.

Summaries of total essential, non-essential and total amino acids and essential PAA/total PAA ratios (Table 17), reflect changes already discussed on an individual basis for several PAA. Decreased total essential PAA and total PAA, relative to the basal diet, occurring with all forms of SAA supplementation support the growth data and increased protein synthesis with increased adequacy of available methionine supply and/or cystine-sparing effect on methionine requirement.

Lower essential PAA/total PAA ratios with methionine + cystine and methionine sulfoxide + cystine supplements suggest improved utilization of essential PAA with first limiting methionine supplied in available forms that

permit protein synthesis. Equal values for this ratio in plasma of rats fed the basal or methionine sulfone + cystine-supplemented diets supports the unavailability of methionine sulfone already demonstrated by growth data. The lowest essential PAA/total PAA ratio occurring with methionine + cysteic acid supplementation reflects the lack of decrease in non-essential amino acids relative to the basal diet.

In general, it would appear that plasma free amino acid levels and in particular, plasma free essential amino acid levels in weanling rats, fasted for only 2 hours, can give good indication of the availability of oxidized SAA in terms of meeting rat growth requirements.

Based upon the results of this study, it is concluded that L-methionine sulfoxide in free form is as available as L-methionine when used to replace up to 50% of the minimal dietary methionine requirement for growth of weanling rats fed 8% protein diets. This assumes that L-cystine supplies 1/3 of total dietary SAA. L-methionine sulfone and L-cysteic acid even in the presence of supplementary unoxidized SAA are unavailable as sources of methionine for the weanling rat fed 8% dietary protein.

VI. EXPERIMENT 4

METHIONINE AVAILABILITY OF COMMERCIAL INFANT FORMULAS

INTRODUCTION

The availability of methionine in commercially-prepared infant formulas is an important area of nutritional concern for a number of reasons. Firstly, while the incidence of breast feeding of infants in North America appears to be on the increase (Fomon 1975, Myers 1979, Martinez and Nalezienski 1979), appreciable numbers of preterm, low birthweight, term and hyperallergic infants continue to be fed infant formulas as sole sources of dietary protein and amino acids during periods of developmentally critical and high demands for growth (Canadian Paediatric Society, Nutrition Committee 1979). Secondly, proteins or amino acids present in infant formulas are often based upon casein, casein hydrolysates or plant proteins (Anderson et al. 1980, American Academy of Pediatrics, Committee on Nutrition 1983) which are inherently low in sulfur amino acids. Finally, processing of materials used in preparation of infant formulas (for purposes of sterilization or drying for example) often involves the application of heat. This can result in loss of availability of SAA due either to influence of Maillard compounds (involving primarily reactions of epsilon amino groups of lysine and reducing sugars also present in formulas) or to direct oxidation of SAA to potentially unavailable forms (Rolls and Porter 1973, Hurrell et al. 1983).

The problem of rat bioassay of available methionine of cow's milk-based infant formulas is exacerbated to some extent by the limited ability of the rat to digest large quantities of lactose, the major carbohydrate of cow's milk

(DeAngelis et al. 1983) and the influence lactose has on absorption of other dietary constituents (Leichter and Tolensky 1975).

It was decided to test the bioavailability of methionine in cow's milk-based infant formulas by using 8% protein diets (based upon casein and crystalline amino acids), low in SAA in which supplemental methionine was supplied by commercial infant formula preparations. In this way extremely high levels of lactose would be avoided without the need for increased processing of the formulas and potential further influence on or modification of methionine availability.

Because the effects of processing are of major concern, it was decided to look at two different preparations of particular infant formulas (powdered and liquid concentrate forms) and to compare these in terms of methionine availability with the relatively less processed fresh condensed skim milk from which they were derived.

MATERIALS AND METHODS

Samples of powdered and liquid concentrate forms of two commercially available infant formulas (denoted PF-I, LC-I, PF-II and LC-II respectively) produced by two different Canadian companies were selected from those of a number of manufacturers obtained in January and February of 1984 for use in a larger study concerned with lysine availability of commercial infant formulas. Fresh liquid condensed skim milk (denoted CSM), used as the starting material by the two manufacturers was also obtained from their source, a large Ontario milk processor.

Individual cans of liquid concentrate from each manufacturer were opened and contents pooled and well mixed in 6 L flasks. The concentrates

were then poured into shallow stainless steel trays, frozen and subjected to freeze drying in a Virtis Model 10-MR-TR Tray Freeze Drier[6]. Freeze dried samples were broken up by hand and ground to fine powders using a Retsch Mill and 1.0 mm mesh seive[7]. At no time during freeze drying or grinding were samples allowed to undergo appreciable heating. The fresh, liquid condensed skim milk samples were similarly treated, while individual 454 g samples of powdered formula from a given manufacturer were removed from vacuum-sealed cans, pooled and also ground using the Retsch Mill. All samples were stored at -20°C in sealed plastic bags until used for diet formulation. Subsamples of each, stored in screw-topped glass jars, were used for nitrogen and amino acid analyses.

Protein (%N x 6.25) for each powdered sample was determined in duplicate by Macro Kjeldahl analysis (AOAC 1975). Total fat was determined by the technique of Bligh and Dyer (1959). Lactose levels were estimated from label claims of the manufacturers and by difference. Subsamples were also hydrolysed under nitrogen for 22 hours at 110°C in 6N HCl both with and without preoxidation by performic acid, to provide hydrolysates for analysis of all amino acids except tryptophan (Moore 1963). Alkaline hydrolysis of subsamples using 4.2N NaOH was also undertaken to permit analysis of tryptophan (Hugli and Moore 1972). Amino acid analyses of the different hydrolysates were undertaken as described in Experiment 1 and outlined in Appendix A.

6 The Virtis Company, Division of Cenco Medical Industries Inc., Gardiner, N.Y. 12525.

7 Retsch KG 5657 HAAN, West Germany.

Based upon the results of the amino acid analyses, 5 supplementary mixtures were prepared such that each infant formula or condensed skim milk preparation would provide 0.05% dietary L-methionine in different 4 kg diets. Also based upon amino acid results, the mixtures were balanced in terms of other essential amino acids by suitable additions of crystalline L-amino acids. These supplements were used in preparation of 4 kg diets in which balance of dietary protein (%N x 6.25) to provide 8.0% total protein was provided by a 50:50 mixture of ANRC casein and crystalline L-amino acids (except methionine and cystine) equivalent to casein. The other diet ingredients were identical to those of the basal diet of Experiment 1 (Table 1) with the following exceptions: total dietary fat was maintained at 10% by suitable reductions in corn oil to account for fat present in the supplements; and lactose contribution of the various supplements was balanced in each diet by addition of pure lactose at the expense of corn starch. Corn starch was also reduced to account for added dietary protein.

A low methionine basal diet was also formulated in which equivalent to 8% dietary protein was provided at the expense of corn starch by a combination of a) the 50:50:ANRC casein:crystalline amino acid mixture (the same amount provided in the test diets) and b) a mixture of essential amino acids (with the exception of methionine) equivalent to those provided by the balanced mixtures of infant formula preparations and amino acids, used as supplements in the test diets. An appropriate amount of a 1:1:1 mixture of non-essential amino acids (glycine, alanine and serine) was added to the basal diet to maintain isonitrogenicity relative to the test diets. Lactose was also added to the basal diet mixture also at the expense of corn starch to balance lactose provided in the test diets. Other dietary components were as described for the basal diet of Experiment 1 (Table 1). An 8% ANRC casein +0.2% L-methionine reference

diet and a diet providing requirement levels of methionine and cystine as well as a non-protein diet exactly as described for Experiment 3 were also made up.

As in Experiment 3, a 2-week rat feeding trial using a randomized complete block design with 8 replicates in which each diet was represented once per block was undertaken. Diets used in the feeding trial thus included: the non-protein diet, the ANRC casein +0.2% L-methionine reference diet, the requirement diet, the low methionine basal diet, and the 5 test diets in which 0.05% supplementary methionine was provided by infant formula or condensed skim milk preparations.

Rat growth parameters, RNPR, RPER (requirement diet = 100), plasma free amino acids (PAA), and Relative Methionine Availabilities (CSM-supplemented test diet = 100) were determined and statistically analysed at the 5% level of significance also as described for Experiment 3.

RESULTS

Protein and amino acid contents of the formulas have previously been described (Sarwar et al. 1985c).

Table 18 reveals that use of infant formula and condensed skim milk preparations as supplemental methionine sources, resulted in significant improvement ($P < 0.05$) in weight gains and feed/gain ratios of rats relative to those fed the basal diet. RNPR and 2 week RPER values (Table 19) similarly indicate significant improvement in animal growth performance relative to the basal diet-fed rats. Supplementation using LC-I consistently gave highest (poorest) feed/gain ratio and lowest feed intake, weight gain, RNPR and RPER of the infant formula preparations tested. Of these growth parameters, only RPER of LC-I was significantly lower than that obtained with the fresh

condensed skim milk (CSM)-supplemented diet. Growth and performance parameters, with the exception of feed intakes and weight gains, obtained with diets supplemented with the other infant formula preparations while improved over those obtained with the CSM-supplemented diet, were not significantly better. It must be mentioned that while there were significant improvements in rat growth performance compared to that of the basal diet, results with each supplement in no way matched normal performance obtained with the reference diet.

As in preceding experiments, concentrations of free SAA in rat plasma samples were relatively low. Methionine was higher relative to basal diet-fed rats in plasma of rats fed all supplemental diets though levels did not differ significantly. Plasma methionine levels were also higher with infant formula-supplemented diets compared to the CSM-supplemented diet though again differences were not significant. The lowest free cystine concentration was obtained with the CSM-supplemented diet; other test diets also resulting in lower plasma cystine than that of rats fed the basal diet. Differences, however, were also not significant. Methionine sulfoxide was detected in plasma of all rats including those fed the basal diet. Lowest methionine sulfoxide concentrations were observed with the CSM-supplemented rats although these did not differ significantly from those fed the basal diet. PF-I- and PF-II-supplemented diets resulted in highest plasma methionine sulfoxide levels and these differed significantly from those fed the CSM-supplemented diet. No significant differences in plasma cysteic acid levels were observed when any of the diets were fed. Plasma taurine was highest in basal diet-fed rats and levels were significantly lower with CSM-, LC-I-, PF-II-and LC-II-supplemented diets. Methionine sulfone was not detected in any plasma samples.

Remaining essential PAA and tyrosine (Table 21), were usually higher in concentration when the various methionine-containing supplements were fed compared to concentrations of rats fed the basal diet. Differences, however, were not significant except in the case of plasma histidine (for diets containing LC-I and PF-II), plasma threonine (for diets containing CSM and LC-I), and for plasma tryptophan (for diets containing CSM, PF-I, LC-I and PF-II). Generally non-significant decreases relative to CSM-containing diet, in essential PAA predominated in the case of the two diets containing powdered formula supplements, while liquid concentrate preparations often produced higher levels of essential PAA. Powdered formula preparations therefore usually resulted in lowest levels of essential PAA compared to their respective liquid concentrate preparations although differences again were not significant.

Plasma free non-essential PAA not yet considered but also detected in rat plasma samples are listed in Table 22. Glycine and serine were significantly lower and α-aminobutyrate non-significantly lower compared to basal diet-fed rats with all supplemental methionine sources used. Other non-essential amino acids, however, were often higher in plasma when rats were fed the various methionine supplements although this was significant only in the case of aspartic acid for all supplements and for glutamic acid in the case of the LC-I-containing supplement.

Compared to the CSM-supplemented diet, the LC-I-containing supplement resulted in higher levels of non-essential PAA with the exception of α-aminobutyric acid. Powdered formula preparations on the other hand resulted in lower levels of most non-essential amino acids; α-aminobutyric acid being a noted exception. Differences, however, were usually slight and not significant. Compared to liquid concentrate forms, powdered formula supplements often resulted in lower plasma levels of non-essential amino acids.

α-Aminobutyric acid was again an exception, being higher in plasma of rats fed powdered formula-supplemented diets versus plasma of rats fed diets supplemented with liquid concentrate preparations from the same manufacturer.

Non-significant increases in total essential PAA and significant decreases in total non-essential PAA occurred with addition of all methionine-containing supplements to the basal diet (Table 23). Significant increases in essential PAA/total PAA ratios also occurred when the various supplements were used. Levels for total essential, total non-essential and total PAA were higher using the CSM-containing supplement relative to those obtained using the more processed forms with the exception of the LC-I-containing supplement. The latter resulted in highest levels of all classes of PAA (total essential, total non-essential and total) and lowest essential PAA/total PAA ratio of all infant formula preparations tested.

Table 24 reveals that supplementary methionine from the LC-I-containing test diet was significantly less available than that provided by the relatively less processed CSM preparation and by the three other infant formula test diets as measured by Relative Methionine Availability. PF-I, PF-II and LC-II-based diets had values not significantly different from that of the CSM-based diet.

Table 18. Effects of supplemental methionine source on rat performance

Supplemental Methionine Source	Feed Intake g	Weight Gain g	Feed/Gain Ratio
Basal	93 ± 3[b]	15 ± 1[c]	6.4 ± 0.38[a]
Condensed Skim Milk	101 ± 6[b]	27 ± 3[b]	3.9 ± 0.16[bc]
MANUFACTURER I			
Powdered Formula	128 ± 6[a]	37 ± 3[a]	3.6 ± 0.20[bc]
Liquid Concentrate	108 ± 5[b]	27 ± 3[b]	4.3 ± 0.40[b]
MANUFACTURER II			
Powdered Formula	135 ± 5[a]	38 ± 3[a]	3.6 ± 0.18[bc]
Liquid Concentrate	124 ± 6[a]	37 ± 3[a]	3.4 ± 0.14[c]

Means ± SE for 8 animals

Means in same column with different letters differ significantly ($P < 0.05$).

Table 19. Effects of supplemental methionine source on rat growth parameters

Supplemental Methionine Source	RNPR* %	RPER* %
Basal	42 ± 1.8^{c}	30 ± 1.6^{c}
Condensed Skim Milk	59 ± 2.1^{a}	47 ± 3.1^{ab}
MANUFACTURER I		
Powdered Formula	59 ± 2.7^{a}	53 ± 2.9^{ab}
Liquid Concentrate	53 ± 2.9^{b}	45 ± 3.8^{b}
MANUFACTURER II		
Powdered Formula	58 ± 1.4^{ab}	53 ± 1.8^{ab}
Liquid Concentrate	60 ± 1.9^{a}	55 ± 1.8^{a}

* RNPR and RPER (Requirement = 100)

Means $\pm$ SE for 8 animals

Means with different letters in the same column differ significantly ($P < 0.05$).

Table 20. Effects of supplemental methionine source on plasma sulfur amino acid concentrations* in growing rats

Supplemental Methionine Source	Met	Cys	Met Sulfoxide	Cysteic Acid	Tau
Basal	2.7	0.5	2.7^{ab}	1.3	5.3^{a}
Condensed Skim Milk	2.9	0.2	2.3^{b}	1.5	3.7^{bc}
MANUFACTURER I					
Powdered Formula	3.4	0.4	3.0^{a}	1.8	4.6^{ab}
Liquid Concentrate	3.4	0.3	2.5^{ab}	1.4	4.0^{bc}
MANUFACTURER II					
Powdered Formula	3.4	0.5	2.8^{a}	1.4	3.8^{bc}
Liquid Concentrate	3.7	0.4	2.7^{ab}	1.4	3.2^{c}
SEM**	0.4	0.2	0.2	0.2	0.3

* μM/100 mL plasma

** SEM calculated from analysis of variance data

Means with different letters in the same column differ significantly ($P < 0.05$).

Table 21. Effects of supplemental methionine source on plasma free essential amino acid concentrations* in growing rats

Supplemental Methionine	Arg	His	Ile	Leu	Phe	Tyr	Thr	Trp	Val	Lys
Basal	16.3	11.2^{c}	8.0	13.9	5.6	6.9	140.4^{b}	4.3^{c}	19.2	77.9
Condensed Skim Milk	17.4	13.2^{a}	9.0	16.1	5.9	9.9	169.2^{a}	6.5^{ab}	23.2	78.9
MANUFACTURER I										
Powdered Formula	17.7	12.2^{abc}	9.6	15.1	5.8	8.6	154.1^{ab}	6.3^{ab}	21.6	88.6
Liquid Concentrate	17.7	13.1^{a}	9.2	16.3	6.1	9.0	167.6^{a}	6.4^{ab}	22.9	79.2
MANUFACTURER II										
Powdered Formula	16.6	13.5^{a}	8.8	14.6	5.1	8.1	133.2^{b}	5.5^{abc}	21.3	87.8
Liquid Concentrate	18.1	12.4^{abc}	9.8	16.1	6.2	9.5	152.4^{ab}	6.6^{a}	23.3	87.3
SEM**	1.3	0.5	0.9	1.6	0.6	1.4	8.4	0.6	2.3	7.4

* μM/100 mL plasma

** SE calculated from analysis of variance data

Means with different letters in the same column differ significantly ($P < 0.05$).

Table 22. Effects of supplemental methionine source on plasma non-essential amino acids* in growing rats

Supplemental Methionine Source	Ala	Asp	Glu	Gly	Pro	Ser	Cit	Orn	α-Aba
Basal	63.8	1.6^{c}	14.7^{b}	69.1^{a}	29.9	182.5^{a}	11.7^{ab}	11.0^{b}	0.4^{a}
Condensed Skim Milk	79.8	1.7^{c}	15.3^{b}	27.6^{b}	39.1	78.4^{b}	11.0^{b}	11.0^{b}	0.2^{ab}
MANUFACTURER I									
Powdered Formula	67.1	2.3^{a}	19.3^{a}	32.1^{b}	36.5	73.7^{b}	13.4^{a}	13.1^{a}	0.4^{a}
Liquid Concentrate	82.6	2.0^{abc}	16.7^{b}	30.7^{b}	43.0	81.7^{b}	11.6^{ab}	12.6^{ab}	0.1^{b}
MANUFACTURER II									
Powdered Formula	68.6	2.1^{ab}	16.8^{b}	26.4^{b}	37.6	69.7^{b}	12.1^{ab}	11.5^{ab}	0.4^{a}
Liquid Concentrate	75.9	1.9^{bc}	16.7^{b}	30.1^{b}	38.0	71.0^{b}	12.2^{ab}	11.7^{ab}	0.2^{ab}
SEM**	6.1	0.1	0.7	3.2	4.8	6.3	0.6	0.5	0.1

* μM/100 ml plasma

** SEM calculated from analysis of variance data

Means with different letters in same column differ significantly ($P < 0.05$).

Table 23. Effects of supplemental methionine source on plasma free amino acid (PAA)* parameters in growing rats

Supplemental Methionine Source	Essential PAA	Non-Essential PAA	Total PAA	Essential PAA / Total PAA
Basal	307	394[a]	701[a]	0.44[b]
Condensed Skim Milk	352	271[b]	624[ab]	0.56[a]
MANUFACTURER I				
Powdered Formula	343	267[b]	610[ab]	0.57[a]
Liquid Concentrate	351	289[b]	640[ab]	0.55[a]
MANUFACTURER II				
Powdered Formula	318	253[b]	571[b]	0.56[a]
Liquid Concentrate	346	265[b]	611[a]	0.57[a]
SEM**	19	15	31	0.01

* μM/100 mL plasma

** SEM calculated from analysis of variance data

Means in same column with different letters differ significantly ($P < 0.05$).

Table 24. Relative Methionine Availability* of commercial infant formulas

Supplemental Methionine Source	Relative Methionine Availability
Condensed Skim Milk	100[a]
MANUFACTURER I	
Powdered Formula	93 ± 15[a]
Liquid Concentrate	66 ± 16[b]
MANUFACTURER II	
Powdered Formula	96 ± 15[a]
Liquid Concentrate	106 ± 14[a]

* Mean ± SE for 8 animals

Means with different letter in same column differ significantly ($P < 0.05$).

DISCUSSION

The presence of high lactose levels in milk and milk by-products has been stated to have a negative effect on rat growth and PER measures of protein quality unless testing involves a casein control diet having similar lactose levels (Steinke 1977). DeAngelis et al. (1983), however, were unable to demonstrate any intolerance in weanling rats to lactose levels $\leq$ 20% of diet, in terms of weight gain, fecal pH, NPR and PER. The freeze-dried infant formula and condensed skim milk preparations contained slightly in excess of 50% lactose after appropriate balancing of lactose and amino acids. When they were used as supplements to provide 0.05% L-methionine, resultant total dietary lactose levels of about 13.5% were under the limit set by the latter authors.

In addition, the test diets contained well below the requirement level of methionine for maximal rat growth making relevant comparison to the reference diet impossible. The interest, however, was in relative methionine availability especially with reference to potential effects of processing. As a result, most of the discussion will be concerned with relative performance of the test diets containing the various infant formulas with respect to the relatively less processed fresh condensed skim milk-containing test diet.

While individual manufacturer's detailed processing methods are trade secrets, generalized descriptions of processing of powdered and concentrates forms of infant formulas reveals that powdered preparations undergo less heat treatment than concentrates.

In the case of powdered formulas, the milk or powdered ingredient mixture might be subjected to high temperature sterilization (pasteurization) for several seconds followed by rapid spray drying in commercial spray driers employing indirect heating. The resultant powder is then vacuum sealed in

cans, likely after nitrogen evacuation of head space to give a product with considerable shelf life due to low potential for oxidative rancidity and perhaps also due to low water activity (Fennema 1976).

Concentrated formulas also derive from high temperature pasteurized condensed skim milk, but are subjected to considerably more heating in the concentrating process. Because of high protein and carbohydrate levels in the condensed material, more rapid decrease in availability of amino acids might be expected due to Maillard reactions and oxidation of amino acids (Cheftel 1979).

The significant improvement in rat growth performance (Table 18, Table 19) over the basal diet with inclusion of the various milk-based supplements was to be expected and indicates that the supplements were effective sources of available methionine. The lower performance of rats fed these diets relative to the casein +0.2% methionine control diet was similarly expected due to the sub-requirement level of methionine in the test diets. The significantly lower RNPR and non-significant but consistently lower RPER and higher feed/gain ratio obtained with the LC-I-supplemented diet relative to the CSM-supplemented diet suggest a lower level of available methionine in the former diet. The significantly lower values for all rat growth performance parameters measured with the LC-I versus the LC-II-containing diet similarly suggest lower available methionine in the LC-I-containing diet perhaps as a result of processing differences.

Despite higher feed intakes and weight gains of rats fed the PF-I, PF-II and LC-II supplements, relative to those fed that containing CSM, the lack of significant difference in terms of feed/gain ratio, RNPR and RPER, suggests that the processing employed did not greatly affect methionine availability of these diets.

As with growth performance, the higher plasma levels of free methionine relative to the basal diet, obtained with the various milk based supplements while not significant, were expected due to higher dietary supply of methionine. Greater differences in sulfur amino acids might have been obtained with a higher level of supplementary methionine but this could have led to problems in terms of excessive dietary lactose.

The general lack of significant differences in plasma sulfur amino acids with the different sources of supplemental methionine after two weeks of feeding makes it difficult to use the data in terms of assessment of methionine availability. It is proposed that the low level of methionine supplementation provided in the face of high demands required for protein synthesis, resulted in the low concentrations measured. Of interest are the consistently higher levels of methionine sulfoxide relative to the CSM-supplemented fed rats of rats fed the four infant formula-containing preparations (significant with PF-I and PF-II supplements). This might be considered indicative of partial SAA oxidation; however, levels did not differ significantly from that of rats fed the basal diet and methionine sulfoxide has been demonstrated to be completely available to the weanling rat when partially replacing dietary methionine (Experiment 3). Methionine sulfone was not detected in plasma of rats fed any of the diets, suggesting that any SAA oxidation that might have occurred was minimal and not likely a factor in terms of reduced methionine availability.

The significantly higher plasma taurine levels in the basal diet relative to the test diets might be indicative of a relative cysteine deficiency in the face of obligatory utilization of methionine for bile acid requirements. This was not, however, supported by the lack of significant differences in plasma cystine and cysteic acid levels in rats fed the different diets and higher food intakes occurring with the test diets.

The trend towards higher levels of many essential PAA and tyrosine (Table 21) that occurred with addition of methionine-containing supplements to the basal diet, while generally consistent, on an individual basis was usually non-significant. It might be indicative of increased protein turnover and higher plasma levels with improvement in methionine status, however, this would seem to contradict the idea of improved utilization of essential amino acids for protein synthesis with provision of higher levels of limiting dietary methionine.

The non-significant increases in many essential amino acids occurring with use of liquid concentrate-based supplements and decreases occurring with powdered formula-based supplements compared to the CSM-based supplement are suggestive of slight detrimental effects of liquid concentrate processing methods on methionine availability.

The significant decreases in glycine and serine and non-significant increase in α-aminobutyrate observed when methionine-containing supplements were fed reflect increased levels of available dietary methionine. As previously stated, these amino acids are involved in major routes of methionine metabolism (Finkelstein and Mudd 1967).

It is proposed that the lower plasma concentration of α-aminobutyrate and the generally higher levels of many other non-essential amino acids (alanine, glutamate, glycine, proline and serine) occurring with liquid concentrate supplements compared to corresponding powdered formula supplements (perhaps most evident in the case of the LC-1 supplement) reflect differential effects of the two processing methods on methionine availability as already suggested based upon differences in essential amino acids.

Summaries of the various classes of PAA (Table 23) reflect results noted on an individual basis for the different amino acids. Significant decreases in total non-essential PAA and non-significant decreases in total PAA (significant

for PF-2 supplement) are proposed to indicate improved utilization of amino acids for protein synthesis relative to the basal diet. The significant increase in essential PAA/total PAA ratios occurring relative to the basal diet would appear to reflect the large drop in non-essential PAA occurring when methionine-containing supplements were fed. Consistently higher levels of all three classes of amino acids obtained when liquid concentrates versus powdered formula preparations from the same manufacturer were fed, while usually not significant, are suggestive of the potentially detrimental effects of increased processing on availability of the methionine. The potential influence of such processing on lysine availability in the same infant formulas, however, must also not be ignored (Sarwar et al. 1985c).

Measures of Relative Methionine Availability (Table 24) suggest that the LC-I-supplemented diet provided the least amount of available methionine of all test diets and support similar conclusions based on growth and amino acid data.

Of interest for subsequent studies of methionine availability of infant formulas is the current practice of an independent company to redilute liquid concentrates of major producers and rebottle the preparations into individual ready-to-use bottles for use by hospitals. The process involves several minutes of heat resterilization as well as increased oxygen exposure of the products. What effect this might have in terms of amino acid availability is an area of ongoing concern.

Breast milk remains the preferred food for most normal infants due to nutritional and immunological characteristics. Nevertheless infant formulas have long been effectively used as sources of nutrition for many infants for a variety of reasons (Cordano 1984). Government regulatory bodies and infant

formula manufacturers themselves continue to study and undertake improvements in safety and nutritional quality of such formulas (American Academy of Pediatrics 1976, Anderson et al. 1982, Cordano 1984).

The infant formulas studied in this experiment were casein-predominant and first limiting in methionine + cystine (Sarwar et al. 1985c). The potential for reduced availability of methionine and cystine due to processing were therefore of major interest. While processing did not prove detrimental in most instances, it would appear that, at least in the case of one liquid concentrate, this concern was justified.

VII. GENERAL CONCLUSIONS

The methionine requirement of the growing rat fed 8% dietary protein in the presence of adequate choline and inorganic sulfate and 0.02% dietary L-cystine was found to be 0.32% of diet. At this level, weight gain, feed/gain ratio, RNPR and LPU reached optimal levels. In general, plasma amino acid changes reflected changes in dietary L-methionine and supported the adequacy of 0.32% dietary L-methionine. Correction for the digestibilities of methionine and cystine in the ANRC casein protein of the test diets suggested that the SAA requirements for the growing rat were 0.33% of diet or 4.1% of dietary protein when 8% protein diets were fed.

Inclusion of L-cystine at the expense of L-methionine in 8% protein diets providing 0.44% total SAA improved overall rat growth performance and plasma indicators of methionine utilization for protein synthesis. Optimal feed/gain ratio, RNPR and plasma amino acid parameters were obtained when L-cystine replaced 33 to 60% of methionine in these 0.44% SAA-containing diets, suggesting that L-cystine could replace up to 60% of dietary L-methionine at the 8% protein intake level.

Supplementation of low SAA diets providing 8% protein, with L-methionine + L-cystine, L-methionine sulfoxide + L-cystine, L-methionine sulfone + L-cystine or L-methionine + L-cysteic acid suggested that free L-methionine sulfoxide was completely available and L-methionine sulfoxide (68%) and L-cysteic acid (55%) less available relative to unoxidized methionine (100%).

Processing was found to have influenced the methionine availability in commercially prepared infant formulas tested. Differences in rat growth parameters and plasma amino acid concentrations were obtained when supple-

mentary methionine was provided from freeze-dried preparations of infant formulas processed to varying degrees. Liquid concentrate forms of infant formulas tested appeared to have greater potential for decreased methionine availability compared to the corresponding powdered preparations made by the same manufacturer.

VIII. LITERATURE CITED

Aguilar, T.S., Benevenga, N.J. and Harper, A.E. (1974) Effect of dietary methionine level on its metabolism in rats. J. Nutr. 104, 761-771.

Allison, J.B. (1964) The nutritive value of dietary proteins. Chapter 12 In: Mammalian Protein Metabolism, Vol. II, Munro, H.N. and Allison, J.B. (Eds.), Academic Press, New York, 41-86.

Almquist, H.J. (1972) Proteins and Amino Acids in Animal Nutrition, 5th ed., S.B. Penick & Co., C.P.C. International, New York.

Aksnes, A. and Njaa, L.R. (1983) Absorption of methionine and methionine sulfoxide in rat intestine and the effect of glutathione. Br. J. Nutr. 50, 583-588.

American Academy of Pediatrics, Committee on Nutrition (1976) Commentary on breast-feeding and infant formulas, including proposed standards for formulas. Pediatrics 57(2)-278-285.

American Academy of Pediatrics, Committee on Nutrition (1983) Soy-protein formulas: recommendations for use in infant feeding. Pediatrics 72(3), 359-363.

American Institute of Nutrition (1977) Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. J. Nutr. 107, 1340-1348.

Anderson, G.H., Ashley, D.V.M., Jones, J.D. (1976) Utilization of L-methionine sulfoxide, L-methionine sulfone and cysteic acid by the weanling rat. J. Nutr. 106, 1108-1114.

Anderson, G.H., Li, G.S.K., Jones, J.D. and Bender, F. (1975) Effect of hydrogen peroxide treatment on the nutritional quality of rapeseed flour fed to weanling rats. J. Nutr. 105, 317-325.

Anderson, S.A., Chinn, H.I. and Fisher, K.D. (1980) A background paper on infant formulas. Life Sciences Research Office, FASEB, 9650 Rockville Pike, Bethesda, Maryland 20014.

Anderson, S.A., Chinn, H.I. and Fisher, K.D. (1982) History and current status of infant formulas. Am. J. Clin. Nutr. 35, 381-397.

Anglemeir, A.F. and Montgomery, M.W. (1976) Amino acids, peptides and proteins. Chapter 5 In: Principles of Food Science, Part 1, Food Chemistry, Fennema, O.R. (Ed.), Marcel Dekker Inc., New York, Basel, 205-284.

Association of Official Analytical Chemists (1976) Official Methods of Analysis, 12th ed., W. Horwitz (Ed.). 1) Biological Evaluation of Protein Quality (28). Official Final Action 14:183 - 14:187, p. 857. 2) Total Nitrogen. Official Final Action 2.047-2.049, pp. 15-16.

Aymard, C., Seyer, L. and Cheftel, J.C. (1979) Enzymatic reduction of methionine sulfoxide. In vitro experiments with rat liver and kidney. Agric. Biol. Chem. 43(9), 1869-1872.

Baker, D.H., Clausing, W.W., Harmon, B.G., Jensen, A.H. and Becker, D.E. (1969) Replacement value of cystine for methionine for the young pig. J. Anim. Sci. 29, 581-584.

Baldessarini, R.T. and Kopin, I.J. (1963) Assay of tissue levels of S-adenosylmethionine. Anal. Biochem. 6, 289-291.

Beare-Rogers, J.L., Nera, E.A. and Craig, B.M. (1972) Cardiac lipids in rats and gerbils fed oils containing C_{22} fatty acids. Lipids 7, 548-552.

Bender, A.E. (1972) Processing damage to protein food: a review. J. Food Technol. 7, 239-250.

Bender, A.E. (1982) Evaluation of protein quality: methodological considerations. Proc. Nutr. Soc. 41, 267-276.

Bernardi, N., Dacke, C.G. and Davidson, N. (1978) Increased in vivo ($1^{-3}H$) taurine efflux from cerebellar cortex evoked by direct electrical stimulation. J. Physiol. 280, 14-17.

Bernhart, F.W. and Tomarelli, R.M. (1966) A salt mixture supplying the National Research Council estimates of the mineral requirements of the rat. J. Nutr. 89, 495-500.

Bhagavan, N.V. (1978) Biochemistry, 2nd ed. J.B. Lippincott Co., Philadelphia, Toronto, 618-627.

Bjarnason, J. and Carpenter, K.J. (1970) Mechanisms of heat damage in proteins. 2. Chemical changes in pure proteins. Br. J. Nutr. 24, 313-329.

Bligh, E.G. and Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.

Bodwell, C.E. (1978) Application of animal data to human protein nutrition: a review. Cereal Chem. 54(4), 958-983.

Bossani, A. and Silano, V. (1978) Control through breeding methods of factors affecting nutritional quality of cereals and grain legumes. In: Nutritional Improvement of Food and Feed Proteins. Adv. in Exp. Med. and Biol., 105, 249-274.

Bressani, R., Viteri, F. and Elias, L.G. (1973) The usefulness of results with human beings in standardizing rat assays. Chapter 19 In: Proteins in Human Nutrition, Porter, J.W.G. and Rolls, B.A. (Eds.), Academic Press, London and New York, 293-316.

Breuton, M.M., Berger, H.M., Brown, G.A., Ablitt, B.L., Iyngkaran, N., Warton, B.A. (1978) Duodenal bile acid patterns and dietary conjugation: sulfur amino acids in the newborn. Gut 19, 95-98.

Bright, S.W.J., Lea, P.J. and Miflin, B.J. (1980) The regulation of methionine biosynthesis and metabolism in plants and bacteria. In: Sulphur in Biology, CIBA Foundation Symposium 72, Excerpta Medica, Amsterdam, Oxford, New York, 101-113.

Brown, R.E. and Gametero, A. (1970) Effect of added dietary sulfate on the utilization of peanut protein by the rat. Can. J. Anim. Sci. 50, 742-744.

Brown, R.G., Button, G.M. and Smith, J.T. (1965) Changes in collagen metabolism by feeding diets low in inorganic sulfur. J. Nutr. 87, 228-232.

Bunce, G.E. and King, K.W. (1969) Amino acid retention and balance in the young rat fed varying levels of lactalbumin. J. Nutr. 98, 159-167.

Byington, M.H. and Howe, J.M. (1972) Effect of level and proportion of methionine and cystine on plasma amino acids of weanling rats. J. Nutr. 102, 995-1000.

Byington, M.H., Howe, J.M. and Clark, H.E. (1972) Effect of different levels and proportions of methionine, cystine, choline and inorganic sulfur on growth and body composition of young rats. J. Nutr. 102, 219-228.

Campbell, J.A. and McLaughlan, J.M. (1971) Applicability of animal assays to humans. In Proc. S0S/70, Third Int. Congr., Food Sci. Technol., Washington, D.C., Ins. Food Technol., Chicago, 336.

Canadian Paediatric Society, Nutrition Committee (1979) Infant feeding. Can. J. Public Health 70, 376-385.

Cantoni, G.L. and Durell, J. (1957) J. Biol. Chem. 225, 1033. As quoted in Meister (1965), 766.

Case, G.L. and Benevenga, N.J. (1976) Evidence for s-adenosylmethionine independent catabolism of methionine in the rat. J. Nutr. 106, 1721-1736.

Chang, K.C., Marshall, H.F. and Satterlee, L.D. (1982) Sulfur amino acid stability. Hydrogen peroxide treatment of casein, egg white and soy isolate. J. Fd. Sci. 47, 1181-1183.

Cheftel, J.C. (1979) Proteins and amino acids. Chapter 6 In: Nutritional and Safety Aspects of Food Processing, S.R. Tannenbaum (Ed.), Marcel Dekker Inc., New York, N.Y., 154-216.

Codex Alimentarius Commission Document Alinorm 85/30 (1984) Working Group's Report to the Third Session of CCVP on Method for Evaluating Protein Quality, Food and Agriculture Organization, Rome, Italy, World Health Organization, Geneva, Switzerland, 22-23.

Cooper, A.J.L. (1983) Biochemistry of sulfur-containing amino acids. Ann. Rev. Biochem. 52, 187-222.

Cordano, A. (1984) Pre-clinical and clinical evaluation of new infant formulas. Nutr. Res. 4, 929-934.

Crawford, L., Finley, J.W. and Robbins, K.R. (1984) Bioavailability of some oxidized cystine derivatives. Nutr. Rep. Int. 24, 781-786.

Cuq, J.L., Besancon, P., Chartier, L. and Cheftel, C. (1978) Oxidation of methionine residues of food proteins and nutritional availability of protein-bound methionine sulfoxide. Fd. Chem. 3, 85-102.

Cuq, J.L., Provansal, M., Guilleux, F. and Cheftel, C. (1973) Oxidation of methionine residues of casein by hydrogen peroxide. Effects on in vitro digestibility. J. Fd. Sci. 38, 11-13.

Daniel, R.G. and Waisman, H.A. (1969) Adaptation of the weanling rat to diets containing excess methionine. J. Nutr. 99, 299-306.

DeAngelis, R.C., Giuli, G.G., Rogano, R.N. and Terra, I.C.M. (1983) Lactose load diet effect in rats. Arq. Gastroent. S. Paulo, 20(4), 166-169.

Dorvil, N., Yousef, I.M., Tuchweber, B. and Roy, C.C. (1983) Taurine prevents cholestasis induced by lithocholic acid sulfate in guinea pigs. Am. J. Clin. Nutr. 37, 221-232.

Duncan, B.D. (1955) Multiple range and multiple F tests. Biometrics 11, 1-42.

du Vigneaud, V., Chandler, J.P., Cohn, M. and Brown, G.B. (1940) J. Biol. Chem. 134, 787. As quoted in Meister (1965), 763.

Edozien, J.C. and Switzer, B.R. (1978) Influence of diet on growth in the rat. J. Nutr. 108, 282-290.

Ellinger, G.M. and Palmer, R. (1969) The biological availability of methionine sulphoxide . Proc. Nutr. Soc. 28, 42A.

Evans, R.J., Bauer, D.H., Sisak, K.A. and Ryan, P.A. (1974) The availability for the rat of methionine and cystine contained in dry bean seed (Phaseolus vulgaris). J. Agr. Food Chem. 22(1), 130-133.

FAO/WHO (1973) Energy and protein requirements. Food and Agriculture Organization, Nutr. Rep. Ser. 52, Rome, World Health Org. Techn. Rep. Ser. No. 522, Geneva.

Featherston, W.R. and Rogler, J.C. (1978) Methionine-cystine interrelations in chicks fed diets containing suboptimal levels of methionine. J. Nutr. 108, 1954-1958.

Fennema, O.R. (1976) Water and ice. Chapter 2 In: Principles of Food Science, Part 1, Food Chemistry, Fennema, O.R. (Ed.), Marcel Decker Inc., New York, Basel, 13-39.

Finkelstein, J.D. (1974) Methionine metabolism in mammals: the biochemical basis for homocystinuria. Metabolism 23, 378-398.

Finkelstein, J.D., Harris, B.J. and Kyle, W.E. (1972) Methionine metabolism in mammals: kinetic study of betaine-homocysteine methyltransferase. Arch. Biochem. Biophys. 153, 320-324.

Finkelstein, J.D., Harris, B.J., Martin, J.J. and Kyle, W.E. (1982) Regulation of hepatic betaine-homocysteine methyltransferase by dietary methionine. Biochem Biophys. Res. Commun. 108(1), 344-348.

Finkelstein, J.D. and Mudd, S.H. (1967) Trans-sulfuration in mammals. The methionine-sparing effect of cystine. J. Biol. Chem. 242, 873-888.

Finley, J.W., Wheeler, E.L., Walker, H.G., Finlayson, A.J. (1982) Cystine oxidation on lysinoalanine formation in proteins. J. Agric. Food Chem. 30, 818-820.

Fomon, S.J. (1975) What are infants fed in the United States? Pediatrics 56, 350-354.

Forsum, E. and Hambraeus, L. (1978) Effects of proteins and their corresponding amino acid mixtures on nitrogen balance and body composition in the growing rat. J. Nutr. 108, 1518-1526.

Fox, P.F. and Kosikowski, F.V. (1967) Some effects of hydrogen peroxide on casein and its implications in cheese making. J. Dairy Sci. 50, 1183-1185.

Friedman, M. and Gumbmann, M.R. (1981) Bioavailability of some lysine derivatives in mice. J. Nutr. 1362-1369.

Girard-Globa, A., Robin, P. and Forestier, M. (1972) Long term adaptation of weanling rats to high dietary levels of methionine and serine. J. Nutr. 102, 209-218.

Gjøen, A.U. and Njaa, L.R. (1977) Methionine sulphoxide as a source of sulphur-containing amino acids for the young rat. Br. J. Nutr. 37, 93-105.

Goswammi, M.M.D., Robblee, A. and McElroy, L.W. (1959) Further observations on factors affecting L-cysteine desulfhydrase activity in chick liver. J. Nutr. 68, 671-676.

Graber, G. and Baker, D.H. (1971) Sulfur amino acid nutrition of the growing chick: quantitative aspects concerning the efficiency of dietary methionine, cysteine and cystine. J. Anim. Sci. 33, 1005-1011.

Graham, G.C., MacLean, W.C., Jr. and Placko, R.P. (1976) Plasma amino acids in infants consuming soybean proteins with and without added methionine. J. Nutr. 106, 1307-1313.

Graham, G.C. and Placko, R.P. (1973) Postprandrial plasma-free methionine as an indicator of methionine adequacy in the human infant. J. Nutr. 103, 1347-1351.

Guy, E.J., Vettel, H.E. and Pallansch, M.J. (1968) Effect of hydrogen peroxide treatment on skim milks for sponge bread making. Cereal Sci. Today 13, 434-437.

Hayes, K.C. (1976) A review on the biological function of taurine. Nutr. Reviews 34(6), 161-165.

Hayes, K.C., Stephan, Z.F. and Sturman, J.A. (1980) Growth depression in taurine-depleted infant monkeys. J. Nutr. 110, 2058-2064.

Hayes, K.C. and Sturman, J.A. (1981) Taurine in metabolism. In: Ann. Rev. Nutr. Darby, W.J., Broquist, H.P., and Olson, R.E. (Eds.) Annual Reviews Inc., Palo Alto, CA, 1, 401-425.

Higuchi, M., Iwami, K., Nakamura, A., Yasumoto, K. and Iwai, K. (1982) In vitro and in situ absorption of methionine sulfoxide in the rat small intestine. Agric. Biol. Chem. 46, 2533-2538.

Hugli, T.E. and Moore, S. (1972) Determination of the tryptophan content of proteins by ion exchange chromatography of alkaline hydrolysates. J. Biol. Chem. 247, 2828-2834.

Hurrell, R.F., Finot, P.A. and Ford, J.E. (1983) Storage of milk powders under adverse conditions. 1. Losses of lysine and other essential amino acids as determined by chemical and microbiological methods. Br. J. Nutr. 49, 343-354.

Inoue, M. and Hayatsu, H. (1971) The interactions between bisulfite and amino acids. The formation of methionine sulfoxide from methionine in the presence of oxygen. Chem. Pharm. Bull. 19(6), 1286-1289.

Itoh, H., Kishi, T. and Chibata, I. (1973) Comparative effects of casein and amino acid mixture simulating casein on growth and food intakes in rats. J. Nutr. 103, 1709-1715.

Itoh, H., Kishi, T. and Chibata, I. (1974) Effect of hydrochloride-free amino acid mixture on growth and food intake in rats. J. Nutr. Sci. Vitaminol. 20, 431-436.

Itoh, H., Kishi, T. and Chibata, I. (1974a) Plasma amino acids and stomach contents of rats fed casein and the corresponding amino acid mixture. J. Nutr. 104, 386-393.

Iwami, K., Nakamura, A., Higuchi, M., Yasumoto, K. and Iwai, K. (1983) Evaluation of L-methionine sulfoxide bioavailability by sulfur incorporation into liver glutathione and urinary excretion as inorganic sulfate. Agric. Biol. Chem. 47(11), 2255-2259.

James, K.A.C. and Hove, E.L. (1978) Effects of inorganic sulfate supplements on the growth of rats fed sulfur amino acid-deficient diets. Can. J. Anim. Sci. 58, 49-54.

James, K.A.C. and Hove, E.L. (1980) The ineffectiveness of supplementary cystine in legume-based rat diets. J. Nutr. 110, 1736-1744.

Johnson, J.L. and Rajagopalan, K.V. (1980) The oxidation of sulphite in animal systems. In: Sulphur in Biology, CIBA Foundation Symposium 72, Excerpta Medica, Amsterdam, Oxford, New York, 119-128.

Kim, K, McMillan, I. and Bayley, H. (1983) Determination of amino acid requirements of young pigs using an indicator amino acid. Br. J. Nutr. 50, 369-382.

Krebs, H.A. (1964) The metabolic fate of amino acids. In: Mammalian Protein Metabolism, Munro, H.N. and Allison, J.B. (Eds.), Vol. I, Academic Press, New York, 175.

Krebs, H.A., Hems, R. and Tyler, B. (1976) The regulation of folate and methionine metabolism. Biochem. J. 158, 341-353.

Kroening, G.H., Pond, W.G., Loosli, J.K. (1965) Dietary methionine-cystine requirement of the baby pig as affected by threonine and protein levels. J. Anim. Sci. 24, 519-525.

Kunachowicz, H., Pieniążek, D. and Rakowska, M. (1976) Changes in the available methionine and lysine content of milk and plant proteins. Nutr. Metab. 20, 415-424.

Kuriyama, K. (1980) Taurine as a neuromodulator. Federation Proc. 39, 2680-2684.

Labuza, T.P. and Saltmarch, M. (1981) Kinetics of browning and protein quality loss in whey powders during steady state and non-steady state storage conditions. J. Fd. Sci. 47, 92-113.

Leichter, J. (1973) Effect of dietary lactose on intestinal lactase activity in young rats. J. Nutr. 103, 392-396.

Leichter, J. and Tolensky, A.F. (1975) Effect of dietary lactose on the absorption of protein, fat and calcium in the postweaning rat. Am. J. Clin. Nutr. 28, 238-241.

Leveille, G.A., Sauberlich, H.E. and Shockley, J.W. (1961) Sulfur amino acid requirements for growth of mice fed two levels of nitrogen. J. Nutr. 75, 455-458.

Malloy, M.H., Rassin, D.K. and Gaull, G.E. (1981) A method for measurement of free and protein bound cysteine. Anal. Biochem. 13, 407-415.

Marshall, H.F., Chang, K.C., Miller, K.S. and Satterlee, L.D. (1982) Sulfur amino acid stability. Effects of processing on legume proteins. J. Fd. Sci. 47, 1170-1174.

Martin, W.G., Truex, C.R., Tarka, M., Hill, K.J. and Borby, W.G. (1974) The synthesis of taurine from sulfate. VIII. A constitutive enzyme in mammals. Proc. Soc. Exp. Biol. Med. 147, 563-565.

Martinez, G.A. and Nalezienski, J.P. (1979) The recent trend in breast-feeding. Pediatrics 64, 686-692.

McBride, W.J. and Frederickson, R.C.A. (1980) Taurine as a possible inhibitory transmitter in the cerebellum. Fed. Proc. 39, 2701-2705.

McLaughlan, J.M. (1974) Evaluation of standard rat assays. Chapter 7 In: Nutrients in Processed Foods. Proteins. AMA, White, P.L. and Fletcher, D.C. (Eds.) Publishing Sciences Group Inc., Acton, Massachusetts, 69-76.

McLaughlan, J.M., Anderson, G.H., Hackler, L.R., Hill, D.C., Jansen, G.R., Keith, M.O., Sarwar, G. and Sosulski, F.W. (1980) Assessment of rat growth methods for estimating protein quality: interlaboratory study. J. Assoc. Off. Anal. Chem. 63(3), 462-467.

McLaughlan, J.M. and Keith, M.O. (1977) Effects of threonine supplementation on the slope ratio assay for protein quality. J. Assoc. Off. Anal. Chem. 60, 1291-1295.

Meister, A. (1965) Intermediary metabolism of amino acids. Chapter VI In: Biochemistry of the Amino Acids, 2nd Edition, Vol. II, Academic Press, New York, London, 754-813.

Meister, A., Griffith, D.W., Novogradsky, A. and Tate, S.S. (1980) New aspects of glutathione metabolism and translocation in mammals. In: Sulphur in Biology, CIBA Foundation Symposium 72, Excerpta Medica, Amsterdam, Oxford, New York, 135-156.

Meister, A. and Tate, S.S. (1976) Glutathione and related γ-glutamyl compounds: biosynthesis and utilization. Ann. Rev. Biochem. 45, 559-604.

Michels, F.G. and Smith, J.T. (1965) A comparison of the utilization of organic and inorganic sulfur by the rat. J. Nutr. 87, 217-220.

Miller, D.S. and Samuel, P.D. (1970) Effects of the addition of sulfur compounds on the utilization of protein in young growing rats. J. Sci. Fd. Agr. 21, 616-618.

Miller, E.L., Carpenter, K.J. and Milner, C.K. (1965) Availability of sulfur amino acids in protein foods. 3. Chemical and nutritional changes in heated cod muscle. Br. J. Nutr. 19, 547-564.

Miller, S.A., Tannenbaum, S.R. and Seitz, A.W. (1970) Utilization of L-methionine sulfoxide by the rat. J. Nutr. 100, 909-916.

Mitchell, J.R., Becker, D.E., Jensen, A.H., Hamon, B.G. and Norton, H.W. (1968) Determination of the amino acid needs of the young pig by nitrogen balance and plasma-free amino acids. J. An. Sci. 27, 1327-1331.

Mokady, S., Viola, S. and Zimmermann, G. (1969) A new biological method for estimating food protein nutritive value. Br. J. Nutr. 23, 491-495.

Moore, S. (1963) On the determination of cystine as cysteic acid. J. Biol. Chem. 238, 235-237.

Morrison, A.B. and Sabry, Z.I. (1963) Factors influencing the nutritional value of fish flour. II. Availability of lysine and sulfur amino acids. Can. J. Biochem. Physiol. 41, 649-655.

Mudd, S.H. (1980) Diseases of sulphur metabolism: implications for the methionine-homocysteine cycle, and vitamin responsiveness. In: Sulphur in Biology, CIBA Foundation Symposium 72, Excerpta Medica, Amsterdam, Oxford, New York, 239-255.

Mudd, S.H., Finkelstein, J.D., Irreverre, F. and Laster, L. (1965) Transsulfuration in mammals. Microassays and distributions of three enzymes of the pathway. J. Biol. Chem. 240, 4382-4392.

Myers, A.W. (1979) A retrospective look at infant feeding practices in Canada: 1965-1978. J. Can. Dietetic Assoc. 40, 200-211.

National Research Council (1978) Nutrient Requirements of Laboratory Animals, Third Revised Edition. National Academy of Sciences, Washington, D.C.

National Research Council (1980) Recommended Dietary Allowances. National Research Council, National Academy of Sciences, Washington, D.C.

Njaa, L.R. (1962) Utilization of methionine sulfoxide and methionine sulfone by the young rat. Br. J. Nutr. 16, 571-577.

Njaa, L.R. and Aksnes, A. (1982) The nitrogen-sparing effect of methionine sulfoxide and some other sulphur-containing amino acids. Br. J. Nutr. 48, 565-570.

Orr, M.L. and Watt, B.K. (1957) Amino Acid Content of Foods. Home Economics Research Report No. 4, United States Department of Agriculture, Washington, D.C.

Paquet, A. and Sarwar, G. (1980) Determination of bioavailability of some long-chain N-substituted derivatives of L-methionine and L-lysine. Can. J. Biochem. 58(7), 577-580.

Pick, R.T. and Meade, R.J. (1971) Amino acid supplementation of Opaque-2 corn diets for growing rats. J. Nutr. 101, 1241-1248.

Pieniazek, D., Rakowska, M., Szkiltadziowa, W. and Grabarek, Z. (1975) Estimation of available methionine and cysteine in proteins of food products by in vivo and in vitro methods. Br. J. Nutr. 34, 175-190.

Rao, M.N. and McLaughlan, J.M. (1967) Lysine and methionine availability in heated casein-glucose mixtures. J. Assoc. Off. Anal. Chem. 50, 704-707.

Rao, P.B. Rama, Metta, V.C. and Johnson, B.C. (1959) The amino acid composition and nutritive value of proteins I Essential amino acid requirements of the growing rat. J. Nutr. 69, 387-391.

Rao, P.B. Rama, Norton, H.W. and Johnson, B.C. (1961) The amino acid composition and nutritive value of proteins. IV Phenylalanine, tyrosine, methionine and cystine requirements of the growing rat. J. Nutr. 73, 38-42.

Rassin, D.K., Sturman, J.A. and Gaull, G.E. (1978) Taurine and other amino acids in milk of man and other animals. Early Human Devel. 2, 1-13.

Rolls, B.A. and Porter, J.W.G. (1973) Some effects of processing and storage on the nutritive value of milk and milk products. Proc. Nutr. Soc. 32, 9-15.

Rose, W.C., Coon, M.J., Lockhart, H.B. and Lambert, G.F. (1955) The amino acid requirements of man. XI. The threonine and methionine requirements. J. Biol. Chem. 215, 101-110.

Sarwar, G. (1984) Available amino acid score for evaluating protein quality of foods. J. Assoc. Off. Anal. Chem. 67(3), 623-626.

Sarwar, G., Blair, R., Friedman, M., Gumbmann, M.R., Hackler, L.R., Pellett, P.L. and Smith, T.K. (1985a) Comparison of interlaboratory variation in amino acid analysis and rat growth assays for evaluating protein quality. J. Assoc. Off. Anal. Chem. 68(1), 52-56.

Sarwar, G., Botting, H.G., Peace, R.W. and Nõel, F.J. (1981) Effect of methionine supplemented legume protein products on liver fat in rats. Nutr. Rep. Int. 24(2), 267-276.

Sarwar, G. and McLaughlan, J.M. (1981) Relative Net Protein Ratio method for evaluating protein quality. Nutr. Rep. Int. 23(6), 1157-1166.

Sarwar, G., Paquet, A. and Peace, R.W. (1985b) Bioavailability of methionine in some tripeptides occurring in dietary proteins. Nutr. Res. (in press).

Sarwar, G., Peace, R.W. and Botting, H.G. (1983) Validity of rat plasma amino acids in predicting first limiting amino acids in protein mixtures. Nutr. Rep. Int. 28(3), 613-620.

Sarwar, G., Peace, R.W. and Botting, H.G. (1985c) Amino acid profile and lysine availability of milk-based infant formulas. Presented at A.O.A.C. Conference on Infant Formulas. May 14-16, 1985, Virginia Beach, Virginia, U.S.A.

Schaffer, S.W., Kramer, J. and Chovan, J.P. (1980) Regulation of calcium homeostasis in the heart by taurine. Fed. Proc. 39(9), 2691-2694.

Schiff, J.A. (1980) Pathways of assimilatory sulphate reduction in plants and microorganisms. In: Sulphur in Biology, CIBA Foundation Symposium 72, Excerpta Medica, Amsterdam, Oxford, New York, 49-63.

Schlenk, F. and DePalma, R.E. (1957) The formation of s-adenosylmethionine in yeast. J. Biol. Chem. 229, 1037-1051.

Seligson, F.H. and Rotruck, J.T. (1983) Tissue nonprotein sulfhydryl content and weight gain of rats as affected by dietary methionine level. J. Nutr. 113, 98-104.

Shannon, B.M., Howe, J.M. and Clark, H.E. (1972) Interrelationships between dietary methionine and cystine as reflected by growth, certain hepatic enzymes and liver composition of weanling rats. J. Nutr. 102, 557-562.

Sjöberg, L. and Bøstrum, S.L. (1977) Studies in rats on the nutritional value of hydrogen peroxide-treated fish protein and utilization of oxidized sulphur-amino acids. Br. J. Nutr. 38, 189-205.

Slump, P. and Schreuder, H.A.W. (1973) Oxidation of methionine and cystine in foods treated with hydrogen peroxide. J. Sci. Fd. Agric. 24, 657-661.

Smith, J.T. (1973) An optimal level of inorganic sulfate for the diet of a rat. J. Nutr. 103, 1008-1011.

Smith, J.T., Acuff, R.V., Bittle, J.B. and Gilbert, M.L. (1983) A metabolic comparison of cysteine and methionine supplements in the diet of a rat. J. Nutr. 113, 222-227.

Smith, R.C. (1972) Acetylation of methionine sulfoxide and methionine sulfone by the rat. Biochem. Biophys. Acta 261, 304-309.

Smith, T.K. (1981) Influence of dietary ornithine and methionine on growth, carcass composition and polyamine metabolism in the chick. Can. J. Anim. Sci. 61, 1005-1012.

Snedecor, G.W. and Cochran, W.G. (1967) Statistical Methods, 6th ed., Iowa State University Press, Ames, Iowa, 258-298.

Sotelo-Lopez, A. and Lucas-Florentino, B. (1978) Determination of net protein utilization using whole carcass, hind leg or liver of the rat and its relationship with Protein Efficiency Ratio determination. J. Nutr. 108, 61-66.

Sowers, J.E., Stockland, W.L. and Meade, R.J. (1972) L-methionine and L-cystine requirements of the growing rat. J. An. Sci. 35(4), 782-788.

Steinke, F.H. (1977) Protein Efficiency Ratio pitfalls and causes of variability: a review. Cereal Chem. 54(4), 949-957.

Steinke, F.H. (1979) Measuring protein quality of foods. In: Soy Protein and Human Nutrition, Wilke, H.L., Hopkins, D.T. and Waggle, D.H. (Eds.), Academic Press, New York, 307-312.

Stipanuk, M.H. (1979) Effect of excess dietary methionine on the catabolism of cysteine in rats. J. Nutr 109, 2126-2139.

Stipanuk, M.H. and Benevenga, N.J. (1977) Effect of cystine on the metabolism of methionine in rats. J. Nutr. 107, 1455-1467.

Stockland, W.L. and Meade, R.J. (1970) Meat and bone meals as sources of amino acids for the growing rat: use of a reference diet to predict amino acid adequacy by levels of plasma free amino acids. J. An. Sci. 31, 1156-1163.

Stockland, W.L., Meade, R.J., Wass, D.F. and Sowers, J.E. (1973) Influence of levels of methionine and cystine on the total SAA requirement of the growing rat. J. An. Sci. 36(3), 526-530.

Strange, E.D., Benedict, R.C. and Miller, A.J. (1980) Effect of processing variables on the methionine content of frankfurters. J. Fd. Sci. 45, 632-637.

Sturman, J.A. (1980) In: Natural Sulfur Compounds: Novel Biochemical and Structural Aspects, Cavallini, D., Gaull, G.E., Zappia, V. (Eds.), Plenum Press, New York, 107-119.

Sturman, J.A., Gaull, G.E. and Räihä, N.C.R. (1970) Absence of cystathionase in human fetal liver: is cystine essential? Science 169, 74-76.

Sturman, J.A. and Gaull, G.E. (1975) Taurine in the brain and liver of the developing human and monkey. J. Neurochem. 25, 831-835.

Sturman, J.A. and Hayes, K.C. (1980) The biology of taurine in nutrition and development. Chapter 9 In: Advances in Nutritional Research, Vol. 3, Draper, H.H. (Ed.), Plenum Press, New York and London, 231-299.

Tannenbaum, S. (1974) Industrial processing. Chapter 14 In: Nutrients in Processed Foods, American Medical Association, White, P.L. and Fletcher, D.C. (Eds.), Publishing Sciences Group Inc., Acton, Massachusetts, 131-138.

Tannenbaum, S.R., Barth,H. and LeRoux, J.P. (1969) Loss of methionine in casein during storage by autooxidation in methyl linoleate. J. Agr. Food Chem. 17, 1353-1357.

Tejani, A., Mahadevan, R., Dobias, B., Varina, P.N. (1979) Total parenteral nutrition of the neonate - a long term followup. J. Pediatr. 94, 803-805.

Torun, B. Viteri, F.E. and Young, V.R. (1981) Nutritional role of soya protein for humans. J. Am. Oil Chem. Soc. 58, 400-405.

University of Nebraska, Food Protein Research Group (1982) The effects of processing on the protein quality of legume based foods and collaborative testing of the C-PER assay. National Science Foundation Project Report.

Visek, W.J. (1984) An update of concepts of essential amino acids. Ann. Rev. Nutr. 4, 137-155.

Whittle, B. and Smith, J.T. (1974) Effect of dietary sulfur on taurine excretion by the rat. J. Nutr. 104, 666-670.

Williams, H.H., Curtin, L.V., Abraham, J., Loosli, J.K. and Maynard, L.A. (1954) Estimation of growth requirements for amino acids by assay of the carcass. J. Biol. Chem. 208, 277-286.

Womack, M. and Rose, W.C. (1941) The partial displacement of dietary methionine by cystine for purposes of growth. J. Biol. Chem. 141, 375-379.

Yanagita, T., Enomoto, N. and Sugano, M. (1984) Hepatic triglyceride accumulation as an index of bioavailability of oxidized methionine to the growing rat. Agric. Biol. Chem. 48(3), 815-816.

Yang, S.F. (1970) Sulfoxide formation from methionine or its sulfite analogs during aerobic oxidation of sulfite. Biochemistry 9, 5008-5014.

Yew, M.-L.S., Gropp, S.T. and Tummins, A.T. (1979) Nutritional improvements of the AIN-76 purified diet in reproductive performance of Holtzman rats. Nutr. Rep. Int. 19, 93-99.

Yoneda, Y. and Kuriyama, K. (1978) A comparison of microdistribution of taurine and cysteine sulfinate decarboxylase activity with those of GABA and L-glutamate decarboxylase activity in the rat spinal chord and thalamus. J. Neurochem. 30, 821-825.

Young, V.R. and Scrimshaw, N.S. (1974) Relation of animal to human assays of protein quality. Chapter 9 In: Nutrients in Processed Foods - Proteins, American Medical Association, White, P.L. and Fletcher, D.C. (Eds.), Publishing Sciences Gp. Inc., Acton, Massachusetts, 85-98.

Young, V.R., Tontisirin, K., Ozalp, I., Lakshmanan, F. and Scrimshaw, N.S. (1972) PAA response curve and amino acid requirements in young men: valine and lysine. J. Nutr. 102, 1159-1165.

Ziegler, D.M. (1985) Role of reversible oxidation-reduction of enzyme thiols-disulfides in metabolic regulation. In: Annual Review of Biochemistry, Richardson, C.C., Boyer, P.D., David, I.B. and Meister, A. (Eds.), Annual Reviews Inc., Palo Alto, CA, 54: 305-309.

Zimmerman, R.A. and Scott, H.M. (1965) Interrelationship of plasma amino acid levels and weight gain in the chick as influenced by suboptimal and superoptimal dietary concentration of single amino acids. J. Nutr. 87, 13-18.

Zlotkin, S.H. and Anderson, G.H. (1982) Sulfur balances in intravenously fed infants: effects of cysteine supplementation. Am. J. Clin. Nutr. 36, 862-867.

IX. APPENDIX A

OPERATING PARAMETERS FOR ANALYSIS OF AMINO ACIDS

a) 121 MB Amino Acid Analyzer, Model 126 Data System, single column (0.28 x 23 cm) system, AA-10 resin, sodium citrate buffers and ninhydrin solutions available from Beckman Instruments[4].

b) Flow rates:

Buffer solutions	8.8 ml/hr
Ninhydrin solution	4.4 ml/hr

c) Buffers used:

1. ph 3.25, 0.16 N sodium citrate buffer
2. pH 3.28, 0.20 N sodium citrate buffer
3. pH 3.90, 0.35 N sodium citrate buffer
4. pH 4.00, 0.44 N sodium citrate buffer
5. Buffer 4:Buffer 6 (8:13 vol/vol)
6. pH 6.40, 1.0 N sodium citrate buffer
7. 0.2 N NaOH regeneration solution

d) System Operating Conditions

Buffer	Buffer Run Times	Total Elapsed Time	Temperature
1	26 mins	26 mins	33°C
2	19 mins	45 mins	51°C
3	3 mins	48 mins	51°C
4	13 mins	61 mins	51°C
5	40 mins	101 mins	51°C
6	18 mins	119 mins	51°C
NaOH	4 mins	123 mins	51°C
1	18 mins	141 mins	51°C

e) Connection of the two independent four-way buffer exchange values in series in front of the buffer pump permitted up to 7 buffer changes including the NaOH regeneration step.

[4] Beckman Instruments, Spinco Division, 1117 California Ave., Palo Alto CA, 94304.