SAFETY OF AMINO ACIDS USED
AS DIETARY SUPPLEMENTS

July 1992

Prepared for
CENTER FOR FOOD SAFETY AND APPLIED NUTRITION
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
WASHINGTON, DC 20204

under
FDA Contract No. 223-88-2124
Task Order No. 8
SAFETY OF AMINO ACIDS USED
AS DIETARY SUPPLEMENTS

July 1992

Prepared for
CENTER FOR FOOD SAFETY AND APPLIED NUTRITION
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
WASHINGTON, DC 20204

under
FDA Contract No. 223-88-2124
Task Order No. 8

edited by
S. A. Anderson R.D., Ph.D.
D. J. Raiten, Ph.D.

LIFE SCIENCES RESEARCH OFFICE
FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY
9650 ROCKVILLE PIKE
BETHESDA, MARYLAND 20814-3998
FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), provides scientific assessments of topics in the biomedical sciences. Reports are based upon literature reviews and the scientific opinions of knowledgeable investigators engaged in work in specific areas of biology and medicine.

This report was developed for the Center for Food Safety and Applied Nutrition (CFSAN), Food and Drug Administration (FDA) in accordance with the provisions of Task Order No. 8 of FDA Contract No. 223–88–2124. It was edited by Sue Ann Anderson, R.D., Ph.D. and Daniel J. Raiten, Ph.D., Senior Staff Scientists, on the basis of discussions of, and materials evaluated by, an ad hoc Expert Panel convened by LSRO. The members of the Expert Panel were chosen for their qualifications, experience, and judgment, with due consideration for balance and breadth in appropriate professional disciplines. Members of the Expert Panel and others who assisted in the preparation of this report are listed in Chapter X.

This study was initiated in September, 1990. In a notice in the Federal Register of November 26, 1990, the FDA announced that FASEB was inviting data, information, and views bearing on the topic under study (Food and Drug Administration, 1990). Accordingly, FASEB provided an opportunity for public oral presentations in an Open Meeting held on February 4, 1991 and for written submissions. Eight individuals made oral presentations at the Open Meeting. Thirty individuals and organizations provided written submissions for consideration by the Expert Panel (FDA Docket No. 90N–0379). These individuals and organizations are listed in Chapter XI. The LSRO also wishes to express its appreciation to the Council for Responsible Nutrition, Washington, DC for its cooperation in this study.

The Expert Panel met five times over the course of the study to assess the available data on exposure to amino acids used in the manner of dietary supplements, identify and review data supplying information on safe levels of intake and endpoints for safety testing, discuss the adequacy of that information for safety evaluations, and develop guidelines for the safety evaluation of amino acids taken as dietary supplements. The Panel members reviewed each draft of the report and provided additional documentation and viewpoints for incorporation into the final report. However, the LSRO accepts responsibility for the study conclusions and accuracy of the report. The listing of individuals in Chapter X does not imply that the individual Panel members specifically endorse all statements in the report.

The final report was reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent Society of FASEB) under authority delegated by the FASEB Board. Upon completion of these review procedures, the report was approved and transmitted to FDA by the Executive Director, FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of the individual members of the FASEB constituent Societies.

August 28, 1992

Kenneth D. Fisher, Ph.D.
Director
Life Sciences Research Office
EXECUTIVE SUMMARY

The eosinophilia–myalgia syndrome (EMS) associated with the use of L-tryptophan has raised concerns about the safety of use of amino acids sold as dietary supplements. Even though investigations suggest that the EMS may be associated with one or more impurities in one specific product, this occurrence raised generic questions about the safety of prolonged daily ingestion of large quantities of amino acids as dietary supplements.

This report provides an assessment by an Expert Panel of current knowledge regarding safety of amino acids as dietary supplements and their recommendations on guidelines for the evaluation of safety of these substances. It includes a summary of information on the extent of use of amino acids as dietary supplements, a review of the scientific literature on safety of free amino acids, and a recommended strategy for evaluation of the safety of amino acids used as dietary supplements. The report was prepared in response to a request from the Center for Food Safety and Applied Nutrition (CFSAN) of the Food and Drug Administration (FDA) which is responsible for ensuring safe use of nutritional supplements. Public input into the process was obtained at an Open Meeting held on February 4, 1991.

STUDY APPROACH

The LSRO ad hoc Expert Panel was charged with the development of guidelines for the evaluation of the safety of amino acids marketed as dietary supplements. However, this charge included the caveat that the Panel should not consider whether the amino acids were consumed for purposes consistent with the definition of a dietary supplement, i.e., for nutritional purposes. That is, amino acids are marketed and used as dietary supplements, as nutrients for the fortification of proteins, as components of medical foods for nutritive and technical effects, as food ingredients for their nutritive and technical effects, and as drugs for their pharmacological effects. These uses are based, in part, on studies that address efficacy for intended effects and, occasionally, safety of use for the intended purpose.

The use as dietary supplements created a dilemma in safety evaluation because amino acid dietary supplements are, in fact, used primarily for pharmacological purposes or enhancement of physiological function rather than for nutritional purposes. This dilemma also encompassed a regulatory incongruity. That is, dietary supplements are currently regulated as foods and as such, they are evaluated only for safety because, from a regulatory perspective, foods are considered efficacious as sources of nutrients. In contrast, evaluation of drugs requires consideration of pharmacological efficacy and potential for adverse effects. If evaluation of amino acids were to include consideration of efficacy, regulatory considerations would require that supplemental amino acids be considered drugs, not foods. Except for uses in protein fortification, medical foods, and food ingredients added for technical effects, the manner of current use appears to be more consistent with the definition of drugs rather than foods. Because of these issues, the Expert Panel focused solely on safety of use of amino acids as dietary supplements and examined

---

1 Major conclusions of the LSRO ad hoc Expert Panel are indicated in bold type face.
2 In accordance with the Scope of Work in the Task Order Contract, this report provides an assessment of the adequacy of the available information on L-tryptophan for evaluation of the safety of the amino acid when used as dietary supplements. The report does not include a review of information on the safety of L-tryptophan products containing 1,1’-ethyldene-bis [tryptophan] (EBT) which has been investigated extensively as a cause of EMS.
published scientific literature related to the several purposes (nutritional, physiological, and pharmacological) of consuming or administering amino acids. However, the conclusions of the Expert Panel pertain only to the use of amino acids as dietary supplements.

The amino acids considered by the Expert Panel were those commonly found in proteins:

- Alanine
- Arginine
- Aspartic acid
- Asparagine
- Cysteine and cystine
- Glutamic acid
- Glutamine
- Glycine
- Histidine
- Isoleucine
- Leucine
- Lysine
- Methionine
- Phenylalanine
- Proline and hydroxyproline
- Serine
- Threonine
- Tryptophan
- Tyrosine
- Valine

Two additional amino acids sold as dietary supplements (ornithine and citrulline) were also included. The Panel focused its evaluation on individual amino acids, with the exception of mixtures of the branched-chain amino acids (BCAA). Leucine, isoleucine, and valine were considered individually and as mixtures of BCAA. Both D- and L-isomers were considered in this review.

Most of the published studies on nutritional effects of added amino acids are investigations of amino acids incorporated into foods to improve protein quality. Little scientific literature was found on most amino acids ingested as single or multiple boluses as dietary supplements, in capsule, tablet, or liquid forms. While the improvement of protein quality by addition of specific amino acids has been well established by scientific investigators, the Expert Panel was not aware of scientific literature that demonstrated a sound rationale for nutritional benefits of supplementation of the diets of healthy individuals with amino acids. Likewise, the members of the Panel were aware of few reports in the scientific literature addressing the safety of amino acid supplementation despite use of large quantities for purported health benefits. However, the published literature is replete with articles reporting or refuting efficacy of ingestion of supplements of amino acids as therapeutic aids or ergogenic aids in a variety of situations. The Expert Panel regarded these types of uses of amino acids not as nutritional uses, but as pharmaceutical applications to attempt to prevent or ameliorate specific physiological or psychological conditions. Nevertheless, the Panel interpreted its charge as the assessment of the safety of ingesting amino acids, either singly or as mixtures, regardless of intended use. To this end, they reviewed studies in which amino acids were administered by an oral route for evidence of safety, without commenting on the rationale or efficacy of such uses.

CONSUMER EXPOSURE AND LABEL INFORMATION

For persons using amino acid supplements, total exposure to amino acids consists of the amounts consumed as dietary protein plus the quantities contained in supplements. However, the effects of an amino acid supplement may not be a simple additive effect of the amount contained in the supplement plus the amount contained in dietary protein. Growth depression, food intake reduction, and other adverse effects are more severe in animals fed diets low in protein than in animals fed diets containing adequate protein. Thus, greater risk for adverse effects of amino acid supplements would be expected in individuals consuming diets low in protein. The Expert Panel regarded consumption of foods supplying adequate amounts of protein as the preferred means of obtaining the quantities of amino acids needed to meet the nutritional needs of the healthy, general population and viewed attempts to compensate for inadequate protein intake with supplements consisting of single amino acids or
incomplete mixtures as an unproven nutritional and potentially harmful practice. The Expert Panel was not aware of evidence for adverse health effects of amino acids when consumed as dietary protein by healthy individuals.

Data on amounts of amino acids sold in the U.S. were provided by the Council for Responsible Nutrition (CRN). These data indicated that amino acids were sold as single amino acids, mixtures containing two or more amino acids, protein powders including intact proteins and partially predigested protein products, and chelated amino acids consisting of complexes of amino acids with metal ions such as zinc, copper, magnesium, or manganese. The largest quantity of amino acids sold was marketed as single amino acids. Mixtures of amino acids accounted for the next largest portion, followed by protein powders. Chelated amino acids, either as single entities or as mixtures of amino acids, were sold in relatively small amounts and were most likely used as sources of the metal ion rather than the amino acid(s) component. Use of chelated forms as sources of supplemental amino acids would contribute large and potentially excessive loads of the metal ions.

Information on quantities of individual amino acids sold by member companies of CRN was calculated by the Life Sciences Research Office from data provided by that organization for the years 1988, 1989, and 1990. These figures do not represent total poundage of amino acids sold as human dietary supplements in the United States because the CRN represents about 50 percent of the companies supplying nutritional supplements in this country. The relative contribution of these companies to overall total quantities of amino acids sold as dietary supplements in the U.S. is unknown. Nevertheless, the data indicated that L-lysine and L-tryptophan were the only individual amino acids sold in quantities greater than 100,000 lb/year. (Figures for L-tryptophan were for 1988 and 1989 only.) One hundred thousand pounds of each of these compounds would provide about 45,000,000 1-g doses of each amino acid yearly. More than 20,000 lb/year of methionine and 10,000 to 20,000 lb/year of arginine/ornithine and glutamate/glutamine were sold; the remaining amino acids were sold in quantities of less than 7000 lb/year. This poundage information could not be used to generate an estimate of per capita exposure to amino acids used as dietary supplements because the fraction of the population using these products is unknown and the actual total quantity used is unknown.

The Expert Panel concluded that the most useful sources of information for estimating probable exposure were the doses suggested on product labels. The information compiled by the LSRO does not include all amino acid products on the market; however, all preparations identified during the course of this study were included. Suggested daily doses ranged from 250 to 4500 mg for single amino acids and from about 1 g to about 15 g for partially predigested protein blends. This compilation was made with the assumptions that persons consumed the products every day and that they adhered to dosage recommendations. Exposure could be much greater for persons not adhering to the suggested doses, as illustrated by reported daily consumption of as much as 15 g of L-tryptophan. The suggested doses may not be much greater than amounts of amino acids provided by generous dietary protein intakes; however, the effects of supplements of amino acids, particularly a single amino acid or a mixture of a few amino acids taken apart from meals, remain to be determined.

Amino acid dietary supplement products sold as single amino acids or as mixtures in capsule, tablet, or powder forms which were available in grocery, drug, and health food stores in the metropolitan Washington, DC area were examined for label information. No effort was made to obtain a comprehensive listing; however, a broad selection of available products was used to examine label information. Overall, labels of amino acid supplement products provided limited information to consumers. Amino acid components and their isomeric and chemical forms were usually specified but rarely was information about the source, grade, or chemical purity of the amino acid(s) included. Dosage suggestions were listed but labels usually did not include information relating the dosage amounts to requirements for indispensable amino acids or rationale for use for dispensable amino acids. Quality control and safety information were not uniformly included. Similarly, information on possible side effects or contraindications for use was rarely found.
REVIEW OF THE SCIENTIFIC LITERATURE ON SAFETY OF AMINO ACIDS AS DIETARY SUPPLEMENTS

The Expert Panel examined the scientific literature on amino acids and assessed the adequacy of those data for making an evaluation of the safety of amino acids used as dietary supplements by humans. The Expert Panel attempted to focus on well controlled studies that involved chronic oral administration of amino acids in amounts that exceeded the World Health Organization (1985) requirements for adults for indispensable amino acids and in amounts of 0.5 g or more for dispensable amino acids. The Panel concentrated on the sufficiency of available data on healthy adults in regard to amino acid safety. In its opinion, such an effort was required to undergird any regulatory effort to determine whether amino acids are safe for use as dietary supplements for persons who might take amino acids for reasons other than diagnosed medical need.

Epidemiological information collected after the occurrence of EMS associated with consumption of L-tryptophan supplements indicated that some individuals had used L-tryptophan for as long as 8 to 10 years. Thus, published reports of controlled animal or human studies in which amino acids were administered orally over a long term in amounts that exceeded dietary requirements of adults for indispensable amino acids and in amounts of 0.5 g or more for dispensable amino acids were regarded as most directly applicable to the safety of amino acids used as dietary supplements. For the majority of amino acids, these types of studies were not available, necessitating consideration of studies in which amino acids were given in single doses or by other routes of administration and studies that lacked adequate controls.

General information on the absorption, transport, and metabolism of amino acids is provided in Chapter IV. The Expert Panel concluded that separate reviews were needed for individual amino acids because of their unique structural properties, metabolic roles, and functions as stimuli for release or precursors of biologically active substances such as hormones and neurotransmitters. Each individual review in Chapter V consists of background information, selected animal and human studies, and summary and conclusions. When available, the experimental endpoints in the models developed in Chapter VII were considered in evaluation of data on each amino acid. Thus, endpoints for animal studies include investigations of changes in food intake, nitrogen balance, and body weight; biochemical, hematologic, endocrine, and behavioral changes; vitamin B6 status; acute pharmacokinetic studies; functional assessments and gross pathology studies; and teratology and developmental studies. Similarly, endpoints for human studies include investigation of changes in food intake and body weight; biochemical, hematologic, endocrine, and behavioral studies; vitamin B6 status; acute pharmacokinetic studies; functional assessments; and studies of inborn errors of metabolism. The summary and conclusions section presents the opinion of the Expert Panel on the adequacy of the information reviewed for evaluation of the safety of the amino acids taken as dietary supplements and for specification of safe levels of intake where possible. In general, there is a paucity of scientific literature addressing safety of amino acid supplements and some evidence of adverse health effects associated with use of some amino acids. The Expert Panel was not able to identify a safe upper level of intake for dietary supplements of amino acids for any of the amino acids reviewed in this report. Use of D-amino acids as dietary supplements is inappropriate as they have not been shown to have nutritional function in humans. Certain D-amino acids, e.g., D-serine and D-proline are potentially quite toxic. Additional conclusions and recommendations are provided in Chapter VIII.

For the most part, the available data with respect to safety of amino acids used as dietary supplements by normal, healthy adult males was considered and the Expert Panel found reason for particular concern about the use of dietary supplements of amino acids by several subgroups of the general healthy population and by patients with certain diseases. The
groups considered to be at higher risk for possible adverse effects included all women of childbearing age, particularly those who are pregnant or lactating; infants, children, and adolescents; the elderly; persons homozygous or heterozygous for inherited disorders of amino acid metabolism; individuals who smoke; and persons with low dietary protein intakes. The rationale for concern about use of amino acid supplements by these groups is detailed in Chapter VI.

In addition, the use of amino acids by patients with various diseases in the absence of responsible medical supervision presents a myriad of potential problems with regard to the safety of amino acid supplements. These range from effects of the amino acid on the disease condition to interactions of amino acids with medications or treatment regimens. For example, administration of methionine has been shown to exacerbate symptom expression in psychotic patients. Tyrosine metabolism has been shown to be abnormal in patients with hepatic cirrhosis and methionine has been implicated in precipitating episodes of hepatic encephalopathy. In addition, several interactions of amino acids with drugs have been identified. The potential gamut of consequences of unrestricted use of amino acid supplements by both healthy and patient population subgroups has not been investigated. Because the Expert Panel did not find sufficient data to be comfortable in establishing safe levels of intake in healthy individuals, it concluded that amino acid supplement use by these special population groups would require responsible medical advice and supervision.

PROPOSED STRATEGY FOR EVALUATION OF SAFETY OF AMINO ACIDS AS DIETARY SUPPLEMENTS

Because the fragmentary nature of the available data provided only a limited basis for the evaluation of the safety of amino acids as dietary supplements, the Expert Panel concluded that a systematic approach to safety testing is needed. Evaluation of safety of amino acids used as dietary supplements either singly or in mixtures should be considered within the larger context of the assessment of safety of food ingredients and nutrients. In general, components for evaluation of the safety of food ingredients include estimation of human exposure, acute and chronic toxicity in animals, and assessment of human responses to exposures at proposed usage levels or higher doses. These latter studies are typically predicated on results of the animal feeding trials. While studies of chronic toxicity in animals usually include reproductive or multigenerational studies, the extent to which mutagenicity, carcinogenicity, and hypersensitivity studies are conducted depends in part on the chemical nature of the substance.

In addition to the general components for evaluation of safety of food ingredients, special considerations that are unique to the assessment of the safety of nutrients include the adequacy of analytical methodology, the availability of appropriate populations for study, and the ethical considerations in such studies. A nutrient–by–nutrient approach would be required for safety evaluation of nutrients and, when possible, consideration should be given to the identification of predictive indicators rather than diagnostic endpoints of toxicity in humans.

The Expert Panel concluded that testing in both animals and humans is needed for evaluation of the safety of amino acids as dietary supplements. Implementation of the components of the testing system would follow evaluation of information in the extant scientific literature. They proposed a two–tiered approach for safety testing of individual or mixtures of amino acids in animals. Studies of acute and chronic effects of ingestion of amino acids and studies of teratologic and developmental effects comprised the first–tier components of the schema. The components of the first tier were viewed as a screening mechanism for identifying adverse effects and as an indicator of the need for further testing. The specialized studies comprising the second tier included functional assessments and gross pathological examinations of
organs and systems reflecting adverse effects in the first tier. In some instances, endpoints for second-tier investigations may be suggested in previously published studies. However, for the most part, necessity and endpoints for second-tier studies would be indicated by results of the first-tier studies.

**Testing in humans was also viewed as requiring a two-tiered approach.** Amino acid supplements eligible for testing would be those for which no serious adverse effects were observed in the animal studies. Epidemiological studies may provide useful information on exposure and populations potentially at risk of adverse effects. Information derived from the literature on effects of large doses of amino acids in humans plus data from safety testing in animals would form the basis for acute and chronic screening studies in the first tier. Outcomes of the screening studies would determine the need for specialized functional studies. This approach is similar to clinical protocols required for Phase 1 testing of investigational new drugs. The general principles for the conduct of clinical trials for drugs apply to the study of the safety of high doses of amino acids.

The proposed strategy for safety evaluation of individual amino acids did not include direct study of mutagenicity and carcinogenicity, endpoints of toxicity of universal significance, nor did it include hypersensitivity testing. The rationale for treatment of these specific endpoints is discussed.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>ado-met</td>
<td>S-adenosylmethionine</td>
</tr>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>ALT</td>
<td>alanine aminotransferase</td>
</tr>
<tr>
<td>AST</td>
<td>aspartate aminotransferase</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<tr>
<td>BCAA</td>
<td>branched-chain amino acids</td>
</tr>
<tr>
<td>BCAAT</td>
<td>branched-chain amino acid transaminase</td>
</tr>
<tr>
<td>BCKA</td>
<td>branched-chain keto acids</td>
</tr>
<tr>
<td>BCKAD</td>
<td>branched-chain keto acid dehydrogenase</td>
</tr>
<tr>
<td>BH$_4$</td>
<td>tetrahydrobiopterin</td>
</tr>
<tr>
<td>BHBN</td>
<td>N-butyl-n(4-hydroxybutyl)nitrosamine</td>
</tr>
<tr>
<td>BUN</td>
<td>blood urea nitrogen</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CoA</td>
<td>coenzyme A</td>
</tr>
<tr>
<td>CRN</td>
<td>Council for Responsible Nutrition</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>CSFII</td>
<td>Continuing Survey of Food Intake by Individuals</td>
</tr>
<tr>
<td>DHPR</td>
<td>dihydropteridine reductase</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DOCA</td>
<td>deoxycorticosterone acetate</td>
</tr>
<tr>
<td>DOPA</td>
<td>dihydroxyphenylalanine</td>
</tr>
<tr>
<td>DOPAC</td>
<td>dihydroxyphenylacetic acid</td>
</tr>
<tr>
<td>EBT</td>
<td>1,1'-ethyldene - bis[tryptophan]</td>
</tr>
<tr>
<td>EMS</td>
<td>eosinophilia myalgia syndrome</td>
</tr>
<tr>
<td>FIGLU</td>
<td>formiminoglutamic acid</td>
</tr>
<tr>
<td>FSH</td>
<td>follicle-stimulating hormone</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>GDH</td>
<td>glutamate dehydrogenase</td>
</tr>
<tr>
<td>GDP</td>
<td>guanosine diphosphate</td>
</tr>
<tr>
<td>GFR</td>
<td>glomerular filtration rate</td>
</tr>
<tr>
<td>γGT</td>
<td>γ-glutamyl transpeptidase</td>
</tr>
<tr>
<td>GTP</td>
<td>guanosine triphosphate</td>
</tr>
<tr>
<td>HVA</td>
<td>homovanillic acid</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>5-hydroxyindole acetic acid</td>
</tr>
<tr>
<td>IAA</td>
<td>indole acetic acid</td>
</tr>
</tbody>
</table>
Ig A  immunoglobulin A
Ig G  immunoglobulin G
KMA  potassium-magnesium aspartate
LDH  lactic acid dehydrogenase
LDL  low-density lipoprotein
LH  luteinizing hormone
LNAA  large neutral amino acids
MAO  monoamine oxidase
MDS  cysteine-homocysteine mixed disulfides
MOPEG  methoxyhydroxyphenylethleneglycol
MSA  monosodium aspartate
MSG  monosodium glutamate
MSUD  maple syrup urine disease
MTP  3-methylthiopropionate
NAD  nicotinamide adenine dinucleotide
NEFA  nonesterified fatty acids
NFCS  Nationwide Food Consumption Survey
NHANES  National Health and Nutrition Examination Survey
NMDA  N-methyl-D-aspartate
OCA  oral contraceptive agents
ORS  oral rehydration solution
OTC  over the counter
PKU  phenylketonuria
PLP  pyridoxal 5'-phosphate
POH  phenylalanine hydroxylase
POMS  Profile of Mood States
RDA  recommended dietary allowance
RNA  ribonucleic acid
RPF  renal plasma flow
SHR  spontaneously hypertensive rats
TCA cycle  tricarboxylic acid cycle
TH$_4$  tetrahydrofolate
TNT  transient neonatal tyrosinemia
TSH  thyrotropin
VAMS  Visual Analogue Mood Scale
VAS  visual analog scales
VLDL  very low density lipoproteins
VMA  vanillylmandelic acid
WHO  World Health Organization
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreword</td>
<td>iii</td>
</tr>
<tr>
<td>Executive Summary</td>
<td>v</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>xi</td>
</tr>
<tr>
<td>I. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>A. Background Information</td>
<td>1</td>
</tr>
<tr>
<td>B. Regulatory Status of Amino Acids</td>
<td>2</td>
</tr>
<tr>
<td>1. Use as drugs</td>
<td>2</td>
</tr>
<tr>
<td>2. Food uses</td>
<td>3</td>
</tr>
<tr>
<td>3. Use as dietary supplements</td>
<td>5</td>
</tr>
<tr>
<td>II. Study Description</td>
<td>7</td>
</tr>
<tr>
<td>A. Purpose of Study</td>
<td>7</td>
</tr>
<tr>
<td>B. Scope of Work</td>
<td>7</td>
</tr>
<tr>
<td>C. Approach to Study</td>
<td>7</td>
</tr>
<tr>
<td>D. Working Premises of the ad hoc Expert Panel</td>
<td>8</td>
</tr>
<tr>
<td>E. Working Definitions of Selected Terms</td>
<td>13</td>
</tr>
<tr>
<td>1. Amino acids</td>
<td>13</td>
</tr>
<tr>
<td>2. Dietary supplement</td>
<td>13</td>
</tr>
<tr>
<td>3. Safety</td>
<td>13</td>
</tr>
<tr>
<td>4. Toxicity</td>
<td>14</td>
</tr>
<tr>
<td>5. Adverse health effects</td>
<td>14</td>
</tr>
<tr>
<td>6. Imbalance</td>
<td>15</td>
</tr>
<tr>
<td>7. Nutritive value</td>
<td>15</td>
</tr>
<tr>
<td>8. Ergogenic aids</td>
<td>15</td>
</tr>
<tr>
<td>F. Organization of the Report</td>
<td>15</td>
</tr>
<tr>
<td>III. Consumer Exposure and Label Information</td>
<td>17</td>
</tr>
<tr>
<td>A. Total Exposure to Amino Acids</td>
<td>17</td>
</tr>
</tbody>
</table>
D. Reviews of Information Pertaining to the Safety of Individual Dispensable Amino Acids

1. Alanine ................................................................. 113
2a. Arginine .............................................................. 117
2b. Ornithine and citrulline ........................................ 126
3a. Asparagine .......................................................... 130
3b. Aspartic acid ....................................................... 131
4. Cysteine and cystine ............................................. 140
5a. Glutamine ........................................................... 147
5b. Glutamic acid ...................................................... 154
6. Glycine ................................................................. 167
7. Proline and hydroxyproline ................................... 172
8. Serine ................................................................. 175
9. Tyrosine ............................................................... 178

VI. Groups Potentially at Higher Risk for Adverse Health Effects Resulting from Use of Amino Acid Supplements ........................................... 193

A. Subgroups of the General Healthy Population .............. 193
   1. Infants and pregnant and lactating women .............. 193
   2. Children and adolescents .................................... 194
   3. Elderly individuals ............................................ 194
   4. Persons homozygous or heterozygous for inborn errors of amino acid metabolism .................. 195
   5. Individuals with low intakes of protein ................. 195
   6. Smokers .......................................................... 195

B. Patient Populations .................................................. 196

VII. Proposed Strategy for Evaluation of Safety of Amino Acids as Dietary Supplements ................................................................. 197

A. Safety Testing of Food Ingredients ............................. 197

B. Approach for Safety Testing of Amino Acids Used as Dietary Supplements ........................................ 198
   1. Test materials .................................................... 198
   2. Types of testing needed ...................................... 198
   3. Approach for safety testing in animals .................. 200
   4. Approach for safety testing in humans ................. 205
   5. Other endpoints of safety testing ......................... 209
VIII. Conclusions and Recommendations ........................................... 213
    A. Conclusions ................................................................. 213
    B. Recommendations ....................................................... 215

IX. Literature Cited .......................................................................... 217

X. Study Participants ......................................................................... 293

XI. Individuals and Organizations Submitting
    Information on Amino Acids .................................................... 295

Appendix A — Regulations for Use of
    Amino Acids as Food Additives ............................................... A-1

Appendix B — Specifications for
    Food-Grade Amino Acids ....................................................... B-1
I. INTRODUCTION

A. BACKGROUND INFORMATION

Amino acids are the individual structural units of proteins and are precursors for or may function as biologically active molecules including some neurotransmitters and hormones. Of the more than 200 amino acids isolated from biological materials, only about 20 occur frequently as constituents of animal and plant proteins. Nine of these amino acids — histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine — must be supplied in the diet and are considered indispensable (National Research Council, 1989) because they are synthesized by humans in amounts inadequate for normal growth or maintenance. The other, or dispensable, amino acids required for protein synthesis can be synthesized endogenously in amounts sufficient to support growth and nitrogen balance and are therefore not specifically required in the diet. For some metabolic disorders (e.g., phenylketonuria) and physiological conditions (e.g., prematurity in infants and critical illness), amino acids that are usually dispensable become conditionally indispensable. It should be noted that these terms and concepts apply only to dietary classification of amino acids. After ingestion, all amino acid constituents of proteins are metabolically essential because each is uniquely necessary for protein synthesis and because metabolic interactions occur between pairs and among groups of amino acids independently of dietary classifications (Steele and Harper, 1990).

Amino acids, with the exception of glycine, are optically active compounds. They are incorporated into nascent proteins almost exclusively as the L-isomers; thus, the L-isomers readily meet the metabolic needs for amino acids. Small amounts of the D-isomers have been identified in proteins and as free amino acids in bacteria and in plant and animal tissues (Felbeck and Wiley, 1987). The D-isomers may also be formed in small amounts from L-amino acids during food processing and preparation (Friedman et al., 1985; Man and Bada, 1987).

Most amino acids are supplied in the normal diet not as free amino acids but rather as constituents of protein. Consumption of foods containing intact proteins ordinarily provides sufficient amounts of amino acids required for growth and development of children and maintenance of adults in the general population of the United States (Life Sciences Research Office, 1989; U.S. Department of Health and Human Services and U.S. Department of Agriculture, 1986). In recent years, knowledge of the nutritional needs for amino acids plus the awareness that some amino acids are precursors of biologically active compounds or stimuli for hormone release have led to public interest in the use of free amino acids such as L-tryptophan and L-lysine marketed over the counter and sold under the rubric of dietary supplements. However, examination of the popular literature such as sports and health-oriented newspapers, books, and magazines, and advertisements in magazines and nutritional supplement product catalogs suggests that dietary supplements of amino acids may be used to produce or support pharmacologic actions rather than meet dietary or nutritional needs.

Recent attention has focused on the safety of use of amino acids sold as dietary supplements because of the eosinophilia–myalgia syndrome (EMS) associated with the use of L-tryptophan. Even though investigations suggest that the EMS may be associated with an impurity that was not removed from one specific product (e.g., Anonymous, 1990; Crofford et al., 1990; Page, 1990; Yamaguchi et al., 1991), this occurrence has raised generic questions about the safety of prolonged daily ingestion of large quantities of amino acids sold as dietary supplements.
B. **REGULATORY STATUS OF AMINO ACIDS**

Amino acids are available as food additives and as drugs. Under current regulations, various amino acids, singly and in mixtures, are marketed as parenteral drugs, components of enteral nutritional products and infant formulas, or as additives to enhance food protein quality. Production of amino acids for food uses must meet provisions of Good Manufacturing Practices (21 CFR 110) (Office of the Federal Register, 1991a) and drug products must meet Good Manufacturing Practices standards that are applicable to drugs on the basis of their intended uses (21 CFR 210 et seq.) (Office of the Federal Register, 1991b).

1. **Use as drugs**

The Food and Drug Administration (FDA) regulates over-the-counter (OTC) and prescription drugs for human use primarily by intended use. The Code of Federal Regulations contains a classification of both categories that is based upon the type of pharmacologic indication for use rather than the type of drug per se. For example, there are over 25 categories of OTC drugs (21 CFR 330.5) (Office of the Federal Register, 1991c) including antacids, analgesics, emetics, and miscellaneous dermatologic products. Prescription drugs are approved for human use after documentation of efficacy for the intended effect (21 CFR Subchapters C and D) (Office of the Federal Register, 1991d,e).

Amino acids, singly and in combination with other ingredients, are found in a number of OTC and prescription drugs for human use. In some instances, the intended use may have some nutritional purpose; in others, it may not. For example, Marlyn Formula 50® is a prescription drug used in combination with or after antibiotic therapy for dermatological purposes where fungal infection has produced nail splitting and peeling. It is also used in controlling excess hair loss following childbirth (Physicians' Desk Reference [PDR], 1992). The drug preparation contains 18 amino acids (all dispensable and indispensable amino acids except asparagine, cysteine, and glutamine) at 0.3 g per capsule along with 1.0 mg pyridoxine hydrochloride. On the other hand, glycine is listed as a generally recognized as safe ingredient in OTC antacid products (21 CFR 331.11[f]) (Office of the Federal Register, 1991f).

The United States Pharmacopeia (1989) provides drug-grade specifications for the following amino acids:

<table>
<thead>
<tr>
<th>L-alanine</th>
<th>L-leucine</th>
<th>L-threonine</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-arginine</td>
<td>L-lysine acetate</td>
<td>L-tryptophan</td>
</tr>
<tr>
<td>L-arginine HCl</td>
<td>L-lysine HCl</td>
<td>L-tyrosine</td>
</tr>
<tr>
<td>L-cysteine HCl</td>
<td>L-methionine</td>
<td>L-valine</td>
</tr>
<tr>
<td>glycine</td>
<td>L-phenylalanine</td>
<td></td>
</tr>
<tr>
<td>L-histidine</td>
<td>L-proline</td>
<td></td>
</tr>
<tr>
<td>L-isoleucine</td>
<td>L-serine</td>
<td></td>
</tr>
</tbody>
</table>
The United States Pharmacopeia (1989) lists specifications for monosodium glutamate. In addition to the Marlyn Formula 50® product, the PDR (Physicians' Desk Reference, 1992) included the following prescription and OTC preparations:

<table>
<thead>
<tr>
<th>Product</th>
<th>Amino Acid(e)</th>
<th>Classification and Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saave+®I</td>
<td>DL-phenylalanine</td>
<td>Medical food – nutrient imbalances</td>
</tr>
<tr>
<td></td>
<td>L-glutamine</td>
<td></td>
</tr>
<tr>
<td>Tropamine+®I</td>
<td>DL-phenylalanine</td>
<td>Medical food – nutrient imbalances</td>
</tr>
<tr>
<td></td>
<td>L-tyrosine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L-glutamine</td>
<td></td>
</tr>
<tr>
<td>Amino-Cerv®</td>
<td>Methionine</td>
<td>Prescription drug for cervicitis, postpartum cervical tears, postcrysurgery</td>
</tr>
<tr>
<td></td>
<td>Cystine</td>
<td></td>
</tr>
<tr>
<td>Aminoplex®</td>
<td>18 L-amino acids</td>
<td>Provides 180 mg N/capsule, OTC</td>
</tr>
<tr>
<td>Catemine®</td>
<td>L-tyrosine</td>
<td>Catecholamine precursor, OTC</td>
</tr>
<tr>
<td>Threostat®</td>
<td>L-threonine</td>
<td>May be helpful in reducing some symptoms of amyotrophic lateral sclerosis, OTC</td>
</tr>
<tr>
<td>Endorphenyl®</td>
<td>D-phenylalanine</td>
<td>Enkephalinase inhibitor, OTC</td>
</tr>
</tbody>
</table>

The information provided in the preceding paragraphs is incomplete in terms of uses of amino acids in OTC and prescription drugs. However, these data are indicative of a wider utilization of amino acids in products for human use that are not classified as food uses. Such uses are tangential to the use of amino acids as dietary supplements; nonetheless, it should be noted that these uses are regulated by the FDA under an approval process separate and distinct from the regulation of food uses.

2. Food uses

The major use of amino acids as direct food additives approved by the FDA is the addition of specific amino acids "to improve the biological quality of the total protein in a food containing naturally occurring primarily-intact protein that is considered a significant dietary protein source..." (21 CFR 172.320) (Office of the Federal Register, 1991g). This use to fortify proteins is classified as a direct food additive use (21 CFR 172.320) (Office of the Federal Register, 1991g). See Appendix A for details concerning this permitted use. In addition, N-acetyl-L-methionine may be added to foods as a source of L-methionine (21 CFR 172.372) (Office of the Federal Register, 1991h).

---

1 Labels of both preparations include the caution that they should not be taken by persons who have phenylketonuria or who are hypersensitive to the ingredients. Pregnant and lactating females, children under 14 years of age, and persons taking monoamine oxidase (MAO) inhibitors are advised to consult their physicians before use.
Other regulated food additive uses of amino acids include the following:

- 21 CFR 172.399  
In tablet form, zinc methionine sulfate, (a chelated amino acid providing a bioavailable source of zinc) is permitted as a food additive. (Office of the Federal Register, 1991i)

- 21 CFR 172.540  
The racemic mixture of DL-alanine may be used as a food additive to enhance the flavor of sweeteners in pickling mixtures. (Office of the Federal Register, 1991j)

- 21 CFR 172.812  
Glycine may be used for its technological effects in masking the bitter taste of saccharin in beverages at levels not to exceed 0.2 percent of the finished beverage and at 0.02 percent as a stabilizer in mono- and diglycerides prepared from edible oils. (Office of the Federal Register, 1991k).


In addition, the FDA has established the following Generally Recognized as Safe (GRAS) uses of various amino acids and certain salts of amino acids:

- 21 CFR 182.1  
Monosodium glutamate is GRAS for its intended uses. (Office of the Federal Register, 1991l)

- 21 CFR 182.1045  
Glutamic acid is GRAS as a salt substitute. (Office of the Federal Register, 1991m)

- 21 CFR 182.1047  
Glutamic acid hydrochloride is GRAS as a salt substitute. (Office of the Federal Register, 1991n)

- 21 CFR 182.1500  
Monoammonium glutamate is GRAS. (Office of the Federal Register, 1991o)

- 21 CFR 182.1516  
Monopotassium glutamate is GRAS. (Office of the Federal Register, 1991p)

- 21 CFR 184.1271  
L-cysteine is GRAS as a source of the amino acid up to 0.009 parts of the total L-cysteine per 100 parts of flour in dough as a dough strengthenener. (Office of the Federal Register, 1991q)

- 21 CFR 184.1272  
L-cysteine monohydrochloride is GRAS for the same uses with the same limitations as L-cysteine. (Office of the Federal Register, 1991r).

All GRAS uses also require use in accordance with Good Manufacturing Practices.
3. **Use as dietary supplements**

Prior to 1973, amino acids were considered as GRAS substances and were so listed by the FDA. In 1973, the FDA modified the regulations to specify the procedures and conditions under which amino acids could be used to fortify proteins (21 CFR 172.320) (Office of the Federal Register, 1991g) and used in special dietary products (21 CFR 105) (Office of the Federal Register, 1991s). In addition, at this time, the GRAS listing of amino acids was deleted from the Code of Federal Regulations except as noted in Section 2 above. Since 1973, the FDA has held that these two categories of regulated uses are considered to be food additive uses (Shank, 1990).

Amino acids are components of various foods for special dietary purposes (21 CFR 105.3) (Office of the Federal Register, 1991t). Special dietary uses as applied to human food include "uses for supplementing or fortifying the ordinary or usual diet with any vitamin, mineral, or other dietary property. Any such particular use of a food is a special dietary use, regardless of whether such food also purports to be or is represented for general use" (21 CFR 105.3 [iii]) (Office of the Federal Register, 1991t). For example, Tropamine® is a preparation containing DL-phenylalanine, L-tyrosine, and L-glutamine along with various vitamins and minerals. It is considered a medical food for correction of nutrient imbalances in patients undergoing treatment for use of illicit drugs (Physicians' Desk Reference, 1992).

Thus, the FDA holds that uses of amino acids except for specific food additive or GRAS uses identified above, for fortification of proteins, or for special dietary purposes are unapproved uses (Shank, 1990). However, it is not clear from the Code of Federal Regulations (21 CFR 105.3) (Office of the Federal Register, 1991t) whether or not single, or mixtures of, amino acids properly labeled would constitute preparations for special dietary purposes.

The Nutrition Labeling and Education Act of 1990 (U.S. Congress, 1990) directs the Secretary of Health and Human Services, and thus presumably the FDA, to promulgate regulations on validity of health claims for certain dietary supplements. The four supplements identified in the law do not include amino acids, but the language of the law refers to "dietary supplements of vitamins, minerals, herbs, or other similar nutritional substances..." Discussion of this provision by the U.S. Senate (1990) suggests that amino acids are included in "other similar nutritional substances." If interpreted in this manner, interested parties could petition the FDA for health claims for labeling of dietary supplements of amino acids. Such petitions would need to document that the totality of scientific evidence supported a relationship between the amino acid and a disease or health-related condition. To date, no petitions for health claim labeling of amino acid supplements have been announced by the FDA.
II. STUDY DESCRIPTION

A. PURPOSE OF STUDY

Because the association of EMS with use of L-tryptophan raised generic questions about the safety of amino acids used as dietary supplements, the Center for Food Safety and Applied Nutrition (CFSAN) of the FDA recognized a need for an evaluation of safety of amino acids as dietary supplements. To obtain this information, the Agency requested that the Life Sciences Research Office (LSRO) of the Federation of American Societies for Experimental Biology (FASEB) prepare a state-of-the-art scientific analysis on evaluation of the safety of amino acids and related products when used as dietary supplements. The purpose of the study was to assess current knowledge regarding the safety of the various forms of amino acids marketed as dietary supplements and to formulate guidelines for evaluating safety of such compounds.

B. SCOPE OF WORK

The Scope of Work provided to the LSRO by the FDA for this study specified that the magnitude of current use of amino acids and related products marketed and sold as dietary supplements be described. This part of the study consisted of three subtasks:

- identification of the products currently available in the marketplace and description of their principal ingredients and total nutrient composition;
- characterization of the current label and labeling information on products identified, including descriptions of recommended intake levels, if any; and,
- collation and assessment of available data on exposures to these products by the U.S. population and subgroups.

In addition, the Scope of Work specified that the available scientific literature on safety of amino acids and products containing free amino acids be reviewed. This part of the study also consisted of three subtasks:

- identification of those animal studies, clinical investigations, and case reports that bear on the safety of the products currently in use;
- characterization and description of the safety endpoints utilized in or derived from those studies having scientific merit; and,
- based on the information obtained, development of a reasonable set of guidelines for interpretation of data for evaluating issues associated with the safety of amino acids and related products.

As a culmination of these analyses, the Scope of Work directed preparation of a documented scientific report that discussed issues relevant to the above efforts.

C. APPROACH TO STUDY

The LSRO conducted a thorough literature review of published scientific studies to determine the availability of information pertaining to safety of individual amino acids (see Chapter V for details) and examined data on amounts of amino acids sold as dietary supplements in U.S. commerce to
estimate exposure to amino acids sold for this purpose. To evaluate the information gathered from these activities and to develop guidelines for evaluation of safety of amino acids, the LSRO convened an ad hoc Expert Panel of scientists with expertise in disciplines relevant to the study. These scientists are identified in Chapter X. Public input into this process was obtained at an Open Meeting held on February 4, 1991. Individuals and organizations making oral or written presentations are listed in Chapter XI. These materials were considered by the Expert Panel and the LSRO staff in the preparation of this report.

D. WORKING PREMISES OF THE AD HOC EXPERT PANEL

The Expert Panel was charged in the contractual Scope of Work with the development of guidelines for the evaluation of the safety of amino acids marketed as dietary supplements. However, this charge included the caveat that the Panel should not consider whether the amino acids were consumed for purposes consistent with the definition of dietary supplement (see page 13). That is, amino acids are marketed and used as dietary supplements, as nutrients for the fortification of proteins, as components of medical foods for nutritive and technical effects, as food ingredients for their nutritive and technical effects, and as drugs for their pharmacological effects. These uses are based, in part, on studies that address efficacy for intended effects and, occasionally, safety of use for the intended purpose.

In developing its approach to evaluation of the safety of amino acids as dietary supplements, the Expert Panel recognized that the manner of use created a dilemma in safety evaluation because amino acid dietary supplements are, in fact, used primarily for pharmacological purposes or enhancement of physiological function rather than for nutritional purposes. This dilemma also encompasses a regulatory incongruity which is beyond the contractual Scope of Work. That is, dietary supplements are currently regulated as foods. As foods, they are evaluated only for safety because foods are considered efficacious as sources of nutrients. In contrast, evaluation of drugs requires consideration of pharmacological efficacy and potential for adverse effects. If evaluation of amino acids were to include consideration of pharmacological efficacy, regulatory considerations would require that supplemental amino acids be considered drugs, not foods. The manner of current use is more consistent with the definition of drugs rather than foods, except for uses in protein fortification, medical foods, and food ingredients added for technical effects.

This regulatory stricture has also been recognized by other expert scientific groups considering uses of dietary supplements. For example, in 1987, the Advisory Panel on Vitamin Preparations as Dietary Supplements and as Therapeutic Agents of the Council on Scientific Affairs of the American Medical Association recognized the possibility of medical consequences of ingestion of high doses of vitamins and recommended that vitamins taken in high doses be treated as therapeutic agents (Council on Scientific Affairs, 1987).

Most of the available research on nutritional effects of added amino acids consisted of studies of amino acids incorporated into foods to improve the protein quality. Little scientific literature was found on most amino acids ingested as single or multiple boluses (i.e., loads) as dietary supplements, (e.g., in capsule or tablet forms). Although the improvement of protein quality by addition of specific limiting amino acids has been well established, the Expert Panel was not aware of scientific literature that demonstrated a sound rationale for nutritional benefits of amino acid supplementation of the diets of healthy individuals. Likewise, the members of the Panel were aware of few reports in the scientific literature addressing the safety of amino acid supplementation despite use of large quantities for purported health benefits. However, the scientific literature was replete with articles reporting or refuting efficacy of ingestion of supplements of amino acids, either singly or as incomplete mixtures, as therapeutic aids or ergogenic aids in a variety of situations. See Table 1 for a compilation of conditions for which amino acids have been used. The Expert Panel regarded these types of uses of amino acids not as nutritional uses but as pharmacological
Table 1. Examples of Investigations of Proposed Therapeutic Effects of Orally Administered Amino Acids

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Effects Investigated</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aliphatic Amino Acids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>Release of growth hormone</td>
<td>Isidori et al. (1981)</td>
</tr>
<tr>
<td></td>
<td>Promotion of weight loss</td>
<td>Schachter et al. (1973)</td>
</tr>
<tr>
<td></td>
<td>Treatment of oligospermia</td>
<td></td>
</tr>
<tr>
<td>Citrulline</td>
<td>Treatment of lysinuric protein intolerance</td>
<td>Rajantie et al. (1980, 1983)</td>
</tr>
<tr>
<td></td>
<td>Treatment of certain hyperammonemias</td>
<td>Brusilow (1984)</td>
</tr>
<tr>
<td></td>
<td>Treatment of Reye's syndrome</td>
<td>DeLong and Glick (1982); Oetgen (1977)</td>
</tr>
<tr>
<td>Ornithine</td>
<td>Release of growth hormone</td>
<td>Evain-Brion et al. (1982)</td>
</tr>
<tr>
<td></td>
<td>Increase of muscle mass</td>
<td>Kang et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>Promotion of weight loss</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment of certain hyperammonemias</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid and</td>
<td>Treatment of drug addiction</td>
<td>Koyuncuoglu (1983)</td>
</tr>
<tr>
<td>asparagine</td>
<td>Improvement of athletic performance</td>
<td>deHaan et al. (1985); Hagan et al. (1982)</td>
</tr>
<tr>
<td></td>
<td>Increased cardiorespiratory function</td>
<td>Hagan et al. (1982)</td>
</tr>
<tr>
<td></td>
<td>Management of chronic fatigue</td>
<td>Kruse (1961); Shaw et al. (1972)</td>
</tr>
<tr>
<td></td>
<td>Treatment of cirrhosis</td>
<td>Erikkson (1985)</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>Relief of hyperammonemina, mental retardation, epilepsy</td>
<td>Canadian Pharmaceutical Association (1988); Mudge (1985); Reynolds (1982)</td>
</tr>
<tr>
<td></td>
<td>Gastric acidification</td>
<td>Canadian Pharmaceutical Association (1988)</td>
</tr>
<tr>
<td></td>
<td>Amelioration of vincristine neurotoxicity</td>
<td>Jackson et al. (1988)</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Treatment of cystinuria</td>
<td>Jaeger et al. (1986); Miyagi et al. (1979)</td>
</tr>
<tr>
<td></td>
<td>Treatment of familial spasmodic torticollis</td>
<td>Korein (1977, 1979)</td>
</tr>
<tr>
<td></td>
<td>Treatment of alcoholism</td>
<td></td>
</tr>
</tbody>
</table>

1 Adapted from Expert Advisory Committee on Amino Acids (Health and Welfare Canada, 1990)

2 Listing of these effects should not be interpreted as implying that the conditions or diseases resulted from a nutritional deficiency of an amino acid or protein. In accordance with the Scope of Work, the Expert Panel did not evaluate the efficacy of these uses.
Table 1. Continued.

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Effects Investigated</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>Treatment and prevention of herpes simplex lesions</td>
<td>DiGiovanna and Blank (1984); McCune et al. (1984); Simon et al. (1985);</td>
</tr>
<tr>
<td></td>
<td>Acidification of urine</td>
<td>Thein and Hurt (1984); Lasser et al. (1960); Rubin et al. (1960)</td>
</tr>
<tr>
<td>Threonine</td>
<td>Treatment of spastic conditions</td>
<td>Barbeau et al. (1982)</td>
</tr>
<tr>
<td></td>
<td>Modification of amyotrophic lateral sclerosis</td>
<td>Patten and Klein (1988)</td>
</tr>
<tr>
<td><strong>Sulfur-containing Amino Acids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>Improvement of inflammatory liver disease</td>
<td>Council on Pharmacy and Chemistry (1947); Prescott and Critchley (1983);</td>
</tr>
<tr>
<td></td>
<td>Treatment of acetaminophen poisoning</td>
<td>Vale et al. (1981)</td>
</tr>
<tr>
<td></td>
<td>Acidification of urine in infants</td>
<td>Goldstein (1952); Kass (1957)</td>
</tr>
<tr>
<td></td>
<td>Lipotropic agent</td>
<td>Higgins et al. (1945); Patek et al. (1948); Wilson et al. (1945)</td>
</tr>
<tr>
<td>Cysteine and cystine</td>
<td>Treatment of acetaminophen poisoning</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mofenson et al. (1992)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prescott and Critchley (1983)</td>
</tr>
<tr>
<td><strong>Branched-Chain Amino Acids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixtures of leucine, isoleucine,</td>
<td>Treatment of noninsulin-dependent diabetes</td>
<td>Rifkin et al. (1966)</td>
</tr>
<tr>
<td>and valine</td>
<td>mellitus with secondary sulfonylurea failure</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment of amyotrophic lateral sclerosis</td>
<td>Plaitakis et al. (1988a,b)</td>
</tr>
<tr>
<td>Leucine</td>
<td>Treatment of Duchenne muscular dystrophy</td>
<td>Mendell et al. (1984)</td>
</tr>
</tbody>
</table>
Table 1. Continued.

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Effects Investigated</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aromatic Amino Acids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Treatment of pain</td>
<td>Balagot et al. (1983a,b)</td>
</tr>
<tr>
<td></td>
<td>Adjunct to acupuncture</td>
<td>Balagot et al. (1983a,b)</td>
</tr>
<tr>
<td></td>
<td>Prevention of depression</td>
<td>Walsh et al. (1986)</td>
</tr>
<tr>
<td></td>
<td>Treatment of depression</td>
<td>Beckmann et al. (1977); Fischer et al. (1975a); Young (1987)</td>
</tr>
<tr>
<td></td>
<td>Treatment of hyperactivity</td>
<td>Zametkin et al. (1987)</td>
</tr>
<tr>
<td></td>
<td>Treatment of attention deficit disorder</td>
<td>Wood et al. (1985)</td>
</tr>
<tr>
<td></td>
<td>Appetite suppressant</td>
<td>Ryan–Harshman et al. (1987)</td>
</tr>
<tr>
<td></td>
<td>Mood changes and arousal</td>
<td>Ryan–Harshman et al. (1987)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Treatment of Parkinson's disease</td>
<td>Cotzias et al. (1973)</td>
</tr>
<tr>
<td></td>
<td>Attention deficit disorder</td>
<td>Nemzer et al. (1986)</td>
</tr>
<tr>
<td></td>
<td>Treatment of narcolepsy</td>
<td>Mouret et al. (1988)</td>
</tr>
<tr>
<td></td>
<td>Treatment of hypertension</td>
<td>Benedict et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>Treatment of obesity</td>
<td>Johnston et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>Treatment of depression</td>
<td>Gelenberg et al. (1980, 1983); Goldberg (1980)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>van Pragg (1983)</td>
</tr>
<tr>
<td><strong>Heterocyclic Amino Acids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>Treatment of rheumatoid arthritis</td>
<td>Pinals et al. (1977)</td>
</tr>
<tr>
<td></td>
<td>Treatment of systemic sclerosis</td>
<td>Henkin et al. (1975)</td>
</tr>
<tr>
<td></td>
<td>Treatment of uremic anemia</td>
<td>Blumenkrantz et al. (1975); Giordano et al. (1973)</td>
</tr>
<tr>
<td>Proline and hydroxyproline</td>
<td>Retardation of progress of gyrate atrophy of choroid and retina</td>
<td>Hayasaka et al. (1985)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Sleep aid</td>
<td>Hartmann (1982/83); Schneider–Helmert and Spinweber (1986)</td>
</tr>
<tr>
<td></td>
<td>Affective disorder</td>
<td>Bender (1982); Chouinard et al. (1983, 1985); D'Elia et al. (1978); van Praag and Lemus (1986); Young (1986)</td>
</tr>
<tr>
<td></td>
<td>Treatment of pain</td>
<td>King (1980); Poloni et al. (1974); Seltzer et al. (1982/83); Shpeen et al. (1984)</td>
</tr>
<tr>
<td></td>
<td>Suppression of appetite</td>
<td>Hrboticky et al. (1985); Leiter et al. (1987); Strain et al. (1985); Wurtman et al. (1981a)</td>
</tr>
<tr>
<td></td>
<td>Nocturnal myoclonus</td>
<td></td>
</tr>
</tbody>
</table>
applications to attempt to prevent or ameliorate specific physiological or psychological conditions. The Panel interpreted its charge as the assessment of the safety of ingesting amino acids, either singly or as incomplete mixtures, regardless of intended use. To this end, they reviewed studies in which large doses of amino acids were given, without commenting on the rationale or efficacy of use in their evaluation.

The amino acids considered by the ad hoc Expert Panel were those commonly found in proteins. These amino acids are listed below:

- alanine
- arginine
- aspartic acid
- asparagine
- cysteine and cystine
- glutamic acid
- glutamine
- glycine
- histidine
- isoleucine
- leucine
- lysine
- methionine
- phenylalanine
- proline and hydroxyproline
- serine
- threonine
- tryptophan
- tyrosine
- valine

Two additional amino acids sold as dietary supplements (ornithine and citrulline) were also included. Compounds similar to amino acids, such as carnitine and taurine, were not included. The information examined included data on free amino acids and on salts of amino acids when any of these compounds were administered in studies contributing information on the safety of amino acids. The Panel focused its evaluation on individual amino acids, with the exception of mixtures of the branched-chain amino acids (BCAA). Leucine, isoleucine, and valine were considered individually and as mixtures of BCAA.

Inherent in the consideration of safety issues on amino acids was the isomeric form of amino acid(s) contained in supplements. In some instances isomers may be by-products formed in the chemical manufacture of amino acids or during chemical hydrolysis of proteins. Because both D- and L-isomers of amino acids may be contained in dietary supplements, both forms were considered by the Expert Panel. For example, safety concerns were evident in the case of serine, as the D-isomer has been shown to produce different and more toxic effects in rodents than the L-isomer of this amino acid. See Berg (1953) and Friedman (1991) for reviews of the metabolism of free D-amino acids.

The Expert Panel recognized the importance of safety issues pertaining to amino acids and to products containing these substances. Although the Scope of Work specified the guidelines for the evaluation of compound safety, the Expert Panel found it necessary to include the following general statements on product safety.

- Amino acid products should not contain amino acids other than the one(s) declared on the label.
- Amino acids should be in the form of the L-isomer unless specifically excepted (e.g., glycine).
- Although supplements that meet these specifications cannot be guaranteed to be without adverse effects as suggested by the association of EMS with use of L-tryptophan supplements (Belongia et al., 1990; Silver et al., 1990; Slutsker et al., 1990), the likelihood of occurrence of adverse health effects may be greatly reduced in products meeting food-grade
or pharmaceutical-grade specifications and manufactured according to provisions of Good Manufacturing Practice [21 CFR 110] (Office of the Federal Register, 1991a).

• In addition, amino acid products sold as dietary supplements should meet standards for dissolution (United States Pharmacopoeial Convention, Inc., 1989) and be in preparations that are biologically available.

In this report, the ad hoc Expert Panel members have approached the issue of the safety of amino acids from a toxicological perspective. That is, they have evaluated the extant literature on amino acids to assess the weight of evidence for safety and absence of adverse health effects for amino acids used as dietary supplements.

E. WORKING DEFINITIONS OF SELECTED TERMS

1. Amino acids

In this report, amino acids are considered to be, with the exception of proline and hydroxyproline, the α-amino acids. α-Amino acids are defined as chemical compounds having a free carboxyl group, a free unsubstituted amino group, and an R-group of varying structure on the α-carbon.

The general structural formula for α-amino acids is shown below.

\[
\begin{align*}
H \\
R & \quad C \quad COOH \\
\downarrow \\
& \quad NH_2
\end{align*}
\]

2. Dietary supplement

In the FDA's implementation of the Nutrition Labeling and Education Act of 1990 (PL 101–535, November 8, 1990) (U.S. Congress, 1990), the agency's proposed definition for dietary supplement was "a food, other than a conventional food, that supplies a component with nutritive value to supplement the diet by increasing the total dietary intake of that substance. A dietary supplement includes a food for special dietary use within the meaning of 101.9(a)(2) that is in conventional food form." [56 FR 60563] (Food and Drug Administration, 1991).

3. Safety

Safety has been defined as "the practical certainty that injury will not result from use of a substance under specified conditions of quantity and manner of use" (Douvall and Bruce, 1986). This definition introduces the issues of quantity (i.e., dose) and manner of use (how used and route of administration). Such definitions reflect the concepts of toxicology that differentiate safety and hazard. In general, guidelines for food safety assurance include a series of experimental tests for the presence or absence of demonstrable toxicity. Thus, safety is defined as the absence of adverse health effects.

Definition of "safe" in the context of food safety regulation is more complex, as definitions have been promulgated for the various types of substances regulated. For example, the Code of Federal Regulations defines color additives as "safe" if "there is convincing evidence that establishes with
reasonable certainty that no harm will result from the intended use of the color additive." [21 CFR 70.3 (i)] (Office of the Federal Register, 1991f). The definition for food additives is more specific and states that

"Safe or safety means that there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance. Safety may be determined by scientific procedures or by general recognition of safety. In determining safety, the following factors shall be considered:

(1) The probable consumption of the substance and of any substance formed in or on food because of its use.

(2) The cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substance or substances in such diet.

(3) Safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food and food ingredients, are generally recognized as appropriate."

Regulatory language includes the manner of use or intended use of the substance. Thus, a strict definition of safety of amino acids must take into account the manner of use. Amino acids, as constituents of proteins consumed as foods, are "safe" because foods are inherently "safe." For example, amino acids added to foods to improve the biological quality of proteins are special dietary and nutritional food additives [21 CFR 172.320] (Office of the Federal Register, 1991g). Amino acids used as dietary supplements, per se, are by definition deemed safe.

Thus, in the current regulatory scheme, amino acids used as dietary supplements are classified as foods; as such they are considered "safe." Nevertheless, from a toxicological perspective, all substances produce injury at sufficiently high doses and expected dosage must be taken into account in any safety evaluation. Thus, the manner of use becomes an important consideration in the evaluation of safety and amino acids must be examined for adverse effects or safety in terms of their potential doses.

4. Toxicity

Toxicity refers to the adverse effects of chemical substances on biological systems (Doull and Bruce, 1986).

5. Adverse health effects

Adverse health effects include unfavorable biochemical, histopathological, physiological, and behavioral changes observed in animals or humans. The Expert Panel considered both reversible and irreversible changes relevant, but weighted irreversible changes more heavily. Although acute (single-dose) effects were of interest, chronic (long-term) effects resulting from multiple exposures were of greater concern because of the manner of use of amino acids taken as dietary supplements.
6. **Imbalance**

*Imbalance* was defined as deviation from the amounts and proportions of indispensable amino acids supporting normal growth, development, and maintenance. The effects of amino acid imbalance typically reported in animals are depression of food intake and growth. The Expert Panel considered that depression of growth and food intake in the absence of other adverse health effects was a safety concern.

7. **Nutritive value**

In the FDA's implementation of the Nutrition Labeling and Education Act of 1990 (PL 101–535, November 8, 1990), the agency proposed that *nutritive value* be defined as "value in sustaining human existence by such processes as promoting growth, replacing loss of essential nutrients, or providing energy" [56 FR 60542] (Food and Drug Administration, 1991).

FDA developed this definition based on the common meaning of the words that make up the term. "Nutrient" is defined in the Random House Dictionary of the English Language (1987) as "a substance capable of providing nourishment or nutriment." This dictionary defines "nutriment" as "any substance or matter that, taken into a living organism, serves to sustain it in its existence, promoting growth, replacing loss, and providing energy." The dictionary defines "nourishment" as "something that nourishes; food, nutriment, or sustenance." Further, the dictionary defines "nourish" as "to sustain with food or nutriment; supply with what is necessary for life, health, and growth." The agency's proposed definition for "nutritive value" encompassed these common definitions except that the definition is specific for humans, for consistency with section 403(t)(1) of the act [56 FR 60542] (Food and Drug Administration, 1991).

8. **Ergogenic aids**

*Ergogenic aids* are substances, techniques, or equipment used to enhance performance by improving energy production, energy control, or energy efficiency during exercise (Williams, 1991). Amino acids have been investigated as one of many groups of ergogenic aids.

F. **ORGANIZATION OF THE REPORT**

In accomplishing the tasks specified in the Scope of Work, the report contains a summary of information on the extent of use of amino acids as dietary supplements, a review of the extant literature on safety of free amino acids, and a recommended strategy for evaluation of the safety of individual amino acids. Sources of available information on amounts of amino acids used as dietary supplements are identified in Chapter III. Use of these sources of data in making estimates of consumer exposure is evaluated. In addition, Chapter III provides observations on the types of products currently available and a summary of information included on product labels. Chapter IV provides general information on the absorption, transport, and metabolism of amino acids. Chapter V consists of summaries of the published scientific literature related to safety of individual amino acids and the conclusions of the Expert Panel regarding the adequacy of that information for evaluation of the safety of amino acids used as dietary supplements by humans. Groups potentially at greater risk for adverse health effects resulting from use of amino acid supplements are specified in Chapter VI. In Chapter VII, generalized schemata for animal and human testing are developed as guidelines for evaluation of safety of amino acids used as dietary supplements. Conclusions and recommendations of the Expert Panel are presented in Chapter VIII.
An estimate of quantities of indispensable and dispensable amino acids ingested by a 70-kg male with a daily protein intake of 100 g, representative of mean protein consumption levels reported in the national surveys cited above, is presented in Table 2. For purposes of this calculation, all protein consumed was assumed to be soybean protein (glycinin). Members of the Expert Panel recognized that methionine intake may be underestimated by use of soybean protein as a proxy for a mixed diet; however, they considered that use of a single "moderate quality" protein such as soybean protein provided a better approximation of the amino acid content of a mixed diet than a single "high quality" protein such as ovalbumin. In addition, use of data on amino acid composition of soybean protein (Lacey and Wilmore, 1990) determined by a gene sequencing method provided more complete information on amino acid composition (e.g., inclusion of glutamine and arginine) than data contained in tables of food composition. As illustrated in Table 2, consumption of usual amounts of dietary protein provides intakes of indispensable amino acids greater than the requirements estimated by the World Health Organization (1985). It was the opinion of the Expert Panel that consumption of foods supplying adequate amounts of protein is the preferred means of obtaining the quantities of amino acids needed to meet the nutritional needs of the healthy, general population. The Expert Panel was not aware of evidence for adverse health effects of amino acids when consumed as protein by healthy individuals.

C. EXPOSURE TO AMINO ACIDS IN PRODUCTS MARKETED AS DIETARY SUPPLEMENTS

Scant data exist for estimation of exposure to amounts of amino acids ingested as dietary supplements. Import figures were considered as potential sources of information because the major producers of amino acids are located outside the United States. Data on amounts of amino acids imported into the United States included amino acids used for pharmaceutical and research purposes, fortification of proteins, and supplementation of animal feeds, as well as those used for human dietary supplements. Because these data cannot be disaggregated according to use, import figures did not supply useful information for estimation of amounts of amino acids marketed as human dietary supplements.

Another potential source of information was data from dietary surveys such as NFCS, 1977–78 and NHANES II, 1976–80. Specialized surveys targeted to use of dietary supplements such as the 1986 supplement to the National Health Interview Survey (Moss et al., 1989) and a western regional project of the U.S. Department of Agriculture on Economic and Behavioral Factors Associated with Food Supplement Usage (Medeiros et al., 1989) also represented potential information sources. Each of the above surveys that included questions about dietary supplements focused on vitamin and mineral supplement usage and did not include collection of data on the use of amino acid supplements.

As part of the information gathering process following the outbreak of EMS associated with use of L-tryptophan, data were collected on amounts of the amino acid ingested by persons taking L-tryptophan supplements. The most commonly reported daily doses for persons who developed EMS were 500 mg to 4 g with a range from 10 mg to 15 g (Swygert et al., 1990). Data were not collected on use of amino acids other than L-tryptophan.

Data on amounts of amino acids sold by member companies of the Council for Responsible Nutrition (CRN) were made available to the Life Sciences Research Office by that organization. The CRN is a trade association that represents about 50 percent of the companies supplying nutritional supplements in the United States. Although not all suppliers of amino acids are members of CRN, these were the most comprehensive data available. This information indicated that amino acids were sold as single amino acids, mixtures containing two or more amino acids, protein powders including intact proteins and partially predigested protein products, and chelated amino acids consisting of complexes of amino acids with metal ions such as zinc, copper, magnesium, or
<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Amount Consumed (g/day)</th>
<th>Dietary Requirements for Indispensable Amino Acids (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>Asparagine</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>1.6</td>
<td>0.56 – 0.84</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>5.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Leucine</td>
<td>7.4</td>
<td>0.93</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.0</td>
<td>0.84</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.4</td>
<td>0.91</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.6</td>
<td>0.98</td>
</tr>
<tr>
<td>Proline</td>
<td>5.6</td>
<td>0.98</td>
</tr>
<tr>
<td>Serine</td>
<td>7.2</td>
<td>0.98</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.0</td>
<td>0.49</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.8</td>
<td>0.25</td>
</tr>
<tr>
<td>Valine</td>
<td>4.8</td>
<td>0.7</td>
</tr>
</tbody>
</table>

1 Intake of 100 g of protein was selected based on mean protein intakes of males 20 – 49 years of age in the National Health and Nutrition Examination Survey (NHANES I), 1971–74; the Nationwide Food Consumption Survey (NFCS), 1977–78; the Second National Health and Nutrition Examination Survey (NHANES II), 1976–80; and the Continuing Survey of Food Intakes by Individuals (CSFII), 1985–86 (Life Sciences Research Office, 1989).

2 Based on consumption of soybean protein (glycinin) with amino acid composition determined by gene sequencing as reported in Lacey and Wilmore (1990).

3 Dietary requirements for a 70-kg person calculated from estimated requirements for dispensable amino acids for adults (World Health Organization, 1985).

4 Requirement for methionine plus cysteine.

5 Requirement for phenylalanine plus tyrosine.
manganese. As shown in Table 3, the greatest quantity of amino acids was sold as single amino acids. Mixtures of amino acids accounted for the next largest portion, followed by protein powders. Information on sources (hydrolyzed protein or synthesized amino acids) and nutritional quality (completeness and balance of the amino acid components) was not specified for mixtures and powders. The large decline in poundage reported in the single and mixtures categories between 1988 and 1990 reflects FDA's ban on the sale of L-tryptophan which began in 1989. Chelated amino acids, either as single entities or as mixtures of amino acids, contributed relatively small amounts; these forms were most likely used as sources of the metal ion rather than the amino acid(s) component. Use of chelated forms as a source of supplemental amino acids would contribute a large and potentially excessive load of the metal ion (e.g., zinc, copper, magnesium, or manganese).

Poundage of individual amino acids sold by about half of the companies marketing dietary supplements in the U.S. was calculated from data provided by CRN. As shown in Table 4, L-lysine and L-tryptophan were the only individual amino acids sold in quantities greater than 100,000 lb/year. (Figures for L-tryptophan were for 1988 and 1989 only.) One hundred thousand pounds of each of these compounds would provide about 45,000,000 1-g doses of each amino acid annually. More than 20,000 lb/year of methionine and 10,000 to 20,000 lb/year of arginine/ornithine and glutamate/glutamine were sold; the remaining amino acids were sold in quantities of less than 7000 lb/year.

The above information on poundage was not used to generate an estimate of per capita exposure to amino acids used as dietary supplements because, in addition to a lack of information about the actual total poundage used, the fraction of the population using these products is unknown. This view was supported by an examination of the scientific literature, advertisements, and product labels (including product names) which suggested that specific amino acids were targeted for use to alleviate certain conditions (e.g., L-lysine for remission of herpes simplex lesions and L-tryptophan for insomnia, pain, or affective disorders), increase muscle mass (e.g., BCAA and predigested protein powders), or enhance athletic performance (e.g., BCAA, aspartic acid/asparagine, and predigested protein powders). (See Table 1 also.) Therefore, the Expert Panel concluded that the most useful sources of information for estimating probable exposure were the doses of amino acids suggested on product labels.

A summary of doses suggested on product labels is shown in Table 5. Ranges of suggested doses for single amino acids and amounts of amino acids contained in products randomly selected from the mixtures category are given. This summary does not include all amino acid products on the market; however, all preparations identified during the course of this study were included. This compilation showed suggested daily doses ranging from 250 to 4500 mg for single amino acids and quantities ranging from around 1 g to about 15 g for partially predigested protein blends. This compilation was made with the assumptions that persons consumed the products every day and that they adhered to dosage recommendations. Exposure could be much greater for persons not adhering to the suggested doses, as illustrated by reported daily consumption of as much as 15 g of L-tryptophan (Swygert et al., 1990). The suggested doses listed in Table 5 may not be much greater than amounts of amino acids provided by generous dietary protein intakes as shown in Table 2; however, as discussed in Chapter VII the effects of supplements of amino acids, particularly a single amino acid or a mixture of a few amino acids taken apart from and in addition to meals, remain to be determined.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>358,635</td>
<td>434,915</td>
<td>303,397</td>
</tr>
<tr>
<td>Mixtures</td>
<td>116,549</td>
<td>115,311</td>
<td>84,028</td>
</tr>
<tr>
<td>Protein Powder</td>
<td>26,137</td>
<td>14,861</td>
<td>20,385</td>
</tr>
<tr>
<td>Chelated (single)</td>
<td>13,062</td>
<td>8,938</td>
<td>9,781</td>
</tr>
<tr>
<td>Chelated (mixtures)</td>
<td>7,666</td>
<td>3,918</td>
<td>4,270</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>522,049</td>
<td>577,943</td>
<td>421,861</td>
</tr>
</tbody>
</table>

---

1 Compiled from poundsage data supplied by the Council for Responsible Nutrition (1991). These figures do not represent total poundsage of amino acids sold as dietary supplements in the United States.

2 The "single" category consisted of products containing individual amino acids. This designation included L-isomers, D-isomers, and DL-combinations of single amino acids.

3 The "mixtures" category consisted of products containing two or more amino acids.

4 "Protein powders" included intact proteins and partially predigested protein products.

5 Chelated amino acids are complexes of amino acids with metal ions such as zinc, copper, or magnesium. "Single" refers to a complex consisting of one kind of metal ion (e.g., zinc) with an individual amino acid (e.g., methionine). "Mixture" refers to a complex of one kind of metal ion with a mixture of amino acids.
Table 4. Reported Poundage of Individual Amino Acids Sold as Dietary Supplements for Human Use 1988–90

<table>
<thead>
<tr>
<th>Poundage Category (lb/year)</th>
<th>Individual Amino Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 100,000</td>
<td>Lysine</td>
</tr>
<tr>
<td></td>
<td>Tryptophan (^2)</td>
</tr>
<tr>
<td>&gt; 20,000</td>
<td>Methionine</td>
</tr>
<tr>
<td>10,000 – 20,000</td>
<td>Arginine – Ornithine</td>
</tr>
<tr>
<td></td>
<td>Glutamate – Glutamine</td>
</tr>
<tr>
<td>3,000 – 7,000</td>
<td>Cysteine – Cystine</td>
</tr>
<tr>
<td></td>
<td>Glycine (^3)</td>
</tr>
<tr>
<td></td>
<td>Isoleucine</td>
</tr>
<tr>
<td></td>
<td>Leucine</td>
</tr>
<tr>
<td></td>
<td>Phenylalanine</td>
</tr>
<tr>
<td></td>
<td>Tyrosine</td>
</tr>
<tr>
<td>1,000 – 3,000</td>
<td>Alanine</td>
</tr>
<tr>
<td></td>
<td>Aspartic Acid (^3)</td>
</tr>
<tr>
<td></td>
<td>Proline (^3)</td>
</tr>
<tr>
<td></td>
<td>Valine</td>
</tr>
<tr>
<td>&lt; 1,000</td>
<td>Histidine (^3)</td>
</tr>
<tr>
<td></td>
<td>Serine (^3)</td>
</tr>
<tr>
<td></td>
<td>Threonine</td>
</tr>
<tr>
<td>No poundage reported</td>
<td>Asparagine</td>
</tr>
<tr>
<td></td>
<td>Hydroxyproline</td>
</tr>
</tbody>
</table>

\(^1\) Compiled from data supplied by the Council for Responsible Nutrition (1991). These figures do not represent total poundage of amino acids sold as dietary supplements in the United States.


\(^3\) Reported in 1990 only.
Table 5. Summary of Daily Doses Suggested on Product Labels of Amino Acid Dietary Supplement Products Marketed in the United States

<table>
<thead>
<tr>
<th>Type of product</th>
<th>Doses suggested on Product Labels (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single Amino Acids</strong></td>
<td></td>
</tr>
<tr>
<td>L-Arginine</td>
<td>500</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>500 – 1500</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>500</td>
</tr>
<tr>
<td>L-Glutamic Acid</td>
<td>500 – 3000</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>Glycine</td>
<td>500 – 4000</td>
</tr>
<tr>
<td>L-Histidine Complex</td>
<td>3000</td>
</tr>
<tr>
<td>(vegetarian formula)</td>
<td></td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>500</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>500</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>500 – 4500</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>500</td>
</tr>
<tr>
<td>L-Ornithine</td>
<td>500 – 4000</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>500 – 3000</td>
</tr>
<tr>
<td>DL-Phenylalanine</td>
<td>500 – 2400</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>500</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>500 – 2000</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>500</td>
</tr>
<tr>
<td>L-Valine</td>
<td>500</td>
</tr>
<tr>
<td><strong>Mixtures of Amino Acids</strong></td>
<td></td>
</tr>
<tr>
<td>BCAA</td>
<td></td>
</tr>
<tr>
<td>L-Leucine</td>
<td>540 – 900</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>360 – 630</td>
</tr>
<tr>
<td>L-Valine</td>
<td>270 – 300</td>
</tr>
</tbody>
</table>

1 Summary compiled from label information on amino acid dietary supplement products sold in the metropolitan Washington, DC area. No attempt was made to cover every product sold.

2 Products contained amino acids in free-base form or as salts. Suggested doses were not adjusted to correct for contributions of the salt components such as hydrochloride.
<table>
<thead>
<tr>
<th>Type of Product</th>
<th>Doses Suggested on Product Labels (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixtures of Amino Acids (Continued)</td>
<td></td>
</tr>
<tr>
<td>Vitamins, minerals, and amino acids packs</td>
<td></td>
</tr>
<tr>
<td>L-Ornithine</td>
<td>250</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>500</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>200</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>100</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>300</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>210</td>
</tr>
<tr>
<td>L-Valine</td>
<td>90</td>
</tr>
<tr>
<td>Other Mixtures</td>
<td></td>
</tr>
<tr>
<td>L-Arginine</td>
<td>500 – 1700</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0 – 1700</td>
</tr>
<tr>
<td>L-Ornithine</td>
<td>0 – 1400</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>250 – 500</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>250 – 500</td>
</tr>
<tr>
<td>Tyrosine (isomer not specified)</td>
<td>0 – 500</td>
</tr>
<tr>
<td>Vegetable soy amino acids,</td>
<td>750 – 1500</td>
</tr>
<tr>
<td>total amino acid content, individual amino acids not listed</td>
<td></td>
</tr>
<tr>
<td>Partially predigested protein blend</td>
<td></td>
</tr>
<tr>
<td>L-Leucine</td>
<td>3213</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>1325</td>
</tr>
<tr>
<td>L-Valine</td>
<td>1361</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>2457</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>567</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>1024</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>1528</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>644</td>
</tr>
</tbody>
</table>
D. LABEL INFORMATION ON AMINO ACID PRODUCTS

Amino acid dietary supplement products sold as single amino acids or as mixtures in capsule, tablet, or powder forms which were available in grocery, drug, and health food stores in the metropolitan Washington, DC area were examined for label information. No effort was made to obtain a comprehensive listing. Rather, labeling information on a wide range of products was sought. The information collected is not amenable to quantitative analyses; however, quantitative data are needed to assess accurately the label information provided to users of these products.

1. Single amino acids

The following single amino acid products were available:

| L-arginine | glycine   | L-ornithine |
| L-cysteine | L-histidine | L-phenylalanine |
| L-cystine  | L-isoleucine | DL-phenylalanine |
| L-glutamic acid | L-leucine | L-threonine |
| D-glutamic acid | L-lysine | L-tryptophan |
| L-glutamine  | L-methionine | L-tyrosine |
|            |            | L-valine |

The name of the amino acid, including designation of the isomer, was the name of the product on most of the single amino acid products examined. The chemical form of the amino acid was described as a salt such as hydrochloride (HCl) or as "free-form," a term initially interpreted as the free-base form of the amino acid; however, the scientific meaning of the term is uncertain. Other product labels included statements that were scientifically incorrect. For example, the label of a DL-phenylalanine product indicated that the product was the pure enantiomeric form of the amino acid and not a mixture of both D and L isomers. Few, if any, product labels included information on the grade or chemical purity of the amino acid.

Most labels did include the name of the distributor or manufacturer of the capsules or tablets; however, no labels included information about the manufacturer or source of the amino acid. A number of labels indicated that certain ingredients, such as corn, soy, yeast, wheat, or milk, were not contained in the products but did not indicate what the actual source of the amino acids was. Labels did not consistently include lists of inert or other ingredients.

Suggested doses were listed (number of capsules or tablets) as well as the amount of amino acid in each capsule or tablet. Some labels directed the user to take the preparation with meals; others specified taking it apart from meals or at bedtime. In a few cases, directions for use included the specification "or as directed by your physician."

Nutritionally indispensable amino acids were correctly labeled as "essential"; labels of dispensable amino acids usually included a sentence such as "Glycine is an amino acid." For indispensable amino acids, labels rarely provided information on the dietary requirement for the amino acid as a component of protein and did not relate the suggested dose to dietary needs such as the information typically included on labels of vitamin and mineral supplements. In the case of L-tryptophan products (no longer on the market), some labels contained statements such as "an amino acid noted for gentle, calming properties," "plays a role in functioning of brain cells," or "products should be evaluated for use in sleep disorders." Labels for some other products included statements such as "should be evaluated for use in patients as a dietary supplement in combination with a proper diet and used only under the recommendation of a physician." For dispensable amino acids, many labels provided no rationale for use or dose and some labels stated "no special dietary claims are made for this product."

25
Information relating to quality control varied among brands of amino acids. Lot numbers and expiration or "use by" dates were not found on labels consistently.

Virtually all products carried a warning to "keep out of the reach of children" and many included storage instructions. However, little safety information was found regarding populations at risk for using the products. For example, not all L-phenylalanine or DL-phenylalanine products included a warning for phenylketonuric patients. Only one product included a warning that the product should not be used by pregnant women. No products included information on interactions of amino acids with drugs (for example, an interaction of tryptophan or tyrosine with monoamine oxidase [MAO] inhibitors) or on possible side effects resulting from use of the products.

2. **Amino acid mixtures**

Mixtures of amino acids were found in several types of products and label contents varied within each category. As with the single amino acid products, labels on products containing mixtures of amino acids included the name of the distributor or the product manufacturer but did not identify the manufacturers of the components or the sources of the amino acids. Use of a particular grade (pharmaceutical) was specified on only one brand of products. Most products contained information on lot numbers but not all included expiration dates. Storage information and warnings to keep out of reach of children were included on most preparations. Some products specified that they were designed for use in households without small children. Labels did not include information about contraindications for use, interactions with pharmaceutical preparations, or possible side effects.

Labels of some amino acid mixtures listed the contents as 2 or 3 amino acids such as L-arginine and L-ornithine; L-phenylalanine and L-glutamine; L-phenylalanine, L-tyrosine, and L-glutamine; or the BCAA (L-leucine, L-isoleucine, and L-valine). Some products contained only amino acids; others contained other ingredients such as lecithin or herbals. In a few cases, the names of products containing two amino acids were the names of the components; more often names of these types of products implied enhancement of mood, mental function (smart drugs), or physical performance (ergogenic aids). Labels of some products containing L-phenylalanine did not include a warning for phenylketonuric patients even though the suggested doses provided more phenylalanine (250 to 500 mg) than an aspartame-sweetened soft drink (100 mg L-phenylalanine in a 12-ounce serving) which must be so labeled. Most labels did not include information regarding grade and purity of amino acids used in these products. Similarly, labels did not specify contraindications for use for groups such as pregnant or lactating women or children, information on interactions with pharmaceutical preparations, or information on possible side effects of the use of these preparations.

Tablets or capsules containing as many as 20 amino acids were also available, primarily as ergogenic aids. Pictures on the labels often implied use for body building. Instructions usually specified that the product should be used with exercise programs and not for weight reduction. Most labels did not include information on grade or purity, contraindications for use, or side effects.

Labels of some products containing mixtures of amino acids with other ingredients such as lecithin or vitamin B6 indicated only that the product was a nutritional supplement containing these ingredients with no further description of the amino acid content. This type of product often included suggested doses and ingredient lists similar to those found on food labels.

Some mixtures of amino acids were sold in tablet or capsule form as components of prepackaged daily doses of vitamins, minerals, and amino acids. Numbers and amounts of amino acids in these preparations varied. Labels provided only information on the amounts of each amino acid contained in the daily package. No information on source, grade, or purity of the amino acids was provided.
"Protein powders" were also sold as sources of amino acids with or without other ingredients such as vitamins, minerals, and carbohydrates. Product names often implied ergogenic properties or enhancement of physical training. These products appeared to consist of hydrolyzed proteins and the source was usually, but not always, specified.

3. **Summary**

Overall, labels of amino acid supplement products provided limited information to consumers. Amino acid components and their isomeric and chemical forms were usually specified but rarely was there any information about the source, grade, or chemical purity of the amino acid(s). Dosage suggestions were listed but labels usually did not include information relating the dosage amounts to requirements for indispensable amino acids or rationale for use for dispensable amino acids. Quality control and safety information were not uniformly included. Similarly, information on possible side effects or contraindications for use were rarely found.
IV. GENERAL CONSIDERATIONS ABOUT METABOLISM OF AMINO ACIDS

The processes of nutrition, particularly digestion, absorption, and homeostatic regulation, that relate to dietary protein have been studied extensively. While many questions remain, especially about the impact of single amino acid ingestion on these processes, there are many aspects that have been well characterized. This section will cover the processes of amino acid nutrition as they relate to dietary protein and regulation of amino acid homeostasis in healthy individuals.

Dietary protein is hydrolyzed in the gastrointestinal tract to constituent amino acids. Digestion occurs as a result of the successive actions of a series of proteolytic enzymes which attach to the polypeptide chain internally (endopeptidases) and externally (exopeptidases). These enzymes recognize the peptide bonds primarily on the basis of the amino acid adjacent to the bond. For example, pepsin from the stomach attacks bonds adjacent to leucine or the aromatic amino acids, whereas pancreatic enzymes have an affinity for bonds adjacent to lysine or arginine (trypsin) or aromatic amino acids (chymotrypsin).

The end result of protein hydrolysis is free amino acids and di- or tripeptides. The absorption of peptides can provide a significant proportion of amino acids and may prevent deficiency in individuals with metabolic problems of individual amino acid absorption. For example, Hartnup's disease is a disorder in which free tryptophan cannot be transported into mucosal cells. Children with this disorder can still achieve normal growth presumably because of the ability of peptides containing tryptophan to be absorbed and thus act as sources of this indispensable amino acid. Regardless of whether amino acids enter the mucosal cells as free amino acids or peptides, they leave the gastrointestinal tract and enter the hepatic portal circulation primarily as free amino acids.

Absorbed amino acids leave the hepatic portal system and liver and enter the peripheral blood as free amino acids or as polypeptides such as albumin. Tissue pools of free amino acids are approximately 0.5 percent of the concentration of bound amino acids (0.01 mol/L free amino acids versus 2 mol/L bound) (Munro and Crim, 1988).

Dietary and recycled amino acids are used for synthesis of body protein, and other physiologically active compounds, such as neurotransmitters, and serve as both gluconeogenic and lipogenic precursors. Consequently, the pool of free amino acids turns over several times daily to meet growth, maintenance, and homeostatic requirements. The daily total body protein turnover has been estimated to be 250 to 300 g in a 70-kg man (Young et al., 1976). Because a daily intake of 46 to 63 g of protein has been established as sufficient to support maintenance in adults (National Research Council, 1989), it is evident that there is a considerable endogenous recycling of amino acids.

The primary mechanism by which utilization of amino acids is regulated is at the level of amino acid catabolism (Peters, 1991). The regulation of amino acid pools occurs at several sites including the gastrointestinal tract, liver, muscle, and kidney. A brief discussion of the role of each of these organs provides a foundation for the extended coverage of potential safety concerns with intakes of individual amino acids. In addition, an abbreviated coverage of the role of vitamin B6 is included to provide additional background about an essential nutrient widely involved in amino acid metabolism. Selected aspects of the metabolism of individual amino acids are included in Chapter V.
A. AMINO ACID TRANSPORT

Unlike certain other nutrients, such as calcium or iron whose whole body utilization is regulated primarily at the level of absorption, there is little regulation of amino acid metabolism exerted at the level of amino acid absorption from the intestine (Peters, 1991). While all of the mechanisms and factors controlling the processes of amino acid absorption have not been elucidated, there are several well characterized generic processes involved. There are separate transmural sodium-dependent active transport systems for different groups of amino acids which are based on the charge of their side chains (Alpers, 1987; Hopfer, 1987). These include: dibasic amino acids (ornithine, histidine, lysine, arginine), acidic amino acids (aspartate, glutamate), imino acids (proline, hydroxyproline), and neutral amino acids (tyrosine, tryptophan, phenylalanine, glycine, alanine, serine, threonine, valine, leucine, isoleucine, methionine, glutamine, asparagine, cysteine). Entrance of free amino acids into the portal circulation may be regulated either at the brush border or intracellularly at the basolateral membrane of the enterocyte by several potential transport mechanisms (Figure 1).

Intestinal digestion of protein results in free amino acids and peptides. While free amino acids are absorbed by the above described sodium-dependent active transport systems, small di- or tripeptides may be absorbed by separate sodium-dependent or independent carrier(s). Larger peptides must be hydrolyzed by brush border peptidase before absorption can occur. According to Castro (1991), the number and specificity of peptide carriers have not been well characterized as yet but are definitely different from free amino acid transport systems. Once in the enterocytes, essentially all peptides are hydrolyzed to free amino acids (Castro, 1991). As noted by Castro (1991) and Scharrer (1989), amino acids presented as peptides are absorbed at a faster rate and result in greater increases in plasma levels than comparable amounts presented in the free form.

The numerous factors that may affect intestinal transport in healthy individuals have been outlined by Scharrer (1989) and include:

- The amount and composition of the diet may influence amino acid transport. For example, food restriction causes an increase in the transport of amino acids. This phenomena may be due to either mechanical or morphological changes, such as gut wall thinning or changes in the functional integrity of the mucosal cells. Another potential contributor may be pancreatic glucagon.

- Circadian rhythms associated with the periodicity of food intake have been demonstrated in pigs and rats and have been shown to be independent of food restriction.

- Protein content of the diet including the amino acid composition influences amino acid transport. High-protein diets have been shown to increase amino acid absorption. Peptide transport is similarly affected.

- The type and quantity of dietary fat can impair amino acid absorption.

- Vitamin status can influence amino acid absorption. There is evidence that a deficiency of vitamin B6 can decrease amino acid and dipeptide absorption through an as yet undefined mechanism. Similarly, a vitamin D deficiency can result in impaired amino acid transport, possibly through changes in calcium homeostasis.

- Excess sodium has been shown to increase colonic amino acid absorption; however, sodium depletion has not been associated with any untoward changes in amino acid transport.

- Hormones such as insulin, glucagon, and growth hormone may affect amino acid absorption.

In addition to the above transmural transport systems that regulate absorption of amino acids from the intestinal lumen across the brush border, there are several other transmembrane carrier
Figure 1. Summary of digestion and absorption of dietary protein. Approximately one-third of the total amino acid is absorbed in free form after luminal digestion. The remaining is absorbed as free acid or dipeptide and tripeptide following membrane digestive processes (Castro, 1991, used with permission).
systems for amino acid transport across cellular membranes. See review by Christensen (1990). It should be noted that, unlike the transluminal carriers, several of these active transport systems are sodium independent, e.g., the leucine or L system. The nomenclature for these seven systems has changed since their original identification and attempts continue to develop a standardized convention for naming new variants as they are identified (Bannai et al., 1984). A listing of the systems and the respective amino acids as outlined by Skeie et al., (1990) follows:

- **System A** Alanine, glycine, proline, serine, methionine;
- **System ASCP** Alanine, serine, cysteine, proline;
- **System L** Leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, methionine;
- **System Ly** Lysine, arginine, ornithine, histidine;
- **Dicarboxylate system** Glutamate, aspartate;
- **β-system** Taurine, β-alanine;
- **System N** Glutamine, asparagine, histidine.

It is not clear whether these transporters are necessary for movement of amino acids across the basolateral membrane into the portal circulation. Furthermore, the nature of the difference (if any) between blood–brain–barrier carrier mechanisms, cellular transport, and amino acid absorption has not been characterized. An additional concern in the interpretation of studies related to neurotoxicity of specific amino acids is developmental differences in blood–brain barrier transport systems. For example, Lefauchonnier and Trouve (1983) found differences in the pattern of amino acid transport into the brains of 5–day-old versus 19–day-old rats.

B. **THE ROLE OF THE LIVER**

Arriving primarily by the portal circulation, six of the indispensable amino acids may be catabolized in the liver with the remaining three, the BCAA (isoleucine, leucine, and valine) being degraded in peripheral tissue, including muscle, kidney, and brain (Munro, 1982).

The liver monitors the amount of dietary amino acids and when dietary intake exceeds needs of the body, there is an induction of enzymes for the breakdown of the excess indispensable amino acids (Harper, 1968). For example, dietary tryptophan beyond metabolic requirements can cause the induction of tryptophan 2, 3 dioxygenase in the liver (Young and Munro, 1973). This enzyme causes the degradation of tryptophan by the kynurenine pathway, thereby reducing the amount of free tryptophan entering the peripheral circulation. The result of ingesting excess lysine was demonstrated by Brookes et al. (1972), who found that intakes beyond those causing optimal growth response resulted in hepatic catabolism of this indispensable amino acid.

While the catabolism of dispensable amino acids increases with increased intake, the system is not as finely controlled as for the indispensable amino acids. Catabolism of dispensable amino acids increases gradually as intake increases. Other factors that affect the induction of liver degradative enzymes include diurnal rhythms related to food intake and several hormones including cortisol, growth hormone, and estrogen.

Even though the liver regulates the amount of amino acids entering the circulation through the induction of catabolic enzymes, there may still be a net efflux of amino acids into the blood following
excess intakes. Other mechanisms by which amino acid concentrations in the blood are regulated include the impact of insulin via the ratio of carbohydrates to protein in the diet. This latter effect, in conjunction with the competition of amino acids for transmembrane transport mechanisms, may influence the amount of amino acids entering certain tissues. For example, tryptophan has been shown to gain a preferential entry into the brain across the blood–brain barrier under certain conditions, e.g., after a meal with a high carbohydrate to protein ratio. These conditions are related to the competitive advantage of tryptophan over competing BCAA for uptake into the brain (Fernstrom and Wurtman, 1972). This mechanism may be more complicated than originally proposed and may involve the interaction of neuroendocrine and hepatic feedback mechanisms.

C. THE ROLE OF MUSCLE

After a meal, the predominant amino acids entering the peripheral circulation are the BCAA. As a result of the activity of insulin, the BCAA enter muscle and adipose tissue. After a carbohydrate-rich meal, the BCAA concentrations are rapidly decreased as a result of the insulin effect. Muscle is the predominant site of BCAA metabolism. As a result of muscle metabolism, large amounts of alanine and glutamine are released. Alanine serves as a nitrogen carrier to the liver for subsequent urea production while glutamine goes mainly to the gut to supply fuel and nitrogen which eventually is delivered to the liver for ureagenesis and to the kidneys to aid in acid–base regulation and ammonia detoxification. The muscle thereby becomes a primary source of nitrogen for urea synthesis and carbon skeletons for gluconeogenesis.

D. THE ROLE OF THE KIDNEY

A major role of the kidney is the elimination of ammonia and urea derived from amino acid catabolism. The primary source of urinary ammonia is the metabolism of glutamine from muscle and the gastrointestinal tract. Glutamine released from muscle can go directly to the kidney where it is metabolized to glutamate and ammonia or to the gastrointestinal tract where similar catabolism takes place.

The kidney responds to both a protein meal and a bolus of individual amino acids with an increase in both renal blood flow (RBF) and glomerular filtration rate (GFR) (Bosch et al., 1983). In the fed state, the pattern of amino acid flux changes. In the case of alanine, the kidney removes it postprandially and secretes it back into the circulation in the postabsorptive state, presumably to serve as a gluconeogenic precursor. The kidney plays a major role in the regulation of several amino acids, including arginine, citrulline, glycine, serine (synthesized from glycine), alanine, and glutamine (Koppel and Fukuda, 1980).

E. VITAMIN B6

Vitamin B6 plays a critical role in amino acid metabolism, predominantly through the activity of the coenzymatic form pyridoxal 5′-phosphate (PLP). While not directly required for protein anabolism, PLP serves as a coenzyme at various points in the metabolism of all of the 20 amino acids involved in protein synthesis. There are three primary types of reactions that require vitamin B6. As outlined in Pike and Brown (1984) these are:

- transamination — e.g., the initial catabolic step in BCAA catabolism or the more minor degradative routes of phenylalanine, threonine, methionine, histidine, and cysteine;
• decarboxylation — e.g., the intermediary step in aromatic amino acid metabolism that results in biosynthesis of neurotransmitters, dopamine (from tyrosine) and serotonin (from tryptophan); and,

• aldol reactions — e.g., conversion of serine to glycine and anhydride condensations such as conversion of glycine to δ-aminolevulinic acid.

These PLP-dependent reactions may be the initial step in the catabolism of an amino acid or they may be necessary for the conversion of intermediary metabolites, e.g., 3-hydroxykynurenine from the tryptophan to niacin pathway. In addition, they may involve the interconversions of amino acids that are essential components of intermediary metabolism as in the reversible transamination of alanine to form glutamate from α-ketoglutarate.

Vitamin B6 deficiency has been found to reduce amino acid absorption and cellular transport (Asatoor et al., 1972; Christensen et al., 1954). The role that vitamin B6 may play in amino acid transport may be indirect, related either to altered intracellular amino acid metabolism or an impact on some specific aspect of the transport mechanism.

The ability of fluctuations in vitamin B6 status to influence plasma amino acid concentrations has been studied in both animals (Swendseid et al., 1964), and humans (Merrow et al., 1966). It should be noted that most studies have examined effects of a deficient intake. However, Kang–Yoon and Kirksey (1992) studied recently the impact of short-term vitamin B6 supplementation (27 mg/day) on amino acid metabolism in women and found evidence of accelerated protein and amino acid metabolism. The functional significance of altered plasma amino acid concentrations due to changes in vitamin B6 status remains to be determined.

Vitamin B6 status has been found to have a significant effect on the expression of several of the steroid hormones involved in the induction of amino acid metabolizing enzymes through a non-enzymatic role in steroid–receptor–binding affinity (Allgood and Cidlowski, 1991; Compton and Cidlowski, 1986). Adrenalectomy in rats has been shown to cause almost complete depletion of liver vitamin B6 (David and Kalyankar, 1986). This latter relationship implies a feedback loop involving steroid induction of vitamin B6 metabolizing enzymes, i.e., pyridoxine phosphate oxidase and pyridoxal kinase, to allow production of PLP which then is involved in steroid–receptor–binding affinity necessary for induction of enzymes, many of which are vitamin B6 dependent. Evidence for this hypothesis is found in the study of DiSorbo and Litwack (1981), who reported an inverse relationship between intracellular levels of PLP and the ability of glucocorticoids to induce the hepatic enzyme tyrosine aminotransferase. This potential mechanism needs to be more fully explored in terms of any potential impact on the safety of amino acid supplementation.

The direct impact of vitamin B6 status on amino acid loading has been studied for only tryptophan and methionine. In both cases the load test has been suggested as a sensitive and specific measure of vitamin B6 status (Sauberlich et al., 1973). There are no studies of the impact of vitamin B6 status on the long-term use of supplemental tryptophan, methionine, or any other amino acid. Similarly, there are no studies of the impact of long-term amino acid supplementation on vitamin B6 status.

Because of the critical role that vitamin B6 plays in amino acid metabolism, the dietary requirement for this essential nutrient is tied to protein intake; consequently, the dietary requirement for vitamin B6 increases as protein intake increases. The RDA for vitamin B6, 2.0 mg/day for men and 1.6 mg/day for women is based on the upper levels of acceptable protein intake, i.e., twice the RDA for protein (126 g/day for men, 100 g/day for women) (National Research Council, 1989). Evidence of impaired vitamin B6 status has been reported in some population groups such as elderly and alcoholic individuals (National Research Council, 1980).
The relationship of vitamin B6 status to the safety of particular amino acid supplements will be discussed in Chapter V.

F. SUMMARY

The preceding discussion reflects the maintenance of homeostasis when balanced amounts of amino acids are ingested in the form of dietary protein. Few data are available to demonstrate how these systems respond to ingestion of single or incomplete mixtures of amino acids, as a single bolus or, more importantly, on a chronic basis. Chapter V will cover available information on selected aspects of metabolism and safety of individual amino acids.
V. REVIEW OF INFORMATION AVAILABLE FOR EVALUATION OF SAFETY OF INDIVIDUAL AMINO ACIDS

A. APPROACH OF THE EXPERT PANEL

The ad hoc Expert Panel examined the scientific literature on amino acids and assessed the adequacy of those data for making an evaluation of the safety of amino acids used as dietary supplements by humans. In surveying the literature, the Expert Panel attempted to focus on well controlled studies that involved chronic administration (preferably by the oral route) of amino acids in amounts that exceeded the World Health Organization (1985) requirements for adults for indispensable amino acids (see Table 2) and in amounts of 0.5 g or more for dispensable amino acids. The Panel members concentrated on the sufficiency of available data on healthy adults in regard to amino acid safety. In their opinion, such an effort was required to undergird any regulatory effort to determine whether amino acids are safe for use as dietary supplements for persons who might consume amino acids for reasons other than diagnosed medical need.

Epidemiological information collected after the occurrence of EMS associated with consumption of L-tryptophan supplements indicated that some individuals had used L-tryptophan for as long as 8 to 10 years (Blauvelt and Falanga, 1991; Slutsker et al., 1990). Thus, published reports of controlled animal or human studies in which amino acids were administered orally over a long term at the above dosage levels were regarded as most directly applicable to the safety of amino acids used as dietary supplements. For the majority of amino acids, these types of studies were not available, necessitating consideration of studies in which amino acids were given in single doses or by other routes of administration and studies that lacked adequate controls.

Many studies of the efficacy of amino acids as treatments for diseases or disorders were found in the published literature. In most of these studies endpoints pertained solely to determination of treatment efficacy. Much of this literature includes studies not conducted with use of Investigational and New Drug Application protocols. Thus, it was not always evident in reports of clinical studies of efficacy, whether the investigators observed no side effects that might bear on safety or simply did not include observations of side effects if they did occur. Further, when side effects were reported, it was usually uncertain whether the observations were related to the disorder, the treatment, or synergistic effects. The more metabolically compromised the subjects (e.g., cancer cachexia), the less relevant the information is to questions of safety of supplementation in healthy subjects. Because evaluation of efficacy as a therapeutic agent (drug use) is not consistent with evaluation of use as a dietary supplement (food use), only a limited number of these studies are cited in this report.

The extensive review on the effects of ingestion of disproportionate amounts of amino acids by Harper et al. (1970) was used as a source for information on animal studies published prior to 1969. The computerized databases of MEDLINE, TOXLINE, CANCERLIT, and DIALOG® were searched for scientific publications bearing on the safety of individual amino acids for the period 1970 to 1992. Oral and written submissions to the Life Sciences Research Office in response to the Federal Register notice of November 26, 1990 (Food and Drug Administration, 1990) provided a third source of information.

In its examination of these sources of information, the ad hoc Expert Panel concluded that separate reviews were needed for individual amino acids because of their unique structural properties, metabolic roles, and functions as stimuli for release or precursors of biologically active substances such as hormones and neurotransmitters. Aspartic acid and asparagine as well as glutamic acid and glutamine were considered separately because of the metabolic differences between the amino acids and their respective amides. An exception was made for the BCAA because few studies were found in which leucine, isoleucine, or valine were given singly and because of the common practice of administering these substances together.
Data on salts of amino acids are included in this review because dibasic and dicarboxylic amino acids are often available as their salts. For example, studies in which lysine hydrochloride was given are included because both the amino acid free base and the hydrochloride salt are sold as dietary supplements. The Expert Panel is aware that the accompanying anion or cation may produce effects on its own. For example, chloride salts of dibasic amino acids in large doses may induce hyperchloremia. Consequently, data associated with anionic or cationic effects are included in discussions of safety of each amino acid as appropriate.

Because both D- and L-isomers of amino acids may be found in dietary supplements, information on safety of both stereoisomers was considered in this review. Separate sections for D- and L-isomers were prepared when appropriate.

In summary, the approach of the ad hoc Expert Panel in evaluating the extant scientific literature weighed the following factors in regard to the usefulness and applicability of the data to the safety of amino acids used as dietary supplements. That is, greater weight was given to human studies than to animal studies and to experimental protocols that included:

- indication of the physical and chemical properties of the administered substance(s);
- oral administration at doses exceeding levels of nutritional need where known;
- long-term rather than short-term administration;
- use of healthy subjects rather than patients with known diseases or disorders;
- adequate experimental controls; and,
- relevance of effects reported in terms of mechanism, reversibility, and duration.

The ad hoc Expert Panel also considered in its evaluation process the number of actual studies conducted, and the consistency of these studies where doses and subject characteristics were comparable. Finally, the ad hoc Expert Panel utilized the schemata developed in Chapter VII as a theoretical model for evaluation of amino acid safety.

B. ORGANIZATION OF REVIEWS OF INFORMATION ON INDIVIDUAL AMINO ACIDS

Reviews of information on individual amino acids are presented for indispensable and dispensable amino acids in Sections C and D of this Chapter, respectively. Each review consists of background information, selected animal and human studies, and summary and conclusions. Background information in each of the reviews includes only a brief description of information relating to aspects of metabolism and function that the available studies suggest may be related to safety. When available, the experimental endpoints in the models developed in Chapter VII were considered in evaluation of data on each amino acid. Thus, endpoints for animal studies include investigations of changes in food intake, nitrogen balance, and body weight; biochemical, hematologic, endocrine, and behavioral changes; vitamin B6 status; acute pharmacokinetic studies; functional assessments and gross pathology studies; and teratology and developmental studies. Similarly endpoints for human studies include investigation of changes in food intake and body weight; biochemical, hematologic, endocrine, and behavioral studies; vitamin B6 status; acute pharmacokinetic studies; functional assessments; and studies of inborn errors of metabolism. The summary and conclusions section presents the opinion of the ad hoc Expert Panel on the adequacy of the information reviewed for evaluation of the safety of the amino acids ingested as dietary supplements and for specification of safe levels of intake where possible.

Abbreviations used in this Chapter are listed on p. xi. Amounts of amino acids added to animal diets are expressed as percent (by weight) of diet. Doses of amino acids given in bolus form are usually expressed as g/kg body weight. Chemical formulas and nomenclature for amino acids may be found in Appendix B.
C. REVIEWS OF INFORMATION PERTAINING TO THE SAFETY OF INDIVIDUAL INDISPENSABLE AMINO ACIDS

1. Branched–chain amino acids (BCAA)

   a. Background

   The BCAA (leucine, isoleucine, and valine) are required for protein synthesis and are metabolically active as either glycogenic or ketogenic compounds. Unlike many other amino acids, they do not act as precursors for other biologically active compounds other than their α-keto analogues. BCAA comprise approximately 35 percent of the indispensable amino acids of muscle, about 40 percent of the preformed mammalian amino acid requirement, and they make up approximately 50 percent of the indispensable amino acids in the food supply (Harper et al., 1984).

   The estimated daily requirements of adults for the BCAA (World Health Organization, 1985) are 14, 10, and 10 mg/kg body weight for leucine, isoleucine, and valine respectively (see Table 2). There is an ongoing debate with regard to these levels of intake as a result of the contention of Young et al. (1989) that studies of amino acid oxidation, as opposed to traditional studies of nitrogen balance, reflect a higher physiological requirement; consequently, this group proposed a daily intake of approximately 20 mg/kg body weight for valine and isoleucine and 40 mg/kg body weight for leucine. The current U.S. guidelines (National Research Council, 1989) accept the WHO standard (World Health Organization, 1985). In a series of studies, Pelletier et al. (1991a,b) examined the impact of individual BCAA on the physiological requirement for other BCAA. Their findings supported the contention of Young et al. (1989) that the requirements for all BCAA should be higher than the World Health Organization's standards.

   According to Harper et al. (1984), three unique features of BCAA distinguish them from other amino acids:

   (1) The initial enzymes in BCAA catabolism are regulated differently than the enzymes that catabolize other amino acids;

   (2) One or more of the BCAA may exert specific regulatory effects on tissue protein metabolism (both anabolic and catabolic processes); and,

   (3) BCAA compete with other amino acids, i.e., aromatic amino acids, for transport mechanisms and this competition can change amino acid availability and subsequent neurotransmitter synthesis.

   These unique aspects of BCAA metabolism are discussed below in order to provide perspective for review of studies related to the safety of BCAA dietary supplements.

Absorption and transport: After a protein meal, BCAA may be absorbed as either free amino acids or as constituents of peptides. The absorption of the BCAA is accomplished via the neutral amino acid carrier system (Alpers, 1987; Hopfer, 1987); although Danner and Elsas (1989) note that leucine transport across the brush border may be accomplished by either sodium–dependent or sodium–independent systems. Movement across the basolateral membrane occurs in a manner similar to other membranes using the sodium–independent system (see Chapter IV). The BCAA compete with other large neutral amino acids (LNAA) for transport across membranes, a factor that has particular relevance to brain neurochemistry (Anderson and Johnston, 1983).

Metabolism: The initial step in catabolism of all BCAA is transamination to form corresponding branched–chain keto acids (BCKA). Each BCKA then undergoes an irreversible decarboxylation to yield acyl CoA derivatives via the action of the mitochondrial enzyme complex, branched–chain ketoacid dehydrogenase (BCKAD). Each of these BCKA are subsequently converted to their respective end–products by the action of specific catabolic enzymes. The end products of isoleucine
catabolism are propionyl CoA and acetyl CoA (which makes it ketogenic and glucogenic), leucine yields acetoacetate and acetyl CoA (making it exclusively ketogenic), and valine is catabolized to succinyl CoA (which makes it glucogenic) (Harper et al., 1984).

BCAA—transaminase (BCAAT) is a ubiquitous, vitamin B6—dependent enzyme found in lowest concentrations in the mammalian liver (Ichihara and Kayama, 1966). In addition to the conversion of BCAA to their respective BCKA, the BCAAT reaction catalyzes the conversion of α-ketoglutarate to glutamate. BCAAT accepts all three BCAA as substrates and is located in both cytosol and mitochondria (Harper et al., 1984).

Ichihara et al. (1975) identified three different BCAAT (Types I, II, and III) in mammalian tissues that differ with respect to organ and cellular location (cytosol versus mitochondria), BCAA substrate specificity, and species specificity. For example, Type II is found only in rat liver and is specific for leucine. Another aminotransferase that accepts leucine and methionine has been found only in rat liver mitochondria. In a survey of human tissues, BCAAT I and III only were found, with Type I being the predominant form in most tissues except brain (Goto et al., 1977). The rat also has a cytosolic ketoleucine—preferring oxygenase in the liver and kidney that provides an alternate pathway for leucine catabolism. As with rat mitochondrial liver aminotransferase, BCAAT II also accepts methionine as a substrate (Harper et al., 1984). The existence of these specific enzymes found only in rat liver raises questions about species specificity and the validity of this animal model for the study of BCAA metabolism.

BCKA—dehydrogenase (BCKAD) is a multi—enzyme complex with three catalytic subunits: thiamin pyrophosphate—dependent α-keto acid decarboxylase, dihydrolipoyl transacetylase, and FAD—dependent dihydrolipoyl dehydrogenase (Harper et al., 1984). Additional cofactors for the reactions are CoA, nicotinamide adenine dinucleotide (NAD) and calcium and magnesium for optimal activity (Harper et al., 1984). BCKAD activity is highest in liver followed by kidney and heart and relatively low in muscle, adipose tissue, and brain (Harper et al., 1984). The differences in relative BCKAD activity are the result of tight regulation by two mechanisms: a reversible kinase/phosphatase process involving the deactivation or activation of the decarboxylase subunit by the addition (deactivation) or subtraction (activation) of an ATP—derived phosphate group (Danner and Elsas, 1989), and end—product inhibition involving competitive inhibition by NADH and branched—chain acyl CoA derivatives (Harper et al., 1984).

In rat skeletal muscle, the BCKAD is almost totally in the inactive form; consequently, BCAA taken up from the circulation are transaminated and subsequently released for metabolism in other tissues containing the active forms of the enzyme, i.e., liver, kidney, and heart (Skeie et al., 1990). In human skeletal muscle, there is a higher proportion of active BCKAD so that a larger amount of BCAA may be catabolized (Skeie et al., 1990). However, comparative studies between humans and rats have demonstrated that the BCKAD activity is minimal in human muscle and maximal in the liver (Wagenmakers and Veerkamp, 1982). It is generally assumed that in light of relatively low BCKAD activity, the primary function of BCAA catabolism in muscle may be to provide glutamine (Harper et al., 1984; Skeie et al., 1990). As with the BCAAT, the species specificity issue related to BCKAD raises questions about the wisdom of cross species extrapolation of study results.

Of the BCAA, leucine has been shown to be the most metabolically active in terms of not only its own effects but in the regulation of other amino acids as well (Hagenfeldt et al., 1980; Hambraeus et al., 1976; Harper et al., 1984). Leucine may be involved in the regulation of protein turnover in muscle (Buse and Reid, 1975; Elia and Livesey, 1983) and has been shown to be a potent stimulator of insulin release (Eriksson et al., 1983) and tissue insulin sensitivity (Frexes—Steed et al., 1990). The release of somatostatin, glycogen, and zinc is also influenced by leucine (Danner and Elsas, 1989).

It should be noted that while the BCAA do not act as precursors for neurotransmitters, they can influence the chemical balance of the central nervous system (CNS) through their competition with
other amino acids for transport carrier across the blood–brain barrier. Fernstrom et al. (1973) demonstrated that brain tryptophan concentrations in rats correlate with the ratio of plasma tryptophan to other LNAA sharing the L-transport system. Hagenfeldt et al. (1980) also demonstrated a competition between leucine and the other BCAA for transport into the brain. Li and Anderson (1987) outlined the interaction between dietary carbohydrate and transport of LNAA into the brain. The functional ramifications of these interactions remain to be determined in humans.

b. Animal studies

Change in food intake and body weight: Consequent to their competition with other LNAA (i.e., tryptophan, tyrosine) for the shared blood–brain carrier system, BCAA have been studied for potential impact on various physiological functions. One area that has received considerable attention is the impact of BCAA and changes in the ratios of tryptophan/tyrosine:BCAA ratio, on macronutrient selection. Based largely on the work of Ashley and Anderson (1975) the premise is that protein intake is regulated by and is inversely proportional to the plasma ratio of tryptophan:LNAA.

Anderson et al. (1990) found that in rats allowed to choose between ad libitum diets of 10 percent and 50 percent casein in agar blocks, or 25 percent and 50 percent casein diet, addition of BCAA (in amounts equivalent to that in the 50 percent casein diet) to the 10 percent casein diet caused the rats to change their preference from the low–protein to the high–protein diet. This was not the case when the 25 percent casein diet was used instead of 10 percent. In rats given no choice, the addition of BCAA to a 15 percent casein diet resulted in depressed intake; however, when given the choice between 15 percent plus BCAA versus 15 percent alone, the animals chose the latter. BCAA addition lowered plasma and brain concentrations of all indispensable amino acids except the BCAA. Anderson et al. (1990) concluded that high plasma and brain concentrations of BCAA were inconsistently associated with protein selection.

In subsequent studies, Peters and Harper (1985, 1987) demonstrated that protein intake is not sensitive to changes in neurotransmitter concentrations or ratios of precursors to other LNAA. However, they pointed out that there may be an association with amino acid levels in general, such that protein intake is somehow regulated within a defined range by the levels of indispensable amino acids in the brain and through the induction of catabolic enzymes. Perhaps this regulation occurs through the bathing of the hypothalamus (which lacks the blood–brain barrier) and consequent release of hormones (e.g., cortisol) capable of enzyme induction. Interestingly, Block and Buse (1990) reported that both acute and chronic administration of glucocorticoid (cortisone acetate) resulted in an increase in skeletal muscle BCKAD activation and increased BCAA oxidation. The impact of supplemental BCAA on the hypothalamic control of glucocorticoid release has not been explored.

While feeding equimolar amounts of BCAA has not been associated with changes in protein intake (Peters et al., 1983), the antagonism between the BCAA has been studied for its potential deleterious effect on both growth and appetite. The antagonism between the BCAA has been described by Harper, who stated that an excess of any 1 BCAA increases the requirement for the other 2 (Harper et al., 1970).

The most widely characterized of these potential antagonisms is the relationship between excess leucine and the other two BCAA. Harper et al. (1984) suggested that the low plasma levels of isoleucine and valine associated with excess leucine in animals fed low–protein diets are the result of increased catabolism. Block (1989) stated that leucine has a direct effect on BCAA metabolism probably occurring predominantly in extrahepatic tissues, especially skeletal muscle. This contention is supported by May et al. (1991) who demonstrated increased catabolism of valine in muscle of rats.
fed a 9 percent protein diet supplemented with 10 percent leucine. May et al. (1991) also noted that while there was an increase in BCAA catabolism in muscle, the incorporation of BCAA into new muscle protein was unaffected and that the depression in growth in the leucine–fed rats was due to some undefined mechanism. Although protein intake may not be specifically regulated by BCAA, the creation of an amino acid imbalance with supplemental BCAA has been shown to depress appetite (Block, 1989). The appetite-suppressing effect of such an imbalance is presumed to be due to a depletion of brain amino acid pools (Harper et al., 1984). The appetite suppressing impact of excess leucine was demonstrated by Feng et al. (1973).

Block (1989) summed up the leucine:BCAA antagonism as an order–dependent sequence involving 1) elevation of the total body pools of free leucine and α–keto isocaproic acid, 2) depletion of the total body free pools of isoleucine and valine, and 3) depression of food intake and growth. It should be emphasized that the antagonism of BCAA for each other, however, has only been demonstrated when the total dietary protein intake is marginal or low (Harper et al., 1984). It should be noted that in the study by Anderson et al. (1990), rats fed a 15 percent casein diet reduced their intake when BCAA were added, and when given a choice between low protein and BCAA and low protein alone, chose the latter. Consequently, based on the animal studies done to date, only in those situations where the adequacy of protein intake is marginal do the potential detrimental effects of a BCAA imbalance from supplementation become a concern with regard to growth and dietary intake.

The D–isomers of the BCAA do not suppress growth in rodents (Borg and Wahlstrom, 1989).

Behavioral studies: A large body of evidence has accumulated regarding the hypothesized impact of fluctuations in the ratio of the various competing LNAA on brain function. (See also sections on tryptophan and tyrosine.) Most of the studies in this area have involved changes resulting from manipulations in dietary macronutrients, i.e., carbohydrate or protein or supplementation with the monoamine precursors, tryptophan and tyrosine, rather than the BCAA. Aside from reports on changes in concentrations of amino acids, no studies were found of the impact of BCAA supplementation on behavior or cognition in animals.

Biochemical studies: As discussed above, several studies have explored the impact of BCAA administration on levels of competing LNAA in plasma and brain. Peters et al. (1983) found that in adult rats receiving either 25 or 75 percent casein plus 4.5 percent equimolar mixture of BCAA, there was an elevation in both plasma and brain BCAA content, with no significant changes in concentrations of other indispensable amino acids. There was a decrease in the plasma ratio of tryptophan:LNAA; however, this change was not associated with any shifts in protein intake.

In the experiments by Anderson et al. (1990), rats adjusted their intakes in response to BCAA additions at low levels of dietary protein. BCAA levels were increased in all animals receiving supplementation, while brain and plasma concentrations of competing LNAA (phenylalanine, tryptophan, tyrosine, methionine) were lowered.

Block and Harper (1991) found that consumption of a 9 percent casein diet supplemented with either leucine or its α–keto acid, α–ketoisocaproate, caused reductions in plasma concentrations of isoleucine, valine, threonine, and tyrosine, and brain concentrations of LNAA (valine, isoleucine, phenylalanine, tyrosine, and methionine). Rats fed excess isoleucine had significant reductions in brain concentrations of leucine, phenylalanine, tyrosine, histidine, methionine, and threonine; whereas, diets with high valine content depressed brain concentrations of histidine, phenylalanine, tyrosine, and threonine but had no effect on the other BCAA. There was no documentation of, or testing for, any functional effects associated with these changes.

The direct impact of BCAA administration on brain neurotransmitter synthesis has been the subject of much discussion (Anderson, 1981). Agharanya and Wurtman (1982) found a significant lowering of serum, heart, and brain tyrosine concentrations in adult male rats given intraperitoneal injections of either isoleucine or valine. Neither isoleucine nor valine had any effect on adrenal tyrosine
content. Concurrent administration of BCAA along with tyrosine attenuated the tyrosine–induced rise in tissue tyrosine content. BCAA tended to decrease urinary excretion of catecholamines (isoleucine significantly reduced urinary epinephrine). Both isoleucine and valine caused nonsignificant rises in brain catecholamines when compared to control levels. Simultaneous dosing with tyrosine and BCAA resulted in reduced catecholamine excretion as compared with tyrosine alone. The relevance of changes in urinary excretion of neurotransmitter to brain metabolism is not clear; moreover, there were no assessments of any functional parameters of either peripheral or central catecholamine metabolism.

**Vitamin B6 studies**: No studies were found of an impact of either acute or chronic feeding of excess BCAA on vitamin B6 metabolism. Similarly, there were no studies of the impact of varying intakes of vitamin B6 on the metabolism of either normal or excess amounts of BCAA.

Shiflett and Haskell (1969) evaluated the changes in activity of leucine transaminase consequent to a vitamin B6 deficiency in chicks. After 8 days of diets supplemented with either 0 or 1.2 mg pyridoxine hydrochloride/100 g diet, chicks on the deficient diet showed overt signs of a vitamin B6 deficiency. After 1.5 days of deficient diet, enzyme activity was significantly decreased in kidney, significant decreases were seen at 4 days in liver and heart and after 9 days in brain. There was no change in enzyme activity in pair-fed controls. In vitro stimulation of enzyme activity by addition of the coenzyme PLP increased activity but did not restore it to control levels. Repletion of chicks fed the deficient diet for 8 days with vitamin B6 resulted in increased activity after 1 day and restoration of activity to control levels in chicks repleted for 5 days. In the latter repletion study, the chicks were pair-fed with the deficient animals so that the increase in activity was not a factor of food intake.

**Teratology and developmental studies**: Thoemke and Huether (1984) bred rats for three consecutive generations on diets enriched with either L–tryptophan (10 g/kg diet), L–phenylalanine (20 g/kg diet), L–tyrosine (20 g/kg diet), or BCAA (10 g/kg diet for each BCAA). Comparisons were made among amino acids and with standard control diet without additional amino acids. The protocol was started with adult rats of both sexes 2 weeks before mating and continued through 3 generations (F1, F2, F3). All litters were reduced to 8 pups (4 males and 4 females). At 5, 10, 15, and 20 days postpartum, 1 male and 1 female from each treatment litter were sacrificed. Remaining viable pups were separated from dams at 5 weeks and bred at 10 weeks. Parameters measured were brain weight, amino acid concentrations in serum, brainstem, and whole brain, and brainstem concentrations of putative neurotransmitters (glycine, glutamate, aspartate, γ-amine butyric acid [GABA], and taurine).

BCAA feeding caused significant increases in serum glycine and decreases in tryptophan and tyrosine in dams. There were no significant differences in serum concentrations of amino acids in either F1 or F2 generations of dams fed amino acid imbalanced diets. There were no significant changes in BCAA levels in any generation. Serum concentrations were reported only for day 5.

Diets supplemented with BCAA caused significant decreases (90 percent of control values) in brain weight at days 5 and 10 postpartum in the F1 generation; there was compensatory gain by day 20 back to normal values. Brain weights of F2 and F3 generations were significantly decreased from day 5 through day 20 postpartum in BCAA pups. While the brain weight in the F2 and F3 pups were less than controls at all 3 time periods, the pattern of brain growth showed most severe decline by day 10 followed by an increase in size by day 20.

Neurotransmitter concentrations were decreased in brainstem tissue of BCAA–fed rats of all three generations. There was a significant increase in concentrations of both aspartate and GABA at 20 days postpartum in the F2 generation only. Aspartate was the most significantly decreased amino acid neurotransmitter at day 5 in all generations. There were no measures of any functional changes associated with any of the reported anomalies; similarly, there was no mention of the impact on reproductive performance or lactation in succeeding generations of rats fed amino acid–supplemented diets (Thoemke and Huether, 1984).
Matsueda and Niyama (1982) examined the impact of excesses of several individual amino acids including valine, isoleucine, and leucine on maintenance and outcome of pregnancy in the rat. Rats were fed a 6 percent casein diet supplemented with 5 percent of each amino acid. There were four control groups given the 6 percent diet in amounts matched to pair-fed amino acid groups. The method for arriving at the amount of diet in the pair-fed groups was not described.

Only 11 out of 20 possible pregnancies were maintained in the rats fed excess L-leucine (2 out of 10) and L-isoleucine (9 out of 10). L-isoleucine fed in excess had no significant effect on maternal food intake or body weight gain. While both pair-fed controls and the L-valine excess group were significantly smaller than ad libitum controls as a result of significantly reduced intake, the addition of L-valine to the 6 percent casein diet led to significant increases in both intake and maternal body weight gain when compared to pair-fed controls. The L-leucine group lost weight (~7 g) during the trial but not as much as pair-fed controls (~20 g). Maternal plasma concentrations of all amino acids measured did not differ from the 6 percent casein control group fed ad libitum.

All fetal weights in the amino acid excess groups were less than those in ad libitum controls; however, while fetal weight in the L-isoleucine and L-valine groups were less than their respective pair-fed controls, fetuses exposed to L-leucine were larger than those in pair-fed controls. This same relationship was evident in the analysis of fetal brain weight. In all 3 BCAA fed groups there were large increases relative to ad libitum controls in brain concentrations of BCAA, histidine, and arginine (>700 percent, >500 percent, and >750 percent increases, respectively). Brain concentrations of other amino acids were also increased except phenylalanine, glutamate, and aspartate. Phenylalanine concentrations were decreased below those of ad libitum control values in the excess valine group.

Carcinogenicity studies: Kakizoe et al. (1984) studied the effects of BCAA on bladder tumor promotion in rats with a short-term assay involving exposure of isolated bladder cells to the suspected carcinogen and measurement of concanavalin A-agglutination. This agglutination process is considered to represent an early stage of carcinogenesis. The test involved culturing isolated cells with low doses of the carcinogen N-n-butyl-N(4-hydroxybutyl)nitrosamine (BHBN), followed by concanavalin A-treatment to maintain agglutinability. The ability of a suspected substance to maintain agglutinability (which would in the absence of a promoter disappear over time) would be indicative of cancer promotion.

Three groups, each with 5 rats, were given the following treatments: 0.01 percent BHBN in drinking water for 1 week, normal diet; 0.01 percent BHBN in drinking water for 1 week followed by normal diet supplemented with 2 percent of L-isoleucine, leucine (D and L), valine (D and L), and L-leucyl-L-leucine for 3 weeks; or water with control diet supplemented with amino acids from weeks 2 to 4. The first treatment was in the form of a pellet diet as opposed to the other two which were in powdered form. In this short-term study, L-isomers of all BCAA maintained and increased agglutinability in cultured bladder cells.

In a long-term study by the same group (Kakizoe et al., 1984), 6-week-old rats were exposed to BHBN and supplemented with L-isoleucine or L-leucine and compared with controls after a 40-week observation period. Incidence of papilloma, a precancerous condition, was found in all groups given pelleted diets supplemented with isoleucine and leucine and the control group (the incidence was 11.1 percent, 23.3 percent, and 16.7 percent for isoleucine, leucine, and controls, respectively). Tumors were found only in animals receiving BHBN plus L-isoleucine and L-leucine, not in controls plus BHBN. The calculated incidence of cancer in isoleucine plus BHBN group was significantly different from control plus BHBN group.

In a follow-up study, Nishio et al. (1986) extended the experimental period from 40 to 60 weeks and included diets supplemented with 2 or 4 percent L-isoleucine or L-leucine. As in the earlier reports, the incidence and numbers of carcinomas were significantly higher in groups treated with BHBN plus amino acids than in animals treated with BHBN or amino acids alone. The higher levels
of both L-isoleucine and L-leucine were associated with a higher incidence of carcinoma; 77 percent of the 4 percent L-isoleucine versus 46 percent of 2 percent group and 74 percent of the 4 percent L-leucine groups versus 52 percent of 2 percent group had carcinomas. No carcinomas, papillomas, pre-neoplastic hyperplasia, or simple hyperplasia were reported for the control group or the groups, receiving amino acids alone.

c. Human studies

Use in special clinical populations: BCAA alone or in BCAA-enriched amino acid mixtures have been used in treatment of a variety of disorders in metabolically compromised patients. Several studies have utilized relatively large daily doses (e.g., 0.24 g/kg body weight for extended time periods). Reports of clinical trials often note presence or absence of toxicity of treatments. Thus, they provide some, albeit limited, information on safety considerations.

Historically, BCAA were first used therapeutically in chronic liver disease and encephalopathy to reduce brain uptake of aromatic amino acids and to raise the low circulating concentrations of BCAA that adversely affect protein nutrition (Marchesini et al., 1990). BCAA have also been used prophylactically in treating other metabolic disorders such as control of plasma phenylalanine concentrations in children with phenylketonuria (PKU) (Berry et al., 1990), hepatic failure and onset of hepatic encephalopathy (Skeie et al., 1990), cancer cachexia (Hunter et al., 1989), as well as metabolic stress in adults (Bower et al., 1985) and children (Coran and Drongowski, 1987).

For example, Marchesini et al. (1990) followed 30 patients with diagnosed chronic hepatic encephalopathy treated with enterally administered supplements containing 0.24 g/kg body weight BCAA in 3 doses daily. Twenty of the patients were treated for 6 months. The investigators monitored portal–systemic–encephalopathy indices as well as blood amino acid levels, nitrogen balance, various liver function tests, and mental state. The report of the study contains no reference to adverse health effects or toxicity of the BCAA treatment over the control (casein) treatment.

Similarly, BCAA have been used in parenteral nutrition of patients with sepsis, stress, and injury. In a report of a workshop on these uses, Brennan et al. (1986) makes no mention of adverse side effects from parenteral solutions containing 12.75 g of nitrogen with up to 50 percent of the amino acid nitrogen as BCAA for periods of several days.

Skeie et al. (1990) reviewed the clinical utility of using BCAA for treatment of various disorders and diseases. While the review provides extensive documentation of the consequences of BCAA administration and issues related to establishing therapeutic efficacy, Skeie et al. (1990) did not mention occurrence of side effects or adverse effects as a parameter of interest in the studies they reviewed. Gil et al. (1990) reported that parenteral infusion of BCAA–enriched solutions increased appetite and food intake in normal subjects. Skeie et al. (1990) suggested that this may occur by decreasing brain uptake of tryptophan, the precursor of serotonin.

Appropriate interpretation of these studies of clinically compromised patients is difficult. On the one hand, BCAA treatments have included relatively high doses both parenterally and enterally for extended periods of time. Indications of adverse effects, i.e., concerns with safety, are essentially absent. On the other hand, the patient populations studied have diagnosed disorders and diseases that are severe in terms of deviation from normal health. Few studies have included BCAA treatment of healthy control populations. Thus, the data available do not raise additional concerns for adverse health effects, but are of limited value in prediction of consequences of the use of BCAA supplements by healthy adults.

Studies of healthy subjects: The use of BCAA for the enhancement of physical performance is an area of research that has focused attention on the use of BCAA in healthy individuals. The
rationale for the focus on BCAA during exercise is based on three primary lines of evidence. Data from exercise studies have shown that BCAA are released from the splanchic bed and taken up by active muscle rather than by the liver. The first step in BCAA oxidation is catalyzed by BCAAT which is found in highest concentrations in muscle, and under catabolic conditions analogous to exercise (e.g., fasting or metabolic stress), BCAA oxidation rates increase (Hood and Terjung, 1990).

Another rationale for BCAA supplementation during exercise has been advanced by Newsholme et al. (1991) who contend both that mental and physical fatigue are caused by increases in CNS concentrations of serotonin. These researchers further postulate that this increase is associated with increased oxidation of BCAA leading to increased clearance from circulation resulting in a concomitant increase in the ratio of tryptophan:BCAA. This latter effect results in a preferential situation favoring tryptophan entry into the CNS via the L-amino acid carrier system and eventual increases in brain serotonin concentrations.

To test this BCAA-fatigue hypothesis, Blomstrand et al. (1991) gave 7.5 or 16 g BCAA in a drink to male subjects running either a 30 km cross-country (n = 25) or marathon racers (n = 193), respectively. Each group was divided into challenge and placebo groups. Blood samples were taken before and after the race from 25 cross-country and 26 marathon racers and assessed for plasma amino acids, glucose, and free fatty acids. Sixteen randomly selected cross-country racers were given the Stroop Test to measure "basic psychological and cognitive processes."

There was no difference in performance of the placebo group on the psychological test before or after the cross-country race; however, the performance after the race of those receiving BCAA improved significantly on specific aspects of the test. There was a significant increase in only plasma valine in the cross-country BCAA group. BCAA concentrations were decreased in both placebo groups. Plasma tryptophan was decreased post–race in the placebo cross–country and both cross–country and marathon runners who received BCAA. There was no difference in tyrosine and phenylalanine concentrations in the marathoners on BCAA, while both amino acids increased in the placebo group. The test and placebo drinks were administered during the race; there was no accounting for compliance or accuracy of intakes. There was no mention of adverse effects nor any assessment of other potential functional changes.

Blomstrand et al. (1991) examined the impact of BCAA supplementation on mental performance of 6 female soccer players given either placebo (a 6 percent carbohydrate drink) or challenge (7.5 g BCAA) during 2 soccer games (1 week apart) in a crossover blinded design. The average amount of drink consumed per subject was 1.3 L which at 7.5 g BCAA/L meant an average intake of 9.75 g BCAA. All subjects were given a psychological test 2 to 2.5 hours before and within 45 minutes after each game. Five subjects had blood drawn at the same time.

There was no change in psychological performance in players given placebo; however, there was a significant improvement in all three parts of the test after exercise with the BCAA treatment. There were numerous ambiguities (i.e., timing of the challenge, lack of analysis by normal protein intake or other potential confounding variables) in the design of this and the previous experiment that minimize their external validity. As in the previous study, no adverse effects were noted or monitored. It should be noted that in the first study Blomstrand et al. (1991) examined only male athletes with conflicting results while in the second report (Blomstrand et al., 1991), all subjects were female and the results were more uniform and consistent. The possibility of a sex-dependent differential response to BCAA has not been explored to date and would be of interest in the examination of potential safety concerns with regard to BCAA supplementation.

Among the BCAA, leucine has received the most attention with regard to its role in exercise because its oxidation rate is quantitatively higher than the other BCAA and because it is associated with the release of glucogenic precursors from muscle (Hood and Terjung, 1990). There may be differences in leucine oxidation that are dependent on the type of exercise performed, i.e., endurance versus resistance training.
Tarnopolsky et al. (1991) gave a total of about 3 g of infused (1 mg/kg body weight/hour for 5 hours) and ingested (6.6 mg/kg body weight/hour for a mean total of an additional 2.6 g) to 6 subjects (mean weight of 77.3 kg) performing circuit resistance training. Blood samples were taken before and incrementally after completion of a series of resistance exercises on a weight machine. Breath analyses were also performed with calculations of $^{13}$CO$_2$ enrichment, VO$_2$, and VCO$_2$. There was no control group or placebo condition reported. Tarnopolsky et al. (1991) found no significant effects on leucine oxidation or flux, whole body protein synthesis, or the rate of appearance of endogenous leucine from the infusion of leucine during exercise. There was no mention of any side effects from the treatments used in this study; whether there was any attempt to document any side effects is not clear. There were no other assessments of functional changes, e.g., hematology, plasma amino acid concentrations, or behavioral changes performed in this study.

Hagenfeldt et al. (1980) administered L-leucine to healthy post-absorptive subjects as a continuous infusion. Three studies were performed. The first measured splanchnic and leg metabolism with L-leucine infusion of 39.3 mg/minute (total dose over 150 minutes was about 5.9 g); the second examined renal clearance of amino acids in response to a 2-hour leucine infusion (dose of about 4.7 g at 39.3 mg/minute); and, the third was a study of brain amino acid exchange (dose equivalent to the second study). The infusions resulted in continual declines in blood concentrations of isoleucine (~55 percent of baseline), methionine (~55 percent of baseline), valine (~40 percent of baseline), tyrosine (~35 percent of baseline), and phenylalanine (~35 percent of baseline). There was no discernible affect of leucine infusion on renal clearance of other amino acids. There was a reduction in the brain uptake (determined via jugular catheterization) of isoleucine and methionine compared to baseline. There was no assessment of any potential functional changes associated with these metabolic responses to leucine infusion. These investigators reported that the leucine infusion was well tolerated by all subjects and there were no subjective symptoms during or after the trials.

Eriksson et al. (1983) also studied the impact of leucine infusion on intermediary metabolism in normal healthy subjects. This investigation involved 18 healthy males divided into 3 groups. The first received a continuous infusion of L-leucine (39.3 mg/minute for 150 minutes, total about 5.9 g). The second group received L-leucine at the same dose as the first group with addition of 2.2 mmol glucose/minute for 150 minutes, and the third group received L-leucine plus somatostatin (7 µg/minute) plus glucagon (1 ng/minute) for 150 minutes. Arterial, femoral venous, and hepatic venous blood samples were collected at timed intervals before and during the infusions for analysis of amino acids, insulin, glucose, and ketone bodies. In subjects receiving only leucine, there was a significant rise in insulin and glucose, and significant decreases in whole blood concentrations of many amino acids. The most pronounced amino acid changes were decreases in valine, isoleucine, methionine, tyrosine, and phenylalanine; there were also significant decreases in threonine, serine, glycine, alanine, and histidine. Infusion of leucine alone resulted in significant splanchnic uptakes of valine and isoleucine. Glutamate uptake in the legs decreased significantly during infusion of leucine. There were no side effects reported, nor was there any mention of attempts to assess any functional changes aside from the measures studied.

In a similar study, Abumrad et al. (1982) examined the effect of leucine infusion on substrate flux in an isolated muscle (forearm) in 13 healthy male volunteers. After an overnight fast subjects were infused with either saline or L-leucine (mean dose 0.275 mg/kg body weight/minute). The average total dose based on a mean body weight of 68 kg for the 5 hour trial was approximately 5.6 g. Blood samples were taken at hourly intervals after baseline and commencement of infusions.

There were significant decreases in whole blood concentrations of glycine, serine, valine, isoleucine, tyrosine, GABA, and methionine. Among the amino acids studied, only leucine and glutamine levels increased significantly. There was a significant output of glutamine from forearm muscle and insulin levels increased by 82 percent over baseline in response to leucine. There was no mention or assessment of any side effects or changes in any other functional parameters.
In one small study of leucine metabolism in muscle, Elia and Livesey (1983) noted no adverse effects in 4 normal male subjects infused with 3.6 g of L-leucine. Again, as with the previous studies, there was no mention of untoward effects of leucine infusion, but no attempts were made to assess any parameters other than the measures studied.

Inborn errors of metabolism: Several metabolic disorders are associated with genetic anomalies in BCAA metabolism. Maple syrup urine disease (MSUD) is the most commonly occurring of these disorders. Although the incidence in the general population remains unknown, occurrence of MSUD in specific populations may range from 1/760 in an inbred Mennonite population to 1/290,000 in a predominantly white sample in New England (Danner and Elsas, 1989).

MSUD, also known as branched–chain ketoaciduria, is a heterogeneous disorder affecting the function of the BCKAD. Seven forms of MSUD have been identified. The distinguishing features of these phenotypic expressions are related to the age of onset, symptoms, and biochemical features (i.e., plasma BCAA levels, characteristics of BCKAD) (Danner and Elsas, 1989).

"Classic" MSUD, which may be diagnosed in the neonatal period or early infancy, is associated with plasma leucine levels of 1000–5000 μmol/L and BCKAD proteins are present (Danner and Elsas, 1989). In addition to the classic form, there are 2 forms, intermediate and intermittent, differentiated by age of onset, symptoms and plasma leucine concentrations, a thiamin–responsive form, and 3 forms, E1β, E2, E3, associated with deficiencies of BCKAD subunits. While the latter 3 forms are lethal, the prognosis associated with the first 4 forms can vary from death to mental retardation to normal development depending on the timing and amount of exposure to BCAA, age at which BCAA restriction is initiated, occurrence of such concurrent insults as infections, anoxia, or hypoglycemia, and effectiveness of continued dietary control (Danner and Elsas, 1989).

Several other disorders of BCAA metabolism are known collectively as the branched–chain organic acidurias. As described by Sweetman (1989), there are inherited deficiencies of all 4 enzymes involved in the catabolism of isovaleryl CoA derived from leucine, 1 disorder related to the catabolism of the isoleucine–derived metabolite 2–methylbutyryl CoA, and 2 disorders of valine catabolism. The biochemical defect, incidence, symptoms, outcomes, and treatment of these disorders were reviewed by Sweetman (1989).

d. Summary and conclusions

Endpoints: The primary index in both animal and human studies employing either individual or combinations of the branched–chain amino acids (BCAA) is either plasma or whole blood amino acid concentrations. The rationale is based on the competition among the BCAA themselves and with other LNAA, i.e., tyrosine, phenylalanine, methionine, and tryptophan. Functional correlates of these relationships have been changes in both total food and protein intake, growth, and changes in brain chemistry (e.g., changes in concentrations of specific neurotransmitter precursors such as tryptophan or tyrosine).

Other endpoints associated with BCAA supplementation in animals include developmental changes, (e.g., decreased brain weight and neurotransmitter concentrations) in successive generations of rats born of dams fed BCAA supplements. Decreases in viable pregnancies and fetal weight were also noted in rats fed excess L-leucine and L-isoleucine.

BCAA, particularly leucine and isoleucine, have been linked to increased risk of bladder cancer. Selected tests of short–term and long–term carcinogenicity have demonstrated a potential role for these amino acids as promoters of carcinogenesis in rats.
Safe levels of human intake: Studies in normal human subjects have employed amounts of individual and combined BCAA well in excess of the World Health Organization estimated requirements listed in Table 2. In the few studies that gave boluses of all of the BCAA together doses ranged from 7.5 g to 9.75 g. For perspective, a person consuming 100 g protein per day would consume about 17.4 g total BCAA. No side effects were reported; however, aside from the specific parameters studied, no attempts to characterize the response to the challenges were reported. The studies examining leucine’s effects infused the amino acid in amounts ranging from 386 mg to 5.9 g/infusion without any reported subjective side effects. However, there were significant changes in intermediary metabolism reported in all leucine infusion studies except the one at the lowest dose. Changes included differences in insulin sensitivity, plasma and brain flux, and concentrations of amino acids. While these changes occurred in apparently healthy individuals and were not associated with any untoward effects, they may be undesirable in certain groups such as pregnant women or individuals on psychotropic medications. Moreover, there was some evidence of gender differences in the response to oral BCAA supplementation. This is a topic that requires further investigation.

The Expert Panel was unable to find reports of studies performed specifically to examine the safety of acute or chronic use of oral supplements of BCAA in healthy humans. Reports of clinical trials often note presence or absence of toxicity of treatments. Thus, they provide some, albeit limited, information on safety considerations. Given the limited database from the studies that have used single boluses of either BCAA or leucine, it is not possible to establish a "safe" level of intake. However, there is a large body of evidence attesting to the impact of these indispensable amino acids, especially leucine, on metabolic processes in brain and other tissues, and development in animals and on the possible efficacy of BCAA as treatment modalities for various metabolic disorders and conditions. Further use of BCAA as dietary supplements by persons susceptible to potentially adverse effects, i.e., women of childbearing age, pregnant women, children, elderly, and individuals on psychotropic medications probably should be avoided.
2. Histidine

a. Background

Histidine is a basic amino acid whose indispensability in humans has not been definitively established. The average amount of histidine consumed by an adult male is approximately 1.6 g/day or 1.6 percent of total protein intake; the dietary requirement for adults as established by the World Health Organization (1985) is 0.5–0.84 g/day. (See Table 2.)

As reviewed by Levy (1989), histidine is indispensable in most animal species with the possible exception of man. Adult humans can apparently synthesize histidine at a low rate, perhaps from endogenous sources such as hemoglobin and carnosine (Sheng et al., 1977). Infants, however, require exogenous histidine for normal growth (Snyderman et al., 1963).

Histidine is transported across cell membranes on the L- or Leucine transporter in a competitive manner with the other LNAA (leucine, isoleucine, valine, methionine, tyrosine, phenylalanine, tryptophan). Using isolated rat brain slices, Lutz et al. (1975) demonstrated reductions in the uptake of histidine as a consequence of competition caused by an unbalanced mixture of amino acids, particularly the LNAA. In addition, Kilberg et al. (1980) have identified a sodium-dependent transporter for histidine, designated system N in rat hepatocytes. This system has been shown to be adaptive to differing physiological states; starvation causes stimulation which may be associated with the increase in serum and tissue histidine concentrations during malnutrition (Mercer et al., 1989).

In the course of its catabolism, histidine is converted ultimately to glutamate through a sequential process including the intermediates imidazolonepropionic acid, and formimino glutamic acid (FIGLU). FIGLU is finally converted to glutamate (which can then enter the TCA cycle after a PLP-dependent transamination) and formimino tetrahydrofolate in a reaction utilizing free tetrahydrofolate (TH$_4$). As pointed out by Benevenga and Steele (1984) the availability of TH$_4$ may be the rate-limiting step in the catabolism of histidine. One pathway for regeneration of TH$_4$ is via the conversion of methyl-TH$_4$ and homocysteine to methionine. Levy (1989) suggested that folic acid deficiency results in significantly increased excretion of FIGLU in response to a histidine load due presumably to reduced TH$_4$; this was supported by the studies by Kohashi et al. (1990).

Another physiologically significant interaction is the relationship between histidine and the trace minerals, zinc and copper. Histidine is a ligand for zinc and copper transport in mammalian blood (Hallman et al., 1971; Prasad and Oberleas, 1970). The latter study by Prasad and Oberleas (1970) documented the competition between amino acids, particularly histidine, and several circulating carrier proteins, (e.g., albumin, haptoglobin, transferrin and IgG) for binding of zinc. The amino acid-bound zinc is the form excreted in urine of animals and humans (Freeman and Taylor, 1977; Zlotkin, 1989).

The potent biogenic amine, histamine (a putative neurotransmitter and mediator of immune responses) is produced via the activity of the ubiquitous aromatic amino acid decarboxylase and histidine decarboxylase, both of which are PLP-dependent enzymes. The nature of the relationship between histidine decarboxylase and vitamin B6 was reviewed by Snell (1990). Administration of L-histidine results in increased levels of histamine in mammalian brains (Schwartz, et al., 1972). As mentioned previously, histidine competes with the other LNAA for blood–brain–barrier transport via the L-transporter; consequently, fluctuations in any of these competing amino acids can cause changes in brain histidine concentrations (Lutz et al., 1975).

D-Histidine was reported to support growth in rodents (Berg, 1953) but no reports were found on utilization of D-histidine by humans.
b. Animal studies

Changes in food intake and body weight: A substantial body of literature attests to the effects on body weight of excess histidine intakes in various animal models. Harper et al. (1970) have summarized those studies performed prior to 1970 that documented decrements in growth and weight gain in animals fed high levels of histidine.

Benevenga and Steele (1984) have reviewed the more recent literature on histidine toxicity in animals. These authors noted studies that demonstrated that low-protein diets in conjunction with supplemental histidine resulted in significant body weight losses within several days. These results were supported by chronic feeding trials in which rats were fed for 3 to 4 weeks. The adverse effects of histidine may be dependent on the amount and quality of the protein in the diet. Effects diminish as higher amounts of high quality protein are fed with the histidine challenge (Benevenga and Steele, 1984).

While the exact mechanism(s) responsible for the weight loss and growth depression associated with excess histidine have not been elucidated, it has been suggested that there is an inhibition of appetite control resulting in decreased intake. Since histidine competes with other LNAA (leucine, isoleucine, valine, tyrosine, phenylalanine, tryptophan, and threonine) for the same carrier system across the blood–brain barrier, this competitive transport may play a role in the appetite suppressive effects of L-histidine (Peng et al., 1973). Whether this effect is a direct result of elevated plasma and brain concentrations of histidine and consequent elevations in the putative neurotransmitter histamine (Sheiner et al., 1985; Mercer et al., 1989), a secondary consequence of concentration changes of other neurotransmitter precursors (Tackman et al., 1990) or any combination thereof remains an open question.

Behavioral studies: Dutra-Filho et al. (1989) reported significantly reduced locomotor activity in male Wistar rats 4 to 5 weeks old given a histidinase inhibitor, nitromethane (0.73 mg/g body weight), and injected with histidine (0.5 mg/g body weight without the inhibitor versus 0.25 mg/g histidine with the inhibitor, isomer not specified). It should be noted that acute histidinemia was chemically induced in these rats.

Pilc et al. (1982) reported a dose-dependent increase in "bizarre behavior" in rats injected peritoneally with L-histidine hydrochloride (dose range 400 to 800 mg/kg body weight). The "bizarre behavior" was not characterized nor was it quantified except to say that similar behaviors have been seen after injection of drugs affecting dopaminergic systems and could be generally described as "mock fighting" (rearing and stereotypic movements of the forepaws). In this experiment the behaviors were regarded as an "all or none" response that did or did not appear in the period 30 to 120 minutes after histidine injection. Upon subsequent injection with various neurotransmitter antagonists, only antihistamines and atropine, a cholinergic receptor blocker, inhibited the histidine-induced behaviors.

Aside from the impact on food intake noted above, there have been no other observations of neurological or behavioral changes associated with feeding high levels of histidine.

Biochemical studies: Several biochemical changes have been associated with L-histidine feeding. These changes include hyperlipidemia (Kerr et al., 1965a), hypercholesterolemia and reduced plasma copper concentrations (Harvey et al., 1981), and increases in both absorption and excretion of zinc (Van Wouwe et al., 1989). In the study by Harvey et al. (1981), the hypercholesterolemia was completely reversed with the addition of copper to the diet, thereby suggesting that the hypercholesterolemia resulted from increased binding and subsequent excretion of copper associated with excess histidine.

There have been several studies of nutrient–nutrient interactions in response to the administration of L-histidine. Onodera and Ogura (1985) documented a dose-dependent interaction between
thiamin deficiency and intraperitoneal injections of L-histidine that is mediated through CNS histidine metabolism. Benevenga and Steele (1984) noted that the rate-limiting step in the metabolism of large loads of histidine is probably due to availability of folic acid (polyglutamyl tetrahydrofolic acid). This opinion was reinforced by Kohashi et al. (1990) who found that the liver's capacity to regenerate these forms of folate was exceeded during excess histidine feeding in rats. The functional consequences of these changes, if any, are not known at this time.

In female mice fed supplemental L-histidine, changes in zinc status were quantitatively and qualitatively different from zinc deficiency (Van Wouwe et al., 1989). Rats were assigned to either zinc deficient, control, or histidine (9 μmol/g/day in their water) plus control diets. Zinc concentrations increased in liver, pancreas and muscle in histidine-supplemented versus either pair-fed control or zinc-deficient animals.

The competition among LNAA for the L-transport system results in the potential for imbalances in any of these amino acids. As described above in the discussion of food intake and demonstrated by Mercer et al. (1989) and Tackman et al. (1990), an imbalance favoring histidine can result in changes in the concentrations of other amino acids in the plasma and the brain. The specific functional ramifications of such imbalances is not clear at this time. It should be noted that many of the changes associated with this type of imbalance in animal models have not been reported in cases of histidinemia, the genetic inborn error of metabolism associated with elevated blood levels of histidine (see p. 54).

There have been numerous studies on the pharmacology of histamine and drugs affecting histamine receptors centrally and peripherally (White and Rumbold, 1988). Schwartz et al. (1972) gave peripheral injections of L-histidine and found increased brain levels of histamine. Hegstrand and Simon (1985) studied L-histidine transport and metabolism in isolated rat brain synaptosomes and found that histidine uptake was regionally distributed and that there was no de novo synthesis of histamine detectable after histidine transport. Hegstrand and Simon (1985) interpreted their data as a demonstration of the inability of histidine transport, per se, to regulate histamine production.

Oishi et al. (1989) studied the in vivo effects of an injected bolus of L-histidine and found dose-dependent changes in brain amino acids. Animals were injected intraperitoneally with an acute dose of either 0.5, 1.0, or 1.5 g/kg body weight of L-histidine and brain amino acids were measured after 1 and 2 hours. After one hour, an increase in the concentration of L-histidine was the only change found. The animals receiving 1.0 g L-histidine/kg had significantly reduced levels of glutamine, and tyrosine; while those receiving 1.5 g/kg had significantly reduced levels of methionine, tyrosine, and phenylalanine when compared to saline injected controls. Histidine levels at all doses were still elevated after two hours; histamine levels were not measured. Serine levels in mice given the highest dose were significantly elevated after two hours.

Hematological studies: Histidine is found in high concentrations in hemoglobin. There have been no studies in either animals or humans of the impact of bolus feeding of histidine on hematological parameters. Similarly, the potential interaction between histidine-induced changes in folate (Kohashi et al., 1990) and/or trace mineral (Van Wouwe et al., 1989) metabolism and hematology have not been explored.

Functional assessments and gross pathology: As discussed previously, Dutra-Filho et al. (1989) noted significantly reduced locomotor activity in rats injected intraperitoneally with histidine (0.5 mg/g body weight without a histidinase inhibitor versus 0.25 mg/g histidine with the inhibitor). Eichelmann et al. (1978) proposed that the suppression of the activities of the liver enzymes fatty acid synthetase and acetyl CoA carboxylase is the cause of the hypercholesterolemic response to histidine in rats. Kang-Lee and Harper (1977) found that dietary L-histidine did not affect liver histidase activity.
Enlarged livers, apparently associated with hypertrophic hepatocytes (Benevenga and Steele, 1984) were observed in rats, rabbits, and monkeys fed L-histidine (Harvey et al., 1981). Data on other types of changes in tissue morphology or histology in response to histidine were not found.

**Teratology and developmental studies:** Burns and Kacsar (1987) discussed genetic susceptibility to behavioral and morphological changes in mice born to histidinemic dams. In certain strains, exposure in utero to high levels of histidine resulted in changes in inner ear morphology that, according to the investigators, could be related to impaired neural tube closure. Levy (1989), in discussing the animal models for histidinemia, stated "the most striking characteristics of this mutant mouse are the clinical changes which result not from the mutant genotype but from prenatal exposure to the maternal biochemical phenotype." He cited studies in which offspring of histidinemic dams had a balance defect characterized by circling behavior and/or head tilting irrespective of whether the offspring had histidinemia. The opinion was shared by Burns and Kacsar (1987) who concluded that adverse outcomes observed in their studies were due to a genetic predisposition to the impact of prenatal exposure to histidine.

c. Human studies

**Changes in food intake and body weight:** In response to an earlier report (Henkin et al., 1975) hypothesizing disturbances in taste and smell consequent to zinc deficiency induced by orally administered histidine (isomer not specified) in 6 patients with progressive systemic sclerosis, Schecter and Prakash (1979) studied the response to 4 g/day L-histidine in 8 healthy men. The study involved a 2-week challenge with a 2-week placebo crossover period. Study order was randomly assigned so that there were 4 subjects in each order group (challenge-placebo versus placebo-challenge). There was no control of dietary intake during the study period. A visual analog scale (VAS) was used to subjectively address changes in appetite, taste, or smell.

These investigators found no differences in the subjective measures of appetite, taste, or smell. There were no differences in urinary histidine between treatment and placebo conditions at any time during the trial; however, there were significant elevations in serum histidine levels. The differences in serum histidine were less in the second week of the trial. After 1 week of challenge, urinary zinc was significantly higher than placebo, but the differences were no longer apparent at 2 weeks. No differences were found in serum zinc after two weeks. The data from this small cohort were highly variable; neither standard deviations nor standard errors were reported.

In a similar report, Geliebter et al. (1981) gave progressively increasing doses of L-histidine ranging from 24 to 64 g/day to 3 obese women and 1 moderately overweight male. In addition to subjective measure of taste and smell, taste and smell thresholds were determined using standards representing the major tastes. As in the Schecter and Prakash (1979) report, there were no subjective changes in taste and smell reported by the subjects; moreover, there were no changes in thresholds of detection of tastants or odorants. Geliebter et al. (1981) did find increases in urinary zinc excretion. At lower doses a number of adverse effects were reported including headache, weakness, drowsiness, and nausea. Anorexia and painful sensations in their eyes and changes in visual acuity were reported in 2 of the female subjects receiving the highest dose, 64 g/day. One subject reported mental confusion, problems with short-term memory and episodes of depression and crying. All symptoms were reported to disappear after discontinuation of L-histidine.

**Biochemical studies:** Zlotkin (1989) added supplemental L-histidine to parenteral solutions fed to term and preterm infants. After a 72-hour adaptation period, urine was collected for 2 48-hour periods. Zinc levels were maintained at a constant intake. There were 2 term infants and 12 preterm (about 30 weeks gestational age) included in the study. Other than a statement to the effect that the subjects could not be fed orally and had no diarrhea, there was no information about the condition of the subjects. All comparisons were made by treatment; all data were pooled within each treatment period.
Urinary zinc excretion during L-histidine infusion was significantly increased in comparison to the low-histidine period. No other clinical or biochemical measures were reported. In an earlier report by this same group (Zlotkin and Buchanan, 1988) a similarly pooled group of term and preterm infants were parenterally fed high and low levels of L-histidine. No increases were found in urinary zinc in the high histidine group. The level of L-histidine fed in the Zlotkin (1989) report was 165 mg/kg body weight/day compared to 124 mg/kg body weight/day in the earlier study (Zlotkin and Buchanan, 1988).

Clinical studies: L-Histidine has been suggested as a treatment for several clinical conditions including, obesity (Geliebter et al., 1981), rheumatoid arthritis (Pinals et al., 1977), and chronic uremia (Blumenkrantz et al., 1975; Giordano et al., 1973; Kopple and Swendsen, 1981). Daily doses in these studies range from 1 to 4.5 g. No adverse effects were reported in any of these trials.

Inborn errors of metabolism: Histidinemia is a genetic disorder characterized by a marked decrease in histidinase activity in both liver and skin (Levy, 1989). Since this enzyme catalyzes the first step in the major histidine catabolic pathway leading to glutamic acid, this deficiency leads to the characteristic elevations in both histidine and certain metabolites in urine and blood.

While early reports of this disorder linked it to mental retardation and other neurological problems in as many as 50 percent of cases (LaDu, 1979), subsequent follow-up reports of untreated infants detected by neonatal screening did not support this association. As outlined by Levy (1989), histidinemia is benign in most affected individuals; however, under certain circumstances (e.g., perinatal hypoxia) there may be neurological problems in a portion of the children affected. The suggestion by Levy (1989) that histidinemic infants are predisposed or "disadaptive" to the influence of perinatal insults is supported in theory by the animal work of Burns and Kacser (1987).

Few biochemical or functional analogies to experimentally induced histidinemia have been found. There are no reported anomalies in the blood profiles of other amino acids, except for several reported occurrences of hyperalaninemia, in individuals with histidinemia. Serum zinc levels are normal in histidinemic children, although urinary zinc is elevated. Urinary concentrations of the product of histidine transamination (e.g., imidazolepyruvate) are elevated in this disorder. Concentrations of histidine are elevated in cerebrospinal fluid of histidinemic cases. Neither hepatomegaly nor hyperlipidemia has been reported in humans with histidinemia (Levy, 1989).

d. Summary and conclusions

Endpoints: L-Histidine administration has been associated with adverse effects in both animals and humans. Changes have been documented in developmental neurobiological measures, appetite regulation, clinical neurological signs, growth, organ morphology, and clinical biochemical parameters. An additional concern is the potential for possibly harmful nutrient-nutrient interactions and nutrient deficiencies.

Safe levels of human intake: There are limited data on human intake of L-histidine. In the studies of parenteral feeding to infants of differing gestational age and clinical condition, the results indicate a possible point beyond which there are changes in zinc metabolism that may be harmful. No clinically obvious effects were reported in 1 study in which 4 g L-histidine (compared with an average daily L-histidine intake of 1.6 g/100 g protein) was given daily for 2 weeks, but after initial elevations in serum histidine and urinary zinc, a return towards placebo values of the parameters studied was noted after 2 weeks of treatment. Anorexia and numerous other neurological side effects were reported within several days in subjects receiving 64 g/day. The available data are insufficient to determine an upper safe limit of histidine intake in humans.
3. **Lysine**

a. **Background**

Lysine is a dibasic amino acid. Its transport across the brush border and cell membranes was reviewed by Alpers (1987) and Hopfer (1987). Lysine, like threonine, does not participate in transamination reactions. The major pathway for L-lysine degradation is the saccharopine pathway in which saccharopine is the intermediate for the transfer of the ε-amino group to α-ketoglutarate. A second pathway for catabolism of lysine is the pipecolic acid pathway which is an overflow pathway for L-lysine and the primary pathway for D-lysine (Dancis and Cox, 1989). Carnitine, which is required for the transport of long-chain fatty acids through the inner mitochondrial membrane, is synthesized from lysine and methionine in liver and kidney (Mayes, 1990). Administration of the hydrochloride salt of L-lysine can result in a decrease of plasma pH.

Replacement of L-lysine by equal or greater amounts of D-lysine resulted in negative nitrogen balance in young male subjects, suggesting that D-lysine is probably not used by humans (Rose et al., 1955a).

b. **Animal studies**

Changes in food intake and body weight: Addition of 4 percent L-lysine as lysine acetate to diets of young pigs resulted in lower food intake and 16 percent growth reduction (not significant) but a significant decrease in food efficiency over a 16-day feeding period (Edmonds et al., 1987a,b).

Body weight gain of chicks and rats was depressed when low-protein diets were supplemented with about 5 or 6 percent lysine (Anderson et al., 1951; Jones et al., 1966; Harper et al., 1970; Muramatsu et al., 1971; Russell et al., 1952; Sauberlich, 1961). Chicks fed diets containing 4 percent lysine exhibited tremors, general weakness, and poor feathering (Anderson et al., 1951). No significant differences were found in liver weight or content of DNA, RNA, or protein in rats fed 5 percent L-lysine in a 10 percent casein diet for 3 weeks (Muramatsu et al., 1971).

Funk et al. (1991) fed diets containing 20 percent casein plus 0.8, 1.6, 7.8, or 15.6 percent L-lysine to female adult Sprague–Dawley rats for 4 weeks. Animals fed diets supplemented with larger amounts of L-lysine (not further specified) were more active than control unsupplemented rats or those fed diets with smaller amounts of added L-lysine. Rats fed the three lower levels of L-lysine "appeared reasonably healthy" but those given the highest level of lysine "developed scaly tails and thinning hair."

Chronic effects of supplemental L-lysine were evaluated in Carworth Farms albino rats in a 2-year feeding study (Haskell Laboratory and Stine Laboratory Reports, 1957). A commercial laboratory diet containing 25 percent protein was supplemented with 1 percent L-lysine hydrochloride for the first 15 weeks and with 3 percent for the remainder of the 2-year period. In the initial part of the experiment, food efficiency was slightly greater in the L-lysine-fed animals because of slightly lower feed consumption but this did not continue in the second year. Male animals fed the L-lysine-supplemented diet ate less than the females. Daily L-lysine intakes for females was 1505 mg/kg body weight and 885 mg/kg for males during the second year. The authors reported no harmful effects of L-lysine supplementation (i.e., no clinical, hematological, or pathological effects) or differences in incidence of spontaneous disease or mortality during the study.

Biochemical studies: A lysine-arginine antagonism has been demonstrated in several species. In mammals, L-lysine and L-arginine share an intestinal transport system (McCarthy et al., 1964; Rosenberg et al., 1966) and arginine has been observed to interfere with absorption of lysine in rats (Kamin and Handler, 1952). Studies in dogs have shown that arginine and lysine compete for
reabsorption from the renal tubules (Kamin and Handler, 1951; Webber et al., 1961). However, later experiments of Jones et al. (1966) provided little evidence that excess dietary lysine significantly affected transport of arginine by gut, tissue, or kidney and those investigators suggested that excess dietary lysine exerted its adverse effects by interfering with the intermediary metabolism of arginine.

In male Holtzman rats force fed a meal (2 g/kg body weight of a 6 percent casein diet supplemented with 5 percent L-lysine), plasma and brain lysine concentrations increased about 400 and 140 percent, respectively, over concentrations in control rats. Plasma arginine concentration did not decrease, but brain arginine concentration decreased about 60 percent. Decreases of 10 to 20 percent were reported in brain concentrations of threonine, histidine, and phenylalanine, with smaller effects on other LNAA. Over a 4-day feeding period, body weight of lysine-supplemented rats decreased slightly (with food intake that was about 55 percent that of control rats) while control rats gained about 20 g (Peng et al., 1973).

In young male Sprague-Dawley rats, feeding of 5 percent L-lysine as L-lysine hydrochloride in 15 percent casein diets for 2 weeks resulted in accumulation of liver lipids (Hevia et al., 1980a,b). Liver triglycerides, cholesterol, and total lipids were increased 600, 200, and 200 percent, respectively, while serum lipids and glucose remained at normal levels in these rats compared with rats fed a 15 percent casein control diet. Feeding of 5 percent L-arginine, L-threonine, L-valine, or L-glutamic acid in the control diet did not produce fatty livers, suggesting that the effect was specific for L-lysine. Addition of 1 percent L-arginine prevented development of fatty livers, suggesting that the relative amounts of arginine and lysine may be an important factor in lipid metabolism. Addition of 1 percent L-methionine partially prevented the fatty livers. Development of fatty livers was also prevented by raising the dietary casein level from 15 percent to 30 percent or by extending the feeding period from 2 weeks to 6 weeks (Hevia, 1980b). Urinary excretion of orotic acid (characteristic of arginine deficiency) was significantly increased with the 5 percent L-lysine, 15 percent casein diet and was reversed with the addition of 1 percent arginine (Ulman et al., 1981). Excretion of orotic acid varied directly with graded levels of dietary L-lysine (up to 4.3 percent) (Fico et al., 1982). Feeding of orotic acid also induced fatty livers (Windmueller, 1964), suggesting that a lysine-arginine antagonism may be a factor in the development of fatty livers with excess L-lysine in low-protein diets.

Behavioral studies: The lysine metabolite, pipelicolic acid, has been shown to influence GABA release and uptake into nerve terminals (Chang and Myslinski, 1985). Impairment was not observed in male Swiss-Webster mice injected intraperitoneally with L-lysine (10 mmol/kg body weight, 37 mg for a 25-g mouse) compared with controls over a 2-hour period when they were tested on a rotating rod. The mice generally exhibited normal exploratory behavior and did not appear sedated or exhibit other side effects related to neurotoxicity. Doses above 2 mmol/kg body weight (about 7 mg) of L-lysine given intraperitoneally protected mice from pentyleneetrazol-induced seizures and prolonged seizure latency. The peak effect dose was 10 mmol/kg body weight. D-Lysine at 10 mmol/kg body weight provided seizure protection and prolonged seizure latency but had a shorter peak effect time than L-lysine (Chang and Myslinski, 1985). Intraperitoneal injection (10 mmol/kg body weight twice daily for 3 days) of both D- and L-lysine were shown to shorten sleep onset time and prolong hexobarbital-induced sleep in rats (Chang et al., 1981).

Endocrine studies: Intravenous infusion of L-lysine (1 mmol/kg body weight) over 15 minutes in fasting, conscious dogs resulted in relatively small and nonsignificant increases in plasma glucagon and insulin concentrations (Rocha et al., 1972). Plasma concentrations were much lower with infusion of L-lysine than with L-arginine, which evoked the greatest response.

Functional assessments and gross pathology: Acute structural and functional effects of intravenously infused L-lysine free base buffered to pH 7.4 with hydrochloric acid (1.9 g/kg body weight or about 0.6 g for a 300-g rat) on kidney were assessed in male Munich-Wistar rats (Malis et al., 1984). After 2 hours of infusion, arterial pH was significantly lower, albumin clearance was
significantly increased and inulin clearance was not different in the L-lysine-infused and control groups. At 4 hours, albumin clearance was significantly higher and inulin clearance was significantly lower than in controls. Medullary casts, outer medullary congestion, and patchy early tubular necrosis in the medulla were reported in the L-lysine-infused group. In longer experiments, a total of 500 mg L-lysine was injected intraperitoneally in 2 injections 2 hours apart. Twenty hours after the second injection, serum urea nitrogen and urinary protein and potassium concentrations were significantly increased while serum sodium, creatinine clearance, and urine volume were significantly lower in L-lysine-treated animals. Light microscopy showed more medullary casts, cortical casts, and tubular necrosis in the lysine group than the control group. Occurrence of medullary casts and tubular necrosis was strongly correlated with creatinine clearance and serum creatinine and urea nitrogen concentrations in the L-lysine group. Light microscopy and transmission electron microscopy revealed giant lysosomes containing crystalline inclusions in the proximal tubules of the L-lysine-treated rats (Malis et al., 1984).

Dose-response studies were also conducted in the Munich-Wistar rat with intravenous infusion of 100, 200, 400, or 600 mg of lysine free base (isomer not specified) at 24 and 48 hours after treatment (Racusen et al., 1985). With the 3 lower doses, serum and urine composition were not significantly different from baseline values. With the highest dose (600 mg), a sustained decrease in renal function was observed. Urine output was slightly elevated at 48 hours, suggesting tubular dysfunction, fractional sodium excretion was significantly increased, serum urea nitrogen and creatinine concentrations were significantly elevated, and clearance of urea nitrogen and creatinine were significantly decreased in this group. Renal morphology at 48 hours was normal by light microscopy in the rats given 100, 200, or 400 mg lysine. However, marked histological changes of the types described in the acute studies (Malis et al., 1984) were observed in the high-dose group. Hyaline casts were observed in the ascending and descending limbs of the loop of Henle in the medulla. These effects were specific for lysine as infusion of 600 mg of arginine, glutamic acid, or glycine did not produce abnormalities in renal function or histology. The 600-mg dose of lysine was described by the authors as comparable to the doses given to human volunteers to block tubular uptake of proteins and measure glomerular filtration and to study nephrotoxicity of single amino acids (Racusen et al., 1985).

Teratology and developmental studies: In Sprague-Dawley rats fed 20 percent casein diets supplemented with 0.8, 1.6, or 7.8 percent L-lysine throughout pregnancy, maternal weight gain and fetal weights were significantly decreased with the largest addition of L-lysine. No increase in resorptions and no teratogenic effects were visible upon gross examination (Funk et al., 1991). The lower levels of supplementation were selected based on L-lysine doses used in treatment of herpes simplex infections in humans.

Feeding of a 6 percent casein diet supplemented with 5 percent L-lysine (chemical form not specified) to Sprague-Dawley rats from day 1 to day 14 or 21 of pregnancy resulted in significantly lower fetal weight than in ad libitum or pair-fed controls but no difference in litter size. Excess L-lysine resulted in a further significant reduction in brain weight and content of DNA and RNA than food restriction alone. Little change was observed in maternal plasma or fetal brain amino acid concentrations with this diet (Matsueda and Niiyama, 1982).

Addition of 10 and 25 percent L-lysine to diets containing 9 or 27 percent casein fed to pregnant CF Wistar rats from day 5 to day 15 of gestation resulted in dose-related reductions in maternal weight gain with both levels of dietary protein. Although fetal mortality increased in a dose-related manner with increased level of L-lysine supplementation, mean weight of surviving fetuses was only 5 to 10 percent less than controls and the fetuses did not exhibit gross malformations. Intraperitoneal injection of L-lysine in doses of 200, 400, or 600 mg/day in the same experimental model resulted in increased maternal and fetal mortality with increasing dose of the amino acid but average weight of surviving fetuses was not affected and gross malformations were not observed (Cohlan and Stone, 1961).
c. Human studies

Biochemical studies: Effects of administration of L-lysine have been investigated to a limited extent in relation to urea cycle and arginine metabolism, carnitine synthesis, and plasma and urine concentrations in humans. Effects of administration of lysine hydrochloride on plasma pH and electrolyte status have also been described.

"Chromatographically pure" L-lysine hydrochloride was injected intravenously at doses of 0.5 mmol/kg body weight (14 to 22 g total dose) in 6 normal fasting volunteer subjects 10 to 14 years of age to assess inhibitory effects of L-lysine hydrochloride on urea cycle metabolism (Kato et al., 1987). Plasma lysine concentration peaked at about 1400 μmol/L 15 minutes after infusion and remained significantly elevated at about 500 μmol/L at 60 minutes (fasting value approximately 130 μmol/L). Plasma arginine increased significantly, rising from 80 μmol/L to about 140 μmol/L at 15 minutes and remaining elevated significantly after 60 minutes; plasma ornithine increased to a lesser but significant extent from an initial value of about 36 μmol/L to 45 μmol/L and approached baseline after 90 minutes. Blood ammonia increased significantly, reaching a plateau of about 540 μg/L at 45 minutes after infusion. Urinary excretion of homocitrulline, putrescine, and orotic acid was significantly increased after 90 minutes. In contrast, plasma concentrations of citrulline and urea did not change (Kato et al., 1987).

Oral administration of a single 5-g dose of lysine (isomer and chemical form not specified) to 6 healthy adult males resulted in higher plasma concentrations of ε-N-trimethyllysine and carnitine and higher urinary excretion of carnitine (Vijayasarithy et al., 1987). Administration of 5 g tryptophan (isomer not specified) did not produce these changes. The report did not mention adverse effects of oral administration of a single 5-g dose of lysine or tryptophan.

Tolerance of infants to L-lysine has been evaluated. In a study of supplementation of whole cow milk with L-lysine hydrochloride, 6 infants 4 to 11 months of age received L-lysine in graded increments of 60, 120, 240, 480, 720, 960, or 1080 mg/8 oz. milk for 3 or 4 days at each increment (Dubow et al., 1958). Intake at the highest concentration was reported to be about 5.2 g/day. Total plasma amino acids increased; however, this increase was maximal with the 120-mg addition and plasma amino acid patterns did not show any marked alterations. The authors reported that unusual nervousness or drowsiness was not observed and the infants remained active with their usual degree of alertness. Anorexia, diarrhea, or other gastrointestinal disturbances that could be associated with the supplement did not occur and no clinical abnormalities were reported. Urinary excretion of lysine was roughly proportional to the amount of supplement and no cystinuria was noted (Dubow et al., 1958). Similarly, urinary lysine excretion was increased but no adverse effects were reported in 6 infants 1 to 5 months of age ingesting about 220 mg/kg body weight (2.2 g for a 10-kg infant) of L-lysine hydrochloride in an amino acid based formula for as long as 15 days (Snyderman et al., 1959). The lysine requirement for infants 3 to 4 months of age was reported to be 103 mg/kg body weight/day (Fomon and Filer, 1967; National Research Council, 1989).

L-Lysine hydrochloride has been given as a source of chloride ions in patients with metabolic alkalosis. Doses of 10 to 40 g/day of L-lysine hydrochloride (8 to 32 g/day of lysine) were given as an adjuvant before mercurial diuresis to increase urinary chloride excretion for 3 to 5 days to 20 patients with fluid retention due to cardiac or liver disease (Lasser et al., 1960). Abdominal cramps and transient diarrhea were reported as side effects but these did not recur when doses were lowered. Ammonia intoxication was not observed in patients with impaired hepatic function.

In 284 patients with metabolic alkalosis, intravenous administration of lysine hydrochloride (20 to 30 mEq/100 ml infusate, total volume not reported and isomer not specified) significantly lowered plasma pH and bicarbonate levels (Bondoli et al., 1980). Similarly, 40 g L-lysine hydrochloride/day given intravenously in 4 divided doses for 2 to 5 days in 14 patients with advanced liver disease or congestive heart failure produced an increase in plasma chloride concentration and decreases in pH and in carbon dioxide combining power (Rubin et al., 1960). Diarrhea in one patient was the only
adverse effect reported in this study. "In no cases" did "flapping tremor, mental change or other significant clinical deterioration" result from the L-lysine hydrochloride administration. No evidence of renal or hematopoietic toxicity was indicated by urinalysis, blood urea, or hematologic studies.

Endocrine studies: Growth hormone release was not stimulated in 8 healthy, fasting male volunteers 15 to 20 years of age given single oral doses of 1.2 g L-lysine hydrochloride (Isidori et al., 1981). Plasma insulin concentration was increased in 9 healthy adults given intravenous infusions of 30 g L-lysine as L-lysine hydrochloride (Floyd et al., 1966). Although side effects were reported in the latter study with infusion of certain amino acids, none were reported with administration of L-lysine.

Clinical studies: Because L-lysine has been shown to inhibit replication of herpes simplex virus in vitro (Tankersley, 1964), use of L-lysine hydrochloride has been investigated as a treatment for herpes infections in humans (DiGiovanna and Blank, 1984, 1985; Griffith et al., 1978, 1987; McCune et al., 1984; Milman et al., 1980; Simon et al., 1985; Thein et al., 1984). Except for the first study of Griffith et al. (1978), these studies were randomized, double-blind, placebo-controlled, and, in some instances, crossover clinical trials of L-lysine hydrochloride administration. In these studies, L-lysine hydrochloride was given in divided doses totaling 624 to 3000 mg/day for 3 to 6 months. In 1 study, McCune et al. (1984) reported that there were no adverse effects on plasma sodium, potassium, and chloride concentrations in 41 patients during 24 weeks treatment with 1248 mg/day L-lysine hydrochloride. In another study, Thein et al. (1984) reported that serum lysine levels were increased with lysine treatment in most subjects and that serum lysine levels greater than 165 μmol/L were associated with a significant decrease in lesion recurrence in 15 L-lysine-treated patients. However, because patterns of response of the two crossover groups were different and because serum concentrations remained within normal range, the significance of this finding is questionable. Only 1 study (Griffith et al., 1987) included mention of an adverse effect, upset stomach, in 3 of 27 patients given 3 g L-lysine hydrochloride daily for 6 months.

A survey of purchasers of lysine at 300 randomly selected retail nutrition stores over a 3-month period indicated that doses of L-lysine given in these studies were similar to the usual dose of 936 mg/day reported by 1543 individuals (Walsh et al., 1983). Questions on side effects were not included in the survey. Ages of the participants were not given; 67 percent were female and 33 percent male, similar to the proportions reported for study subjects.

A trial of L-lysine supplementation of pregnant women was reported by Terry (1957). In a pilot test of 12 women with hemoglobin levels of less than 105 g/L, supplementation with 600 mg/day L-lysine plus "adequate iron supplementation" resulted in a rise in hemoglobin level (no data given). The chemical forms of the supplement components were not reported. The author reported this response as better than the hematocrit response of women supplemented with iron alone. In a subsequent unblinded experiment of 204 obstetric patients given L-lysine plus vitamins and iron and 92 patients given vitamins and iron only, the women given supplemental L-lysine also had higher hemoglobin values during pregnancy and at 3 days postpartum than the control group (118 and 102 g/L, respectively). Little experimental detail and few data were presented.

Functional assessments: Of 14 amino acids tested, L-lysine most strongly inhibited renal tubular reabsorption of protein. Amino acids with a terminal, positively-charged amino- or guanidino-group such as lysine, arginine, and ornithine were the only amino acids that inhibited tubular protein reabsorption. (See arginine section for comparisons and further details.) In a further investigation of this effect, L-lysine hydrochloride supplying 2.7, 5.0, 16.5, 24.8, 33.0, or 41.3 g L-lysine was injected intravenously over a 2-minute period in 5 nonfasting young male subjects (Mogensen and Sølling, 1977). Twenty minutes after injection, urinary excretion of albumin, β-microglobulin, light chains (kappa and lambda chains), transferrin, and immunoglobulin G increased significantly in a dose-related manner, indicating a strong inhibitory effect of L-lysine on renal tubular reabsorption of proteins. Maximal values after excretion were reported as a 15- to
20-fold increase for the light chains, about a 40-fold increase for albumin and immunoglobulin G, a 74-fold increase for transferrin, and a 1500-fold increase for β-microglobulin; however the mean values after injection were not given. With the 2 lowest doses, protein excretion returned to baseline levels by 40 minutes; with the higher doses, only the maximal values for protein excretion were reported with no indication of how long excretion remained elevated. Protein clearance, as a percent of GFR, showed a higher correlation with serum lysine concentration than with urinary lysine. Nausea occurred in two subjects given the highest dose of L-lysine hydrochloride (Mogensen and Sølling, 1977).

In a subsequent study of functional changes in the kidney of Type I diabetic patients, the effect of L-lysine injection (0.4 g/kg body weight or 25 g for a 70-kg subject) on albumin excretion was studied in 9 control and 32 Type I diabetic subjects (Mogensen et al., 1979). These investigators found that diabetic subjects with normal baseline albumin excretion values responded similarly to control subjects whereas those with abnormal albumin excretion at baseline had much higher levels of albumin excretion (2 to 3 times that of controls). However, L-lysine injection did not increase excretion of light chain proteins and β-microglobulins significantly above that of control subjects (Mogensen et al., 1979).

Gastric intubation of 21 healthy young adults (14 females, 7 males) with 5 g L-lysine hydrochloride (a product identified as Darvyl and described as containing more than 95 percent L-lysine hydrochloride) together with a test meal of homogenized, toasted white bread resulted in increased pepsin activity in gastric juice (Sackler and Sophian, 1957). Little description of experimental protocol and results was included; the authors reported that a 5-g dose of L-lysine hydrochloride was well tolerated with a test meal of bread and water.

Inborn errors of metabolism: Inherited hyperlysinemia is an autosomal recessive disease in which lysine accumulates in abnormally high concentrations in serum and urine (Dancis and Cox, 1989). The metabolic defect responsible for the disease is a defect in the bifunctional protein α-aminoacidic semialdehyde synthase. Activities of two associated enzymes, lysine α-ketoglutarate reductase and saccharopine dehydrogenase, begin the degradation of lysine by removal of the α-aminogroup in normal individuals. In familial hyperlysinemia, activities of both enzymes are no more than 10 percent of normal with resultant hyperlysinemia and lysinuria, sometimes with elevated urinary levels of saccharopine. The less active piperolic acid pathway is then largely responsible for lysine degradation (Dancis and Cox, 1989). Plasma piperolic acid concentration is elevated in patients with familial hyperlysinemia (approximately 30 μmol/L, normal 2.1 μmol/L) as well as plasma lysine concentration (about 1000 μmol/L [146 mg/L], normal 100 to 280 μmol/L [15 to 41 mg/L]).

Although the metabolic disorder was first detected as a result of diagnostic studies for neurologic damage and mental retardation, a study in which patients were identified during routine newborn screening and familial surveys of affected individuals and confirmed by enzymatic assays of skin fibroblasts reported that no damage was observed which could be attributed to hyperlysinemia (Dancis et al., 1983). This study included a normal infant born to a woman with familial hyperlysinemia. Although hyperlysinemia seems to be tolerated without recognized adverse effects, it remains to be shown that chronic hyperlysinemia is benign.

d. Summary and conclusions

Endpoints: Ingestion of 4 to 6 percent added L-lysine in low-protein diets has resulted in decreased food intake and growth in chicks, rats, and pigs in short-term experiments; however, ingestion of a diet containing a total of 3.75 percent L-lysine over a 2-year period did not result in adverse effects in rats. Effects on sleep latency and length were noted with intraperitoneal injection of L-lysine in rats; however, the functional significance of these changes is unknown. Plasma and
brain concentrations of lysine increased and fatty livers developed in rats fed diets containing graded concentrations up to 5 percent added L-lysine. Structural and functional changes which lasted at least 48 hours were observed in rat kidney with intravenous infusions of 1.9 g/kg body weight of L-lysine hydrochloride. No teratologic effects were observed in fetuses of rats fed diets containing as much as 25 percent L-lysine; however, fetal body weights and brain weights were significantly decreased when pregnant rats were fed diets containing less than 10 percent L-lysine.

Results of human studies are generally similar to those of animal studies. Intravenous infusions of L-lysine hydrochloride have resulted in lowering of plasma pH in patients with metabolic alkalosis. Urinary excretion of lysine was increased in infants fed formulas with added L-lysine. Investigations in patients with inherited hyperlysinemia suggest that high plasma levels of lysine (about 1000 μmol/L) do not usually result in mental retardation or developmental delays in infants or children. Plasma lysine concentrations at least as high as those reported in inherited hyperlysineinemia were reported with single intravenous doses of 0.5 mmol/kg body weight of L-lysine hydrochloride in children 10 to 14 years of age. Total doses in that study were calculated to range from 14 to 22 g. Although intravenous injection of L-lysine in doses ranging from 3 to 41 g strongly inhibited tubular resorption of proteins and resulted in increased urinary excretion of protein, it is not known whether the oral ingestion of L-lysine would produce this result.

Safe levels of human intake: Few side effects (upset stomach) have been associated with oral consumption of L-lysine by patients with herpes infections in doses as high as 3 g daily for 3 to 6 months; however, no biochemical or other endpoints useful in assessing the safety of L-lysine were measured in these studies. For reference, the daily intake of L-lysine by an adult consuming 100 g protein is about 5 g. Nausea was the only side effect reported in some subjects given 41 g L-lysine hydrochloride intravenously; however, protein excretion was significantly increased in these subjects and subjects given lower doses as well. Intravenous injection of L-lysine hydrochloride in rats has also resulted in abnormal renal structure and function. In light of the consistent association between intravenous infusion of L-lysine and renal effects, possible effects of large oral doses of L-lysine on tubular reabsorption of protein should be investigated.

Any adverse effects of excess lysine consumption may be exacerbated by low protein intake or low dietary arginine intake. Maintenance of a lysine/arginine intake ratio of less than 1.5 is desirable to minimize any adverse effects due to lysine–arginine antagonism; however, there are no experimental data to indicate whether there would be any tempering effect of large doses of arginine on possible adverse effects of large doses of lysine. In the absence of a systematic evaluation of the safety of lysine, the data are insufficient to determine a safe level of intake.
4. Methionine

a. Background

Humans, as well as other mammals, cannot fix inorganic sulfur into organic molecules and must rely on ingested sulfur amino acids (methionine and cysteine) for synthesis of protein and biologically active sulfur-containing compounds such as taurine (Cooper, 1983). The metabolism of the sulfur-containing amino acids is closely interrelated; however, because the effects of excesses of methionine and cysteine are distinctly different, they are treated separately in this review. The reader is also referred to the section on cysteine and cystine.

Methionine is metabolized by a methylation and transsulfuration pathway and by a transaminative pathway. Although both ultimately lead to production of carbon dioxide from the methyl carbon and sulfate from the sulfur atom, experimental evidence has shown that the two pathways are separate and distinct and suggests that the metabolic basis for methionine toxicity, at least in rats, is related to metabolism of the methyl moiety by the transaminative pathway (Benevenga and Steele, 1984).

Methylation and transsulfuration pathway: This pathway has been well characterized (Cooper, 1983). The initial step in the pathway is activation of methionine to S-adenosylmethionine (ado-met), the major donor of methyl groups in the body. With loss of the methyl group, ado-met is converted to S-adenosylhomocysteine which is then sequentially metabolized to homocysteine, cystathionine, cysteine, cysteinesulfenic acid, hypotaurine, and taurine. Homocysteine can be remethylated by either N5-methyltetrahydrofolate or betaine to regenerate methionine and tetrahydrofolic acid or dimethylglycine, respectively. Increased utilization of this pathway in the presence of excess methionine has been suggested by the observation that either glycine or serine decreases the adverse effects of consumption of methionine-supplemented diets in rats, presumably by increased conversion of homocysteine to cystathionine (Benevenga and Harper, 1967).

By giving up the methyl group in the formation of S-adenosylhomocysteine, ado-met also serves as a methyl donor in transmethylation reactions for synthesis of such biologically active compounds as betaine, choline, creatine, melatonin, epinephrine, sarcosine, N-methylated amino acids, or methylation of phospholipids, proteins, polysaccharides, and nucleic acids. Ado-met is also an important intermediary in the synthesis of polyamines. Other reactions include the transfer of the adenosyl portion to enzyme protein or the 3-amino-3-carboxypropyl group to tRNA and the hydrolysis of ado-met to 5'-methylthioadenosine and homoserine lactone (Cooper, 1983).

Transaminative pathway: In a series of reactions independent of ado-met formation, methionine is transaminated to α-keto-γ-methylthiolbutyrate which is decarboxylated to 3-methylthiopropionyl CoA. The methylthiol moiety of 3-methylthiopropionate is cleaved to form methanethiol which is subsequently metabolized to carbon dioxide and sulfate. Intermediates in the formation of the final oxidation products are formaldehyde, formate, and hydrogen sulfide (Benevenga, 1984; Cooper, 1983).

Utilization of D- and L-isomers: Two feeding experiments in healthy men indicate that D-methionine is less effective than L-methionine in maintaining nitrogen balance. Supplementation of a soy protein and amino acid diet for 9 days with 420 mg D-methionine did not result in significantly greater nitrogen retention than the unsupplemented diet (nitrogen balance was negative with both diets); however, supplementation of the diet with the same amount of L-methionine resulted in positive nitrogen balance (Zezulka and Calloway, 1976). Similar results were reported by Kies et al. (1975) with an oat-based diet supplemented with D-, L-, or DL-methionine.

Plasma concentrations and urinary excretion of methionine also differ after ingestion or TPN infusion of the 2 isomers. After infusion of 1.5 or 4.5 g DL-methionine, Kinsell et al. (1948) found
that D–methionine, but not L–methionine, was excreted in the urine by normal individuals and patients with liver damage. Stegink et al. (1986) reported that mean peak plasma methionine concentrations were 98 and 144 μmol/L after loading with L– and D–methionine, respectively, at levels of 0.0605 mmol/kg body weight (630 mg for a 70–kg subject). Urinary excretion of methionine after ingestion of D–methionine was 20 times higher than after ingestion of L–methionine; the D–isomer was the form excreted. Poor utilization of D–methionine by infants was suggested by biochemical studies of Stegink et al. (1971). (See p. 69.) A study in which L–methionine or the racemate was infused intravenously in a TPN solution in morbidly obese patients after gastric bypass surgery also indicated poor utilization of D–methionine as evidenced by elevated plasma methionine concentrations and increased urinary excretion of the D–isomer (Printen et al., 1979). However, when energy intake was restricted to 15 to 22 percent of the requirement, normal adults infused with amino acid solutions not containing glucose (protein sparing therapy) appeared to utilize a larger proportion (67 percent) of D–methionine, as evidenced by decreased urinary excretion of D–methionine (Brummel et al., 1977; Stegink, 1977).

In a 2–week randomized crossover study, monkeys appeared to utilize D–methionine poorly (Stegink et al., 1980a). Supplementation of the diet of 4 female adult cynomolgus monkeys (Macaca fascicularis) with 0.14 percent DL–methionine resulted in a significant increase in urinary excretion of methionine; 97 percent of the excreted methionine was in the form of the D–isomer and 27 percent of the supplemental D–methionine was excreted in the urine. Postprandial total plasma methionine concentrations were similar when the animals were fed the diet supplemented with DL–methionine and the control diet but 24 percent of plasma methionine was in the form of the D–isomer when the supplemented diet was fed. Administration of a bolus of 150 mg DL–methionine in water by stomach tube to fasted monkeys resulted in urinary excretion of 17 percent of the dose in the first 24 hours after administration (Stegink et al., 1980a).

In contrast to the poor utilization of D–methionine by humans and monkeys, rats utilize D–methionine efficiently. At a low level of supplementation (0.5 percent) in a low–protein (10 percent casein) diet, addition of D–methionine resulted in greater weight gain in rats than L–methionine (Muramatsu et al., 1971). Similarly, rats infused with TPN solutions containing DL–methionine utilized 99 percent of the D–isomer (Cho and Stegink, 1979). However, at low levels of dietary addition (less than about 1 percent), weight gain in mice fed D–methionine appeared to be about half that of mice fed L–methionine (Friedman, 1991). Reports differ on the relative utilization and toxicity of the D– and L–isomers of methionine in rats. Van Pilsum and Berg (1950) reported that addition of 1.2 percent L–methionine to a diet containing 18.6 percent of an indispensible amino acid mixture produced only about 70 percent of the weight gain resulting from feeding of equal amounts of D– or DL–methionine and Muramatsu et al. (1971) observed that with addition of either isomer at levels of 1, 2, 3, or 5 percent to the 10 percent casein diet, weight gain decreased sharply and to a similar extent. Tissue damage was reported to be greater with L– than with D–methionine in rats although no difference in weight gain was observed when the individual isomers were added at levels of 0.5, 1, 2, and 4 percent to a high–protein (24 percent casein) diet (Stekol and Szaran, 1962).

b. Animal studies

Of the amino acids required for protein synthesis, methionine is the most toxic in terms of depressed food intake and weight gain and tissue damage in animals (Benevenga, 1974; Benevenga and Steele, 1984). Harper et al. (1970) reviewed in detail studies published prior to 1969 demonstrating effects of excess methionine on growth and gross pathology.

Changes in food intake and body weight: Numerous studies have shown that food intake and weight gain are decreased to about 30 to 80 percent of control values in rats fed low–protein diets supplemented with about 1 to 5 percent DL–methionine (e.g., Beaton, 1967; Benevenga and Harper,
1967; Cohen et al., 1958; Daniel and Waisman 1968; DeBey et al., 1952; Hardin and Hove, 1951; Roth and Allison, 1949; Rottruck and Boggs, 1977; Sauberlich, 1961). Weight gain and food consumption were more severely depressed with the L-isomer than with D-methionine or the racemate. Consumption of diets containing excess methionine and deficient in vitamin B6 resulted in more severe depression of growth of rats than consumption of diets containing adequate amounts of the vitamin; supplements of vitamin E, vitamin B12, and folacin were reported to moderate growth depression in animals fed low-protein diets supplemented with methionine (Harper et al., 1970).

More recently, Muramatsu et al. (1971) demonstrated that weight gain of rats decreased linearly with increasing dietary additions of 1, 2, 3, and 5 percent methionine over a 3-week period; the decreases were similar for both the D- and L-isomers. Liver weight and content of DNA, RNA, and protein were also significantly less in the methionine-fed animals. Similarly, body weights of rats fed a 10 percent casein basal diet supplemented with 2 percent DL-methionine for 2 years were significantly less than those of control animals fed the basal diet; however, there was no decrease in survival rate (Fau et al., 1988).

Kittens fed an 18 percent casein diet supplemented with 4 percent L-methionine lost weight and had lower food intakes than control animals over a 6-week period. At the end of the experimental period, the plasma concentrations of methionine and cystathionine were 80 times and 3 times greater, respectively, than in control kittens. Addition of lower levels of L-methionine (2 or 3 percent) resulted in an initial reduction in food intake and body weight. After 10 days, kittens fed these diets gained weight at a much slower rate than controls. Plasma concentrations of methionine showed a dose-related elevation of about 66 and 75 times control levels for the 2 and 3 percent levels of added L-methionine, suggesting a limited capacity to metabolize these levels of L-methionine. Weight changes in kittens showed a clear inverse, dose-related response to 2, 3, or 4 percent added dietary L-methionine. Addition of 4 percent glycine to the 4 percent L-methionine diet increased food intake and allowed some weight gain (Fau et al., 1987).

Weight gain and feed intake over a 27-day experimental period were significantly depressed in weanling pigs fed corn-soybean meal diets containing 20 percent protein and supplemented with 2 and 4 percent DL-methionine but not in those fed diets supplemented with 0.5 and 1 percent of the racemate (Edmonds and Baker, 1987).

Biochemical studies: In weanling pigs fed diets containing 0, 0.5, 1, 2, or 4 percent added DL-methionine for 27 days, plasma levels of methionine showed a dose-related linear increase, reaching over a 100-fold increase at the 4 percent level of addition (Edmonds and Baker, 1987).

In rats fed a commercial laboratory diet supplemented with 3 percent L-methionine for 14 days, methionine concentrations exhibited 30, 15, and 10-fold increases in serum, liver, and brain, respectively. Taurine concentrations increased 2 and 5-fold in serum and liver and only slightly in brain. Glutamic acid levels in brain were decreased but GABA levels were not changed (Daniel and Waisman, 1969). In rats force fed a meal (2 g/kg body weight of a 6 percent casein diet supplemented with 3 percent L-methionine), brain methionine concentration increased nearly 13-fold while concentrations of LNAA (valine, isoleucine, leucine, phenylalanine, and tyrosine) fell to about 50 percent of control values (Peng et al., 1973). For technical reasons, no information was available on possible changes in brain concentration of tryptophan. However, increased urinary excretion of xanthurenic acid and total indoleacetic acid was reported in rats fed a diet supplemented with 4 percent DL-methionine for 4 weeks, suggesting that excess dietary methionine may also influence tryptophan metabolism (Sprince et al., 1969).

Benevenga and Harper (1970) found that oxidation of methionine methyl groups was increased in rats fed diets supplemented with L-methionine, possibly indicating that the animals showed some metabolic adaptation to the increased L-methionine load. Metabolic adaptation has also been implied by the decreased severity in pathologic abnormalities over time in some studies; however, in
a 2-year feeding study in rats, pathologic abnormalities were present at the end of the trial (Fau et al., 1988). (See p. 67–68).

The biochemical mechanism of methionine toxicity in rats has been investigated extensively. Consumption of diets containing excess methionine caused a greater deficit in weight gain and histopathological damage than diets containing the same amounts of homocyst(e)ine, suggesting that metabolism of the methyl group of methionine, rather than accumulation of metabolites in the transsulfuration pathway, is responsible for development of toxicity in this species (Benevenenga, 1974; Benevenenga and Steele, 1984; Harper et al., 1970). Adverse effects (seizures) resulting from injection of L-homocysteine also differ from effects of L-methionine injections in rats (see p. 66). Addition of some methyl-containing compounds (S-methyl-L-cysteine, 3-methylthiopropionate [MTP], or dimethylthetin) to low-protein diets also caused reduced food intake and weight gain and histopathologic changes in spleen like those resulting from excess L-methionine intake whereas addition of methylated methionine metabolites produced in the methylation and transsulfuration pathway (choline, betaine, or sarcosine) at levels providing methyl loads greater than or equal to those of L-methionine did not produce those results (Benevenenga and Steele, 1984; Benevenenga et al., 1976; Case and Benevenenga, 1976; Case et al., 1976;). Also supporting the evidence that the methylthiol group may be responsible for the toxicity of methionine was a study by Yokota et al. (1978) in which addition of 2.5 percent glycine to a diet supplemented with 2.5 percent L-methionine decreased the excess iron deposition in spleen and restored the increased activity of δ-aminolevulinic acid synthetase in bone marrow observed with methionine excess to control levels. Supplemental serine and retinol have also shown protective effects against the growth suppression and damage observed with methionine excess. See review by Benevenenga and Steele (1984).

In vivo and in vitro experiments indicated that α-keto-γ-methylbutyrate and MTP are intermediates in the transaminative pathway of methionine metabolism (Benevenenga, 1984; Mitchell and Benevenenga, 1978; Steele et al., 1979). Supplementation of diets with 2.57 percent MTP for 2 weeks depressed weight gain and food intake in rats similar to that observed in rats fed an equimolar level of L-methionine. Splenes of the MTP-supplemented animals also showed histopathologic changes similar to those of L-methionine-supplemented animals (Steele et al., 1979). In vitro experiments indicated that MTP was converted to two extremely toxic intermediates, methanethiol and hydrogen sulfide, in its metabolism to carbon dioxide and sulfate in rat liver; formaldehyde and formic acid were also produced in the degradation of methanethiol to carbon dioxide (Steele and Benevenenga, 1979). The capacity of rats to metabolize MTP to urinary sulfate increased from 50 percent in 15-day-old rats to 80 percent in 24, 50, and 100-day-old animals (Finkelstein and Benevenenga, 1984).

As summarized by Benevenenga and Steele (1984), methanethiol has been shown to suppress the activity of a number of enzymes in brain, muscle, and liver (Maggio et al., 1977; Quarfoth et al., 1976; Vahlkamp et al., 1979; Waller, 1977; Wilms et al., 1980); sulfhydryl reagents have also been shown to alter erythrocyte deformability and increase their susceptibility to hemolysis (Fischer et al., 1978; Jacob and Jandl, 1962). The collective results of these studies have led to the hypothesis that the adverse effects of consumption of diets supplemented with L-methionine may be caused by production of protein-bound methanethiol-cysteine mixed disulfides (MDS) and possibly by a direct action of methanethiol on enzyme-bound metals (Benevenenga and Steele, 1984).

Changes in activity of peroxidative enzymes in rats given excess methionine has been investigated as a mechanism for methionine toxicity. In rats fed a diet supplemented with 3 percent L-methionine for 3 or 7 days, activities of enzymes involved in peroxidative damage were decreased in liver, spleen, and erythrocytes and the concentration of malondialdehyde in liver did not increase, suggesting that lipid peroxidation was not increased (Finkelstein and Benevenenga, 1986). However, in rats fed a diet containing 2 percent supplemental DL-methionine for 7 weeks, activities of hepatic and erythrocyte copper–zinc dismutase were significantly decreased and activities of catalase and glutathione peroxidase were significantly increased. Hepatic lipid peroxidation, measured as thiobarbituric acid reactive substances, was also significantly increased (Lynch and Strain, 1989).
Additional effects of methanethiol and the other intermediates in mediating the toxicity of L–methionine remain to be investigated.

**Hematologic studies:** Hemoglobin concentration, hematocrit, and other hematologic values were lower in rats fed diets supplemented with about 2 or 4 percent DL–methionine for 1 to 3 months than in control animals. Increased numbers of reticulocytes in peripheral blood, erythroid hyperplasia in bone marrow, and increased erythrocyte turnover were also reported (Cohen and Berg, 1956; Klavins et al., 1963a; Mengel and Klavins, 1967). In rats fed 2.57 percent MTP for 2 weeks, hemoglobin concentrations and hematocrits were normal although significant morphologic alterations were observed in developing and peripheral red cells (Steele et al., 1979). (See p. 67.)

Severe hemolytic anemia accompanied by a marked increase in methemoglobin concentration and Heinz–body formation was observed in cats given 1 g/kg body weight of DL–methionine (1.6 to 3.2 g) daily for 10 days (Maede et al., 1987). Cats given 0.5 g/kg body weight DL–methionine had less severe hemolytic anemia with methemoglobinemia and moderate Heinz–body formation after 17 and 31 days but, after 52 days, appeared to recover from the anemia. In vitro incubation of erythrocytes with 10 mM MTP, but not an equimolar amount of DL–methionine, increased the concentration of methemoglobin and formation of Heinz bodies, suggesting that MTP or another intermediate of the transaminative pathway produces tissue damage by an oxidative mechanism.

In weanling pigs fed 20 percent protein diets supplemented with 0.5, 1, 2, or 4 percent DL–methionine for 27 days, hemoglobin concentrations and hematocrits were similar to those of control animals (Edmonds and Baker, 1987); however, red cell morphology was not examined.

**Behavioral studies:** In a study of the mechanism of action of L–methionine in inducing acute psychotic reactions in some schizophrenic patients, Beaton et al. (1975) assessed the effects of methionine on behavior of rats and mice injected subcutaneously with 250 mg/kg body weight of L–methionine or other selected compounds daily for 21 days. Discriminated Sidman Avoidance performance in the rats and slow wave and rapid eye movement (REM) sleep were monitored in the mice. In comparison with saline–injected controls, L–methionine induced avoidance behavior and REM sleep disturbances; the latter were significantly different (although the multiple statistical comparisons were not properly adjusted) and the former were not analyzed statistically. DL–Homocysteine (5.5 or 7.4 mmol/kg body weight; free base, pH 6.6) has been reported to induce convulsions and death when injected intraperitoneally in male Charles River CD rats; however, equimolar doses of DL–methionine (820 or 1100 mg/kg body weight; 287 or 385 mg for a 350–g rat at the 2 dose levels) did not produce convulsions or result in any marked behavioral change (tests not specified) or death (Sprince et al., 1970).

**Endocrine studies:** Intravenous infusion of methionine (1 mmol/kg body weight or 1.5 g for a 10–kg dog) over 15 minutes resulted in small but significant increases in plasma insulin and glucagon concentrations in 4 fasting dogs (Rocha et al., 1972).

**Vitamin B6 studies:** Aggravation of methionine toxicity and increased requirement for vitamin B6 have been suggested by more rapid occurrence of acrodynia, more severe growth depression, and shorter survival times in rats fed diets deficient in vitamin B6 and containing excess methionine (Cerecedo and DeRenzio, 1950; DeBey et al., 1952; Sarma et al., 1947).

Impaired vitamin B6 status has been associated with metabolic changes in the methylation and transsulfuration pathway in rats. Smolin and Benevenga (1982) demonstrated the accumulation of plasma free and protein–bound homocysteine and erythrocyte membrane–bound homocysteine and decreased plasma cysteine in vitamin B6–deficient animals. These metabolic changes occurred when animals were fed a high–protein (60 percent casein) diet without added L–methionine. The methionine content of the diet was calculated to be about 1.7 to 2.5 percent, based on a methionine content of 1 to 1.5 percent in casein. Supplementation with the amount of L– or DL–methionine usually added to casein diets to meet the methionine requirement for rats (0.3 percent) resulted in a
more marked increase in urinary cystathionine excretion in vitamin B6-deficient Wistar rats than in Wistar rats fed a vitamin B6-deficient diet without supplemental methionine; cystathionine was not detected in the urine of the control groups fed diets adequate in vitamin B6 either with or without supplemental methionine (Shannon et al., 1975). These experiments also showed that L-methionine supplementation resulted in greater cystathionine excretion than the racemate and strain differences in the excretion of this metabolite, i.e., Wistar and Long-Evans strains excreted significantly more cystathionine than Sprague-Dawley rats.

**Functional assessments and gross pathology:** Dietary excesses of about 2 to 5 percent D-, L-, and DL-methionine have been associated with several histologic changes in rats, in particular, enlargement and darkening of the spleen, caused by increased hemosiderin deposition (e.g., Benevenga et al., 1976; Celandar and George, 1963; Klavins et al., 1963b; Steele et al., 1979; Van Pilsum and Berg, 1950). Benevenga et al. (1976) found accumulation of hemosiderin in phagocytes and accumulation of erythrocytes in the sinusoids of spleens of rats fed diets containing 2.7 percent L-methionine; no ionizable iron was found in erythrocytes in the areas of sinusoidal dilation. Supplements of MTP (2.57 percent) also resulted in development of these splenic abnormalities, suggesting that the histologic changes were caused by metabolic intermediates formed by the transaminative pathway (Steele et al., 1979). Detailed histologic examination of the spleens of MTP-fed animals by transmission electron microscopy also showed fewer reticulocytes, erythroblastic islets, megalakrococytes, and immature leukocyte forms. Despite the sequestration of erythrocytes and evidence suggesting decreased hematopoiesis in spleen, hemoglobin concentrations and hematocrits of rats fed MTP were normal. In the MTP-fed rats, bone marrow contained increased numbers of erythroblastic islands, suggesting accelerated erythropoiesis. However, reticulum cells in the erythroblastic islands showed evidence of abnormal development and peripheral erythrocytes contained ferritin and were altered in size and distorted in shape (Steele et al., 1979).

Pancreatic damage including distortion of acinar architecture and shrinking and loss of individual acinar cells was reported in male Sprague-Dawley rats fed diets supplemented with 2 or 4 percent DL-methionine for 30 days (Kaufman et al., 1960; Klavins et al., 1963b). However, no abnormalities in cellular organelles or pigment deposition were observed in pancreatic tissue of rats fed diets containing 2.7 percent L-methionine for 20 days (Benevenga et al., 1976). Kidney enlargement, apparently resulting from dilation of the renal tubules, was reported by several investigators (Benevenga et al., 1976; Klavins et al., 1963b; Roth and Allison, 1949). Although increased liver weights have been reported, no abnormalities in liver morphology were found in rats supplemented with L- or DL-methionine (Benevenga et al., 1976; Earle et al., 1942; Klavins et al., 1963a,b; Van Pilsum and Berg, 1950).

Evidence of adaptation to dietary methionine supplementation has been reported by some investigators (Benevenga et al., 1976; Stekol and Szaran, 1962) but not by others (Fau et al., 1988). In animals fed a high-protein (24 percent casein) diet supplemented with 2 or 4 percent D- or L-methionine, histological changes occurred during the first 2 months of feeding but tissues appeared normal after 3 months (Stekol and Szaran, 1962). In rats fed low-protein (10 percent casein) diets supplemented with 2.7 percent L-methionine, pathological changes in spleens were less marked at 21 days than at 14 days (Benevenga et al., 1976). However, in rats fed a low-protein diet (10 percent casein) supplemented with 2 percent DL-methionine for 2 years, increased weight and hypertrophy of spleens were reported but specific morphological characteristics were not described (Fau et al., 1988). The regression of the morphological changes in the study of Stekol and Szaran (1962) may be related to the higher protein content of the diet. Without longer follow-up, it is not known whether the decrease in morphological abnormalities reported by Benevenga et al. (1976) would have been sustained.

Experimental homocystinemia has been associated with development of endothelial lesions and thrombosis in rats (Hladovec, 1979). Methionine supplementation (2 percent DL-methionine) of a low-protein (10 percent casein) diet fed to Wistar CF male rats for 2 years resulted in aortic lesions
associated with a decrease in aortic wall distensibility that were more severe than aortic lesions in rats fed a high–protein (50 percent casein) diet (Fau et al., 1988). In these rats, the aortic intima was 10–fold thicker than in control rats. Smooth muscle cells in the aortic media showed hypertrophy and increased collagen content with replacement by chondroid cells (representative of advanced arteriosclerotic changes normally found only in much older rats) and foci of fibrosis. Significant increases in triglycerides in serum, aorta, heart, liver, kidney, and adipose tissue together with significant increases in serum LDL and VLDL cholesterol and a significant decrease in HDL cholesterol were reported in male Sprague–Dawley rats fed diets containing 1.1 percent methionine (isomer not specified) for 14 days (Kurup et al., 1982). Fecal excretion of deoxycholic acid was also significantly decreased. The authors suggested that decreased activity of triglyceride lipase in liver and aorta and decreased activity of lipoprotein lipase in aorta and heart may have been involved in the accumulation of triglycerides in tissues.

Feeding of methionine–supplemented diets high in protein and fat and adequate or deficient in vitamin B6 for 14 months resulted in higher serum triglycerides in male monkeys (Macaca radiata) than usually reported for that species (Krishnaswamy and Rao, 1977). The DL–methionine content of the diets was about 0.7 percent by weight. Gross and histological examinations of organs and tissues were normal. Total lipid content of the arterial wall was not significantly different between the 2 groups although cholesterol and phospholipids were significantly higher and triglycerides were significantly lower in this tissue in the vitamin B6–deficient animals. This study has been described as providing evidence of species differences in response to methionine supplementation; however, there was no comparison of a methionine–supplemented group with a nonsupplemented group.

In crossbred pigs which were not fed excess methionine but which were given a vitamin B6–deficient diet for 12 weeks in an attempt to increase plasma homocysteine levels, plasma methionine was elevated 10–fold. Plasma concentrations of free and protein–bound homocysteine increased but were quite variable and cystathionine was decreased (Smolin et al., 1983). At the end of the 12–week period, arteries of pigs deficient in vitamin B6 stained heavily with Evan’s blue dye indicating an increased permeability of the endothelium to proteins. Other than a large area of medial necrosis in the aorta of one pig fed the vitamin B6 diet, evidence of aortic lesions was not observed. Mural thickening of the arterioles in the kidneys of pigs given the vitamin B6 deficient diet was suggestive of hypertension, but blood pressure was not measured. Given the results reported by Fau et al. (1988), it is not clear whether the length of the experimental period was sufficient for vascular lesions to develop.

Teratology and developmental studies: No pregnancies were maintained in Sprague–Dawley rats fed a 6 percent casein diet supplemented with 5 percent L–methionine from day 1 to 14 or 21 of pregnancy (Matsueda and Niiyama, 1982). Injection of estrone and progesterone allowed maintenance of pregnancy with the methionine–supplemented diet but maternal serum concentration of methionine increased significantly (about 22–fold) and food intake and body weight decreased significantly during pregnancy. Fetal body and brain weights and brain content of RNA were significantly decreased and to a greater extent than by maternal food restriction alone. Fetal brain concentration of methionine was about 20–fold higher than that of control animals.

Rat pups injected subcutaneously with methionine (5 g/kg body weight daily in 3 divided doses, isomer not specified) from birth to 18 days of age incorporated significantly less $^{35}$S–sulfate into the sulfatide fraction of myelin lipids (only about 10 percent of the controls) and had a high mortality rate (50 percent). This treatment also resulted in significantly lower body weight, brain weight, and total brain lipid at 18 days of age (Chase and O'Brien, 1970).
c. Human studies

Changes in food intake and body weight: In a study of urinary and plasma methionine levels in infants fed commercial soy formulas fortified with 0.02 percent DL-methionine, Stegink et al. (1971) reported that urinary methionine excretion was 5 to 7 times higher than baseline in 4- to 13-day-old infants and 20 times higher than baseline in older infants. About 90 percent of the excreted methionine was in the form of the D-isomer. Total plasma methionine levels were not significantly increased, but 25 percent of the circulating methionine was in the form of the D-isomer 4 hours postprandially, indicating that tissue amino acid pools of young infants fed diets fortified with DL-methionine might contain substantial quantities of D-methionine. Growth was reported to be normal and ill effects of hypermethioninemia such as hypoglycemia or acidosis were not observed although the supporting data were not reported.

Biochemical studies: Administration of equimolar loads of L-methionine or N-acetyl-L-methionine to fasting normal adults and infants in amounts similar to the adult requirement and the quantity ingested by infants (0.065 mmol/kg body weight; i.e., 90 mg for a 10-kg infant and 630 mg for a 70-kg adult) resulted in significantly increased plasma concentrations of methionine and alanine and significantly decreased plasma concentrations of the BCAA and aromatic acids (tyrosine and phenylalanine) (Stegink et al., 1980b, 1982). Neither report included mention of side effects with this relatively small dose of L-methionine. In 3 healthy adult males, a daily supplement of 13.9 g DL-methionine given orally in a liquid formula diet for 5 days resulted in a decrease in serum CO2 and potassium concentration but no change in venous pH or serum concentrations of sodium or chloride. Urinary excretion of inorganic sulfate rose 5-fold with a concomitant decrease in pH. About 70 percent of the sulfur fed as DL-methionine was excreted in the urine as inorganic sulfate. Nitrogen, potassium, and calcium excretion increased. Sodium and chloride excretion did not change (Lemann et al. 1959). The report included no mention of side effects of ingestion of DL-methionine.

Horowitz et al. (1981) administered intravenous infusions of 50 mg L-methionine/kg ideal body weight to 9 normal control subjects and 14 patients with hepatic cirrhosis (3 g for a person with an ideal body weight of 60 kg). In the normal subjects, the fasting plasma methionine concentration was 26 μmol/L and maximal plasma concentration was reached 30 minutes after infusion. Less than 1 percent of the administered dose was excreted as methionine. Cystathionine excretion was increased but cystine and taurine excretion were not significantly altered. Homocysteine was not detected in the urine during the 24-hour period. In patients with cirrhosis, the fasting plasma methionine concentration was 65 μmol/L. The peak plasma concentration was similar to that of control subjects and was reached 30 minutes after infusion but plasma concentrations declined more slowly, resulting in a significantly longer half-life and a significantly slower metabolic clearance rate for methionine. Urinary excretion of methionine was significantly greater in cirrhotic patients than in controls. Cystathionine, cystine, and taurine excretion did not change significantly over the 24-hour period. As in the control subjects, homocysteine was not detected in the urine. A slower rate of disappearance of methionine from plasma and increased excretion of methionine was also reported in patients with acute hepatitis or cirrhosis resulting from chronic alcoholism who were given 1.5 or 4.5 g DL-methionine by intravenous infusion (Kinsel et al., 1947, 1948).

Perry et al. (1965) reported urinary excretion of small amounts of α-keto-γ-methylbutyrate in 6 subjects following an L-methionine load of 100 mg/kg body weight (7 g for a 70-kg subject). This dosage of L-methionine produced lethargy for several hours but no other untoward effects. However, oral administration of 150 mg/kg body weight of L-methionine to 1 subject (10.5 g for a 70-kg subject) produced severe nausea and vomiting beginning 2 hours after ingestion.

Kaji et al. (1980) also reported increased excretion of α-keto-γ-methylbutyrate in urine of 3 healthy subjects following 1 or 3-g loads of D-methionine but not after 3-g loads of L-methionine. Metabolites in the transaminative pathway have been identified in humans (Blom et al., 1989a,b) but no studies were found in which intermediates of this pathway were measured following a load of
L-methionine in humans. Methionine metabolites (homocysteine, homocystine and cysteine-homocysteine MDS, cystathionine, cysteinesulfinic acid, and taurine) produced by the methylation and transsulfuration pathway have been measured in plasma and urine following loading doses of L-methionine. (See p. 72 also.)

In fasting normal subjects given a single load of L-methionine (100 mg/kg body weight or 7 g for a 70-kg subject), plasma concentrations of methionine and MDS increased more than 20-fold and 3.5-fold, respectively. Plasma cysteine concentrations increased only slightly after the L-methionine load (Brattstrom et al., 1984). In a comparison of younger and older men and pre- and postmenopausal women, Boers et al. (1983) found no differences in peak levels and clearances of methionine after an oral L-methionine load (100 mg/kg body weight). Slight increases in plasma homocystine and MDS concentrations were observed in both groups of men and marked increases in these compounds were found in postmenopausal women. In contrast, premenopausal women showed no increase in plasma homocystine and a small increase in MDS (Boers et al., 1983). In 5 of 16 women who had given birth to infants with neural tube defects, peak levels of plasma total homocysteine following a methionine load were more than 2 standard deviations above the mean peak increase for 15 control women, also of childbearing age (Steegers-Theunissen et al., 1991). Traces of homocysteine were found in urine following a 3-g load of L-methionine in healthy men and women after vitamin B6 depletion (Park and Linkswiler, 1970; Shin and Linkswiler, 1974) and in 7 of 9 healthy women not depleted of the vitamin (Miller et al., 1978), although other investigators did not detect this compound (Horowitz et al., 1981; Leklem et al., 1977).

Homocysteine is highly reactive and has been shown to induce injury to vascular endothelium (Harker et al., 1976; Wall et al., 1980), potentiate the auto-oxidation of LDL cholesterol (Heinecke et al., 1987; Parthasarathy, 1987), and promote thrombosis (Graebner et al., 1982; McCully and Carvalho, 1987; Panganamala et al., 1986; Rodgers and Kane, 1986; Rodgers and Conn, 1990). Homocysteine thiolactone was used to induce homocystinemia in some of these studies and questions have been raised about the specific cause of the tissue damage, for example, the acid produced by hydrolysis of the thiolactone near the endothelium, the thiolactone itself, or one of its metabolites or acylation products (Dudman and Wilcken, 1982). However, the association of elevated plasma concentrations of homocysteine with cardiovascular disease strengthens the weight of evidence that homocysteine is responsible for the tissue damage. (See discussion on p. 72.)

Oral administration of methionine supplements (8 g given in divided doses to 5 normal subjects daily for 4 days, isomer not specified) resulted in a significant decrease in serum folate concentration over the experimental period and a progressive rise in white blood cells which was statistically significant at the end of the experimental period. Inclusion of 5 mg folic acid with the 8-g methionine supplement for 4 days resulted in a large and steady increase in plasma folate concentration in 2 subjects given both substances (Connor et al., 1978). It should be noted that the RDA for folate for an adult male is 200 μg/day (National Research Council, 1989).

Increased urinary excretion of cystathionine has been reported in young males and females following a 3-g load of L-methionine (Leklem et al., 1977; Miller et al., 1978; Park and Linkswiler, 1970; Shin and Linkswiler, 1974). Pre- and postload urinary cystathionine excretion was further increased in the presence of vitamin B6 depletion in men and women. Urinary excretion of methionine, cysteinesulfinic acid, and taurine was not altered significantly by administration of an L-methionine load. Use of oral contraceptive agents (OCA) did not alter excretion of these metabolites in women.

Behavioral studies: As reviewed by Cohen et al. (1974), 10 studies have shown that administration of methionine in combination with a monoamine oxidase (MAO) inhibitor produced functional psychosis in 62 of 107 chronic schizophrenic patients. In these studies, daily doses of L- or DL-methionine ranged from 2 to 40 g and were given for periods of 1 week to 2 months. Psychiatric symptoms were assessed by a variety of methods. Administration of L-methionine alone for 2 weeks (20 g daily during the first week and 10 g daily during the second week) also resulted in
production of functional psychosis in 7 of 11 patients with schizophrenia (Antun et al., 1971). These results may reflect increased methylation of catecholamines as a result of increased production of ado-met in brain.

**Endocrine studies:** Intravenous infusion of 30 g of L-methionine buffered to pH 7.4 in 2 fasting healthy adults resulted in small increases in plasma insulin concentration. One subject developed nausea and vomiting after the infusion. However, the other subject developed a more severe reaction that began with nausea, vomiting, increased sweating, chill followed by fever, hypotension, tachycardia, and intermittent disorientation. After 18 hours, there was mild azotemia and biochemical evidence of moderately severe hepatic dysfunction. Although intravenous glucose and electrolyte solutions and oral fluids were given, hypotension and fever persisted for about 36 hours. Symptoms disappeared completely after 4 days and subsequent liver function tests were normal (Floyd et al., 1966).

**Inborn errors of metabolism:** Perry et al. (1965) reported that autopsy of 3 siblings who died at 11 or 12 weeks of age revealed that the liver, kidney, and pancreas showed many of the histopathological changes induced in animals fed diets supplemented with methionine. The plasma concentration of methionine in 1 infant was 30 times that of control infants and the brain concentration of methionine, but not cystathionine, was also elevated. Plasma amino acids were not measured in the other 2 siblings but all 3 patients were described as having the same "fishy" or "boiled cabbage" odor. It was reported that the urine of the mother had the same odor after administration of a load of methionine. The cause of the elevated plasma methionine concentration was not determined but it is possible that these infants had an inherited metabolic disorder of methionine metabolism.

Since 1974, persistent hypermethioninemia resulting from a partial deficiency of hepatic methionine adenosyltransferase has been identified in 6 individuals. Plasma concentrations of methionine ranged from 250 to 1270 μmol/L at diagnosis and often remained above 400 μmol/L (normal <30 μmol/L) when methionine–restricted diets were fed. All of the patients had some residual activity of methionine adenosyltransferase in the liver and had normal activity in other tissues. No clinical abnormalities other than breath odor have been identified in these patients, 1 of whom was 31 years of age at diagnosis. Data available for 3 patients indicated normal development during childhood (Mudd et al., 1989).

Highly elevated concentrations of homocysteine and methionine are present in patients with homocystinemia, an autosomal recessive error of methionine metabolism, usually resulting from a deficiency of cystathionine β-synthase. Fasting plasma concentrations of methionine as high as 2000 μmol/L have been reported in patients with this disorder. Individuals with this disorder have ocular, skeletal, and neurologic symptoms and often develop premature arteriosclerotic vascular disease and venous thrombosis. Moderately elevated plasma concentrations of homocysteine are present in persons heterozygous for the disorder; in these individuals, cystathionine synthase activity is only about 50 percent that of normal (Mudd et al., 1989). Concentrations of cysteine–homocysteine MDS in plasma have been shown to rise dramatically in heterozygotes following an L-methionine load (100 mg/kg or 7 g for a 70-kg individual); in contrast, plasma MDS levels increased only moderately in normal individuals after a methionine load and homocysteine was not detected at all (Sardharwalla et al., 1974).

In a retrospective epidemiological study, Mudd et al. (1981) found that the risk of premature heart disease and strokes was not increased in 930 obligate heterozygotes (parents and grandparents of children homozygous for homocysteinemia); i.e., it was estimated that fewer than 5 percent of heterozygotes may have a fatal or nonfatal heart attack by age 50. In another study of 629 individuals with cystathionine β-synthase deficiency, 253 thromboembolic events were identified in 158 patients and of these events 130 occurred in peripheral veins or arteries (Mudd et al., 1985).
Clinical studies: Moderately elevated fasting concentrations of plasma homocysteine have been reported in patients with premature vascular disease in several studies (Araki et al., 1989; Boers et al., 1985; Brattstrom et al., 1984, 1990; Israelsson et al., 1988; Kang et al., 1986; Malinow et al., 1989; Murphy–Chutorian et al., 1985; Olaszewski and Szostak, 1988; Wilcken and Wilcken, 1976). Methionine intolerance (excessive elevation of plasma homocysteine and/or MDS following a 100 mg/kg body weight L-methionine load) has also been reported in 14 of 50 patients with premature peripheral and cerebral arterial disease but in 0 of 25 patients with myocardial infarction (Boers et al., 1983); in 4 of 19 and in 26 of 72 patients with arteriosclerotic cerebrovascular disease (Brattstrom et al., 1984, 1990); in 16 of 38, 7 of 25, and 18 of 60 patients with cerebrovascular, peripheral vascular, and coronary vascular diseases, respectively (Clarke et al., 1991); in 3 of 21 patients after myocardial infarction (Israelsson et al., 1988); and in 16 of 99 subjects with coronary vascular disease (Murphy–Chutorian et al., 1985). In contrast with their earlier findings (Wilcken and Wilcken, 1976), Wilcken et al. (1983) reported that plasma methionine, homocysteine, and cysteine concentrations were not significantly different for patients with premature ischemic heart disease and normal controls; however, there was also considerable overlap in the plasma total homocysteine concentrations of a group of obligate heterozygotes for homocysteinemia and their control subjects in this study. In the one study in which plasma methionine concentrations were reported, this amino acid was elevated to a similar extent (a 20-fold increase) 4 hours after the L-methionine load in healthy control subjects and patients with arteriosclerotic cerebrovascular disease (Brattstrom et al., 1984), similar to the 30- and 20-fold increases at 1 and 4 hours after a 5-g load of L-methionine earlier reported for healthy subjects by Block et al. (1969). Based on their own findings plus the results of the other studies cited here, Clarke et al. (1991) concluded that elevated plasma homocysteine concentration may be an independent risk factor for vascular disease, including coronary disease.

Homocysteinemia can also result from a reduced capacity for remethylation of homocysteine to methionine, a reaction dependent on folate and vitamin B12. Limited evidence suggests that folate status may also moderate the response to an L-methionine load in normal individuals and patients with vascular disease. In the study of Israelsson et al. (1988), erythrocyte folate concentrations were low in the 5 patients who had elevated fasting total homocysteine concentrations and in 3 normal subjects with relatively high MDS values before and after a 100 mg/kg body weight L-methionine load. Oral administration of 5 mg folic acid daily for 4 weeks resulted in a substantial reduction of plasma MDS concentrations before and after a subsequent load of L-methionine (Brattstrom et al., 1984). Fasting homocysteine concentrations were decreased by 53 percent and peak homocysteine levels by 39 percent after an oral L-methionine load (100 mg/kg body weight or 7 g for a 70-kg person) in 20 patients with premature cerebral and peripheral occlusive arterial disease after 4 weeks of treatment with 240 mg pyridoxine hydrochloride and 10 mg folic acid (Brattstrom et al., 1990). Wilcken et al. (1983) also reported that administration of 5 mg folic acid daily for 2 and 6 weeks decreased concentrations of homocysteine (both fasting and after a methionine load) in monozygotic twins with ischemic heart disease. In 1 twin, elevated fasting plasma concentration of homocysteine and an exaggerated response to a methionine load were verified on 2 occasions 4 years apart.

Development of hepatic encephalopathy has been precipitated by administration of methionine to patients with hepatic cirrhosis. About 15 percent of individuals with chronic alcoholism have impaired liver function due to hepatic cirrhosis (Podolsky and Isselbacher, 1987). Reports include neurological deterioration in 1 patient after administration of 8 g methionine (Watson, 1949), in 1 patient given 9 g DL-methionine daily for 3 days (Kinsell et al., 1949), and in 1 patient after 10 g DL-methionine (Singh et al., 1954).

Tolerance for methionine of patients with hepatic insufficiency and hepatic encephalopathy was greater when the amino acid was infused intravenously as compared with oral administration or when an oral antibiotic (chlorotetracycline) was administered simultaneously (Phear et al., 1956). In this study, DL-methionine was given daily in divided doses (mean dose 10 g, range 8 to 20 g) to
patients with cirrhotic liver disease. Neurologic deterioration graded on a scale of 1 to 5 by 2 observers occurred after total doses of 11 to 46 g taken over 1 to 4 days in 7 of 9 patients with portal cirrhosis and pre-existing neurologic complications associated with an extensive portal systemic collateral circulation. The neurologic changes followed a pattern similar to those seen during spontaneous deterioration. In 7 patients with less severe impairment of liver function (portal cirrhosis without neurological complications), 50 to 102 g methionine was given over an 8-day period without producing neurological changes (Phear et al., 1956). Mercaptans (methanethiol) derived from intestinal metabolism of methionine may contribute to the development of the encephalopathy (Podolsky and Isselbacher, 1987; Victor and Martin, 1987).

d. Summary and conclusions

Endpoints: Methionine is the most toxic of the amino acids required for protein synthesis in terms of depressed food intake and weight gain in animals. Plasma concentrations of methionine increased 30 to 100-fold in rats, pigs, and kittens fed diets containing 2 to 4 percent L- or DL-methionine. Liver and brain concentrations of methionine increased in rats as well, although not to such a marked extent.

Dietary excesses of about 2 to 5 percent D-, L-, and DL-methionine have been associated with enlargement and darkening of the spleen caused by increased hemosiderin deposition, pancreatic abnormalities, and kidney enlargement in rats. Significant morphologic changes in developing erythrocytes in bone marrow have been described in rats given supplements of the methionine metabolite 3-methylthiopropionate. Aortic lesions were found in rats fed a diet supplemented with 2 percent DL-methionine for 2 years; splenic hypertrophy was also reported in these animals after 2 years. Evidence from some studies has suggested that animals may adapt to diets supplemented with methionine; however, adaptation has not been shown in all studies.

Female rats fed a low-protein diet supplemented with 5 percent L-methionine were not able to maintain pregnancy. Pregnancy could be maintained with this level of methionine by injection of progesterone and estrone but body weight decreased during pregnancy and serum methionine increased 20-fold in dams; fetal body and brain weights were significantly decreased and the concentration of methionine in brain was increased 22-fold. Subcutaneous injection of methionine resulted in abnormal composition of myelin lipids in rat pups.

Impaired vitamin B6 status has been associated with impairment of the transsulfuration pathway in rats, resulting in the accumulation of homocysteine in plasma and increased cystathionine excretion. The consequences of these metabolic alterations are not known, but are of interest in view of the association of increased plasma homocysteine concentrations with cardiovascular disease in humans.

Limited evidence on behavioral effects of methionine suggests that it may have some effect on sleep-wake cycles in rats.

Studies of the biochemical mechanism of methionine toxicity suggest that depression of food intake and growth and spleen damage in rats may be caused by intermediates in the transaminative pathway of methionine metabolism. Enzymes in the transaminative pathway have been identified in humans but no studies were found in which this metabolic pathway was studied following a methionine load. However, morphologic abnormalities similar to those found in spleen, kidney, and liver of rats supplemented with methionine or 3-methylthiopropionate were reported in 3 infant siblings with elevated plasma concentrations of methionine. In an apparently different form of hypermethioninemia, no adverse developmental effects or clinical abnormalities have been identified in patients with plasma methionine concentrations persistently elevated to 8 to 40 times normal plasma concentrations.
In humans, single loads of L-methionine (100 mg/kg body weight or 7 g for a 70-kg person) have been associated with increased plasma concentrations of methionine and cysteine-homocysteine mixed disulfides (MDS). Methionine intolerance (excessive elevation of plasma homocysteine and/or MDS following a methionine load) has been reported in many patients with vascular disease and it has been suggested that elevated plasma homocysteine concentration may be an independent risk factor for vascular disease. Traces of homocysteine have been found in urine following a 3-g L-methionine load in healthy men and women depleted of vitamin B6 and in healthy women not depleted of the vitamin. Increased urinary excretion of cystathionine has also been reported in healthy males and females following a 3-g load of L-methionine.

DL-Methionine administered in daily doses of 13.9 g for 5 days to 3 healthy adults produced changes in serum pH and potassium concentration and increased urinary excretion of nitrogen, potassium, and calcium; no mention of the presence or absence of side effects was included in the study. With administration of 8 g methionine daily for 4 days, serum folate levels fell significantly and leukocytosis developed. Administration of 2 to 40 g L- or DL-methionine daily for periods of 1 week to 2 months with or without monoamine oxidase (MAO) inhibitors has resulted in production of functional psychoses in schizophrenic patients and administration of as little as a single 8-g dose of the amino acid has precipitated hepatic encephalopathy in patients with impaired liver function.

Numerous studies have shown that D-methionine is metabolized to a very limited extent, if at all, by humans.

Safe levels of human intake: Single oral doses of about 0.6 g L-methionine in adults and 0.09 g in infants produced increases in plasma methionine concentrations. No side effects were reported with these doses which were less than the amount of methionine that would be consumed in protein daily by these groups. Oral challenges of 3 g L-methionine have been associated with increased urinary excretion of cystathionine and possibly homocysteine in healthy individuals. This amount is only twice the daily methionine intake (approximately 1.4 g) of persons consuming 100 g protein per day.

Single oral doses of 7 g L-methionine have been associated with increased plasma concentrations of methionine and MDS in healthy individuals and with excessive elevations of plasma homocysteine and/or MDS in persons with vascular disease. Elevated plasma homocysteine concentration has been suggested as an independent risk factor for vascular disease, including coronary disease. Single oral doses of 100 mg/kg body weight (7 g for a 70-kg person) and 150 mg/kg body weight (10.5 g for a 70-kg person) have produced lethargy and nausea with vomiting, respectively. With administration of 8 g methionine per day for 5 days, serum folate concentration decreased and leukocytosis developed in healthy individuals. Doses of 13.9 g DL-methionine given for 5 days have been associated with biochemical changes in serum and urine.

In persons with a metabolic error in which hepatic methionine adenosyltransferase is deficient, plasma concentrations of methionine that are persistently elevated to 8 to 40 times normal plasma concentration have not been associated with developmental problems or clinical abnormalities. However, in patients with elevated plasma concentrations of methionine and homocysteine resulting from a deficiency of cystathionine β-synthase, ocular, skeletal, neurologic, and cardiovascular abnormalities result. The reason for an apparent lack of effect of high circulating concentrations of methionine in the first disorder is not understood; however, the apparent absence of adverse effects in this special population should not be interpreted as evidence for safety of methionine used as a dietary supplement by the general population.

Administration of 10 or 20 g L-methionine daily for 2 weeks produced functional psychoses in one study of patients with schizophrenia. This effect has been demonstrated in many studies in which 2 to 40 g/day of methionine was given with a monoamine oxidase (MAO) inhibitor to schizophrenic patients. Administration of as little as a single 8-g dose of methionine to patients with impaired liver function has been shown to induce hepatic encephalopathy. About 15 percent of individuals with chronic alcoholism have impaired liver function due to hepatic cirrhosis.
Although limited data are available on long-term ingestion of methionine by humans, the adverse effects observed in animal studies together with the metabolic changes observed in healthy individuals after single doses of methionine as low as 3 g suggest that use of methionine as a dietary supplement may be associated with adverse effects.
5a. L-Phenylalanine

a. Background

L-Phenylalanine is a nutritionally indispensable amino acid. It shares a transport system for uptake from the intestinal lumen (system L) with other LNAA --- leucine, isoleucine, valine, tyrosine, tryptophan, and methionine (Skeie et al., 1990). Mechanisms regulating uptake into the portal circulation are not known.

Upon reaching the liver, L-phenylalanine may be converted to tyrosine by phenylalanine hydroxylase (POH), a substrate-induced mixed-function oxidase present only in liver. This hepatic localization of POH suggests that tyrosine may be an "essential" nutrient for tissues, such as the brain, where entry is limited (see below). The basal activity of POH varies among species. The activity is much higher in rats than in humans, resulting in greater conversion of a phenylalanine load to tyrosine in rats than in humans (Clarke and Bier, 1982; Moldawer et al., 1983). Typically, about 16 percent of L-phenylalanine is converted to tyrosine in humans (Clarke and Bier, 1982). A minor pathway of L-phenylalanine metabolism is its transamination to phenylpyruvate, resulting in excretion of phenylpyruvate, phenylacetate, and phenyllactate. This pathway becomes important when POH activity is severely limited or overloaded. A third pathway, conversion to phenylethylamine, does not seem to occur to any extent in humans (Scriver et al., 1989).

Movement of L-phenylalanine among peripheral tissues is largely under hormonal control. Uptake of L-phenylalanine by skeletal muscle is facilitated by insulin (Schauder et al., 1983), and mobilization of amino acids from peripheral tissues is enhanced by cortisol (Guyton, 1991). Conversely, work of Floyd et al. (1966) suggests that increased plasma phenylalanine, or possibly catecholamine, concentration may influence the plasma concentration of insulin.

L-Phenylalanine shares a facilitated diffusion transport system with LNAA in the capillary endothelial cells of the blood-brain barrier and thus competes with these amino acids for brain uptake (Choi and Partridge, 1986). The transport system appears to be essentially alike in humans and rats (Partridge, 1988). Because phenylalanine has a greater affinity than other LNAA for binding sites in the LNAA transport system, ingestion of a bolus of phenylalanine can have a disproportionately large effect on monoamine synthesis by decreasing the entry of other LNAA into brain, including tyrosine and tryptophan which are, respectively, the precursors for the monoamine neurotransmitters catecholamines and serotonin. Phenylalanine is not readily converted to tyrosine in the brain. High concentrations of phenylalanine may also inhibit the tyrosine and tryptophan hydroxylase enzymes that catalyze the synthesis of monoamine transmitters in neurons (Caballero and Wurtman, 1988).

b. Animal studies

As stated above, the activity of POH differs substantially among species. Benevenga and Steele (1984) summarized results of studies in which amounts of L-phenylalanine ranging from 3 to 7 percent were fed to rodents and noted that in some of these experiments, rats developed symptoms similar to those seen upon addition of excess tyrosine to the diet. For example, Dolan and Godin (1966) observed signs of tyrosine toxicity, including lesions of the eyes and paws, in rats given a diet containing 7 percent L-phenylalanine. More recently, it has been shown that administration of a phenylalanine load as aspartame (L-aspartyl-L-phenylalanine methyl ester) (200 mg/kg body weight aspartame or 100 mg/kg L-phenylalanine) by gavage to rats resulted in a 2.4-fold increase in plasma tyrosine and a 1.6-fold increase in phenylalanine (Yokogoshi et al., 1984), whereas oral administration of a load of L-phenylalanine as aspartame (also 200 mg/kg body weight) to normal adult humans produced about a 9-fold increase in plasma phenylalanine (Filer and Stegink, 1988). Hjelle et al. (1992) reported that rodents require about 2 to 6 times more L-phenylalanine
(administered as a single oral bolus of aspartame) to achieve increases in plasma phenylalanine:LNAA ratios similar to those in humans given lower doses. Because of the major difference in disposition of excess L-phenylalanine between rodents and humans and the possibility that effects observed in rats following L-phenylalanine administration are due, at least in part, to its conversion to tyrosine, studies in which phenylalanine was given to rodents were not reviewed as direct evidence of adverse effects of L-phenylalanine in humans. Readers interested in biochemical and behavioral effects of L-phenylalanine in rodents are referred to studies such as those of Gibson et al. (1982a), Hommes and Matsuo (1987), Johnson and Shah (1984), McKean et al. (1967), and Okano et al. (1986).

**Endocrine studies:** Intravenous infusion of L-phenylalanine (1 mmol/kg body weight or 1.65 g for a 10-kg dog) over 15 minutes resulted in significant increases in plasma insulin, glucagon, and glucose concentrations in 4 fasting dogs (Rocha et al., 1972).

**Teratology and developmental studies:** Waisman and Harlow (1965) experimentally induced hyperphenylalaninemia in 6 infant rhesus monkeys (*Macaca mulatta*) fed milk-based formulas containing added L-phenylalanine at a level of 3 g/kg body weight/day from a few days after birth until 2 or 3 years of age. Physical growth, as evaluated by weight gain, was very similar to that of normal animals. Plasma phenylalanine concentrations, measured weekly, varied considerably but were elevated to concentrations between 610 and 2720 μmol/L (100 and 450 mg/L) for more than half of the determinations. Plasma phenylalanine concentrations were not reported for animals not given the L-phenylalanine supplement. Phenylketones were excreted in the urine when the plasma level of phenylalanine reached about 610 μmol/L, and the urine had a musty odor typical of that of children with PKU.

All of the monkeys with hyperphenylalaninemia had convulsant seizures beginning 4 to 8 months after the high L-phenylalanine diet was initiated. EEGs for these monkeys were similar to those of patients with epilepsy or PKU. With one exception, the animals did not show gross neurological abnormalities such as incoordination in walking, climbing, or prehension although all were somewhat awkward in prehension and in avoiding obstacles (Waisman and Harlow, 1965).

An extensive behavioral testing program was administered over the course of the study. Animals fed the high L-phenylalanine diet required about 35 to 65 trials in a Hebb-Williams maze whereas normal animals required about 12. The percent correct responses on the delayed-response tests were about 50 percent throughout a series of 900 trials. The percent correct responses in an object-discrimination test was similar to normal monkeys for 5 of the 6 monkeys with hyperphenylalaninemia, and the percent correct responses in the learning-set test was lower than that of normal monkeys. In delayed-response and object-discrimination learning-set testing, hyperphenylalaninemic monkeys took at least 4 times as long to complete a session as normal animals and occasionally required 3 or 4 hours to complete a session which normal animals of the same age finished in 10 to 15 minutes. Hyperphenylalaninemic animals also took a greater number of trials and a greater number of days to reach a specified criterion on successive discrimination reversal tests. The percentage of correct responses on the parallel strings test did not reach that of normal monkeys until 300 tests had been given. When the behavioral testing battery was administered, the animals supplemented with L-phenylalanine demonstrated "a high incidence of balking, long latencies before responding, failure to orient themselves to the test stimuli, and frequent lack of interest in the reward foods." Further difficulties in testing of these animals were caused by "[t]he frequent occurrence of hyperactivity and the somewhat less frequent occurrence of psychological withdrawal, as seen in body-clasping, lying down in the test cage, and huddling in a corner" (Waisman and Harlow, 1965).
c. Human studies

Few studies were found in which L-phenylalanine was administered in large doses to humans. Studies of the effects of the artificial sweetener aspartame constitute most of the scientific literature on ingestion of large doses of L-phenylalanine by humans. Phenylalanine constitutes 50 percent by weight of aspartame, and free phenylalanine enters the portal circulation after ingestion of aspartame. Specifically, methanol is cleaved from aspartame by esterases in the small intestine, and the dipeptide is split at the mucosal surface to form aspartic acid and phenylalanine and the individual amino acids and methanol are absorbed into the portal circulation (Matalon et al., 1988). Because plasma phenylalanine concentrations rise after ingestion of aspartame without a rise of similar magnitude in plasma aspartate (Steigink et al., 1980c), potential effects of ingestion of aspartame have been generally examined in relation to the phenylalanine component.

Doses of L- or DL-phenylalanine ranging from 500 to 3000 mg/day have been suggested on labels of products sold as dietary supplements (see Table 5). Some of the doses of aspartame administered in human studies have provided doses of L-phenylalanine similar to the doses suggested on amino acid product labels. For example, aspartame in doses of 50 and 100 mg/kg body weight provides 25 and 50 mg L-phenylalanine/kg body weight. Intake of 3000 mg/day of phenylalanine would correspond to daily intakes of 43, 50, and 60 mg/kg body weight for 70-, 60-, and 50-kg individuals, respectively. In comparison, a 12-ounce serving of soft drink sweetened with aspartame contains 200 mg of the sweetener or 100 mg L-phenylalanine (1.4 mg/kg body weight for a 70-kg person).

Changes in food intake and body weight: In studies of the human dietary requirement for phenylalanine, Rose et al. (1955b) initially fed a diet containing a total of 4.29 g/day of L-phenylalanine to 1 young man for 6 days without report of side effects. Although this amount is about 4 times the requirement for phenylalanine, it is similar to amounts of the amino acid that might be consumed as a component of dietary protein (see Table 2).

Inborn errors of metabolism: Because many of the studies of L-phenylalanine or aspartame administration include subjects homozygous or heterozygous for PKU, a brief overview of this group of metabolic disorders precedes the review of studies of biochemical and behavioral effects of L-phenylalanine or aspartame.

The extensive investigations of hereditary errors of phenylalanine metabolism were recently reviewed by Scriver et al. (1989). The hyperphenylalaninemias comprise a group of autosomal recessive disorders of phenylalanine hydroxylation resulting in chronically elevated plasma phenylalanine concentrations above 120 µmol/L (20 mg/L). Normal plasma phenylalanine levels are 58 ± 15 µmol/L with an upper limit of 120 µmol/L in infants. In this group of disorders, observed mutations that have resulted in a deficiency of POH, tetrahydrobiopterin (BH$_4$), or dihydropteridine reductase (DHPR) also resulted in high circulating levels of phenylalanine. PAH-deficient metabolic phenotypes are designated as PKU and non-PKU hyperphenylalaninemia. Deficiencies in the cofactor system are termed BH$_4$-deficient and DHPR-deficient hyperphenylalaninemas. Hyperphenylalaninemias resulting from POH deficiency are discussed in the following paragraphs.

Clinical manifestations of the hyperphenylalaninemias range in severity depending on the degree of deficiency of POH. Their development requires exposure to an environmental factor (phenylalanine) as well as the presence of the genetic mutation. Without dietary restriction of phenylalanine, the classic PKU syndrome is manifested as severe mental retardation; growth retardation; and dermatologic abnormalities including eczema, pigment dilution, and scleroderma-like lesions. Plasma concentrations of phenylalanine above 1000 µmol/L adversely affect brain development and function. Elevated plasma phenylalanine concentrations have been associated with quantifiable impairment of higher integrative brain functions and electroencephalographic abnormalities in PKU patients. As described above, elevated concentrations of plasma phenylalanine during pregnancy in PKU patients cause intraterine growth retardation, congenital malformations, microcephaly, and mental retardation (Scriver et al., 1989).
Much of the irreversible brain damage of untreated PKU can be prevented by restriction of dietary phenylalanine within one month of birth and continuation at least through childhood and adolescence. Termination of the treatment results in recurrence of higher plasma phenylalanine concentrations. Performance deficits including decreased IQ scores, abnormal EEG patterns, decreased neurotransmitter synthesis, impaired vigilance, and deficits in social quotients have been observed in many, but not all, PKU patients following cessation of treatment. Abnormal brain development in PKU is probably a multifactorial process possibly associated with a concomitant tyrosine deficiency, effects of phenylalanine on cellular transport mechanisms, and distribution of metabolites or secondary effects on neurochemical reactions (Scriven et al., 1989). Several alternative metabolites of phenylalanine accumulate in blood and urine including phenylpyruvate, phenyllactate, phenylacetate, phenylacetylglutamine, dihydroxyphenylacetate, and phenylethylamine. However, definitive roles for these substances in the pathology of PKU remain to be determined.

In non-PKU hyperphenylalaninemia, POH deficiency is less severe than in PKU and results in less severe disturbances in phenylalanine homeostasis. Excessive production of phenylpyruvate may occur minimally or not at all. Infants with non-PKU hyperphenylalaninemia have developed normally both with and without phenylalanine restriction (Scriven et al., 1989).

**Biochemical studies:** Plasma concentrations of phenylalanine and tyrosine were compared in 2 subjects with normal POH activity after simultaneous oral and intravenous administration of L-phenylalanine labeled with stable isotopes of nitrogen or hydrogen (Lehmann and Heinrich, 1985). Equal doses were administered by each route: 1.5 g L-[15N]phenylalanine was given orally, and 1.5 g L-[2H5]phenylalanine was given intravenously. During the 4 hours after administration, plasma concentrations of the intravenously administered phenylalanine were higher than plasma concentrations of the orally administered amino acid. In both subjects, plasma concentration peaked at about 20 μg/ml and 50 μg/ml for the orally and intravenously administered amino acids, respectively. Concentrations of labeled tyrosine were similar for both isotopes.

Ford and Berman (1977) administered an L-phenylalanine load of 100 mg/kg body weight to 115 parents and 40 of their children who were PKU heterozygotes and to 24 of their normal children. Plasma phenylalanine concentrations increased significantly in the normal children and to a greater extent in the heterozygote adults and children. Plasma concentrations returned to baseline concentrations in 4 hours in normal subjects but decreased more slowly in the heterozygote subjects. These findings suggest that persons heterozygous for PKU may clear phenylalanine from plasma more slowly than normal individuals.

Filer and Stegink (1988) summarized studies of intake of aspartame under various conditions by normal individuals, heterozygote carriers for PKU, and persons with PKU. Dose-related increases in plasma phenylalanine in normal human adult subjects have been observed following oral administration of single bolus doses of aspartame ranging from 4 to 200 mg/kg body weight (2 to 100 mg/kg body weight phenylalanine) (Filer and Stegink, 1988). As doses increased, plasma phenylalanine concentrations rose over longer time intervals (e.g., 30, 45, or 90 minutes to reach peak concentration). In contrast to the changes in plasma phenylalanine concentration, only small changes were reported in plasma aspartate concentrations following a single dose of aspartame (100 mg/kg body weight) in normal subjects (Stegink et al., 1980c, 1987a). Dose-related increases in plasma phenylalanine were observed following ingestion of solutions containing 34, 50, or 100 mg/kg body weight of aspartame by 24 infants 1 year of age. Peak plasma concentrations in these infants were similar to those of adults ingesting the same doses in solution (Filer et al., 1983).

Plasma phenylalanine concentrations rose in a dose-related manner but were increased significantly more in adults heterozygous for PKU than in normal adults given 10, 34, or 100 mg/kg body weight of aspartame (5, 17, or 50 mg/kg body weight L-phenylalanine) (Caballero et al., 1986; Stegink et al., 1979a, 1980c). Oral administration of 10 mg/kg body weight of aspartame (5 mg/kg body weight of L-phenylalanine) to adults and children with classic PKU and to patients with
atypical hyperphenylalaninemia did not result in significant increases in the already elevated plasma concentrations of plasma phenylalanine or phenylalanine:LNAA (Caballero et al., 1986). Similar results were reported in 7 adolescents with PKU who ingested 200 mg aspartame as a diet beverage (Wolf-Novak et al., 1990).

Matalon et al. (1988) reported changes in plasma levels of phenylalanine and tyrosine and in urinary excretion of organic aromatic acid metabolites and phenylethylamine with single loads of aspartame at 50 or 100 mg/kg body weight (25 or 50 mg/kg L-phenylalanine) in 25 normal subjects and 28 heterozygote carriers for PKU. Aspartame was given as a slurry in applesauce. With single loads at both dose levels, blood phenylalanine levels were increased significantly in normal and heterozygote subjects after 1 and 3 hours; increases were significantly higher in heterozygotes than in normal subjects. With the higher dose, blood phenylalanine rose 5- to 10-fold in heterozygotes and 2.5 to 6-fold in normal subjects. Blood levels of tyrosine increased only slightly, but the mean ratios of phenylalanine:tyrosine were much higher in heterozygotes than in normal subjects and were much higher with the higher dose than the lower dose. Hydroxy derivatives of aromatic acid metabolites of phenylalanine were elevated in urine of normal and heterozygote subjects in a dose-related manner; however, differences between groups were not significant because of considerable variation in values for individuals.

These investigators also conducted a 12-week study in which effects of chronic intake of aspartame (100 mg/kg body weight daily in 2 divided doses or 50 mg/kg daily of L-phenylalanine) were measured biweekly for 12 weeks (Matalon et al., 1988). No information was reported on dietary composition or intake during the study or whether the doses of aspartame given as a slurry in applesauce were administered with or apart from other foods. Plasma concentrations of phenylalanine tended to be higher in 18 heterozygote subjects than in 22 normal controls. Most subjects had plasma concentrations less than 360 μmol/L (6.0 mg/dL), the upper limit advised for pregnant women with phenylketonuria (Matalon et al., 1991). However, about 20 to 30 percent of heterozygotes and 5 percent of controls had plasma concentrations of 360 μmol/L or greater at 1 or more sampling times. Concentrations of aromatic acid metabolites of phenylalanine in urine samples collected randomly over the chronic study ranged widely in both normal and heterozygote subjects and intermittently were more than 8 times higher than baseline concentrations in 40 and 73 percent of these groups, respectively. Phenylethylamine concentrations in urine were also elevated intermittently in both groups, but differences between groups were not significant (Matalon et al., 1988). No measures of neurological or cognitive function were included in this study.

Free phenylalanine concentrations in human milk have been shown to reflect plasma concentrations of mothers. Concentrations of phenylalanine in milk were increased slightly but significantly by administration of a single oral dose of 50 mg/kg body weight of aspartame (25 mg/kg L-phenylalanine) to 6 lactating normal women (Stegink et al., 1979b). However, phenylalanine concentrations were reported to be about 24 times higher in milk of 1 PKU mother than in milk of 12 normal women (Valdivieso et al., 1973). L-Phenylalanine was not administered nor was information given on collection of milk samples or on dietary intake of the subjects in the latter study.

Behavioral studies: Oral administration of L-phenylalanine in doses of 0.8, 2.5, 5, or 10 g to 13 normal weight men did not result in changes in mean energy intake or macronutrient selection from a subsequent meal or in response to VAS that assessed hunger, mood, and arousal. The VAS used in these experiments included headache, dizziness, stomachache, nausea, drowsiness, alertness, and depression. At 45 or 90 minutes after ingestion, plasma phenylalanine concentrations and plasma phenylalanine:LNAA ratios rose significantly at all doses in comparison with control values in subjects given a 5 or 10 g L-alanine placebo. Plasma tyrosine:LNAA ratios rose significantly after the 2.5 and 5-g doses of phenylalanine doses but not after the 10-g dose (Ryan-Harshman et al., 1987). It should be noted that administration of a higher dose of L-phenylalanine (500 mg/kg body weight) suppressed food intake in Rhesus monkeys (Macaca mulatta) (Gibbs et al., 1976). Effects of
administration of individual amino acids on food consumption are discussed in greater detail in the section on aspartic acid.

Stokes et al. (1991) evaluated effects of a single 50 mg/kg body weight dose of aspartame in capsules (1.75 g L-phenylalanine for a 70-kg person, given with a doughnut and fruit juice drink) on cognitive performance of pilots (8 male and 4 female). A 90-minute SPARTANS 1.0 cognitive test battery consisting of subtests on perceptual–motor abilities, spatial abilities, working memory, attentional performance, risk taking, processing flexibility, and planning and sequencing ability was administered beginning 45 minutes after ingestion. Subjects were instructed not to use aspartame-containing products during the study or alcohol within 24 hours of the test period. Results were compared with placebo and positive alcohol controls given in a randomized double-blind protocol. No discernable performance decrements associated with aspartame ingestion for tasks related to complex cognitive performance were detected. Plasma amino acid concentrations were not measured.

A human behavioral test battery (reaction time, vigilance, Digit Symbol, tapping, mood) sensitive to effects of a number of drugs and food constituents that appear to act by altering plasma amino acid levels was used to assess mood and performance effects of aspartame in 20 healthy adult males in a double-blind, placebo-controlled, crossover study (Lieberman et al., 1988). None of the participants had reported side effects from ingestion of aspartame before the study. Subjects were given 60 mg/kg body weight aspartame (2.1 g of L-phenylalanine for a 70-kg subject) with either 36 g carbohydrate or a carbohydrate placebo, 20 mg/kg aspartame (0.7 g L-phenylalanine for a 70-kg subject) with a carbohydrate placebo, or aspartame placebo and carbohydrate. All substances were given in capsules. No behavioral effects of aspartame with or without added carbohydrate were apparent on any of the performance or mood parameters assessed. No adverse effects were reported on the side-effects questionnaire. Plasma amino acid concentrations of 4 subjects were measured before administration of the test substances and 3 hours later, well after peak phenylalanine concentration would have been reached. At this time, plasma phenylalanine:LNAA was still significantly increased with both doses of aspartame but more so with the higher dose. Addition of carbohydrate to aspartame potentiated the increase in phenylalanine:LNAA. The tyrosine:LNAA ratio was significantly increased with the higher dose both with and without carbohydrate, but the tryptophan:LNAA was not affected (Lieberman et al., 1988).

In a randomized, double-blind crossover study in 30 preschool boys, Kruesi et al. (1987) evaluated effects of aspartame (30 mg/kg body weight aspartame supplying 270 mg L-phenylalanine for an 18-kg boy), sucrose (1.75 g/kg body weight), and glucose (1.75 g/kg body weight), or saccharin (dose not stated). Eighteen of the subjects were boys whose behavior was perceived as worsening after sugar consumption; 10 of these had psychiatric diagnoses, 4 of which were described as attention deficit disorder with hyperactivity. The other 12 subjects were familiar male playmates who did not appear to respond to sugar consumption. Behavior was assessed following challenges in a playroom setting by teachers and independent observers and at home by parents. Increased motor activity with sucrose or glucose in comparison with either aspartame or saccharin was the only significant difference reported. No significant differences were found in measures of aggression and emotional reactivity (Kruesi et al., 1987).

Wolraich (1988) reviewed studies in which aspartame was used as a placebo in assessment of behavioral effects of sugar in normal, hyperactive, and psychotic children (Ferguson et al., 1986; Mahan et al., 1988; Milich and Pelham, 1986; Wolraich et al., 1985). Aspartame was given in doses of about 10 mg/kg body weight in these studies. None of the studies provided evidence of adverse behavioral effects with ingestion of aspartame.

Endocrine studies: Intravenous injection of 30 g L-phenylalanine in 6 fasting healthy adults resulted in a maximal increase in plasma insulin concentration of 28 μU/ml. Of seven amino acids infused individually at this dosage level, phenylalanine ranked third as measured by the maximal increase of plasma insulin, after arginine and lysine (81 and 52 μU/ml, respectively). No adverse
effects were reported with intravenous administration of this dose of phenylalanine although side
effects were reported for some other amino acids (Floyd et al., 1988).

**Functional assessments:** Cotzias et al. (1967) administered DL-phenylalanine to 8 patients with
Parkinsonism and 2 control subjects (1 with congenital hydrocephalus and 1 with rheumatoid
arthritis). Doses for patients ranged from 1.6 g given for 1 day to 12.6 g given for 50 days. Doses for
control subjects were 8 g for 5 days and 4.8 g for 7 days. Tremor and rigidity were exacerbated with
oral administration of DL-phenylalanine in 7 of the 8 patients. No physical or mental changes
(criteria for evaluation not given) were observed in the 2 control subjects.

Some case reports have associated aspartame ingestion with migraine headaches, seizures, and other
neurologic effects (Drake, 1986; Ferguson, 1985; Johns, 1986). In a double-blind, placebo-
controlled, crossover study, Schiffman et al. (1987) reported no increase in incidence of headaches
and no changes in vital signs, blood pressure, or plasma concentrations of cortisol, insulin, glucagon,
histamine, epinephrine, or norepinephrine following an oral challenge of 30 mg/kg body weight
aspartame in 40 overweight subjects. However, in a double-blind, placebo-controlled crossover
study in 10 children with newly diagnosed but untreated generalized absence seizures, administration of aspartame (40 mg/kg body weight or 1.4 g for a 35-g child) resulted in an increase
in EEG spike-wave discharge activity (Camfield et al., 1992). Tolleson and Barnard (1992)
concluded that Food and Drug Administration passive surveillance reports did not support the claim
that seizure occurrence was linked to consumption of aspartame.

**Developmental studies:** Effects of high circulating levels of phenylalanine on fetal and childhood
development have been studied in offspring of mothers and fathers with PKU. Microcephaly, mental retardation, congenital heart disease, and low birth weight have been described in offspring of mothers with PKU (Lenke and Levy, 1980). Screening of umbilical cord blood of mothers with
PKU indicated that IQ levels of the offspring were significantly and inversely correlated with
maternal blood concentrations of phenylalanine (Levy and Waisbren, 1983). Recently, results of a
prospective, controlled investigation of pregnant women with PKU, hyperphenylalaninemia, or
normal controls (the Maternal PKU Collaborative Study) have shown that high maternal blood
concentrations of phenylalanine during gestation were correlated with smaller head circumference
in infants (Matalon et al., 1991). Men with PKU have fathered children although one study
indicated that serum phenylalanine levels were inversely correlated with sperm count and semen
volume in males with PKU (Fisch et al., 1981). Children of fathers with PKU whose diets were not
restricted in phenylalanine did not have the physical or mental abnormalities associated with
maternal PKU (Fisch et al., 1991; Levy et al., 1991), strengthening the evidence that fetal
abnormalities in maternal PKU result from intrauterine exposure to high levels of phenylalanine
rather than genetic damage.

### d. Summary and conclusions

**Endpoints:** Because of major differences in phenylalanine metabolism between rodents and
humans, studies in which high doses of L-phenylalanine were administered to rodents were not
included in the evaluation of safety of L-phenylalanine for humans. Intravenous infusion of
L-phenylalanine has been shown to produce significant increases in plasma insulin, glucagon,
and glucose concentrations in dogs. Studies in monkeys have demonstrated that irreversible brain
damage results from oral ingestion of 3 g/kg body weight/day of L-phenylalanine beginning in early
infancy.

Occurrence of inherited hyperphenylalaninemia has provided an opportunity for observation of
effects of chronic exposure to high concentrations of plasma phenylalanine in humans. These
studies indicate that persistently elevated levels of phenylalanine in plasma before birth and during
infancy and childhood result in irreversible brain damage, growth retardation, and dermatologic
abnormalities in persons with phenylketonuria (PKU). The effects of less drastic chronic elevations of plasma phenylalanine such as those found in non–PKU hyperphenylalaninemia appear to be more heterogeneous and appear to be related to the degree of elevation in plasma phenylalanine concentrations.

Four studies in which L-phenylalanine was given to humans were included in this evaluation. Plasma phenylalanine concentrations were shown to increase after oral challenge with 100 mg/kg body weight of L-phenylalanine in normal children and adults and children heterozygous for PKU. Plasma phenylalanine concentrations were elevated to a greater degree and returned to prechallenge concentrations more slowly in heterozygote subjects than in normal subjects. Intravenously infused L-phenylalanine at a dose of 30 g produced an increase in plasma insulin concentration in normal adults. Behavioral indices measured in adult males showed no significant changes when evaluated after a single oral dose of as much as 10 g of L-phenylalanine. No adverse effects were reported when 4.3 g/day of L-phenylalanine was fed to 1 subject for 6 days; however, it should be noted that this was the total phenylalanine content of the diet and is similar to the amount consumed as a component of dietary protein.

Use of aspartame as a food additive has required studies of safety of that compound. Increases in plasma phenylalanine concentrations have been demonstrated with single oral doses of aspartame in normal adults and infants, heterozygote carriers for PKU, and patients with PKU. Daily ingestion of 100 mg/kg body weight of aspartame (50 mg/kg body weight of L-phenylalanine) resulted in increased plasma phenylalanine concentrations. Small increases in phenylalanine concentration in human milk have been reported following aspartame ingestion by lactating normal women. Ingestion of as much as 60 mg/kg body weight of aspartame (about 2 g L-phenylalanine) by normal adults did not adversely affect behavior or cognitive function. Similarly, as much as 30 mg/kg body weight of aspartame (about 260 mg L-phenylalanine) had no effects on the limited behavioral measures employed in the normal, hyperactive, or psychotic children studied. No side effects of aspartame ingestion were reported in these studies. One recent study reported increased EEG spike-wave discharge activity after aspartame ingestion in children with newly diagnosed but untreated generalized absence seizures.

Safe levels of human intake: Adverse effects were not reported with acute single oral doses of L-phenylalanine as high as 10 g or with intakes of 8 g daily for 5 days in normal individuals. In comparison, a person consuming 100 g protein per day would consume about 4.6 g L-phenylalanine. Oral ingestion of 100 mg/kg body weight of aspartame (50 mg/kg body weight of L-phenylalanine or 3.5 g for a 70-kg individual) daily for 12 weeks resulted in increased plasma levels of phenylalanine in normal individuals and heterozygous carriers for PKU. No side effects were reported, but no other measures of possible effects of chronic ingestion of L-phenylalanine were included in the study.

The safety of chronic ingestion of L-phenylalanine as dietary supplements by normal adults can not be determined from the extant data. While adverse effects observed in individuals with PKU may partly reflect their genetic defect, it seems clear from studies of this disease that L-phenylalanine itself has the potential for severe toxic effects, particularly to the fetus. Knowledge that increases in plasma phenylalanine concentration are exaggerated in persons heterozygous for PKU (most of whom are not aware of their heterozygosity) indicates concern for use of L-phenylalanine by that population. The prevalence of PKU heterozygotes in the population is estimated to be as high as 1 in 100. The high frequency of undetected heterozygotes implies a large number of the population are potentially at risk for effects of excessive phenylalanine intake. Likewise, knowledge that elevated plasma levels of phenylalanine are associated with increased transfer of phenylalanine from mother to fetus and with slightly increased levels of phenylalanine in human milk suggests that pregnant and lactating women should not use amino acid dietary supplements containing L-phenylalanine.
5b. D−Phenylalanine

a. Background

D−Phenylalanine has been shown to inhibit the activity of enkephalinase with a resultant accumulation of met−enkephalin in brain of mice (Balagot et al., 1983a). In mammals D−phenylalanine may be metabolized by oxidative deamination by D−amino acid oxidase to form phenylpyruvic acid which can then be reaminated to L−phenylalanine (Friedman, 1991). The amounts and specificities of D−amino acid oxidase differ among animal species. Humans appear to be able to invert about 0.5 g/day of D−phenylalanine to the L−isomer (Rose et al., 1955b).

b. Animal studies

Changes in food intake and body weight: Berg (1953) and Friedman (1991) summarized findings from studies in chicks, rats, and mice fed diets containing D−phenylalanine. Weight gain of animals fed the D−isomer increased from 28 to 81 percent of the gain of animals fed the L−form as the amount of D−phenylalanine in the diet increased. D−Phenylalanine did not appear to have "marked antinutritional effects or toxicity" when fed to mice at twice the optimal dietary level for L−phenylalanine (Friedman, 1991).

Neurologic studies: In studies of the analgesic effect of D−phenylalanine, mice given 158 mg/kg body weight (4 mg for a 25−g mouse) of this compound as a single dose or as 10 daily doses did not develop tolerance or dependence on the drug (Dove et al., 1985). This report did not include any mention of side effects. Likewise, no untoward effects were reported in mice given 125 or 250 mg/kg body weight of D−phenylalanine as a single intraperitoneal injection (Balagot et al., 1983a; Cheng and Pomeranz, 1979). Met−enkephalin levels in CNS tissue of mice were significantly increased for as long as 6 days after injection of 250 mg/kg of D−phenylalanine (Balagot et al., 1983a). Ehrenpreis (1982) reported that D−phenylalanine given to mice at a dose of 1 g/kg body weight/day (about 25 mg/day for a 25−g mouse) for 30 days did not produce toxic effects but they did not specify the criteria for that assessment.

c. Human studies

Nitrogen balance: Feeding of diets containing 1.1, 1.5, or 2.2 g/day of D−phenylalanine in the absence of L−tyrosine resulted in negative nitrogen balance over a 6−day period in young men, but no other adverse effects were reported. Feeding of 1.1 g/day of L−phenylalanine or the racemate resulted in slightly positive nitrogen balance in these studies (Rose et al., 1955b). The authors concluded that humans can invert as much as 0.5 g/day but not as much as 1.1 g/day of the D−isomer.

Clinical studies: D−Phenylalanine administered orally as the D−isomer or the racemate to patients with depression in doses ranging from 50 to 500 mg/day for 15 days to 6 months was associated with transient and minor side effects such as vertigo and headache and, in 1 patient, insomnia with doses greater than 300 mg/day (Beckmann et al., 1977, 1979; Fischer et al., 1975a; Heller, 1976). Plasma phenylalanine concentrations were not reported in these studies. Beckmann et al. (1977) reported that there were no biochemical changes in blood or urine and no changes in blood pressure, pulse rate, or body temperature with D−phenylalanine administration.

In an open trial with no placebo, oral administration of D−phenylalanine in divided doses totaling 750 to 1000 mg/day for 4 to 5 weeks to 78 patients with chronic pain was associated with side effects in 22 (28 percent) including drowsiness in 7, nausea in 4, increased frequency of bowel movements in 4, headaches in 3, hyperirritability in 2, sweating in 1, and an allergic rash in 1 (Balagot
et al., 1983b). Three double-blind, placebo-controlled, crossover studies of oral administration of D-phenylalanine included some mention of side effects. In the first, 2 of 22 adult patients with intractable pain given 750 mg/day of D-phenylalanine in divided doses for 3 weeks experienced mild and temporary vomiting, nausea, and drowsiness. Two additional patients experienced these side effects with both D-phenylalanine and the placebo (Budd, 1983). No adverse hematologic, hepatic, or neurological changes were reported in 30 patients given 1000 mg/day of D-phenylalanine in divided doses for 4 weeks for chronic pain (Walsh et al., 1986). Mild sedation and fatigue in an unspecified number of patients but no abnormal laboratory or physical findings were reported when doses greater than 600 mg/day were given to 19 adult patients with attention deficit disorder (Wood et al., 1985).

Urinary excretion of phenylethylamine did not increase with a single infusion of 100, 300, or 600 mg DL-phenylalanine in 6 healthy adult volunteers (Beckmann and Kasper, 1983). Similar results were observed in 22 hyperactive boys 6 to 12 years of age given D-phenylalanine orally (20 mg/kg body weight/day or 600 mg/day for a 30-kg boy) for 2 weeks (Zametkin et al., 1987). There were no side effects reported in the latter investigation which was a double-blind, placebo-controlled, crossover study.

d. Summary and conclusions

Endpoints: D-Phenylalanine given to mice at levels of 1 g/kg body weight/day for 30 days did not produce toxic effects. Side effects observed in humans given D-phenylalanine in doses as high as 1000 mg/day for 4 to 5 weeks included drowsiness and fatigue, nausea and vomiting, increased frequency of bowel movements, headaches, hyperirritability, and sweating in a small proportion of patients, and an allergic rash in 1 patient. No adverse hematologic, hepatic, or neurological changes were reported in one study in which patients were given 1000 mg/day of D-phenylalanine.

Safe levels of human intake: In limited human studies, doses of D-phenylalanine ranging from 50 to 1000 mg/day for periods as long as 6 months have been associated with occurrence of side effects. In the absence of a systematic evaluation of the effects of administration of D-phenylalanine, it is not possible to estimate a maximal safe level of oral intake of D-phenylalanine.
6. Threonine

a. Background

L-Threonine is one of the LNAA. As such, it competes for the same transport system at the blood-brain barrier as phenylalanine, tryptophan, tyrosine, methionine, and the BCAA. L-Threonine has a relatively low affinity for the carrier but its concentration in cerebrospinal fluid (CSF) is increased when large doses of the amino acid are given (Growdon, 1988; Growdon et al., 1991; Lee et al., 1990).

Like L-lysine, L-threonine does not participate in transamination reactions. The initial step in the catabolism of L-threonine is its cleavage to form acetaldehyde and glycine. The acetaldehyde is metabolized to form acetyl CoA and the glycine is catabolized via the glycine-cleavage system or freely interconverted to L-serine (Rodwell, 1990a). Thus, the metabolism of L-threonine is closely linked with that of L-serine and glycine and any consideration of adverse effects of L-threonine should take into account effects of elevated levels of glycine and L-serine as well. (See also sections on glycine and serine.)

b. Animal studies

Changes in food intake and body weight: Incorporation of excess amounts of L- or DL-threonine into diets has resulted in comparatively less growth depression in rats and pigs than high levels of other amino acids. Growth depression in rats fed low-protein diets containing 3.2, 5, and 6 percent of DL-threonine increased with increasing level of addition of the amino acid (Harper et al., 1966, 1970; Salmon, 1958; Sauberlich, 1961). Weight gain was also decreased in rats fed a low-protein diet containing 1.25 percent of added L-threonine (Alam et al., 1966a) but not in rats fed an adequate diet containing 2.4 percent of L-threonine (Benevenga and Harper, 1967). Some evidence from studies in which dietary levels of more than one amino acid were manipulated has suggested that excess threonine may precipitate a tryptophan deficiency resulting in cataract formation in rats (Harper et al., 1970). Because threonine competes with other LNAA for uptake at the blood-brain barrier (Fernstrom et al., 1973), availability of tryptophan to ocular tissues may be decreased when threonine is present in excess.

Addition of 5 percent L-threonine to a 10 percent casein diet resulted in a 27 percent reduction in food intake and a 37 percent reduction in weight gain over a 3 week period in male weanling Donryu rats compared with control animals fed the 10 percent casein diet. No significant differences were found in liver weight, or in hepatic DNA, RNA, or protein contents (Muramatsu et al., 1971).

Approximately 3 times the minimal level of L-threonine required for growth in the rat (0.8 percent in a diet containing 5 percent of an amino acid mixture) did not result in depression of food intake or growth when fed for 6 days; however, free threonine levels in plasma, liver, and muscle were 12 to 23 times greater than the levels in rats fed the basal diet. Radiotracer recovery studies indicated that the excess threonine was metabolized to carbohydrate (blood sugar and liver glycogen), liver lipids, and carbon dioxide; levels of U-14C-threonine were much higher in the amino acid pools of liver and muscle and much lower in the protein fractions of these tissues when excess threonine was fed (Yamashita and Ashida, 1971).

Addition of 0.5, 1, 2, or 4 percent excess of L-threonine to a diet containing 20 percent crude protein as corn-soybean meal did not depress growth performance (weight gain, food intake, and gain:feed ratios) in weanling pigs (Edmonds and Baker, 1987; Edmonds et al., 1987a). As might be expected from the close relationship of the metabolism of L-threonine, L-serine, and glycine, plasma levels of serine and glycine, as well as threonine, increased linearly with increased levels of dietary L-threonine supplementation (Edmonds and Baker, 1987).
Biochemical studies: Intraperitoneal injection of L-threonine at doses of 0, 50, 100, 200, or 400 mg/kg body weight to male Sprague-Dawley rats resulted in a dose-dependent and significant increase in threonine concentration in brain and spinal cord. Spinal cord glycine concentrations were also increased significantly at the 100, 200, and 400 mg/kg doses. Glycine in brain did not increase to any significant extent. Tyrosine concentrations were decreased when brain and spinal cord glycine levels were increased, suggesting that L-threonine competed with other LNAA for uptake at the blood-brain barrier (Maher and Wurtman, 1980).

Functional assessments and gross pathology: Injection of 2 nmol (238 ng) of L-threonine bilaterally into the prepyriform cortex of male Sprague-Dawley rats which had been fed a diet limiting in threonine for 14 days resulted in significantly increased intake of the threonine-imbalanced diet in preference to a protein-free diet (reversal of the usual situation when an imbalanced amino acid diet is fed). However, identical treatment with 4 nmol (476 ng) of L-threonine resulted in reduced intake of the threonine-imbalanced diet (Beverly et al., 1991). The investigators suggested that two separable responses (recognition of an amino acid imbalance in a diet, as indicated by dietary choice, and decreased food consumption) may be responsible for changes in food consumption resulting from imbalanced amino acid mixtures. They further suggested that the results of their study indicated that dietary preference and food intake were affected separately by the concentration of the dietary limiting amino acid in the prepyriform cortex.

Teratology and developmental studies: Feeding of a 6 percent casein diet containing 5 percent added L-threonine to pregnant Sprague-Dawley rats from day 1 to day 14 or 21 of pregnancy resulted in significantly lower fetal weight than in ad libitum or pair-fed controls but no change in litter size (Matsueda and Niiyama, 1982). Excess L-threonine produced a significant reduction in brain weight, DNA, RNA, and protein that was not produced by food restriction. The maternal plasma concentration of threonine increased about 13-fold. Fetal brain concentration of threonine increased about 16-fold and concentrations of phenylalanine, aspartic acid, and glutamic acid were also appreciably increased in this tissue.

c. Human studies

Nitrogen balance studies: Twenty young men fed amino-acid formula diets containing a total of 1.2 or 1.6 g of DL-threonine per day for 6-day periods remained in positive nitrogen balance (Rose et al., 1955c). In contrast, addition of the D-isomer to these diets resulted in negative nitrogen balance, suggesting that humans metabolize D-threonine to a limited extent, if at all. No mention of adverse effects other than negative nitrogen balance with D-threonine was included in the report. For perspective, an adult consuming 100 g of protein daily would consume about 4 g of threonine. (See Table 2.)

Biochemical studies: Oral loads of L-threonine (0.85, 2, or 6 g as a single dose) in 4 fasted obese subjects and 1 or 2 g given intravenously to 3 healthy controls resulted in increased excretion of threonine and its oxidation product aminoacetone, a slight increase in glycine excretion, and no change in 6-aminolevulinic acid excretion (von Studnitz, 1967). This experiment was too limited to generalize about use of aminoacetone excretion as a marker for excess threonine intake.

L-Threonine has been given as an experimental therapeutic agent with the aim of increasing concentrations of glycine in CSF in patients with spasticity. The compound has orphan drug status for this use. In 2 pilot studies, daily doses ranged from 500 mg given once a day to 6 patients for 12 months (Barbeau et al., 1982) to 500 to 1500 mg given in divided doses to 6 patients for 1 month (Growdon, 1988). In a single-blind, open-label, crossover study with 2-week observation periods, 3 or 6 g of L-threonine was given daily in divided doses to 6 patients (Lee et al., 1990). Threonine concentrations in plasma and CSF were shown to increase as threonine intake increased (Growdon, 1988; Growdon et al., 1991; Lee et al., 1990). Barbeau et al. (1982) and Growdon (1988) reported
that there were no signs of clinical or biochemical toxicity associated with L-threonine therapy. Lee et al. (1990) reported headaches in one patient and worsening of stiffness and spasms in another during L-threonine therapy.

In a randomized, double-blind, placebo-controlled, crossover study with 2-week treatment periods, 4.5 or 6.0 g of L-threonine was given daily in divided doses to 18 patients with familial spastic paraparesis (Growdon et al., 1991). Treatment with L-threonine resulted in similar and significantly increased levels of threonine in plasma and CSF at both dose levels. However, plasma and CSF glycine concentrations were not increased by L-threonine administration. The investigators reported that there were "no noticeable clinical side effects of L-threonine administration; the results of tests of hematological, hepatic and renal function did not change during treatment" (Growdon et al., 1991).

Plasma threonine concentrations have been observed to increase with increasing threonine intake, especially in premature infants. Higher plasma concentrations of threonine are of potential concern in infants because CSF levels increase with increasing plasma concentrations and because of the extensive neurologic development occurring during early infancy. Plasma and urinary threonine concentrations were higher in term infants fed whey-predominant formulas (n = 10) than in term infants fed casein-predominant formulas (n = 11) or human milk for 12 weeks (n = 22) (Jarvenpää et al., 1982a). Whey-predominant formulas have been shown to contain higher levels of threonine than casein-predominant formulas and human milk (98, 72, and 47 mg/100 ml, respectively) (Rassin et al., 1977a, 1978). Although BUN concentration was significantly higher with both types of formula, feeding of these formulas or human milk did not result in other consistent differences in rate of weight gain, head circumference, or crown–heel length (Jarvenpää et al., 1982b).

Plasma concentrations of threonine were about twice as high in 8 premature infants fed whey-predominant formulas than in an equal number of premature infants fed casein-predominant formulas (460 and 240 μmol/L or 55 and 29 mg/L, respectively). Biochemical variables including BUN concentration, blood acid–base status, plasma albumin concentrations, and plasma electrolyte concentrations did not differ significantly with the type of feeding (Kashyap et al., 1987).

In another group of 163 low-birth-weight infants fed two formulas differing in threonine content, a TPN formula, or human milk, serum threonine concentration was directly related to threonine intake (Rigo and Senterre, 1980). In this study, serum threonine concentrations were more markedly increased in the infants born more prematurely. With high threonine intakes, the most premature infants had serum threonine levels twice as high as term infants (69 and 37 mg/L, respectively). Based on cord blood concentrations for full-term infants of 32 mg/L and the regression of serum threonine concentrations in premature infants on threonine intake, the authors suggested that threonine intakes should not exceed 143 mg/kg body weight/day in premature infants (Rigo and Senterre, 1980). A recent study in which human milk was fed to premature infants by nasogastric tube has provided evidence suggesting that the composition of human milk, rather than breastfeeding and associated parental behaviors, may have an effect on IQ at age 7.5 to 8 years (Lucas et al., 1992). Although effects of specific components of human milk on neural development have not been identified, these results raise concerns about effects of high circulating levels of amino acids in all infants, full-term as well as premature.

Endocrine studies: Intravenous administration of 7.5, 8.5, 15, and 22.5 g L-threonine to 5, 1, 2, and 1 fasting healthy adults, respectively, resulted in increased plasma levels of insulin. Several side effects were reported with these treatments. One subject experienced a headache 3.5 hours after receiving an infusion of 7.5 g L-threonine. Another subject experienced transient back pain after infusion of 8.5 g, which resulted in discontinuation of the infusion. A third subject experienced severe headache and chill followed by mild elevation of body temperature which began about 30 minutes after infusion of a 22.5-g dose of L-threonine and subsided about 2 hours later (Floyd et al., 1966).
Vitamin B6 studies: Substantial elevations in fasting and postprandial plasma concentrations of threonine, serine, and glycine were reported in 6 healthy male subjects fed a high-protein diet and depleted of vitamin B6. Marked increases in urinary excretion of serine and threonine were also noted in these subjects (Park and Linkswiler, 1971). These results suggest that individuals with low or marginal intakes of vitamin B6 may have less capacity to metabolize loads of L-threonine.

Inborn errors of metabolism: Reddi (1978) published a case report of a male infant 8 months of age who exhibited growth retardation and convulsions and whose serum concentration of threonine was 71 mg/L (about 10 times higher than the normal range of 3.7 to 10.5 mg/L). Urinary excretion of threonine was elevated (620 mg/g creatinine/24 hour, normal 10 to 60 mg/g creatinine/24 hour). The infant's hemoglobin concentration was low (103 mg/L), fasting blood glucose concentration was 750 mg/L, and liver function tests were normal. The infant's serum and urine concentrations of threonine were further elevated with oral administration of 540 mg of L-threonine. Williams and Hillman (1979) reported the occurrence of hypoglycemic ketoacidosis and coma following an oral load of threonine (150 mg/kg body weight or 3 g for a 20-kg child; isomer not specified) in a child with hypotonia and growth failure. Administration of the same amounts of valine, isoleucine, or lysine did not produce this result.

d. Summary and conclusions

Endpoints: Limited data from animal studies indicate that L-threonine supplementation of low-protein diets depresses growth in rats and pigs, but to a lesser extent than many other amino acids. Growth was not depressed in rats fed L-threonine at 3 times the established level for optimal growth for 6 days and plasma, liver, and muscle levels of the amino acid were increased significantly. No adverse effects were noted in the feeding studies.

L-Threonine competes with other LNAA for uptake into the brain and large doses of threonine produce differences in concentrations of amino acids in the brains of rats. Intraperitoneal injection of L-threonine results in increased levels of threonine and glycine, but lower levels of tyrosine in the spinal cord of rats and L-threonine introduced directly into the prepyriform cortex of rats results in altered dietary preference. Supplementation of diets of female rats during pregnancy with 5 percent L-threonine resulted in lower fetal weights, lower brain weight and concentrations of DNA, RNA, and protein, and increased concentrations of threonine and other amino acids that are neurotransmitters or neurotransmitter precursors in brain.

In humans, consumption of the racemic mixture of DL-threonine at the level of 1.6 g/day for 6-day periods apparently did not result in noticeable side effects. L-Threonine has been used in treatment of spasticity at daily doses from 0.5 to 6.0 g. No clinical or biochemical indicators of possible toxicity were reported in these studies. However, with intravenous infusion of doses of L-threonine ranging from 7.5 to 22.5 g, headaches and other side effects lasting several hours were reported in 3 of 6 healthy adults receiving the amino acid.

In contrast to the results in rats, oral administration of 4.5 or 6.0 g/day of L-threonine for 2 weeks resulted in elevated levels of threonine but not of glycine in CSF in humans; however, because L-threonine competes with other LNAA for uptake in brain, effects of large doses of L-threonine are of concern. In infants, plasma levels of threonine increase with feeding of formulas containing high levels of L-threonine. This effect is more marked in premature infants who are born at a time of especially rapid neural development and in whom the plasma levels of threonine may be double those of term infants.

Safe levels of human intake: A systematic evaluation of the effects of oral administration of L-threonine on various endpoints has not been conducted and limited information is available on biochemical changes in individuals given large doses of L-threonine for an extended period. Side
effects have not been reported with daily intakes of L-threonine (e.g., 4.5 and 6 g) over a 14-day experimental period. These intakes are similar to the amount (4 g) that would be consumed by an adult consuming 100 g of protein per day. Oral administration of 1 g/day of L-threonine in divided doses for 1 year did not result in toxicity. These results are from clinical studies with a relatively small number of patients and data from chronic feeding trials in healthy subjects have not been reported. Based on the data reviewed above, L-threonine intakes at levels up to 6 g/day for 2 weeks have not been associated with adverse effects; however, consequences of intakes at that or other levels have not been investigated systematically.

Because of the possibility of alterations in brain and CSF concentrations of amino acids and potential effects on neuronal development in infants, high intakes of L-threonine are of concern. Some evidence has suggested that total daily intake of L-threonine should not exceed 143 mg/kg body weight for premature infants who may be most susceptible to CNS effects of high concentrations of threonine in plasma.
7a. L-Tryptophan

In accordance with the Scope of Work, this section provides an assessment of the adequacy of the available information on L-tryptophan for evaluation of the safety of the amino acid. It does not include a review of information on the safety of L-tryptophan produced by one manufacturer in which the compound 1,1'-ethylidene-bis[tryptophan] (EBT, also known as peak 97 or peak E) was identified as an impurity (Anonymous, 1990; Page, 1990). The association of this compound with EMS has been investigated extensively. It should be noted that Yamaguichi et al. (1991) have reported that EBT breaks down in artificial gastric juice to form 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid. Ito et al. (1992) recently synthesized EBT by the reaction of L-tryptophan with acetaldehyde and confirmed the instability of EBT in artificial gastric juice with the formation of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid. Adachi et al. (1991a) have reported the endogenous formation of this compound in humans. The structures of EBT and its β-carboline breakdown product are shown below.

![Chemical structures of EBT and its β-carboline breakdown product]

Although the emphasis of investigations has centered on EBT, it has been reported that L-tryptophan may contain as many as 60 impurities (Swinbanks and Anderson, 1992). A second impurity, 3-phenylamino-L-alanine, was recently identified in the suspect L-tryptophan (Goda et al., 1992). This compound is very similar to the compound in cooking oil that was associated with a disease very similar to EMS in Spain in 1981. The structure of the newly identified compound is shown below.

![Chemical structure of 3-phenylamino-L-alanine]

Studies of adverse effects of L-tryptophan containing EBT were not included in this review. However, selected epidemiological studies examining the association of L-tryptophan with development of eosinophilic fasciitis were considered relevant to the safety of L-tryptophan. For recent reviews of information on EMS associated with L-tryptophan, see Belongia et al. (1992) and Roufs (1992).

a. Background

L-Tryptophan is the least abundant, generally only about 1 percent, of the amino acids in protein. As one of the LNAA, L-tryptophan shares a transluminal sodium-dependent active transport system in the gastrointestinal tract with other neutral amino acids (Alpers, 1987; Hopfer, 1987). However, unlike the BCAA, tryptophan is extensively metabolized in the liver upon arrival from the portal circulation (Ross et al., 1973). For uptake into other tissues including brain, L-tryptophan must compete for carrier system L with leucine, isoleucine, valine, phenylalanine, tyrosine, and methionine (Pardridge and Oldendorf, 1977; Skeie et al., 1990). In addition to its incorporation into tissue proteins, L-tryptophan is metabolized primarily in liver by an oxidative pathway leading to complete oxidation through acetyl CoA or to the synthesis of nicotinic acid or by the 5-hydroxyindole pathway leading to formation of the neurotransmitter serotonin (5-hydroxytryptamine) in brain (Bender, 1982).
Oxidative metabolism: The major route of tryptophan metabolism in liver is an irreversible oxidative pathway. This pathway is not present in muscle. Under normal conditions some 95 to 99 percent of tryptophan metabolism occurs along this route (Bender 1982; Peters, 1991).

The initial and rate-limiting step in this pathway, conversion to N-formyl-L-kynurenine, is catalyzed by tryptophan 2,3-dioxygenase, which is induced by L-tryptophan and by glucocorticoids (cortisol). The amino acid may be oxidized to CO₂ and water or may be metabolized to form nicotinic acid by this route. The availability of the vitamin B₆ coenzyme PLP appears to be the limiting factor for the subsequent conversion of kynurenine to other metabolites along this pathway.

Several intermediates of L-tryptophan generated from kynurenine along the oxidative pathway also participate in side reactions to produce end products excreted in the urine (kynurenic acid, anthranilic acid, xanthurenic acid, and quinolinic acid). These are of interest in the assessment of adverse health effects associated with oral ingestion of L-tryptophan. Kynurenine metabolites of L-tryptophan may modulate excitatory amino acid transmission. Freese et al. (1990) reviewed evidence for neurotoxic activity of these compounds. L-Kynurenic acid and 3-hydroxyanthranilic acid have been shown to induce the accumulation of neutral lipids in livers of rats (Hirata et al., 1967). Differences in metabolism of L-tryptophan to these metabolites have been shown between males and females of several species, with differences in vitamin B₆ status, and with use of OCA (Leklem, 1971; Rose, 1966). Differences in excretion of these metabolites has generally been ascribed to altered hepatic metabolism of L-tryptophan; however, kidney, which contains high levels of several kynurenine-metabolizing enzymes, may also play a role in production of metabolites as well as exert an effect by tubular reabsorption (Rose, 1972).

Kynurenic acid has been demonstrated to be an antagonist of the excitatory amino acid N-methyl-D-aspartate (NMDA) excitatory amino acid receptors in the CNS (Ganong and Cotman, 1986; Perkins and Stone, 1982). In extrahepatic tissues including brain (Young, 1983), kynurenine can be formed by the action of indoleamine 2,3-dioxygenase. This enzyme, unlike the tryptophan 2,3-dioxygenase of liver, is not inducible by tryptophan or corticosteroids, but is induced in certain pathological conditions, including viral infections and endotoxic shock (Taylor and Feng, 1991). One study in rat brain slices suggests that under normal conditions the cerebral kynurenine pathway is minor, representing about 25 percent of the activity of the more commonly studied 5-hydroxyindole pathway (Rizza et al., 1983). Possible regulatory functions in brain and convulsant effects of kynurenic acid were reviewed by Gál and Sherman (1980) and Lapin (1981).

Under normal conditions, xanthurenic acid is a minor by-product of tryptophan metabolism. Kotake and Murakami (1971) have shown that xanthurenic acid combines with insulin in vitro to form a complex that had little insulin-like activity when injected into rabbits and dogs. It has been suggested that a high circulating concentration of xanthurenic acid would undergo a similar reaction with circulating insulin in vivo (Bender, 1982), but this has not been investigated in humans.

Quinolinic acid is an endogenous agonist of the NMDA receptor in the CNS and, as such, can be neurotoxic (Stone and Perkins, 1981). This compound normally does not cross the blood-brain barrier (Kitt and Spector, 1987) and is presumably synthesized from kynurenine in the central nervous system. Heyes (1991) has questioned whether tryptophan loading might have neurological or behavioral consequences.

5-Hydroxyindole pathway: The first step in this pathway is the hydroxylation of L-tryptophan to form 5-hydroxytryptophan. This compound is subsequently decarboxylated to form serotonin (5-hydroxytryptamine), a potent vasoconstrictor found chiefly in the brain, intestinal tissues, blood platelets, and mast cells (Sidransky, 1986). Hydroxylation of tryptophan is the rate-limiting step in the synthesis of serotonin in the CNS. Because the enzyme is not ordinarily fully saturated with tryptophan, higher brain levels of tryptophan increase the rate of serotonin synthesis. Serotonin is oxidized to 5-hydroxyindole acetic acid (5-HIAA), which is excreted in the urine (Bender, 1982; Peters, 1991; Wurtman et al., 1981b; Young, 1986).

92
Only a small fraction of L-tryptophan is metabolized by the 5-hydroxyindole pathway; however, because it has been shown that plasma concentrations of L-tryptophan influence synthesis of 5-hydroxytryptophan and serotonin (Fernstrom and Wurtman, 1971), effects of L-tryptophan loads on this system have been studied extensively. Competition among all the LNAA for the L carrier system is an important factor controlling brain tryptophan levels (Fernstrom and Wurtman, 1972; Wurtman et al., 1981a). Protein and carbohydrate composition of meals affects the levels of plasma tryptophan and ratios of tryptophan:LNAA and thus influences the amount of tryptophan available for uptake by brain (Fernstrom, 1986). Because L-tryptophan is the amino acid usually present in smallest quantity in proteins (0.5 to 1.6 percent), the relative increase in plasma tryptophan concentration is less than that of the other LNAA and consumption of a high-protein meal results in a fall in the tryptophan:LNAA ratio. In contrast, consumption of a meal high in carbohydrates stimulates insulin secretion, removing the competing BCAA from circulation and increasing tryptophan availability by increasing the tryptophan:LNAA ratio (Gibson et al., 1982b). When L-tryptophan is ingested as a dietary supplement apart from meals, competition from other LNAA for uptake in brain would be expected to be reduced.

Other pathways of L-tryptophan metabolism: L-Tryptophan is also converted in brain to tryptamine, the pineal indoles (products of the action of indoleamine 2,3-dioxygenase), and products such as tetrahydro-β-carbolines formed from the condensation of L-tryptophan with an aldehyde (e.g., acetaldehyde produced during the oxidation of ethanol) (Young, 1986). Tryptamine, which can cross the blood–brain barrier, is also produced in the liver and kidney and by the gastrointestinal flora (Young and Gauthier, 1981).

The metabolism and function of tryptamine in brain was summarized by Young (1986). The rate of tryptamine metabolism appears to be about 15 percent that of serotonin. In contrast to serotonin which increased only about 2-fold following administration of L-tryptophan, the concentration of the tryptamine metabolite indoleacetic acid (IAA) in the cisternal CSF of humans increased in proportion to the size of an oral L-tryptophan load (3 or 6 g). However, the increase in the concentration of 5-hydroxytryptamine (5-HT), the intermediate in the synthesis of tryptamine from tryptophan, was similar following both loads, suggesting that some of the tryptamine in brain may be derived from peripheral sources (Young and Gauthier, 1981). Release of tryptamine does not depend on the rate of neuronal firing (Héry et al., 1979). Young and Anderson (1982) have shown that tryptophan administration (100 mg/kg body weight injected intraperitoneally) can increase tryptophan, serotonin, 5-HIAA, 5-hydroxytryptophol, and melatonin contents in pineal glands of rats, but effects of enhanced tryptophan availability on pineal function remain to be determined.

The presence of tetrahydro-β-carbolines has been reported in brain, adrenal, and platelets of rats (Barker et al., 1979; Honecker and Rommelspacher, 1978; Rommelspacher et al., 1978, 1979; Shoemaker et al., 1978) and human urine following loading with 5-hydroxytryptophan (Rommelspacher et al., 1979). More recently Adachi et al. (1991b) identified tetrahydro-β-carbolines in human milk and human urine. Urinary excretion of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid was reported to increase following oral administration of 0.5 g L-tryptophan to adult males (Adachi et al., 1991b). This compound was suggested as a probable precursor of the benzodiazepine receptor antagonist, β-carboline-3-carboxylate (Braestrup et al., 1980). Buckholtz (1980) reviewed evidence for possible effects of these compounds on neurotransmitter metabolism, behavior, and neuroendocrine function.

These compounds are of interest because evidence suggests that 1,1'-ethyldiene-bis[tryptophan], which was an impurity not removed in the manufacture of L-tryptophan by the process used by a single company, breaks down in artificial gastric juice to form 1-methyl-1,2,3,4-tetrahydro-β-carboxylic acid (Yamaguichi et al., 1991). It has been suggested that this compound may be responsible for development of the EMS associated with consumption of L-tryptophan supplements from that source (Anonymous, 1990; Belongia et al., 1990). To date, possible metabolic and functional effects resulting from production of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid
following a load of L-tryptophan in humans have not been investigated. Two retrospective studies of the association of eosinophilic fasciitis and localized scleroderma with L-tryptophan ingestion have suggested that some types of cutaneous fibrosis may have developed following L-tryptophan ingestion prior to the outbreak of L-tryptophan-associated EMS (Blauvelt and Falanga, 1991; Hibbs et al., 1992).

Skatole (3-methylindole) is formed by the action of bacteria on L-tryptophan. Both skatole and tryptophan have been shown to cause pulmonary edema and emphysema in ruminants (Carlson and Bray, 1983). This could possibly be of concern to humans with conditions such as achlorhydria where bacteria may convert tryptophan to skatole in the upper part of the intestine or malabsorption where tryptophan may reach the flora of the lower gastrointestinal tract (Young, 1986).

b. Animal studies

Changes in food intake and body weight: Harper et al., (1970) and Benevenga and Steele (1984) reviewed the literature on effects of orally administered L-tryptophan on food intake and growth depression in animals. Feeding of low-protein diets (6 or 10 percent casein) containing 5 percent added L-tryptophan for periods of 4 days to 4 weeks resulted in reduced food intake and markedly decreased weight gain in rats (Muramatsu et al., 1971; Peng et al., 1973; Sauberlich, 1961). The growth depressing effect of 5 percent L-tryptophan was counteracted completely by increasing the protein content of the diet to 25 percent (Muramatsu et al., 1971).

Rapid weight loss and deterioration in general appearance occurred in adult female Sprague-Dawley rats fed 20 percent casein diets supplemented with 28.5 percent L-tryptophan for 10 days. Supplementation with 14.3 percent L-tryptophan for 4 weeks resulted in development of scaly tails and thinning hair; adverse effects were not reported in rats supplemented with 1.4 or 2.9 percent of the amino acid (Funk et al., 1991).

In 30-day subacute toxicity studies in male and female Wistar rats injected intraperitoneally with L-tryptophan (500, 1000, or 2000 mg/kg body weight/day), body weight gain and food intake were significantly decreased with the 2 higher doses (Kawaguchi et al., 1980). Histopathological and biochemical findings in this study are described on p. 98.

In an 80-week carcinogenicity study, male Fischer 344 rats were fed diets containing 2 percent added L-tryptophan with 2 levels of vitamin B6. Compared with rats consuming diets containing no added L-tryptophan, rats consuming diets containing 2 percent added L-tryptophan had significantly lower body weights only at the end of the study. Dietary vitamin B6 and L-tryptophan levels did not influence food consumption (Birt et al., 1987). Similarly, body weights of male and female B6C3F1 mice and male Fischer 344 rats (but not female rats) were lower than those of matched control animals in a 2-year carcinogenicity study in which 2.5 and 5.0 percent L-tryptophan was added to the diet (U.S. Department of Health, Education, and Welfare, 1978). No dose–related differences in mortality rates were observed.

In a study of the safety of L-tryptophan supplements for pigs, weight gain was neither increased nor decreased over a 40–day period with supplementation of the diet with 0.1 or 1 percent feed–grade L-tryptophan. These levels of L-tryptophan were 3 and 30 times the usual levels of L-tryptophan supplementation for pigs. The intakes of L-tryptophan were about 3.6 and 36 g/day at the 0.1 and 1 percent levels of supplementation. Initial weight of the animals was about 79 kg. Supplementation with 2 or 4 percent L-tryptophan resulted in decreased weight gain; feed intake was also decreased with the 4 percent supplement. Diarrhea did not occur with any level of L-tryptophan supplement (Chung et al., 1991). Histopathologic and hematologic findings from this study are presented on p. 98.
Biochemical studies: As reviewed by Sidransky (1985), administration of L-tryptophan by feeding or intraperitoneal injection has been shown to induce the activities of a variety of nutritionally and hormonally sensitive hepatic enzymes which may or may not be related to tryptophan metabolism (Sidransky, 1985).

L-Tryptophan induces a rapid increase in protein synthesis and polyribosomal aggregation in liver of rats and mice. These actions are specific for L-tryptophan and are not duplicated by analogs, metabolites or compounds related to L-tryptophan or by other indispensable amino acids (Kurl et al., 1987; Sidransky, 1985; Sidransky et al., 1992). Additionally, L-tryptophan has been reported to increase protein synthesis in blood, lung, brain, and kidney (Sidransky, 1985). Possible mechanisms for these actions were reviewed by Sidransky (1986).

Administration of L-tryptophan has been associated with induction of hypoglycemia in rats; however, studies in other species suggest that there may be considerable interspecies variation in this effect (Munoz-Clare et al., 1981). Sidransky (1985) has summarized proposed mechanisms for a role of L-tryptophan in hepatic carbohydrate metabolism. L-Tryptophan has also been reported to induce changes in hepatic lipid metabolism. Hepatic lipid changes are discussed in greater detail on p. 98–99. It should be noted that Aviram et al. (1991) reported that addition of 0.4 percent L-tryptophan to atherogenic diets did not affect plasma cholesterol concentration in rats, but it significantly increased plasma lipid peroxidation and enhanced the macrophage cholesterol esterification rates, probably through increased serotonin production. However, in male Wistar rats fed 10 percent L-tryptophan in a 23 percent casein diet for 2, 3, or 4 months, plasma total cholesterol concentration was 35 percent lower than in the control group (Sérougine and Rukaj, 1983).

Neurotransmitter studies: Serotonin synthesis in the brain can be influenced by the availability of L-tryptophan in several species including monkeys (Leathwood and Fernstrom, 1990), dogs (Moir and Eccleston, 1968), and rats (Ashcroft et al., 1965; Eccleston et al., 1965; Fernstrom and Wurtman, 1971; Fregly et al., 1989a; Green et al., 1962; Marsden and Curzon, 1976). Because of the similarity and supportive nature of results across species, only the results from 2 studies conducted over extended time periods (Fregly et al., 1989a; Leathwood and Fernstrom, 1990) will be given in detail. It should be noted that, in contrast to results obtained in studies of catecholamine synthesis from L-tyrosine (see p. 181), the influence of L-tryptophan availability on serotonin release appears to be independent of the rate of neuronal firing (Collard et al., 1982; Karobath, 1972; Nagatsu et al., 1983h; Schaechter and Wurtman, 1990).

Effects of oral L-tryptophan administration on brain tryptophan, serotonin, and 5-HIAA concentrations were studied in 24 adult male and female cynomolgus monkeys (Macaca fascicularis) given a mixture of L-tryptophan and maltodextrin daily for 13 weeks in a study designed to evaluate the chronic toxicity of a combination of carbohydrate and L-tryptophan (Leathwood and Fernstrom, 1990). Each morning monkeys were given one of the following treatments: distilled water, 20 mg/kg body weight L-tryptophan and 2 g/kg body weight maltodextrin, 90 mg/kg body weight L-tryptophan and 3.2 mg/kg body weight maltodextrin, and 400 mg/kg body weight L-tryptophan and 5 g/kg body weight maltodextrin. Daily L-tryptophan intakes were 60, 270, and 1.2 g for a 3-kg monkey. Baseline values and response to a load of L-tryptophan and maltodextrin were measured at the end of the 13-week period. For this load, fasting monkeys were given an oral bolus of L-tryptophan at the dose level they had received during the study. Fasting plasma concentrations of tryptophan and other LNAA did not show an effect of the preceding 13–weeks dosing. The concentration of tryptophan in plasma increased in a dose-related manner to nearly 16 times the control values with the highest dose. Plasma phenylalanine showed a dose-related decrease in concentration. Other LNAA (tyrosine, leucine, isoleucine, and valine) also showed significant decreases in plasma concentrations but did not show greater decrements with the larger doses. Plasma tryptophan:LNAA ratios rose in a dose-related manner after the amino acid/carbohydrate treatment and the ratios reflected the decrease in the LNAA concentrations brought about by insulin-mediated uptake of these amino acids, mainly into muscle. Plasma levels
of aspartate, but not glutamate, also decreased significantly after the L-tryptophan load. In cerebral cortex, the tryptophan concentration increased significantly and in a dose-related manner. Cortical levels of the other LNAA showed a dose-related decrease, significant at the highest dose, but the concentrations of aspartate and glutamate did not decrease significantly. Essentially similar increments and decrements in hydroxyindoles and LNAA in plasma and brain were also reported in rats given L-tryptophan (Peng et al., 1973; Yokogoshi et al., 1987).

Leathwood and Fernstrom (1990) measured serotonin and 5-HIAA concentrations in corpus striatum, brain stem, cerebral cortex, and hypothalamus. Separate statistical analyses for each brain region showed that the increases in serotonin and 5-HIAA levels were not comparable among brain regions. Both serotonin and serotonin plus 5-HIAA increased significantly with increasing dose in the corpus striatum and concentrations of both serotonin and 5-HIAA separately increased in a dose-related and significant manner in the brainstem. In the cerebral cortex, serotonin and 5-HIAA concentrations increased only slightly and did not show a dose-related effect. In the hypothalamus, 5-HIAA increased in a dose-related manner, significant only at the highest dose but serotonin concentration did not increase significantly. A global analysis of variance for all the brain regions indicated a significant dose-effect for 5-HIAA and for serotonin plus 5-HIAA as well as significant differences between brain regions and a significant dose by region interaction for serotonin and serotonin plus 5-HIAA (Leathwood and Fernstrom, 1990). No behavioral parameters were reported in this study.

Concentrations of serotonin and 5-HIAA, but not norepinephrine, were significantly increased in the lower brain stem of female Sprague-Dawley rats fed a powdered commercial laboratory ration supplemented with 5 percent L-tryptophan for 6 weeks. Daily urinary excretion of norepinephrine and dopamine were not changed, but excretion of epinephrine was almost doubled. Systolic blood pressure, plasma renin activity, and plasma aldosterone concentration were not affected by L-tryptophan supplementation, and weights of heart, kidney, adrenals and thyroid gland did not differ significantly in the control and L-tryptophan supplemented groups (Fregly et al., 1989a).

Behavioral studies: As summarized by Wurtman et al. (1981b), evidence from animal studies suggests that serotonergic neurons may play a role in many aspects of behavior including sleep, feeding, temperature control, pain sensitivity, locomotor activity, aggression, and myoclonus. Effects of L-tryptophan administration on several of these responses have been investigated.

Sleep latency was significantly reduced in rats with a single injection of 450 or 600 mg/kg body weight L-tryptophan but not with lower doses. However, overall time spent in synchronized sleep, desynchronized sleep, and waking over an 8-hour period was not affected by L-tryptophan (Hartmann and Chung, 1972).

Injection of L-tryptophan was also observed to reduce food intake in rats allowed free access to food (Latham and Blundell, 1979). In food-deprived rats given access to food, some investigators (Latham and Blundell, 1979) have shown decreased food intake while others (Weinberger et al., 1978) have shown no effect of L-tryptophan injection. Studies in dogs and cats have suggested that gastric emptying is delayed with administration of L-tryptophan (Cooke, 1978; Stephens et al., 1975).

Motor activity, as measured by interruption of a beam between photoconductive sensors, was significantly decreased in male NMRI mice at 1 to 1.5 hours after intraperitoneal injection of 800 mg/kg body weight L-tryptophan but not by doses of 50 to 400 mg/kg body weight (Modigh, 1973). At the highest dose, brain tryptophan, serotonin, and 5-HIAA concentrations were significantly elevated over the same time period that motor activity was depressed. Pretreatment of L-tryptophan-injected mice with inhibitors of certain reactions in tryptophan metabolism did not antagonize the L-tryptophan-induced depression of motor activity, but some agents (e.g., allopurinol) potentiated and prolonged the depressive effect and prolonged the elevations of brain tryptophan and serotonin concentrations, suggesting that L-tryptophan itself and not tryptophan
metabolites may be responsible for the decrease in motor activity (Modigh, 1973). Further investigations of the effects of compounds inhibiting tryptophan metabolism were not found. Subsequently, Gibson et al. (1982a) demonstrated in male Charles River CD-1 mice that intraperitoneal injection of 320 mg/kg body weight of L-tryptophan (but not lower doses) increased the duration of immobility in a swim test, but they did not observe a significant decrease in open field activity at doses as high as 320 mg/kg body weight. Brain concentrations of tryptophan and serotonin showed a dose-related increase with the amount of L-tryptophan administered.

Spontaneous locomotor activity of male gerbils, as measured by interruption of a photocell beam was decreased significantly after intraperitoneal injection of 200 mg/kg body weight of L-tryptophan. This dose of L-tryptophan also significantly decreased locomotor activity in gerbils whose locomotor activity had been greatly increased by a 5-minute period of cerebral ischemia when measured at 6 and 24 hours post–ischemia (Carney, 1986).

In rats, doses of 100 mg/kg body weight of L-tryptophan given intraperitoneally did not alter shuttle escape behavior (Brown et al., 1982). Doses of 120 mg/kg body weight given intraperitoneally did not alter EEG or EMG recordings in rats (Wojcik et al., 1980) and did not alter lever pressing responses in an FR–40 food reinforcement paradigm (Lyness, 1983).

Male Sprague-Dawley rats trained to press a lever to self-administer d-amphetamine significantly decreased their daily amphetamine self-injections in a dose-related manner when their diet was supplemented with 2 or 4 percent L-tryptophan for 3 days (Smith et al., 1986). When the original diet (AIN-76 with an L-tryptophan content of 0.26 percent) was substituted for the L-tryptophan-supplemented diets, the animals returned to baseline (pre-supplementation) level of self-administration of d-amphetamine. Control experiments suggested that amphetamine self-administration did not reduce food intake and that L-tryptophan supplements did not affect responding in a related paradigm without amphetamine (i.e., motor performance was not affected by L-tryptophan, only amphetamine self-administration). Brain concentrations of serotonin and 5-HIAA were significantly increased over a 3-day period by 4 percent L-tryptophan supplementation in d-amphetamine-naive animals, but concentrations of dopamine and norepinephrine were not affected. Serotonin and 5-HIAA concentrations increased to a lesser extent with the 2 percent L-tryptophan supplement (Smith et al., 1986). Neurotransmitter concentrations were not measured in the brains of L-tryptophan–supplemented rats exposed to d-amphetamine.

Supplementation of a powdered commercial laboratory ration with 2.5 or 5 percent L-tryptophan for 12 weeks produced a dose-related reduction in the exaggerated spontaneous salt intake induced by deoxycorticosterone acetate (DOCA) of female Sprague-Dawley rats given a choice of water or 0.15 M sodium chloride solution (Fregly et al., 1989b). However, without DOCA treatment, spontaneous salt intake was not affected by addition of 5 percent L-tryptophan to the diet for a 4-week period.

Muricidal (mouse-killing) behavior of male rats was significantly inhibited and the latency to kill significantly increased with a single 50-mg/kg body weight dose of L-tryptophan given intraperitoneally. A daily 25-mg/kg body weight dose given for 4 days did not significantly affect either parameter (Broderick and Bridger, 1984). Similar results in muricidal activity following L-tryptophan administration were also reported in rats by Gibbons et al. (1979) and Valzelli et al. (1981).

Maintenance of weanling Sprague-Dawley rats on a diet supplemented with 5 percent L-tryptophan for 2 weeks resulted in significantly improved performance (fewer errors) in a water maze test and a significant increase (141 percent of controls) in brain serotonin concentration. Feeding of the 5 percent L-tryptophan supplement produced a 10-fold increase in plasma tryptophan concentration and a 35 percent decrease in weight gain at the end of the 2-week period (McKean et al., 1967).
Administration of L-tryptophan in drinking water (about 2 mg/day or 800 mg/kg body weight) for up to 57 days did not result in significant quantitative behavioral or neurologic changes in 8 rhesus monkeys (Macaca mulatta). Behavioral tests included tests of visual shape discrimination and spatial alternation but little experimental detail was provided. Urinary excretion of tryptophan, kynurenine, kynurenic acid, xanthurenic acid, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, and indole acetic acid was increased in one of the monkeys; excretion studies were not reported in the other animals (Curzon et al., 1963).

Endocrine studies: Intravenous infusion of L-tryptophan (1 mmol/kg body weight or 2.04 g for a 10-kg dog) over a 15-minute period resulted in significant increases in plasma glucose, insulin, and glucagon concentrations in 4 fasting dogs (Rocha et al., 1972). Infusion of this dose of L-tryptophan induced vomiting in all dogs. Intraduodenal administration of L-tryptophan (in incremental doses of 0.09, 0.28, 0.83, 2.5, and 7.5 mmol given over a 45-minute period) produced a dose-related increase in cholecystokinin-like activity in the dog (Singer et al., 1989). Intestinal administration of amino acids has been shown to stimulate pancreatic enzyme secretion by releasing cholecystokinin from the intestine (Konturek et al., 1986). L-tryptophan was found to be the most potent essential amino acid for pancreatic enzyme secretion in the dog (Singer et al., 1979).

Subcutaneous injection of 10 mg L-tryptophan with 0.5 mg bovine growth hormone daily for 6 days significantly increased weight gain in adult female rats; injection of either compound alone did not have this effect (Ottaway, 1973).

Functional assessments and gross pathology: Effects of supplementation of diets with 0.1 and 1 percent feed-grade L-tryptophan were studied in 16 crossbred pigs. These supplements were 3 and 30 times the usual intake of tryptophan for the pig. Blood counts and enzyme assays (AST, creatine phosphokinase, and LDH) were done weekly and a pathological examination was done at the end of the 40-day feeding trial. Comprehensive pathological studies showed no differences between control and L-tryptophan-supplemented animals. Total blood leukocytes, relative eosinophil counts, and enzyme activities were not significantly affected by L-tryptophan supplementation. All bone marrow specimens were extremely cellular and all cell lines were represented. There were no atypical or unusual findings in the bone marrow and no abnormal accumulation of eosinophils and/or their precursors (Chung et al., 1991).

In 30-day subacute toxicity studies in male and female Wistar rats injected intraperitoneally with 500, 1000, or 2000 mg/kg body weight L-tryptophan daily, the mortality rate was higher with the highest dose (4 of 10 males and 2 of 10 females compared with no deaths in the saline-injected control groups). Histopathological examination revealed hepatocyte hypertrophy, thymic atrophy, lipid depletion of zona fasciculata cells in the adrenal, and reticular cell hyperplasia in the lymph nodes with the two higher doses (Kawaguchi et al., 1980). Thymus weights decreased as L-tryptophan dose increased in both sexes. The differences were significant with the two higher doses. Liver weights increased with increasing dose only in females; the increases were significant with all doses of L-tryptophan. The only serum biochemical values showing significant differences were the activities of ALT and AST, which were increased in both sexes. No hematological values in the rats were significantly different from controls. These included hemoglobin concentration, hematocrit, and counts of red cells, platelets, white cells, and a differential count of basophils, eosinophils, neutrophils, lymphocytes, and monocytes.

Development of fatty livers, mainly an increase in triglyceride content in the peripheral zones of the lobules, was described when L-tryptophan was given intraperitoneally in single doses (500 to 1000 mg/kg) to fasted male rats (Hirata et al., 1967; Rao et al., 1981; Sidransky, 1985) or administration of 250 mg/kg body weight by gastric gavage on alternate days over a 14-day period (Trulson and Sampson, 1988). Increased hepatic lipogenesis but no fatty deposits were reported with intraperitoneal doses (50 or 500 mg/kg body weight) (Fears and Murrell, 1980; Sakurai, 1974; Sakurai et al., 1973) or with a diet containing 0.25 percent L-tryptophan fed for 7 days (Fears and Murrell, 1980) or when L-tryptophan was given to rats in drinking water, providing about
200 mg/kg body weight of L-tryptophan daily for 60 days (Bucci and Chiavarelli, 1979). The role of L-tryptophan-induced changes in hepatic lipid metabolism and pathology has not been determined. Sidransky (1985) has reviewed and discussed possible mechanisms.

Spermatogenesis was significantly decreased and spermatid degeneration significantly increased in adult male Wistar rats given intraperitoneal injections of 200 mg/kg body weight daily for 21 days (Das et al., 1982).

Platelet aggregation was markedly enhanced in rats fed diets containing 0.4 and 1 percent L-tryptophan for 21 days (Mokady et al., 1990). A lack of change in plasma concentrations of triglycerides, total cholesterol, and HDL-cholesterol suggested that plasma lipid levels were not related to this effect. Excretion of high levels of 5-HIAA with this treatment suggested the possible involvement of serotonin.

The Lewis rat is susceptible to many inflammatory diseases and has been used to study the effects of administration of L-tryptophan containing various impurities associated with EMS. Love et al. (1991) reported that treatment of young female Lewis rats with noncase-associated L-tryptophan (L-tryptophan not containing EBT) resulted in a mild but significant increase in the thickness of the myofascia and significant pancreatic pathology including periductal and perivascular fibrosis and acinar changes (reactive and hyperplastic acinar epithelium). These effects were similar to the pathological changes noted in animals receiving EMS-case-associated L-tryptophan or chemically synthesized EBT. However, only those animals receiving the EMS-case-associated L-tryptophan showed evidence of immune activation of their peripheral mononuclear cells. In this study, rats were given a 2-g bolus of L-tryptophan/kg body weight in a methyl cellulose vehicle by gavage 6 days/week for 6 weeks. In an earlier study, young female Lewis rats were given a 1.6 g/kg body weight bolus of noncase-associated L-tryptophan by gavage daily for 38 days. These animals did not develop the pathologic changes in small intestine produced by EMS-case-associated L-tryptophan (DeSchryver-Kecskemeti et al., 1991) nor did they develop eosinophilia; histology of muscle, lung, and skin was similar for the vehicle control group and the group given noncase-associated L-tryptophan (Crofford et al., 1990). Crofford et al. (1990) also reported that plasma kynurenine concentrations were increased to a similar extent for animals given both sources of tryptophan.

Chronic (6-week) administration of a diet supplemented with 2.5 percent L-tryptophan supplying 850 mg L-tryptophan daily prevented development of cold-induced hypertension, attenuated cardiac hypertrophy, and did not affect body weight in Harlan Sprague-Dawley rats exposed to cold (Rieselsmann et al., 1991). However, supplementation of the diet with 5 percent L-tryptophan supplying 1690 mg L-tryptophan daily was associated with the development of some adverse effects in that addition of this amount of L-tryptophan only partially prevented the rate of blood pressure increase, did not affect cardiac hypertrophy, slowed the rate of body weight gain, and increased the urinary excretion of epinephrine. Fregley and coworkers have also shown an association of L-tryptophan-supplemented diets with prevention of DOCA-induced hypertension, renal hypertension, and spontaneous hypertension in rats (Fregley and Fater, 1986; Fregly et al., 1988, 1989b).

Carcinogenicity studies: A possible association between L-tryptophan and liver and bladder cancers was first reported in Fischer female rats in 1950 (Dunning et al., 1950). Because L-tryptophan exerts unique effects on liver RNA and protein metabolism and because several metabolites of L-tryptophan are similar to aromatic amines that are known bladder carcinogens (e.g., 2-naphthylamine, benzidine, and 4-aminobiphenyl), possible carcinogenic effects of L-tryptophan on these organs have been studied in detail. Results of these extensive investigations were reviewed by Sidransky (1986).

Liver tumors: Feeding of diets containing 1 to 2 percent added L- or DL-tryptophan for 6 months to 3.5 years in combination with administration of known carcinogens has resulted in a higher incidence of liver tumors in rats in some studies (Boyland et al., 1954; Dunning et al., 1950;
Kawachi et al., 1968) and a lower incidence in others (Evarts and Brown, 1977; Okajima et al., 1971). Reasons for the disparity of results is not clear but may be related to differences in commercial laboratory rations (Sidransky, 1986).

Ingestion of purified diets containing 2 percent added L-tryptophan has been used to study the role of L-tryptophan in development of \( \gamma \)-glutamyl transpeptidase \( (\gamma-GT) \)-positive foci (precursors of neoplastic nodules and hepatomas) in livers of Sprague-Dawley rats exposed to diethylnitrosamine. Results from these studies suggest that addition of 2 percent L-tryptophan to purified diets may have a promoting effect on liver carcinogenesis. Possible mechanisms for promotional effects of L-tryptophan on hepatocarcinogenesis were reviewed by Sidransky (1986). Fat level of the diet did not affect the promotional effect of elevated dietary L-tryptophan (Sidransky et al., 1986).

**Urinary bladder tumors**: In general, urinary bladder tumors have not been induced in experimental animals by administering L-tryptophan or L-tryptophan metabolites alone, either orally or subcutaneously. For example, in a study by the National Cancer Institute, no evidence of carcinogenicity was found when L-tryptophan was added to a commercial laboratory diet at levels of 2.5 and 5 percent and fed to Fischer 344 rats and B6C3F1 mice of both sexes for 1 year (U.S. Department of Health, Education, and Welfare, 1978). Similarly, Birt et al. (1987) reported that the incidence of urinary bladder tumors was not increased by excess dietary L-tryptophan in male Fischer 344 rats in an 80-week study.

**Teratology and developmental studies**: A trend of decreasing fetal weight and maternal weight gain (greater than that resulting from decreased food intake alone) was observed with increasing levels of L-tryptophan supplementation when Sprague-Dawley rats were fed diets containing 1.4, 2.9, or 7.1 percent L-tryptophan throughout pregnancy (Funk et al., 1991). Dams supplemented with 7.1 percent L-tryptophan had a significantly increased number of late resorptions although the number of viable fetuses and early resorptions were not significantly different from controls. Teratogenic effects were not observed upon gross examination. It should be noted that results from a pilot study indicated that nonpregnant rats had "difficulty maintaining body weight and health" when given higher levels of supplementation (14.3 and 28.5 percent) for 4 weeks (Funk et al., 1991). (See p. 94.)

Similar effects of L-tryptophan supplementation on fetal weight and litter size were reported with a lower level of L-tryptophan supplementation in a low-protein diet. Feeding of a 6 percent casein diet containing 5 percent added L-tryptophan to pregnant Sprague-Dawley rats from day 1 to day 14 or 21 of pregnancy resulted in significantly lower fetal weight than in ad libitum or pair-fed controls but no difference in litter size (Matsueda and Niiyama, 1982). Excess L-tryptophan produced a further significant reduction in DNA, RNA, and protein in fetal brain than food restriction alone; however, brain weight was not further decreased by excess L-tryptophan. Acidic and aromatic amino acids accumulated in fetal brain, reaching levels 2- to 3-fold higher than in controls, although no differences were found in concentrations of free amino acids in maternal plasma (Matsueda and Niiyama, 1982).

Supplementation of a 40 percent casein diet with 0.66 or 1.86 percent L-tryptophan did not result in a decrease in body or brain weights of pups (Leprohon and Anderson, 1982). Dose-related increases in maternal plasma tryptophan concentrations during gestation and lactation and maternal brain concentrations of tryptophan, serotonin, and \( 5\)-HIAA concentrations at the end of lactation were reported. Likewise, plasma tryptophan and brain concentrations of indoleamines also increased with maternal dietary L-tryptophan increments in weanling pups. The biochemical changes resulting from L-tryptophan supplementation did not influence the feeding behavior of the pups during the first three days after weaning.

Thoemke and Huether (1984) fed a diet containing 1 percent added L-tryptophan to male and female Wistar rats beginning 2 weeks before initial mating and continued for three consecutive generations (F1, F2, and F3). Brain weights, concentrations of amino acids in plasma, and
concentrations of putative amino acid neurotransmitters in the brainstem were measured at 5, 10, 15, and 20 days postpartum and compared with those of rats fed a control diet over the three generations. Brain weight decreased with each generation fed the L-tryptophan supplement, reaching about 90 percent of that of controls in the F3 generation but plasma tryptophan levels were not significantly different from controls in any generation.

c. Human studies

Changes in food intake and body weight: Effects of L-tryptophan administration on food intake have been examined by several investigators including Hrboticky et al. (1985), Leiter et al. (1987), and Wurtman et al. (1981a). In a study of the effects of L-tryptophan on carbohydrate craving, doses of 2.4 g L-tryptophan (given in 3 divided doses immediately after meals to 8 subjects of both sexes) decreased carbohydrate intake in 3 subjects, increased carbohydrate intake in 1 subject, and did not change snacking patterns in the total group (Wurtman et al., 1981a). Hrboticky et al. (1985) found that administration of 2 g L-tryptophan given 45 minutes before a self-selected luncheon reduced energy intake by 13 percent in 17 men of normal weight. Similarly, 2 or 3 g, but not 1 g, L-tryptophan given in the same manner diminished food intake by at least 19 percent in 15 normal weight men. Plasma tryptophan concentrations showed a dose-related increase and tryptophan:LNAA ratios were elevated with L-tryptophan consumption but to the same extent with doses of 1, 2, or 3 g. No effect on macronutrient selection was observed; however, the 2 and 3-g doses of L-tryptophan resulted in decreased subjective hunger ratings and alertness as measured by VAS, with increased dizziness scores and faintness. With 3 g L-tryptophan dizziness scores did not return to baseline after lunch (Hrboticky et al., 1985).

In contrast to the results reported with men, administration of L-tryptophan (probably 2 g but dose not specified) did not result in decreased energy intake of 14 women at various stages of the menstrual cycle (Leiter et al., 1987). Measurements of energy and carbohydrate consumption with L-tryptophan or a placebo during the follicular and luteal phases of the menstrual cycle led the investigators to conclude that the metabolic disposal of L-tryptophan and its effects on food consumption in women were influenced by the menstrual cycle; however, the lack of experimental detail and results do not permit an accurate assessment of these conclusions. Metabolism of tryptophan via the kynurenine pathway is affected by the phase of the human menstrual cycle. Plasma concentration and urinary excretion of kynurenine were significantly greater after ingestion of an oral load of 3 g L-tryptophan in the luteal phase of the menstrual cycle than in the follicular phase (Hrboticky et al., 1989).

Administration of 1 g L-tryptophan with 10 g carbohydrate before meals (3 g L-tryptophan daily for 8 weeks) resulted in significantly higher levels of plasma tryptophan but no significant differences in Beck Depression Inventory, SCL 90 rating, POMS, or body weight in 8 obese subjects (Strain et al., 1985).

Biochemical studies: The transport of tryptophan into brain is somewhat more complex than that of other LNAA, for which the relative concentrations of LNAA competing for the transport system appear to be the primary determinant of brain uptake. About 75 to 80 percent of nonpeptide tryptophan is bound by a low-affinity, high-capacity mechanism to albumin in the peripheral circulation (McMenamy and Oncley, 1958). A major regulator of the extent to which tryptophan binds to albumin is the concentration of nonesterified fatty acids (NEFA) in plasma; i.e., when the concentration of NEFA is high, less tryptophan is bound to albumin (Madras et al., 1974). As detailed by Sved (1983) and Pardridge (1979), the apparent affinities of albumin and the transport carrier for tryptophan appear to be affected by the concentrations of NEFA and LNAA, respectively. Thus, the transportable pool of L-tryptophan may depend on physiological changes in blood levels of LNAA and NEFA. For example, under conditions where plasma concentrations of NEFA are low and/or LNAA are high such as fasting or after a high-protein, low-fat meal, the transportable pool
will be similar to the free pool measured in vitro. However, when NEFA are high and/or LNAA are low such as after a high-fat, low-protein meal, the total serum tryptophan pool may provide a better estimate of the transportable fraction (Sved, 1983).

Green et al. (1980) have shown a linear increase in total plasma tryptophan and a hyperbolic increase in free tryptophan after a single oral tryptophan load of 10, 25, and 50 mg/kg body weight (about 0.7, 1.75, and 3.5 g for a 70-kg individual) in nonfasted healthy male subjects. Both the volume of distribution and rate of clearance for total and free tryptophan decreased as the load increased, suggesting that the total body clearance and apparent volume of distribution were saturable (Green et al., 1980, 1985). With repeated oral doses of 100 mg/kg body weight in 2 divided doses daily for 7 days, basal total and free plasma tryptophan concentrations increased considerably. After 7 days, the dissociation constant for the albumin-bound tryptophan decreased markedly and the rate of plasma clearance increased significantly. However, with a final loading dose of 50 mg/kg body weight L-tryptophan at the end of 7 days, plasma total tryptophan values were similar but free tryptophan values were significantly lower than those found after a single 50 mg/kg body weight load. Administration of L-tryptophan produced little change in urinary excretion of tryptophan or 5-HIAA (Green et al., 1980, 1985).

In studies of the effect of L-tryptophan on blood pressure, liver function tests (including ALT, AST, alkaline phosphatase, total and indirect bilirubin, and serum albumin) did not change from baseline values during 8 weeks treatment of 16 subjects with 1.5 to 4 g L-tryptophan given in divided doses. Routine urine analysis, plasma creatinine concentration, and creatinine clearance were affected by L-tryptophan treatment and no changes in heart or lungs were discernable from chest x-rays (Cade et al., 1990).

**Plasma concentrations of kynurenine metabolites:** Substrate induction of hepatic tryptophan 2,3 dioxygenase activity may increase levels of kynurenine metabolites produced in the oxidative pathway following large or repeated doses of L-tryptophan. Metabolic and/or functional changes resulting from increased production of these compounds remain to be determined.

L-tryptophan administration increased the plasma concentration of kynurenine, with the increase approximately proportional to the dose given (Green et al., 1980). After 7 days supplementation, both basal and peak kynurenine concentrations in plasma were markedly elevated. Administration of a single dose of 10 or 25 mg/kg body weight L-tryptophan was reported to have little effect on kynurenine excretion although a single dose of 50 mg/kg produced a nearly 10-fold rise. Continued supplementation for 7 days resulted in a further significant increase in excretion of kynurenine but not 5-HIAA. Urinary excretion of the tryptamine metabolite, IAA, increased with increasing L-tryptophan doses given singly, but IAA excretion did not increase above baseline levels with the final 50-mg/kg body weight load of L-tryptophan given after 7 days (Green et al., 1980).

Effects of single (50 and 100 mg/kg body weight, 3.5 and 7 g for a 70-kg individual) and repeated (50 mg/kg body weight daily for 14 days) oral doses of L-tryptophan on blood serotonin, tryptophan and kynurenine concentrations were also examined by Yuwiler et al. (1981). Serum total tryptophan peaked 2 hours after administration of the 50 and 100 mg/kg body weight doses at levels about 8- and 11-fold the basal levels, suggesting the possibility that induction of tryptophan 2,3 dioxygenase occurred with the resultant removal of some of the administered L-tryptophan on the first pass through the liver. After 9 days of supplementation, the peak blood level was about 16 percent lower than it had been after a single dose of 50 mg/kg body weight. In contrast to the results reported by Green et al. (1980), free tryptophan concentrations followed the changes in total tryptophan concentration, a result also reported by Møller (1981). Concentrations of serum kynurenine peaked after the peak of L-tryptophan (3 to 4 hours after loads of 50 mg/kg L-tryptophan). Peak concentrations were similar with single and repeated doses. However, with a single 100 mg/kg body weight dose of L-tryptophan, the peak in serum kynurenine was more than double that following the 50 mg/kg dose at 4 hours. Plasma tryptophan and kynurenine concentrations approached baseline levels after 6 to 8 hours. The investigators reported that the
rate of response varied among individuals and that the subjects with the most sluggish responses were the ones reporting the most side effects including drowsiness, difficulty in concentrating, and weakness, faintness, and mild nausea (Yuvalier et al., 1981).

In studies of Møller (1981), on the pharmacokinetics of a 100–mg/kg body weight load of L-tryptophan, peak tryptophan and kynurenine concentrations were similar in magnitude and followed the same time course as in the study of Yuvalier et al. (1981). The area under the curve (AUC) of kynurenine correlated better with the linear disappearance rate of total tryptophan than free tryptophan; it was not correlated with the load of L-tryptophan. Pretreatment with nicotinamide resulted in decreases in the AUC in plasma and in urinary excretion of kynurenine and 3-hydroxykynurenine, suggesting that the administration of nicotinamide may inhibit the substrate-induced increase in tryptophan 2,3 dioxygenase activity (Møller, 1981).

The metabolism of tryptophan is impaired in patients with liver disease. In acute hepatic failure, an increase in brain tryptophan concentration has been suggested as a factor in the development of hepatic coma (Mans et al., 1979). Rössle et al. (1986a) reported that the activity of tryptophan 2,3 dioxygenase was significantly reduced in patients with cirrhosis. These investigators also reported that following intravenous infusion of a 1.5 g load of L-tryptophan, patients with cirrhosis showed a significant elevation in fasting free tryptophan in plasma, a significant reduction in hepatic clearance of total and free tryptophan (34 and 64 percent, respectively), a significant 3-fold increase in the half-life of circulating tryptophan, and a significant increase in the volume of distribution of total tryptophan compared with normal patients. Free tryptophan clearance was significantly reduced but other changes in pharmacokinetic parameters were not apparent in patients with noncicrhotic liver disease (Rössle et al., 1986b).

Urinary excretion of kynurenine metabolites: Watanabe et al. (1979) reported that mean excretion of kynurenic acid, xanthurenic acid, 3-hydroxykynurenine, and 3-hydroxanthranilic acid doubled in healthy women when tryptophan intake was doubled by addition of an L-tryptophan supplement. Marked individual differences and daily fluctuations were observed, particularly in 3-hydroxanthranilic acid excretion. In this study, basal diets containing 770 or 850 mg L-tryptophan in protein were supplemented with 900 mg/day L-tryptophan (3 divided doses taken with meals) for 7 days.

Administration of a single 2-g loading dose of L-tryptophan to fasting young men resulted in increased urinary excretion of anthranilic acid glucuronide, o-aminohippuric acid, kynurenic acid, acetylkyurenine, kynurenine, 3-hydroxykynurenine, and xanthurenic acid (Wolf and Brown, 1971). Addition of 100 mg pyridoxine hydrochloride did not further alter the excretion of any of these metabolites. After ingestion of the same dose of L-tryptophan by elderly men with prostatic disease, excretion of one or several tryptophan metabolites was abnormal in about 25 percent of the subjects. Excretion levels returned to normal after administration of pyridoxine hydrochloride, suggesting that the 2-g dose of L-tryptophan had exceeded the capacity of the kynurenine pathway in these subjects (Wolf and Brown, 1971).

A deficiency of vitamin B₆ has been shown to result in increased excretion of kynurenine metabolites of healthy adults following an L-tryptophan load. After challenge with a 2-g load of L-tryptophan, 6 men who had consumed a vitamin B₆-deficient diet for 33 to 46 days excreted significantly greater amounts of kynurenic acid, xanthurenic acid, acetylkyurenine, kynurenine, and hydroxykynurenine and a significantly smaller amount of o-aminohippuric acid compared with loading tests before depletion (Yess et al., 1964).

In women who were not pregnant and not using OCA and who had consumed a vitamin B₆-deficient diet for 12 days, oral ingestion of 2 g L-tryptophan, increased excretion of xanthurenic acid over 300-fold and excretion of 3-hydroxykynurenine, kynurenic, and kynurenine acid about 20 to 30-fold. Intraindividual patterns of excretion of the metabolites were quite constant at 6 and 13 days but interindividual patterns varied greatly (Shin and Linkswiler, 1974). In a 28-day study
of vitamin B6 depletion, Leklem et al. (1975) reported similar increases in urinary excretion of 3-hydroxykynurenine, kynurenic acid, and xanthurenic acid and decreases in excretion of niacin metabolites after a 2-g L-tryptophan load in women not using OCA.

Elevated baseline excretion of tryptophan metabolites and an exaggerated response after L-tryptophan loading has been consistently reported in women using OCA compared with nonusers. A number of studies ranging in length from 1 to 31 days have reported significant increases in excretion of 3-hydroxykynurenine and xanthurenic acid and a tendency toward increased excretion of 3-hydroxyanthranilic acid in OCA users following single or multiple L-tryptophan loads ranging from 2 to 5 g (Adams et al., 1973; Green et al., 1978; Leklem et al., 1975; Luhby et al., 1971; Rose, 1966). With the exception of the study of Green et al. (1978), increased excretion of kynurenic acid was usually reported (Adams et al., 1973; Leklem et al., 1975; Price et al., 1967; Rose and Adams, 1972). In one study, administration of a 2-g loading dose of L-tryptophan resulted in a significantly decreased excretion of anthranilic acid but not 3-hydroxyanthranilic acid by OCA users compared with women not using OCAs or men (Van der Poll et al., 1986); however, this was not reported in another study (Leklem et al., 1975).

Green et al. (1978) reported that increases in plasma tryptophan concentrations following a 50 mg/kg body weight load of L-tryptophan were similar in OCA users and nonusers. Plasma peak kynurenic acid concentration and excretion were lower in the OCA group than in controls. Excretion of the tryptamine metabolite, IAA, but not the serotonin metabolite, 5-HIAA, showed a small but significant increase in OCA users compared with nonusers. The volume of distribution, plasma clearance, and plasma half-life of tryptophan was similar in both groups.

**Neurotransmitter studies:** Concentrations of serotonin and 5-HIAA measured in blood and CSF following administration of single or multiple doses of L-tryptophan provide indirect evidence that serotonin synthesis in human brain, like that in several animal species, is influenced by availability of L-tryptophan.

In the study of Yuwiler et al. (1981), baseline blood serotonin concentrations showed large interindividual and small intraindividual variance. After a single 50 or 100-mg/kg body weight load of L-tryptophan, blood serotonin concentrations increased but individuals differed in both the form and magnitude of their responses. On the ninth day of supplementation, the basal serotonin concentration was increased about 32 percent over baseline and the average increase following the L-tryptophan load (50 mg/kg body weight) was similar to the increase following the single-dose load. Before supplementation, serotonin was not detected outside of platelets. After a single dose of 100 mg/kg body weight or repeated doses of 50 mg/kg for 9 or 14 days, platelet serotonin was increased but extraplatelet serotonin accounted for more than half of the total increase. Small quantities of extraplatelet serotonin were also found before loading on days 9 and 14 of the repetitive experiment (Yuwiler et al., 1981). Increased platelet serotonin concentrations were also reported with L-tryptophan treatment by Murphy et al. (1974).

In studies of Young and Gauthier (1981), CSF concentrations of tryptophan and 5-HIAA were increased similarly after loads of 3 and 6 g L-tryptophan although there was a tendency for the elevation to be sustained for a longer period with the higher dose. The rise in CSF IAA was greater with the higher dose. Similar results were reported with doses of 50 mg/kg body weight (3.5 g for a 70-kg subject) L-tryptophan (Eccleston et al., 1970) and 9 g/day L-tryptophan given in divided doses (Dunner and Goodwin, 1972). An infusion of L-tryptophan that was described as producing plasma concentrations similar to those found after oral administration of L-tryptophan in the treatment of depression resulted in a 6-fold elevation in cortical tryptophan levels in neurosurgical patients (Gillman et al., 1981). Significant rises of CSF 5-HIAA suggested that serotonin concentration was increased.

In a double-blind, placebo-controlled trial, Benedict et al. (1983) investigated the effect of an oral supplement of L-tryptophan (3 g/day in divided doses given with meals) in 6 normal men on plasma
and platelet concentrations of serotonin and plasma and urinary concentrations of free and conjugated catecholamines. Plasma and urine samples were collected after the subjects had taken the supplement with a small carbohydrate-containing breakfast on the morning of the fourth day of supplementation. Although plasma tryptophan concentration increased 113 percent after taking the supplement for 3 days, there were no significant changes in platelet or extraplatelet serotonin concentrations or in plasma or urinary free or conjugated catecholamines. No significant changes in blood pressure, heart rate, plasma sodium levels, or 24-hour urinary sodium excretion were reported in these subjects. Likewise, ingestion of the L-tryptophan supplements with meals did not result in any adverse effects or drowsiness (Benedict et al., 1983).

In another double-blind, placebo-controlled study, Rasmussen et al. (1983) found that ingestion of a 10-g load of L-tryptophan by 6 healthy men did not alter plasma concentrations of dopamine, epinephrine, norepinephrine, or 3,4-dihydroxyphenylacetic acid (DOPAC, a deaminated metabolite of dopamine), suggesting that an L-tryptophan supplement did not interfere with brain uptake of L-tyrosine. The investigators reported that there were no discernible physical or psychological effects following L-tryptophan ingestion.

**Behavioral studies:** Many clinical studies and case reports were found on efficacy of administration of L-tryptophan given as a therapeutic agent to affect metabolism of neurotransmitters in patients with depression. Except for side effects such as drowsiness, headache, and nausea included in some of these studies, no adverse effects were reported. However, these studies were designed to evaluate efficacy of L-tryptophan as a therapeutic agent and therefore did not include examination of the types of endpoints that would be important in the evaluation of safety. Because evaluation of efficacy as a therapeutic agent is not a part of the evaluation of safety of a food or food component, these studies were not included in the present review.

Sleep patterns in newborn infants were modified by administration of tryptophan in a 10 percent glucose solution in comparison with administration of valine in a 10 percent glucose solution or a commercially available formula. The amount of tryptophan in the treatment feeding (223 mg/L) was similar to the amount in the formula (201 mg/L). The infants ingested about 20 mg of tryptophan and about 27 mg valine with the respective amino acid solutions. The infants in the tryptophan group entered quiet sleep about 20 minutes earlier and entered active sleep about 14 minutes earlier than after the commercial formula. The infants in the valine group entered quiet sleep about 40 minutes later and active sleep about 16 minutes later than after commercial formula. These differences were significant although the findings on total duration of sleep and waking states over the 3-hour observation period were not significantly different (Yogman and Zeisel, 1983).

Oral ingestion of L-tryptophan by adults has been associated with increased drowsiness and psychopharmacological activity regarding human mood. This effect has been attributed to increased serotonergic activity resulting from increased tryptophan uptake into brain. Results from studies of effects of L-tryptophan on sleep in humans were reviewed by Hartman and Greenwald (1984) and Schneider-Helmert and Spinweber (1986). Overall, these indicated that L-tryptophan reduced sleep latency and had little effect on the stages of sleep. Behavioral and physiological effects of single doses of L-tryptophan administered orally to healthy adults have been measured in several controlled studies (Greenwood et al., 1975; Lehnert et al., 1989; Lieberman et al., 1985; Smith and Prockop, 1962; Spinweber et al., 1986).

In a double-blind, placebo-controlled study, Greenwood et al. (1975) employed self-rating scales to measure changes of mood and bodily symptoms; reaction time and key tapping tests to measure behavioral performance changes; and EEG, mean evoked response, finger tremor, palmar skin conductance, and radial pulse to measure physiological changes after administration of 5 g L-tryptophan to 10 healthy subjects. With this dose of L-tryptophan, plasma concentrations of total and free tryptophan peaked 2 hours after ingestion, reaching increases of 8 and 20-fold, respectively. Severe nausea and headache occurred soon after ingestion. An increase in drowsiness but no euphoria was reported on the mood self-rating scales. Activity in the slow wavebands of the
EEG was increased but other measures of physiological changes were not affected. No significant differences were found in performance tests of reaction time and key tapping. Effects were maximal 1 hour following ingestion and returned to baseline by 3 hours. With administration of single doses of 50 and 100 mg/kg body weight L-tryptophan, Yuwiler et al. (1981) briefly reported that all subjects experienced drowsiness which lasted 2 to 4 hours and that some experienced an additional 3 to 6 hours of lethargy. Subjects were able to continue normal activities but reported that concentration was difficult. During the 14-day study, behavioral effects were similar but the lethargy was more pronounced sometimes lasting into the evening.

Lieberman et al. (1985) reported similar results of increased subjective fatigue and decreased self-ratings of vigor and alertness but no performance impairments in 20 men given a single oral dose of L-tryptophan (50 mg/kg body weight or 3.5 g for a 70-kg subject given in the morning) in a double-blind, placebo-controlled, crossover study. Two mood scales, Visual Analogue Mood Scale (VAMS) and POMS and 4 performance tests, Simple Auditory Reaction Time, Two-Choice Visual Reaction Time, Grooved Pegboard Test, and Thurstone Tapping Test, were used in this study.

Although serotoninergic neurons may play a role in the regulation of pain sensitivity and L-tryptophan has been given to patients with chronic pain, effects of L-tryptophan on pain perception have not been consistent in 3 double-blind, placebo-controlled trials in individuals not experiencing pain. Alterations in pain perception could be a beneficial effect in some situations and an adverse effect in others. Oral administration of 50 mg/kg body weight L-tryptophan reduced thermal and pain sensitivity at moderate intensities but not at low or very high intensities in 8 healthy men as evaluated by subjects' abilities to discriminate among different intensities of thermal stimuli (Lieberman et al., 1983). The responses measured in this study were the subjects' abilities to discriminate among different intensities of thermal stimuli delivered by a dolorimeter and the subjects' sensitivity in discriminating each pair of stimulus intensities and their response bias towards describing the intensity of stimuli were evaluated separately.

Daily ingestion of 2 g L-tryptophan in 2 divided doses by 20 women for 14 days was not associated with increased threshold to burning pain. Pain threshold in this study was evaluated by the length of time a subject could tolerate a heat stimulus (infrared lamp) of constant intensity. The occurrence of side effects was not mentioned (Seltzer et al., 1982). In contrast, administration of 2 g L-tryptophan daily in 5 divided doses for 7 days (together with instructions to consume a high-carbohydrate diet) was associated with increased pain tolerance thresholds in response to electrical stimulation of dental pulp in 14 adults. Perception threshold for pain was not changed with L-tryptophan treatment. Side effects including nausea, skin itching, weight loss, and mood elevation were more commonly reported in the group ingesting L-tryptophan (Seltzer et al., 1982).

Military personnel were given 2 g L-tryptophan every evening for 4 days during and after a flight from the United States to Okinawa and were administered tests of subjective mood (analog scale, the Stanford Sleepiness Scale, and the POMS). Performance measures included 4-choice reaction time, addition task, the Williams Word Memory Test, and the Digit Symbol Substitution Test. Significantly increased length of sleep time and less slowing of reaction time in the L-tryptophan-treated than in placebo controls were reported at selected times (Spinweber et al., 1986).

Administration of 3 g L-tryptophan daily for 4 weeks to hypertensive patients in a randomized, double-blind, placebo-controlled study resulted in improved performance on the d-2 concentration task but no significant changes in subjective reports of mood. High-carbohydrate or high-protein diets did not have a significant effect on performance or mood when fed in conjunction with L-tryptophan treatment (Lehnert et al., 1989).

Observation of 7 healthy subjects following oral administration of graded doses (30, 50, 70, and 90 mg/kg body weight) of L-tryptophan revealed sustained nystagmus in all subjects at the 2 higher doses and a dose-related increase in drowsiness (Smith and Prockop, 1962). Urinary excretion of tryptamine and xanthurenic acid was also increased in these subjects.
Adverse effects including headache, dizziness, and nausea have been reported consistently when doses of 6 to 18 g DL-tryptophan were taken in conjunction with MAO inhibitors. In addition, Pope et al. (1985) reported mild to severe delirium with doses of 1 to 6 g L-tryptophan and MAO inhibitors. Other neurologic effects often observed such as changes in verbalization and locomotion resembling ethanol intoxication, drowsiness, hyperreflexia, and clonus were first reported by Oates and Sjoerdmsa (1960) after administration of 20 to 50 mg/kg body weight L-tryptophan with an MAO inhibitor. The symptoms are reversed when tryptophan is withdrawn and long-term adverse effects have not been associated with the treatment (Young, 1986).

Endocrine studies: Effects on pituitary, adrenal, and pancreatic hormones have been examined following intravenous and oral administration of L-tryptophan. Significant transient increases in plasma growth hormone have been reported in healthy fasting adults of both sexes following intravenous infusion of 5 to 10 g L-tryptophan (Charney et al., 1982; Cowen et al., 1985; Smith et al., 1991; Winokur et al., 1986) and single oral doses of 100 mg/kg body weight (about 7 g) and 2 or 10 g (Hedone et al., 1977; Hyypa et al., 1979; Woolf and Lee, 1977). The magnitude of increase was generally similar for both routes of administration. A smaller and nonsignificant increase in plasma growth hormone was reported following administration of 70 mg/kg body weight (about 3.5 to 5 g) (Müller et al., 1974).

Significant transient increases in plasma prolactin concentration were also reported after intravenous administration of 5 to 10 g L-tryptophan in fasting healthy subjects of both sexes (Charney et al., 1982; Cowen et al., 1985; MacIndoe and Turkington, 1973; Smith et al., 1991; Winokur et al., 1986) but not with doses of 4 g or less (MacIndoe and Turkington, 1973; Winokur et al., 1986). However, oral administration of about 5 to 10 g L-tryptophan as a single dose to fasting healthy adults did not produce an increase in serum prolactin concentration (Glass et al., 1980; Hyypa et al., 1979; Wiebe et al., 1977; Woolf and Lee, 1977). Neither was plasma prolactin concentration significantly increased with postprandial administration of 0.25, 0.5, or 1.0 g L-tryptophan even though the plasma tryptophan concentration and tryptophan-LNAA concentrations were increased significantly (Finer et al., 1987). Likewise, serum prolactin levels were not elevated with oral administration of 6 g L-tryptophan daily for 3 weeks (Faber et al., 1977).

No significant increases in plasma thyrotropin (TSH), follicle-stimulating hormone (FSH) or luteinizing hormone (LH) were reported following intravenous or oral administration of 2 to 10 g L-tryptophan (Hyypa et al., 1979; MacIndoe and Turkington, 1973; Winokur et al., 1986; Woolf and Lee, 1977).

Reported effects of L-tryptophan on plasma cortisol in normal subjects have been mixed and some evidence suggests that it may produce different effects in control subjects and patients with major depression. Woolf and Lee (1977) reported an initial decrease in plasma cortisol concentration followed by a rebound above baseline levels with oral administration of 10 g L-tryptophan in fasting normal subjects. Modlinger et al. (1979) reported that oral administration of 2 or 10 g L-tryptophan resulted in a significant increase in plasma cortisol in fasting normal subjects; however, higher plasma tryptophan levels were reported in this study. In other studies, plasma cortisol and urinary excretion of free cortisol and 3-methoxy-4-hydroxyphenylglycol (MOPEG) were not changed in normal subjects by intravenous administration of a single 10-g dose (Winokur et al., 1986) or oral administration of a single dose of 2 to about 7 g L-tryptophan (Hyypa et al., 1979; Maes et al., 1989). However, in patients with major depression, Maes et al. (1989) reported that urinary free cortisol was significantly increased and MOPEG significantly decreased following L-tryptophan ingestion, suggesting that L-tryptophan administration may be associated with decreased conversion of cortisol to MOPEG in severely depressed individuals.

Go et al. (1970) measured pancreatic enzyme secretion (trypsin, lipase, and amylase) in 45 fasted, healthy subjects after duodenal perfusion with single amino acids, separate mixtures of indispensable and dispensable amino acids, and a standard amino acid mixture that was a
combination of the indispensable and dispensable mixtures. Perfusion with the mixture of indispensable amino acids caused enzyme secretions similar to those induced by perfusion with the standard mixture but perfusion with the dispensable amino acid mixture had no effect. In comparison with the enzyme output following infusion of isotonic saline, phenylalanine, methionine, and valine elicited increases of 2.5 to 3 times the response to saline in pancreatic enzyme secretion when the dispensable amino acids were tested individually. Tryptophan also elicited an increase that was less than twice that of saline. The effect of tryptophan was considered equivocal by the investigators. The pancreatic enzyme response was smaller following infusion of phenylalanine than following infusion of the mixture containing all of the indispensable amino acids. The individual amino acid solutions infused in these experiments were given in physiological concentrations (20 mmol/L); studies of effects of tryptophan given as a larger bolus were not found.

Intravenous infusion of 2.5, 5.0, or 7.5 g of L-tryptophan in 3 fasting healthy adults resulted in small, dose-related increases in plasma insulin concentrations. One of 2 subjects receiving an infusion of 7.5 g of L-tryptophan experienced transient facial flushing, nausea, and brachycardia beginning 20 minutes after administration of the amino acid (Floyd et al., 1966). Oral ingestion of 10 g L-tryptophan by healthy male subjects was followed by gradual and significant rises in blood glucose and plasma insulin and glucagon concentrations (Hedo et al., 1977). These authors suggested the increased glucagon concentration as the probable cause of the increased blood glucose concentrations and postulated that L-tryptophan, through conversion to alanine, may have provided substrate for increased gluconeogenesis as well as increasing glucagon concentration. Oral ingestion of a smaller dose of L-tryptophan (70 mg/kg body weight or 4.9 g for a 70-kg individual) was not associated with an elevation of insulin or blood glucose (Müller et al., 1974). Ajdukiewicz et al. (1968) reported an increase in plasma insulin concentration only when L-tryptophan was given postprandially and inconsistent changes in blood glucose concentrations in hospitalized patients, most of whom had various forms of liver disease.

Developmental studies: Fetal breathing activity was assessed after 40 women with normal term pregnancies continued a 9-hour fast (control group) or were given oral doses of 1 g tryptophan, 100 g glucose, or both substrates together. The isomer was not specified. The incidence of fetal breathing movements decreased in the control group but rose significantly in the other three groups; however, tryptophan alone did not produce as great an increase as glucose or glucose plus tryptophan. Breathing rates were similar in the control group and the group given tryptophan and increased significantly in the groups given glucose or glucose plus tryptophan. The concentration of tryptophan in maternal plasma increased from about 32 μmol/L before ingestion to about 75 μmol/L from 1 to 3 hours after ingestion. The addition of glucose did not modify the increase in plasma tryptophan concentration (Devoe et al., 1986).

Epidemiologic studies: Two retrospective studies of a time period prior to the 1989 EMS outbreak have reported an association of L-tryptophan use with eosinophilic fasciitis, a condition that shares many of the clinical characteristics of EMS. In the first study, history of use of L-tryptophan was examined in 49 patients with cutaneous fibrosis (Blauvelt and Falanga, 1991). L-Tryptophan use was reported prior to the onset of symptoms by 11 of 17 patients with eosinophilic fasciitis, 2 of 10 patients with localized scleroderma, and none of 22 patients with systemic sclerosis. The 11 patients with eosinophilic fasciitis had taken L-tryptophan in doses of 500 mg to 5 g for 1 month to 10 years before the onset of symptoms. Disease onset occurred from September 1986 to October 1989. Five of the 11 patients had initial clinical manifestations before October 1989 when the EMS was recognized. The 2 patients with localized scleroderma had taken 1.5 and 2 g of L-tryptophan daily for 3 and 10 months before onset of symptoms, respectively. Both of these patients reported initial clinical manifestations prior to October 1988. Given the probable use of L-tryptophan by 11 of the 17 patients with eosinophilic fasciitis and by none of the 22 patients with systemic sclerosis, the results of this study seem quite significant.
In the second study, 9 of 45 patients with eosinophilic fasciitis recalled use of 500 mg to 2.5 g of L-tryptophan daily for periods of 1 month to 10 years before disease onset (Hibbs et al., 1992). Eight of the 9 patients reported the onset of symptoms of eosinophilic fasciitis between April 1, 1986 and March 31, 1987. The ninth patient reported onset in 1984. No control patients (individuals with polymyositis diagnosed during the same period) recalled use of L-tryptophan.

d. Summary and conclusions

Endpoints: L-Tryptophan performs many metabolic roles. It serves as the precursor for the neurotransmitter serotonin. Several biologically active metabolites including kynurenine, xanthurenic acid, anthranilic acid, and quinolinic acid are produced during its oxidative degradation and other physiologic substances including tryptamine, pineal indoles, and condensation products such as tetrahydro-β-carbolines are produced by other pathways of tryptophan metabolism. L-Tryptophan directly induces the activities of many nutritionally and hormonally sensitive hepatic enzymes and also increases plasma levels of several pituitary, pancreatic, and adrenal hormones. It has also been shown to influence hepatic metabolism of carbohydrate and lipid and to stimulate protein synthesis in liver, brain, and other tissues. Because of its direct involvement and regulatory role in many aspects of metabolism, ingestion of L-tryptophan as a dietary supplement might reasonably be expected to alter metabolic processes and affect the integrity and function of tissues. To date, most studies of effects of L-tryptophan ingestion have focused on effects on brain or liver separately. Studies which examine multiple endpoints occurring with ingestion of large amounts of L-tryptophan are needed to evaluate the safety of L-tryptophan.

Decreased body weight gain and food intake are well-documented effects of short- and long-term ingestion and intraperitoneal injection of L-tryptophan in experimental animals. Because food intake is reduced following injection of the amino acid, it suggests that L-tryptophan has a physiologic role in control of food intake. Plasma and brain concentrations of tryptophan are increased by ingestion of L-tryptophan. Likewise, serotonin synthesis is increased in several species including rats, dogs, and monkeys. Presumably as a result of increased serotonin synthesis, several aspects of behavior have been modified in rats following L-tryptophan administration. These include decreases in sleep latency, food intake, motor activity, self-injection of d-amphetamine, salt intake in DOCA hypertensive rats, and muricide behavior. In contrast, maze performance was improved in weanling rats fed a diet containing added L-tryptophan. No behavioral or neurologic effects were observed in monkeys orally administered L-tryptophan; however, the lack of experimental detail provided in this study make the results difficult to evaluate.

In one study, no adverse biochemical, hematologic, or histopathological abnormalities were found in pigs fed diets containing 0.1 and 1.0 percent L-tryptophan for 40 days. In another study, administration by gavage of L-tryptophan not containing EBT as an impurity produced pathological changes in muscle and pancreas of Lewis rats similar to the changes produced by administration of EBT alone or L-tryptophan containing EBT as an impurity. The differences reported in these studies may be due, in part, to differences in route of administration. However, it is not known whether pigs are susceptible to EMS or a similar condition.

Two lifetime carcinogenicity studies in rats and mice have not produced evidence that L-tryptophan is a carcinogen; however, a number of studies suggest that it may be a promotor for development of liver and urinary bladder tumors.

Decreased maternal weight gain and fetal weights in rats have been observed with addition of 1.4 to 7.1 percent L-tryptophan to low-protein diets. Supplementation of a high-protein diet with 1.86 percent L-tryptophan did not decrease body or brain weights of pups although tryptophan concentrations in plasma and brain of progeny were increased. In a multigeneration study, brain weight and concentrations of amino acid neurotransmitters were decreased in the third generation.
Administration of L-tryptophan has been found to influence spermatogenesis and platelet aggregation in rats.

In humans, ingestion of L-tryptophan has resulted in a range of effects on food intake which are not readily explained. Its administration has been reported to stimulate secretion of several hormones including growth hormone, glucagon, and possibly insulin and cortisol. Increased plasma tryptophan concentrations have been reported following L-tryptophan ingestion in many studies as have plasma concentrations and urinary excretion of kynurenine and other metabolites from the oxidation of L-tryptophan. Metabolic and/or functional changes resulting from chronically increased production of these compounds need to be determined as part of the assessment of safety of prolonged use of L-tryptophan as a dietary supplement.

Concentrations of serotonin and 5-HIAA in blood and CSF following administration of L-tryptophan provide indirect evidence that serotonin synthesis in human brain, like that in several animal species, is influenced by the availability of L-tryptophan. Double-blind, placebo-controlled human studies suggest that oral administration of L-tryptophan results in increased drowsiness and possibly increased pain tolerance but has little effect on performance or mood. Adverse side effects reported with ingestion of L-tryptophan include headache, dizziness, faintness, and nausea. Increased drowsiness and difficulty in concentrating may be adverse effects in some cases but desirable outcomes in others.

In 2 retrospective epidemiological studies, use of L-tryptophan was associated with development of eosinophilic fasciitis as early as 5 years before the presence of EBT as an impurity in L-tryptophan was recognized. It has been estimated that L-tryptophan may contain as many as 60 impurities. With the possibility that EBT or other impurities may play a role in the etiology of EMS associated with use of L-tryptophan, it is not possible to determine whether L-tryptophan itself is involved in the disease process.

Safe levels of human intake: Single doses ranging from about 0.7 to 7 g in humans have been associated with increased plasma tryptophan concentrations and increased concentrations of kynurenine and serotonin metabolites. Controlled experiments in which more than 3 g/day L-tryptophan was administered orally to healthy humans for periods longer than 4 weeks did not include reports of adverse effects. A person consuming 100 g protein per day would ingest about 0.8 g tryptophan.

Retrospective epidemiological studies, case reports, and clinical studies suggest that L-tryptophan has been used in quantities of 500 mg or more for prolonged periods, in some cases as long as 10 years. Ingestion of doses as low as 500 mg/day for periods of 1 month to 10 years before development of symptoms of eosinophilic fasciitis prior to October 1988 were reported. It is not known whether impurities were present in products taken prior to 1988 and the identity of such impurities, if present, is unknown. Because the etiology of EMS in persons taking L-tryptophan is uncertain, it is not possible to conclude that use of L-tryptophan is not associated with adverse health effects in the general population. L-Tryptophan should be used only under responsible medical supervision.

The Expert Panel had additional concerns about use of L-tryptophan by several subgroups of the population. Because of the possibility of decreased maternal weight gain and birth weight of infants and alterations in CSF concentrations of amino acids, including neurotransmitters and precursors for neurotransmitters in infants, high intakes of L-tryptophan are of concern for pregnant and lactating women. Evidence of impaired L-tryptophan metabolism in hepatic disease suggests that persons with impaired liver function should not use L-tryptophan supplements. Likewise, the occurrence of severe side effects with concurrent use of monoamine oxidase (MAO) inhibitors and L-tryptophan provides a contraindication for its use in persons taking this class of antidepressants.
7b. **D-Tryptophan**

a. **Background**

D-Tryptophan is inverted to the L-isomer in rat kidney and liver (Loh and Berg, 1971), tissues shown to be rich in D-amino acid oxidase activity (Krebs, 1935; Meister, 1965). As discussed below, D-tryptophan is probably metabolized to a very limited extent, if at all, by humans.

b. **Animal studies**

*Changes in food intake and body weight:* Friedman (1991) reported that the ability of D-tryptophan to promote weight gain in mice over a 2-week period reached a maximum (87 percent that of L-tryptophan) at about 0.44 percent by weight of the diet. D-Tryptophan was reported to be almost equal to L-tryptophan in its effect on weight gain in rats (Oesterling and Rose, 1952).

*Biochemical studies:* Indole-3-carboxaldehyde was identified as a major metabolite in urine of normal, vitamin B6-deficient, and germfree rats following intraperitoneal injection of $^{14}$C-labeled D-tryptophan. This compound was not identified after injection of L-tryptophan. Indoleacetic acid was also formed in much larger quantities from D- than from L-tryptophan (Chen et al., 1974).

*Neurotransmitter studies:* Concentrations of serotonin and 5-HIAA in brains of female Long-Evans rats rose following intraperitoneal injection of 0.1 g L- or D-tryptophan. Brain concentrations of tryptophan and 5-hydroxyindoles increased more slowly after administration of D-tryptophan than after L-tryptophan. Activity of tryptophan aminotransferase in brain and liver were stereospecific for L-tryptophan and the investigators found no evidence for a tryptophan racemase in brain. Evisceration prevented a rise in brain 5-hydroxyindoles following intraperitoneal administration of D- but not L-tryptophan, suggesting that the increase following D-tryptophan administration occurred only after its deamination and subsequent transamination in peripheral tissues (Yuwiler, 1973).

*Carcinogenicity studies:* Administration of diets containing 2 percent D- or DL-tryptophan to rats maintained the increased agglutinability of bladder cells caused by administration of a subcarcinogenic dose of BHBN. L-Tryptophan and its metabolites did not produce this effect, suggesting that the D-isomer may be responsible for promotion of bladder cancer in this model. Neither D- nor DL-tryptophan induced bladder tumors without prior initiation by BHBN (Kakizoe et al., 1982, 1984).

c. **Human studies**

*Nitrogen balance:* Rose et al. (1954) reported that negative nitrogen balance occurred when 150 mg/day of DL-tryptophan was fed to 2 healthy male subjects consuming an amino acid diet. Because this amount of L-tryptophan maintained positive nitrogen balance, the investigators concluded that D-tryptophan was utilized to only a slight extent, if at all, by humans.

*Biochemical studies:* D-Tryptophan was reported to be the primary indole excretion product, and D-kynurenine was identified as a second excretion product following 1 or 2-g doses of D-tryptophan in 3 subjects (Langner and Berg, 1955). In a more detailed analysis of the urinary excretion of D-tryptophan metabolites in 4 normal male subjects, Price and Brown (1956) reported that ingestion of 2 g D-tryptophan resulted a large increase in urinary excretion of kynurenine (about 10 times higher than with the same dose of L-tryptophan), smaller increases in kynurenic acid, xanthurenic acid, and pyridone, and no change in anthranilic acid glucuronide, and o-aminohippuric acid. Ingestion of 4 g DL-tryptophan resulted in a pattern of excretion of urinary
metabolites intermediate between those found when 2 g of each isomer was given separately. D-Kynurenine was the primary metabolite following ingestion of DL-tryptophan.

d. Summary and conclusions

Endpoints: Brain 5-hydroxyindoles are increased in rats with D-tryptophan administration. D-Tryptophan may also be a promoter for carcinogenesis in mice and rats. In contrast to these species which utilize D-tryptophan, humans appear to utilize D-tryptophan to a very limited extent. D-Tryptophan and D-kynurenine are excreted in the urine by humans following ingestion of D-tryptophan or the racemate. No information is available on formation of serotonin from D-tryptophan in humans.

Safe levels of human intake: Data are available from only 7 individuals consuming D-tryptophan or the racemate in single doses of 2 and 4 g. These data are not sufficient to permit an estimate of the safe intake of D-tryptophan.
D. REVIEWS OF INFORMATION PERTAINING TO THE SAFETY OF INDIVIDUAL DISPENSABLE AMINO ACIDS

1. Alanine

   a. Background

L-Alanine is a dispensable amino acid that is synthesized in muscle by the transamination of pyruvate and generated in the gut and kidney from glutamine removed from the circulation by these organs. L-Alanine plays a major role in the postabsorptive maintenance of steady state concentrations of circulating amino acids. Together with glutamine, L-alanine accounts for about half of the total α-amino acid nitrogen released from muscle. A smaller but significant quantity of alanine is released from kidney.

L-Alanine released from muscle and kidney provides a means for nitrogen transport in plasma. It is primarily removed from the plasma by the liver where it may be used for the synthesis of glucose being metabolized for energy via the TCA cycle. In the liver, the rate of glucose synthesis from alanine and serine is much higher than that of all other amino acids combined. The liver has an enormous capacity to synthesize glucose from alanine. An alanine concentration of about 9 mmol/L, about 20 to 30 times higher than its physiologic concentration, is required to saturate this pathway (Rodwell, 1990b).

The enzyme responsible for transamination of alanine (ALT) is dependent on vitamin B6 (Merrill and Burnham, 1990). In vitamin B6 deficiency, the activity of this enzyme is decreased, diminishing the capacity to convert alanine to pyruvate.

   b. Animal studies

Changes in food intake and body weight: Little or no depression in weight gain or food intake has been observed in rats and chicks fed low-protein diets containing as much as 5 percent added DL- or L-alanine for as long as 2 months (Adkins et al., 1962; Anderson and Combs, 1952; Fishman and Artom, 1945; Hsu and Combs, 1952; Sauberlich, 1961; Yokota et al., 1978). DL-Alanine depressed growth to a greater extent than L-alanine (Adkins et al., 1962). In male and female Wistar rats fed 22 percent protein diets containing 20 percent added DL-alanine for 26 weeks, weight gain was decreased although food consumption was increased in comparison with rats fed diets containing 0, 5, or 10 percent added DL-alanine (Chow et al., 1976).

Biochemical studies: Biochemical changes were measured after 26 weeks in growing male and female Wistar rats fed 22 percent protein diets with 0, 5, 10, or 20 percent DL-alanine substituted for the carbohydrate component. Urinary excretion of alanine increased about 100– to 1000–fold with the increasing doses of DL-alanine at 13 weeks, but the increase was much less at 26 weeks. Serum alanine concentrations were about 4 to 6 times higher in animals fed the highest level than in unsupplemented animals at 26 weeks. Blood ammonia concentration increased significantly (doubled) only in male rats fed 20 percent DL-alanine. Blood pyruvate levels were decreased significantly in rats of both sexes fed 20 percent DL-alanine while blood lactate concentrations were significantly decreased in female rats fed all 3 levels of the amino acid. Blood triglyceride concentrations were significantly decreased in rats of both sexes fed 10 and 20 percent DL-alanine and cholesterol concentrations were decreased significantly only in male rats fed the 10 percent DL-alanine. No abnormalities or lesions were found upon gross examination of all organs at the end of the experimental period (Chow et al., 1976).

Urine composition (protein, reducing substances, total amino acids, ammonia, and bisulfite-binding substances) and kidney histology were normal in rats given 100 mg of DL-alanine by stomach tube daily for 12 days; in addition, the rats had normal growth and food intake (Fishman and Artom, 1945).
Hematologic studies: Addition of 2.5 percent L-alanine to a basal diet containing 9 percent casein produced no significant changes in hematocrit, hemoglobin concentration, number of erythrocytes, or splenic iron concentration in weanling rats fed the diet ad libitum for 2 months (Yokota et al., 1978).

Endocrine studies: In adult mongrel dogs, rapid intravenous administration of L-alanine (0.25 mmol/kg body weight followed by 0.05 mmol/kg/minute for 15 minutes, total dose 89 mg/kg body weight) produced a significant rise in plasma glucagon and a small increase in plasma insulin concentration. With administration of the same dose over 60 minutes, a significant but more gradual rise in plasma glucagon concentration was observed and plasma insulin concentrations rose intermittently (Müller et al., 1971). No parameters other than the endocrine changes were measured in this study.

Functional assessments and gross pathology: A single intracisternal injection of 10 μmol (890 μg) of alanine (isomer not specified) decreased arterial blood pressure and heart rate significantly in conscious normotensive control male Wistar rats (Takemoto, 1991). Larger decreases in these parameters were observed in renovascular hypertensive rats. Intravenous infusion of L-alanine in single doses ranging from 10 to 200 mg/kg body weight (2 to 40 mg for a 200-g rat) was observed to have a pressor effect in rats made hypotensive by hemorrhage. The blood pressure increase followed a time course similar to the rise in plasma alanine concentration (Conlay et al., 1990).

Intramuscular injection of 1 to 2.5 g/kg body weight of D-alanine produced ataxia, paralysis, and in some cases death in pigeons. Injections of this amount of L-alanine did not produce untoward effects. Doses of 7 g/kg body weight of D-alanine were required to produce corresponding adverse effects in rats and guinea pigs (Edlbacher and Wiss, 1944).

c. Human studies

Changes in food intake and body weight: Addition of L-alanine to the standard World Health Organization (WHO) oral rehydration solution (ORS) at the level of 30 mmol/L did not result in adverse effects on food intake, body weight, or stool or urine output when given to 20 male infants less than 1 year of age with acute diarrhea and dehydration (Da Costa Riberio and Lifshitz, 1991). Maximal potential intakes of L-alanine by these infants was calculated to be about 46 mmol (4 g) over a 2-day period. Similarly, addition of 90 mmol/L of this amino acid to the ORS given to 48 male adults and children older than 6 years of age was not associated with clinically adverse effects (Patra et al., 1989). Average intake of L-alanine by these patients was calculated to be about 1479 mmol (132 g) over the duration of the therapy which ranged from 0.5 to 4 days.

Biochemical studies: In a study of the effect of alanine (isomer not specified) on uric acid production, one fasting adult male was given a single 12.1-g dose of alanine (Lewis et al., 1918). No side effects of alanine administration were reported although side effects were reported with ingestion of aspartic and glutamic acids; thus, it is inferred that ingestion of a single 12.1-g dose of alanine by this individual did not result in adverse effects.

L-Alanine was shown to have an antiketogenic effect in humans independent of its effect on glucagon and insulin release (Nosadini et al., 1981). To suppress glucagon and insulin secretion, 6 healthy male subjects were infused intravenously with somatostatin for 3 hours or for 1 hour prior to and during 2 hours of L-alanine infusion. With intravenous infusion of L-alanine (0.03 mmol/kg body weight over 2 minutes and 0.01 mmol/kg body weight/minute for 118 minutes; total dose, 7.5 g over a 2-hour period for a 70-kg subject), β-hydroxybutyrate and acetoacetate concentrations, which were markedly elevated by the somatostatin infusion, remained at that plateau for 1 hour and then decreased in concentration. Concentrations following the alanine infusion were significantly lower than the β-hydroxybutyrate and acetoacetate concentrations in the somatostatin control
experiment. Plasma NEFA concentrations and blood glycerol levels were similar with both treatments. Blood glucose was slightly increased (statistically significant at only 1 sampling point), but lactate and pyruvate concentrations were not significantly different with the alanine infusion. Ratios of β-hydroxybutyrate:acetoacetate and lactate:pyruvate as well as concentrations of glucagon, insulin, C–peptide, and C–peptide:insulin ratio were similar with both treatments (Nosadini et al., 1981). The effect on ketone bodies was significant at physiologic concentrations of plasma alanine (<0.6 mmol/L, or 53 mg/L). Plasma alanine concentrations during infusion reached almost 1 mmol/L (89 mg/L). An earlier study in rats (Nosadini et al., 1980) suggested that the effects observed in humans were caused by a direct effect on hepatic ketone body production.

Endocrine studies: A dose-dependent increase in plasma growth hormone concentration was observed in normal adults after intravenous administration of L-alanine at levels of 0.1 and 0.5 g/kg body weight over 5 minutes (7 and 35 g, respectively, for a 70-kg person); only the increases with the higher dose were statistically significant. In insulin-dependent diabetic subjects given these doses of L-alanine, changes in growth hormone were inconsistent but tended to change inversely with changes in free fatty acid concentrations (Gennuth and Castro, 1974).

Oral (10 g in 1 minute) and intravenous (10 g in 60 minutes, 0.5 g/kg body weight, or 0.1 g/kg body weight in 2 to 5 minutes) administration of L-alanine resulted in significantly increased secretion of the pancreatic hormones insulin and glucagon in obese and normal weight subjects (Asano et al., 1989; Gennuth and Castro, 1974; Rossini et al., 1975). In untreated diabetic subjects given 0.5 g/kg body weight of L-alanine, plasma insulin concentration increased significantly and free fatty acid and β-hydroxybutyrate levels decreased significantly (Gennuth and Castro, 1974). Changes in GFR, which would indicate renal stress, were not measured.

Compared with a soft drink containing aspartame, a 50-g load of alanine dissolved in the soft drink resulted in a significant increase in plasma glucagon and a significant decrease in 3-hydroxybutyrate whether the alanine was given to carbohydrate-starved athletes either immediately before or after a period of controlled exercise; changes in concentrations of glucose, insulin, and pancreatic polypeptide were not significantly different from those of controls (Koeslag et al., 1985a,b). These authors reported that the ingestion of L-alanine did not result in abdominal discomfort, nausea, or hypoglycemia in any of the subjects.

Oral administration of a single L-alanine load (200 mg/kg body weight, about 10 to 13 g total dose) produced a significant increase in plasma insulin level, no change in plasma glucose concentration, and little increase in serum triglycerides in 13 subjects not using OCA. Plasma insulin levels were elevated to a greater extent and serum triglycerides were significantly increased following the L-alanine load in 23 subjects using OCA (Rose et al., 1977). Following the L-alanine load, plasma alanine levels were increased significantly less in OCA users than in nonusers and both fasting and post-load levels of blood pyruvate were higher in OCA users, a difference possibly resulting from an estrogen-induced increase in ALT activity in OCA users (Rose et al., 1976). Side effects were not reported in either study.

In 5 massively obese subjects, a 50-g load of L-alanine given orally over a 5-minute period after an overnight fast resulted in an increase in plasma insulin and decreases in plasma glucose and β-hydroxybutyrate (Gennuth, 1973). A 50-g load of L-alanine given to the same subjects in the same manner after a 14-day fast produced smaller changes in plasma insulin and β-hydroxybutyrate and an increase in plasma glucose. Over an additional 7-day fast, 50 g/day of L-alanine given orally in divided doses (10 g every 4 hours) to 4 of the subjects produced no change in plasma insulin, a decrease in β-hydroxybutyrate, and an increase in serum glucose. Total nitrogen excretion and BUN increased during the period of chronic L-alanine administration. Occasional transient nausea and abdominal cramping were the only side effects observed with acute or chronic administration of L-alanine (Gennuth, 1973).
Acute doses of L-alanine as high as 0.5 g/kg body weight given intravenously over a 10-minute period to healthy adult males were reported to cause less severe subjective side effects (not further described) than acute doses as high as 1 g/kg body weight given orally over 20 minutes (Kay et al., 1986). Side effects were not reported following intravenous injection of an L-alanine load (0.1 g/kg body weight, 4.9 to 5.4g total dose) in fasting control and hyperthyroid patients before and after control of thyroid function (Ida et al., 1987).

d. Summary and conclusions

Endpoints: Studies of food intake, growth, and hematologic changes resulting from oral ingestion of L-alanine in animals and humans provide few data to suggest that L-alanine is unsafe. Several studies of endocrine effects in adults indicate that oral and intravenous doses of L-alanine up to 50 g/day for 1 or several days can increase plasma growth hormone, glucagon, and insulin levels and possibly alter plasma glucose and β-hydroxybutyrate levels. One metabolic study of intravenous infusion of L-alanine suggests that it may have antiketogenic effects independent of its endocrine effects. Occasional nausea and gastrointestinal upset was observed in obese subjects receiving 50 g/day of L-alanine over a 7-day period. Further evaluation of the safety of alanine should include study of hormonal responses and gastrointestinal distress with chronic administration of the amino acid.

Safe levels of human intake: Limited data suggest that a single bolus ingestion of L-alanine at levels of up to 50 g/day by adults has not been associated with adverse effects. However, the safety of continued ingestion of L-alanine doses greater than the amount consumed in dietary protein (e.g., a person consuming 100 g protein per day would consume about 5.8 g L-alanine) as a dietary supplement cannot be determined from the available data.
2a. **Arginine**

a. **Background**

Arginine is a glycogenic amino acid metabolized via ornithine to α-ketoglutarate which can either be used for glucose synthesis or catabolized to produce energy in the TCA cycle (Rodwell, 1990a). Arginine is needed for tissue protein synthesis, biosynthesis of amino acids and polyamines, provision of the amidino group in creatine synthesis, and ammonia detoxification via the urea cycle (Rodwell, 1990b,c). The amino acid is also a secretagogue for several endocrine glands, stimulating the secretion of pituitary growth hormone and prolactin, pancreatic insulin, somatostatin, and polypeptide, and adrenal catecholamines. Arginine is a dispensable amino acid; however, in children with congenital defects of one of the urea cycle enzymes (other than arginase), arginine becomes an essential amino acid, and supplementation of the low-protein diets prescribed for these children is required indefinitely (Brusilow and Horwich, 1989).

L-Arginine is synthesized from a urea cycle intermediate, citrulline, primarily in the liver and to a lesser extent in the kidney. Many of the biologic and pharmacologic effects of L-arginine are shared with the urea cycle intermediates L-ornithine and L-citrulline. L-Arginine shares many transport mechanisms in the body with L-lysine. For example, together with ornithine and histidine, they share the system Y carrier for transport across cellular membranes (Skeie et al., 1990). Investigations in pigs have shown that arginine and lysine, as free amino acids, compete for absorption in the small intestine (Buraczewski et al., 1970). Similarly, studies in dogs have shown that arginine and lysine compete for reabsorption from the renal tubules (Kamin and Handler, 1951; Webber et al., 1961). Urinary excretion of lysine was also increased by excess dietary arginine in pigs (Southern and Baker, 1982).

L-Arginine, but not D-arginine, has been reported to lead to the generation of nitric oxide and citrulline via a deaminase-like enzyme system (Calver et al., 1990; Moncada et al., 1989). Nitric oxide may act as a neurotransmitter in brain. In abnormally high concentrations, it can be toxic to neurons (Bretdt and Snyder, 1992). Investigations in vitro systems, animals, and humans indicate that arginine can serve as a nitrogen source for formation of nitric oxide in brain and other cells and in endothelium of blood vessels (Bretdt and Snyder, 1992; Leaf et al., 1989; Palmer and Moncada, 1990). However, it remains to be determined whether manipulation of oral intakes of L-arginine can affect nitric oxide synthesis. It has been speculated that arginine might exert some effects on the immune system by increasing nitric oxide levels (Barbul et al., 1990).

Oral or intravenous administration of the hydrochloride salt of arginine may result in acidosis. Thus, in any situation in which arginine is administered as the hydrochloride salt, evaluation of responses or effects due to acidosis need to be distinguished from possible effects of the amino acid itself. Intraperitoneal injection of arginine hydrochloride has been reported to protect rats (duRuisseau et al., 1956; Greenstein et al., 1956; Gullino et al., 1956) and dogs (Najarian and Harper, 1956) against ammonia intoxication induced by administration of ammonium acetate or toxic doses of other amino acids. A neutralized solution of L-arginine given intraperitoneally at a dose of 6 mmol/kg prevented the rise in blood and brain ammonia concentrations in rats induced by insulin, pentylenetetrazol, or ammonium salts and protected the animals against convulsions (Roberge and Charbonneau, 1969). In premature infants, supplemental arginine (free base) at 0.5 to 2 mmol/kg body weight daily has been shown to counteract the hyperammonemia commonly seen in these children (Batshaw et al., 1984; Heird et al., 1972).

b. **Animal Studies**

**Changes in food intake and body weight:** Growth depression has resulted in rats fed low-protein diets containing 4, 5, and 7.5 percent of added arginine (Harper et al., 1966; Sauberlich, 1961;
Schimke, 1963). Growth depression associated with excess arginine was lessened when the protein content of the diet was increased and when the protein quality was improved (Harper et al., 1970; Muramatsu et al., 1971).

Muramatsu et al. (1971) demonstrated that weight gain over a 3-week feeding period was depressed by 16 percent in male weanling Donryu rats fed a 10 percent casein diet with a 5 percent L-arginine supplement although food intake was similar to that of control animals. Liver weight and protein, DNA, and RNA content were not significantly different from controls. Supplementation of a 25 percent casein diet with 2 percent L-arginine as L-arginine hydrochloride improved post-trauma growth but did not result in improved nitrogen retention in male ARS Sprague-Dawley rats traumatized by hind leg fracture compared with traumatized rats fed the 25 percent casein diet without an amino acid supplement (Pui and Fisher, 1979).

Addition of 0.94 to 1.63 percent L-arginine as L-arginine hydrochloride to diets containing 1.03 and 1.2 percent lysine did not affect weight gain or feed intake of young growing pigs over a 28-day period unless lysine was insufficient in the diet (Hagemeier et al., 1983). However, addition of 4 percent L-arginine free base to diets of young pigs decreased weight gain by 31 percent and food intake by 22 percent over a 16-day feeding period (Edmonds et al., 1987a,b).

Biochemical studies: Feeding of 5 percent L-arginine in a 6 percent casein diet to male weanling Sprague-Dawley rats for 4 weeks resulted in a 5-fold increase in plasma arginine (Sauberlich, 1961). Hyperkalemia and hypermagnesemia in the presence of acidosis developed in bilaterally nephrectomized male Sprague-Dawley rats injected intraperitoneally with 400 mg arginine hydrochloride, but not in nephrectomized controls. The isomer was not specified in this study. The authors interpreted this finding as a demonstration of an arginine-induced flux of these ions from intracellular to extracellular compartments (Whang et al., 1988).

Behavioral studies: Male rats of the Wistar and inbred CDR strains were given a saline control solution or L-arginine hydrochloride orally at dose levels of 100, 500, or 1000 mg/kg body weight (about 12, 60, and 120 mg L-arginine for a 150-g rat) 1 hour before behavioral trials for 5 or 7 days. CDR rats (a strain with poor learning capacity) were trained to avoid footshock presented on one side (the side the rat was in) of a two-compartment shuttle-box. A buzzer preceded the footshock by 5 seconds signalling the animal to move from one side of the box to the other. Avoidance behavior was greater in CDR rats given the highest dose than in controls. A similar finding was reported in a passive avoidance task (in which not making a response was associated with avoiding a shock) after 7 days of treatment with the highest dose of L-arginine. In the Wistar rats, conditioned avoidance behavior was not affected although there was an increase in ambulation (Drago et al., 1984).

Endocrine studies: Intravenous infusion of L-arginine (1 mmol/kg body weight or 1.7 g for a 10-kg dog) over 15 minutes resulted in significant increases in plasma insulin and glucagon concentrations in 4 fasting dogs (Rocha et al., 1972). However, in fasting male Sprague-Dawley rats, intragastric administration of arginine (142 mg/kg body weight or 36 mg for a 250-g rat) or an equimolar amount of aspartic acid did not result in an increase in plasma insulin, glucagon, or growth hormone although administration of an equimolar amount of arginine aspartate (250 mg/kg body weight or 62 mg for a 250-g rat) produced a significant increase in the concentration of growth hormone (Francimont et al., 1984).

Immunological studies: Solutions for intravenous hyperalimentation supplying about 290 or 540 mg/day of L-arginine were administered to adult male Sprague-Dawley rats for 7 days (Barbul et al., 1985). With the higher level of arginine, wound healing was accelerated and thymic function was improved (increased thymic weight, total number of thymic lymphocytes/gland, and mitogenic reactivity of thymic lymphocytes to phytohemagglutinin and concanavalin A) (Barbul et al., 1985). Ingestion of L-arginine hydrochloride as dietary supplements at levels of 0.5, 1, 2, and 3 percent for 6 days significantly increased thymic weight, thymic lymphocyte content, and the in vitro reactivity
of thymic lymphocytes in CBA/J mice (Barbul et al., 1980). Responses were similar at all levels of supplementation (Barbul et al., 1980). Also in the CBA/J mouse strain, arginine supplementation (1, 2, or 4 percent in drinking water providing 60, 120 or 240 mg/day assuming 6 ml consumed; isomer not specified) resulted in significantly increased thymus weight, spleen cell mitogenesis, and inducible natural killer cell activity with 1 and 2 percent but not 4 percent supplementation (Reynolds et al., 1990). However, in other studies (Ronnenberg et al., 1991), ingestion of L-arginine hydrochloride at levels of 3 percent of the diet did not increase thymus weights and had little effect on lymphocyte proliferation or IL-2 production in healthy young or aged rats.

Survival rates decreased with increasing level of arginine supplementation (0, 2, 4, or 6 percent arginine hydrochloride in a parenteral formulation containing 5 percent protein) in 8 female Hartley guinea pigs with established sepsis (Gonce et al., 1990). Previous work by this group indicated greater resting metabolic expenditure and lower mortality rates with intragastric tube feedings supplemented with 1 and 2 percent arginine but not 4 percent arginine in burn-traumatized female Hartley guinea pigs for 14 days (Saito et al., 1987). Ear thickness response to a dinitrofluorobenzene challenge on post burn day 12 showed the best response in the group given 2 percent arginine. The authors reported that the lack of improvement seen with 4 percent arginine might be considered an adverse effect and suggested that amino acid imbalance and disturbed protein metabolism might be important mechanisms for an adverse effect of excess arginine.

Gross pathology: Mizunuma et al. (1984) reported marked pathological changes in pancreas and adipose tissue of male Wistar rats given a single intraperitoneal injection of L-arginine (5 g/kg body weight or 0.75 g for a 150-g rat). Over the 24 to 72 hours after injection, pancreatic acinar cells were destroyed selectively; no changes were observed in the islets of Langerhans. The changes were described as similar to pancreatic changes observed after excess L-methionine. The authors concluded that a decrease in protein synthesis in acinar cells resulting from an amino acid imbalance was responsible for the pancreatic damage. Over the same time course, peripancreatic, epididymal, omental, and retroperitoneal adipose tissue became necrotic and infiltrated with polymorphonuclear leukocytes. Serum lipase activity was significantly increased 24 hours after L-arginine injection.

Teratology and developmental studies: Daily intraperitoneal injection of 15 mg/kg body weight of L-arginine hydrochloride to 18 pregnant rats (about 2.5 mg/day in a 200-g rat) on days 1 to 6 of gestation resulted in hindlimb malformations in 45 percent of the fetuses of the arginine-treated dams (Naidu, 1973). Information on effects in the control group was not reported. The report included little experimental detail and it may not be reasonable to attribute teratogenic effects to the small amount of arginine administered.

c. Human Studies

Biochemical studies: The arginine salt of glutamic acid (arginine glutamate) has been used to treat acute hepatic encephalopathy in doses of 50 to 100 g given intravenously (Tobe, 1961; Davey, 1964). In these studies, 1 or 2 infusions of arginine glutamate were given to each patient. No side effects were seen apart from blisters at the site of infusion in 2 of 40 patients and mild side effects were reported at infusion rates exceeding 25 g/hour (Davey, 1964). However, oral administration of 25 g arginine glutamate to 3 fasting patients (2 with chronic alcoholism and 1 with episodic encephalopathy) resulted in an increase in blood ammonia concentration of at least 10 percent within 2 hours in all patients. Because of the consistent rise in blood ammonia, the investigators concluded that oral and intravenous administration of arginine glutamate did not produce equivalent effects. They further advised against oral administration of arginine glutamate to patients with liver disease.
Intravenous loading of L-arginine hydrochloride (0.5 g/kg body weight/30 minute supplying 0.4 g arginine/kg body weight) 4 hours after feeding in 7 infants 2 to 6 weeks of age (about 2 g of arginine in a 5-kg infant) resulted in an immediate marked increase in plasma arginine concentration, with a peak of about 7000 μmol/L (normal range 50 to 100 μmol/L). Ornithine concentrations also increased markedly but lagged behind the increase in arginine; plasma concentrations of other amino acids did not change significantly. Ammonia and urea concentrations and acid–base balance (pH and standard bicarbonate levels) did not change significantly with arginine infusion (Kraus et al., 1976). It should be noted that the peak plasma concentration in this study was about 5 times higher than plasma levels in patients with hyperargininemia (see p. 123) and remained above 1000 μmol/L for 2 hours after infusion. Lack of increase in plasma ammonia and urea levels was interpreted by the authors as evidence that infants were capable of metabolizing this load of arginine without overt adverse effects.

Metabolic effects of arginine administration and protein restriction in five patients with liver cirrhosis were studied by Baertl and Gabuzda (1959). Patients were infused intravenously over 3-hour periods with 20 g L-arginine hydrochloride daily for 6 days. Infusion of L-arginine hydrochloride did not have a significant effect on serum sodium, chloride, or potassium concentrations; however, effects on blood urea nitrogen and nonprotein nitrogen concentrations varied among the patients. Urinary chloride excretion was greatly increased and nitrogen, ammonium, and potassium excretion were also increased. In one patient also given 20 g L-arginine hydrochloride daily for 6 days or 16.5 g L-arginine base daily for 6 days, metabolic changes were similar to those described for intravenous infusion. Baertl and Gabuzda (1959) reported that there was no clinical intolerance to L-arginine hydrochloride infused intravenously or to L-arginine free base given orally. Daily oral ingestion of 20 g of L-arginine hydrochloride resulted in diarrhea in the one patient studied. In the absence of a control group of patients or administration of a placebo compound, these results should be interpreted with caution.

Intravenous administration of arginine hydrochloride (0.5 g/kg body weight given over 30 minutes or 35 g for a 70-kg person) to 15 normal adults resulted in a rise in blood potassium (from 4 mEq/L initially to 4.9 mEq/L 90 minutes after infusion) and a fall in blood phosphorus (from 3.3 mg/L initially to 2 mg/L 60 minutes after infusion) (Massara et al., 1979). Administration of this dose of arginine to 14 insulin-dependent diabetic subjects resulted in a pronounced increase in blood potassium concentration to pathological levels (5.6 to 6.5 mEq/L in 9 subjects) and a smaller but significant decrease in blood phosphorus concentration (from 3.5 to about 3.3 mg/L). No significant changes were observed in blood pH, plasma osmolality, or plasma aldosterone (Massara et al., 1981). In patients with renal and hepatic insufficiency, infusion of arginine hydrochloride also resulted in life-threatening hyperkalemia (Bushinsky and Gennari, 1978; Hertz and Richardson, 1972). Isomers were not specified in these studies.

Oral ingestion of L-arginine hydrochloride (100 mg/kg body weight [83 mg arginine/kg body weight]) by 10 fasted healthy human subjects 20 to 49 years of age resulted in significantly increased concentrations of ornithine and arginine compared with 8 control subjects. Glucogenic precursors (proline and alanine) did not accumulate (Iwasaki et al., 1987). Serum insulin concentration was significantly increased 20 minutes after ingestion and serum glucose and free fatty acid levels were significantly decreased after 1 hour. In contrast, ingestion of L-ornithine hydrochloride (100 mg/kg body weight [79 mg ornithine/kg]) by 13 subjects resulted in significantly increased plasma concentrations of proline, alanine, ornithine and arginine and decreased concentrations of valine and urea; however, serum insulin, glucose, and free fatty acid levels were not significantly different from the control group 1 hour after administration. Serum levels of growth hormone and cortisol were reported unchanged with both treatments but the data were not presented (Iwasaki et al., 1987). The report did not include mention of side effects resulting from administration of a single bolus of L-arginine or L-ornithine.
Endocrine studies: Intravenous administration of 30 g arginine (0.5 g/kg in children) over a 30-minute period has been given routinely in tests of human insulin response or pituitary function (Alba-Roth et al., 1988; Barbul, 1986; Casanueva et al., 1984; Cordido et al., 1960; Fajans and Floyd, 1972; Ferrero et al., 1980; Page et al., 1988; Goodner and Porte, 1972). Intravenous injection of 30 g L-arginine in 13 fasting healthy adults produced the largest increase in plasma insulin concentration of any of 10 single amino acids given. No adverse effects were reported (Floyd et al., 1966). Differences in arginine-induced responses in insulin and glucagon concentrations were reported for young adult and elderly subjects by Ferrero et al. (1980). No observations of adverse effects were noted in the reports of these studies.

In 8 healthy male subjects without history of cardiovascular disease, consumption of a meal containing 20 percent soybean oil, 20 percent casein, and 60 percent corn syrup solids supplemented with 3 g of arginine and 2 g glycine resulted in a significant increase in plasma glucagon and a nonsignificant decrease in plasma insulin in comparison with consumption of an ununsupplemented meal; postprandial plasma cholesterol and triglyceride concentrations were similar with both meals (Sanchez et al., 1988).

Arginine aspartate was administered orally at a level of 250 mg/kg body weight (17.5 g/day arginine aspartate for a 70-kg male; 9.9 g/day of arginine and 7.6 g/day of aspartate) daily for 7 days to 5 healthy male subjects (Bessett et al., 1982). The slow wave sleep-related growth hormone peak was about 60 percent higher after 7 days of arginine aspartate administration than in the control period. Individual increases ranged from 24 to 162 percent. Nocturnal mean plasma prolactin concentration was also higher after arginine aspartate infusion. The hormonal changes were not accompanied by any detectable alteration of sleep organization (Bessett et al., 1982).

Immune function studies: Park et al. (1992) reported that the rate of tumor protein synthesis more than doubled in breast cancer patients given oral doses of L-arginine free base (30 g/day in 4 divided doses for 3 days) compared with breast cancer patients not given the amino acid. In addition to the significant increase in protein synthesis, the investigators reported a marked stimulation in the expression of the activation antigen Ki67 in the tumors of the L-arginine-supplemented patients. No difference was found in the plasma insulin concentration, which was considered an index of arginine-induced endocrine stimulation. Most of the tumors in these patients were categorized histologically as invasive ductal cell carcinomas. Mild diarrhea, which subsided when the supplement was no longer taken, was reported as a side effect in 2 of the 10 women given the supplement. These results are in direct contrast to studies in animals in which administration of L-arginine suppressed tumor growth (e.g., Barbul, 1986; Levy et al., 1954; Milner and Stepanovich, 1979; Tachibana et al., 1985).

In a 7-day study of the effect of oral ingestion of 30 g/day arginine hydrochloride on lymphocyte immune response measured in vitro as peripheral blood lymphocyte mitogenic reactivity in 21 healthy human volunteers, no differences in liver function, BUN, creatine, or blood glucose were found (Barbul et al., 1981). Nausea and diarrhea were reported by 2 and 3 subjects, respectively. These side effects responded to lowering the dose ingested at any one time; the total daily dose was not decreased. There was no control group for the study and dietary intake was not controlled or monitored.

The effect of oral arginine supplementation on wound healing and lymphocyte immune responses was studied in 36 healthy, nonsmoking human volunteers (Barbul et al., 1990). For 2 weeks, volunteers were given 30 g/daily arginine hydrochloride (24.8 g arginine), 30 g/day arginine aspartate (17 g arginine), or a placebo. Diet was not controlled during the experimental period. In the subjects supplemented with 30 g/day of arginine hydrochloride, mild hyperchloremic acidosis developed with significant increases in serum chloride and significant compensatory decreases in serum potassium, calcium, and bicarbonate. No increases in blood urea nitrogen or creatinine levels were found with either form of arginine supplement. Plasma amino acid analyses showed significant increases in arginine and ornithine concentrations, and a decrease in lysine.
concentration with both arginine supplements. Concentrations of other amino acids were not altered by the arginine supplements. During the study, several subjects reported side effects such as bloating, mild anorexia, and diarrhea (Barbul et al., 1990). These were not severe enough to interfere with regular activities. Incidence of all occurrences was 1 of 12 subjects given placebo, 3 of 12 given arginine aspartate, and 6 of 12 given arginine hydrochloride. Subjects were instructed to space intake of supplements throughout the day but this was not rigorously controlled. The investigators attributed the occurrence of side effects to the large osmotic load.

In studies of mitogenic reactivity of peripheral lymphocytes of normal subjects fed 30 g of L-arginine hydrochloride daily for 1 to 2 weeks, no adverse effects on liver function were identified. Diarrhea and nausea that occurred at higher doses did not recur with lower doses (Barbul et al., 1981).

In postsurgical patients, supplementation of an enteral diet with 25 g of L-arginine daily for 7 days significantly improved the mean CD4 phenotype (percent T-cells) and the mean T-lymphocyte response to concanavalin A compared with a glycine-supplemented group given the same diet (Daly et al., 1988).

Tiwary et al. (1973) reported an apparent anaphylactic reaction in a child ten years of age following infusion of 100 ml of a commercially available 5 percent arginine hydrochloride solution (approximately 5 g arginine given over 12 minutes). All signs and symptoms of the reaction had abated in 2 hours. No description of treatment for the reaction was given.

Functional assessments: Intravenous infusion of 60 g of L-arginine in 500 ml of infusate over 40 to 50 minutes resulted in an increase in excretion rate of albumin from a baseline level of 8.6 μg/minute to 142 μg/minute in 5 healthy men (Mogensen et al., 1975). Administration of graded doses of L-arginine (3, 6, 9, or 12 g) to 6 healthy subjects resulted in significant increases in albumin excretion at each dose level (5.8 μg/minute baseline, 9.4 μg/minute with 3 g, 13.2 μg/minute with 6 g, 21.6 μg/minute with 9 g, and 33.9 μg/minute with 12 g arginine). Dose-related significant increases in light chain immunoglobulin and β-microglobulin were also seen with increasing doses of L-arginine (Mogensen et al., 1975).

A single intravenous injection of 6 g L-arginine in 5 nonfasting young male subjects caused an immediate increase in urinary excretion of tubulin protein reabsorption, resulting in significantly increased urinary excretion of albumin, light chains, and beta-2-microglobulin (Mogensen and Sølling, 1977). The authors reported that the single injection of this amount of arginine did not result in untoward effects. The response to arginine was lower than the response to an equimolar injection of L-lysine, the most active of the compounds tested. Injection of 1.5 g L-arginine also significantly increased β-microglobulin excretion but injection of equimolar amounts of other L-amino acids (leucine, valine, proline, histidine, methionine, aspartic acid, glycine, serine, phenylalanine, or tryptophan) did not increase excretion of this protein. Intravenous injection of 2.4 g L-ornithine, which shares many of the biological effects of L-arginine, resulted in a significant elevation in excretion of light chains and β-microglobulin but not albumin. Injection of an equimolar amount (3.2 g) of citrulline, a precursor of arginine in the urea cycle, had no inhibitory effect on tubular reabsorption of any of the proteins (Mogensen and Sølling, 1977).

GFR, RPF, and plasma glucagon concentrations were compared when 30 g arginine hydrochloride (24.9 g arginine) was given orally or the same amount of arginine hydrochloride or glucagon (10 ng/kg body weight/minute) was given intravenously to 6 normotensive subjects with no history of renal disease (Smoyer et al., 1991). The peak GFR was higher with oral administration of arginine than with intravenous arginine or glucagon, despite a lower peak glucagon concentration. Both oral arginine and intravenous glucagon, but not intravenous arginine, significantly increased RPF. Plasma concentrations of the gastrointestinal hormones (gastrin, neurotensin, and pancreatic polypeptide) did not differ with either route of arginine administration (Smoyer et al., 1991). These findings suggest that when arginine supplements are given, the route of administration may be
important with respect to changes in renal function; however, care must be exercised in extrapolating between effects observed with bolus intravenous infusions and oral intakes of similar or smaller amounts.

Intravenous injection of both isomers of arginine have been reported to induce hypotension in humans (Calver et al., 1990; Nakaki et al., 1990). Doses producing hypotensive effects were 640 μmol (1.11 mg) given in 4 minutes to 10 healthy normotensive subjects and 2.4 mmol (415 mg)/kg body weight (30 g for a 70-kg subject) given over 30 minutes to 5 normotensive and 5 hypertensive subjects, respectively. Suggested mechanisms have included vasodilation mediated by endothelium-derived relaxing factor (EDRF) (Nakaki et al., 1990), by nitric oxide formed endogenously from L-arginine (Hishikawa et al., 1981), by other unspecified arginine-mediated vasodilation mechanisms (Calver et al., 1990), and arginine-mediated histamine release (Paton, 1990).

Eighteen males aged 27 to 57 years ingested 1 g each of arginine and ornithine daily in 2 divided doses taken apart from meals 5 days per week for 5 weeks in a randomized placebo-controlled, double-blind study of the effects of these amino acids on body composition in combination with a weight training program (Elam, 1988). With the combined treatment body mass and body fat were reduced significantly more than with weight training and placebo. No information was given on dietary intakes of the subjects.

In another study with the same experimental protocol, 22 males with a mean age of 37 years consumed the same doses of L-arginine and L-ornithine while participating in a strength-training program (Elam et al., 1989). Subjects were asked to maintain their usual eating habits. Total strength and lean body mass were increased significantly and urinary hydroxyproline excretion was decreased significantly in the group taking the amino acid supplements. Neither study included mention of side effects of the L-arginine and L-ornithine supplements (Elam, 1988; Elam et al., 1989).

Investigations of orally administered arginine (0.5 to 4 g/daily) for as long as 12 weeks as a treatment for male infertility have not shown consistent effects of arginine on sperm count or motility (De Aloysio et al., 1982; Pryor et al., 1978; Schachter et al., 1973; Tanimura 1967). Weight increase, digestive troubles, and sleepiness were reported as reversible, dose-related side effects in subjects treated with 9 or 18 g arginine aspartate (5 or 10 g arginine) daily for 80 days (De Aloysio et al., 1982).

Solomons et al. (1971) reported that there were "no important side effects" attributable to oral administration of buffered arginine with treatment of L-arginine (mixed in a ratio of 0.9 g L-arginine free base to 15 g of L-arginine hydrochloride) for 14 patients with cystic fibrosis (1 to 19 years of age) for 10 days. The maximum daily dose was 25 g, given in an attempt to improve fat absorption.

Side effects of nausea in 2 patients with chronic liver disease and slight facial stiffness and paresthesia in 1 of these patients were reported with intravenous infusion of arginine glutamate (13.5 g L-arginine and 12 g L-glutamic acid) at a more rapid rate than 25 g/hour (Davey, 1964). No side effects occurred when the rate of infusion was reduced.

Inborn errors of metabolism: Argininemia is a metabolic disorder resulting from a deficiency of arginase activity. In addition to high plasma levels of arginine, the enzyme deficiency results in increased plasma and urine concentrations of ornithine, and intermittently, mild hyperammonemia (Brusilow and Horwich, 1989; Yoshino et al., 1982). Clinical manifestations of the disorder include recurrent vomiting, seizures, spastic diplegia, psychomotor retardation, and delayed physical growth (Kang et al., 1983). Elevated plasma levels of both arginine (as high as 1500 μmol/L or 261 mg/L, normal range 50 to 100 μmol/L or 9 to 17 mg/L) and ammonia (3 to 4 times normal levels) may be responsible for these occurrences. CSF concentrations of arginine (normal range 10 to 30 μmol/L or 1.7 to 5 mg/L), as well as ornithine, aspartate, threonine, glycine, and methionine, are also
The dibasic amino acids share common transport systems at the blood–brain barrier and renal tubules. CSF lysine concentrations were reported to be low in one patient before, but not during, treatment with a diet low in arginine (Brockstedt et al., 1990). However, lysine loads (250 mg/kg body weight; isomer not specified) given to normal subjects or patients with hyperargininemia did not result in decreased plasma arginine concentrations (Kang et al., 1983; Michels and Beaudet, 1978; Snyderman et al., 1977). Oral lysine treatment (250 mg/kg body weight/day) of one child with hyperargininemia for 6 months resulted in an increase in arginine and a decrease in ornithine in CSF (Kang et al., 1983).

Results of biochemical and hematological investigations were reported in 1 untreated patient with hyperargininemia (plasma and CSF arginine concentrations of 907 μmol/L and 78 μmol/L, respectively) (Brockstedt et al., 1990). Blood chemistry, including electrolytes, creatinine, protein–electrophoresis, creatine kinase, alkaline phosphatase, LDH, glucose and ceruloplasmin was within normal limits. The patient exhibited a mild hyperammonemia and a low plasma concentration of urea. Hemoglobin concentration, hematocrit, erythrocyte sedimentation rate, red cell morphology, platelet count, and leukocyte differentiation were reported to be normal in this subject.

d. Summary and conclusions

Endpoints: In animal studies, addition of 4 to 7 percent of L-arginine to the diet has resulted in growth depression of rats and pigs. One study in rats and one study in humans have shown little behavioral effect of arginine given orally; however, these studies were very limited in scope. Administration of arginine resulted in elevated plasma potassium concentrations in animals and humans, reaching pathological levels when about 30 g was given to persons with diabetes mellitus or renal insufficiency. Arginine is known to be a secretagogue for several endocrine glands; intravenous infusion and oral administration of 30 g arginine as a single dose resulted in hypertensive effects, stimulation of insulin, glucagon, and growth hormone secretion, and increased glomerular filtration rate in humans.

The rate of tumor protein synthesis and expression of an activation antigen were significantly increased in women with breast cancer given 30 g L-arginine for 3 days. These results are in direct contrast to results of animal studies showing decreased tumor growth. In studies of immune function, administration of 25 and 30 g/day of arginine has not produced changes in liver function or plasma biochemical parameters in healthy humans, although a mild metabolic acidosis was reported following oral administration of arginine hydrochloride in humans. Administration of arginine to rats orally and intravenously has enhanced certain aspects of immune function at levels of dietary addition as high as 3 percent by weight, but not at 4 percent, suggesting that there may be an upper limit for augmentation of immune function by arginine.

Investigations in children with argininemia suggest that high plasma and cerebrospinal fluid concentrations of arginine early in life result in adverse effects on neurological development and growth. It appears that effects of high levels of arginine may result from competition of arginine with lysine for uptake and utilization in tissues as well as by other mechanisms.

Safe levels of human intake: The safety of excess arginine may be affected by lysine intake as well as the total amount of protein consumed. Daily intakes of arginine and lysine from dietary protein are about 5.4 and 5.0 g, respectively, for a person consuming 100 g protein.

Arginine glutamate given intravenously in doses of 50 to 100 g had few side effects in patients with hepatic encephalopathy; however, oral doses of 25 g arginine glutamate resulted in increased blood ammonia concentration in patients with liver disease. Long-term supplementation of the diets of children with certain inherited disorders of the urea cycle with 0.4 to 0.7 g/kg daily of arginine free base has not resulted in adverse effects. Side effects were not reported with daily doses of 1 g
L-arginine in combination with 1 g L-ornithine given 5 days per week for 5 weeks. Intravenous infusion of 30 g of L-arginine hydrochloride in a single bolus has been used clinically to evaluate insulin and pituitary hormone secretion. Although intravenous administration appears to be well-tolerated, increased plasma concentrations of potassium, particularly in diabetic individuals and persons with renal insufficiency, is of concern.

Oral intake of arginine aspartate (5 and 10 g/day of arginine) over an 80-day period has been reported to result in dose-related weight increase, digestive troubles, and sleepiness. Oral intakes of about 20 to 30 g/day of L-arginine hydrochloride for 7 to 14 days have resulted in gastrointestinal side effects (nausea, bloating, mild anorexia, and diarrhea). However, results of a recent study indicated that ingestion of 30 g/day of L-arginine as the free base resulted in stimulation of tumors in women with breast cancer. Although most studies have suggested that ingestion of as much as 30 g/day of arginine produced few side effects, the report that tumor growth increased with daily ingestion of 30 g L-arginine raises concern about its use as a dietary supplement. The Expert Panel considered these results of particular importance because it is likely that a study of this type will not be repeated because of ethical considerations. Therefore, it is not possible to conclude that use of L-arginine as a dietary supplement is not associated with adverse health effects. The Expert Panel is well aware that L-arginine has numerous uses medically, including treatment of inborn errors of metabolism and other disorders. Such treatments under medical supervision are outside the scope of this report. L-Arginine as a dietary supplement should be used only under responsible medical supervision.
2b. Ornithine and citrulline

a. Background

L-Ornithine and L-citrulline are amino acids that are not incorporated into protein and that are intermediates in the mammalian metabolism of urea, creatine, and polyamines. L-Arginine from dietary protein provides the main source of these amino acids. Both ornithine and citrulline are metabolized by pathways closely related to those of proline and, together with proline, provide a means for exchange of molecules between the urea and tricarboxylic acid cycles. More specifically, carbon atoms of ornithine and citrulline may be incorporated into protein as arginine, proline, glutamate, or dispensable amino acids derived from \( \alpha \)-ketoglutarate; converted to the polyamines and GABA; or oxidized in the TCA cycle (Valle and Simell, 1983; Windmueller and Spaeth, 1981).

b. Animal studies

Changes in food intake and body weight: Addition of ornithine to an arginine-free diet did not promote growth of rats (Levenson et al., 1980; Milner and Visek, 1975); however, continuous intragastric infusion of an arginine-free mixture of amino acids, including ornithine, plus other nutrients led to nearly normal increases in weight and tail length in rats (Abram and Walser, 1982a). The explanation of these disparate results is not clear. Kittens fed an arginine-free basal diet with added ornithine lost weight, although not as fast as kittens fed the basal diet (Morris et al., 1979). No impairment of growth was observed in weanling rats fed ornithine (as salts of BCKA) at doses up to 4 g/kg body weight (Funk et al., 1987).

Biochemical studies: Feeding of ornithine \( \alpha \)-ketoglutarate to burn-traumatized rats at 5 g/kg body weight (1.25 g daily for a 250-g animal) attenuated muscle protein catabolism (Vaubourdolle et al., 1991). In rats made hyperammonemic by giving ammonium acetate, intraperitoneal injection of 0.5 mmol ornithine acetate or ornithine glutamate infusion counteracted the hyperammonemia (Zieve et al., 1989). Ornithine aspartate has similar effects (Salvatore et al., 1964) as does ornithine \( \alpha \)-ketoglutarate (Michel et al., 1971; Camatte et al., 1966). In dogs made hyperammonemic, ornithine \( \alpha \)-ketoglutarate lowered blood ammonia and increased oxygen uptake by brain (James et al., 1972).

Functional assessments and gross pathology: Intravitreous injection in rats of either D- or L-ornithine hydrochloride (10 amol or 1.3 mg ornithine) caused destruction of the retinal pigment epithelium followed by permanent degeneration of outer retinal layers similar to the pathologic process seen in gyrate atrophy caused by hyperornithinemia resulting from a deficiency of ornithine-\( \delta \)-aminotransferase in humans. Injection of equimolar amounts of L-citrulline, L-proline, or L-arginine hydrochloride did not result in permanent damage to the retinal pigment epithelium (Ishikawa and Kuwabara, 1982).

c. Human studies

Biochemical studies: Patients with chronic renal failure given ornithine (as salts of BCKA at 0.2 g/kg body weight/day) by continuous nasogastric infusion for several weeks (Abram and Walser, 1982b) or by mouth for many months (Mitch et al., 1982; Modification of Diet in Renal Disease (MDRD) Study Group, 1989; Walser et al., 1987) had no increase in fasting plasma ornithine concentration and showed no adverse effects. Boys with muscular dystrophy fed ornithine (also as salts of BCKA at 0.2 g/kg body weight/day) for 4 days exhibited a small but significant decrease in urinary 3-methylhistidine excretion and no change in fasting plasma ornithine concentration (Stewart et al., 1982).
Burn patients given 20 g ornithine α-ketoglutarate daily for 24 days exhibited pronounced hyperornithinemia on day 13, but by day 24 the plasma concentration had returned to normal (Cynober et al., 1984). In postoperative patients, parenteral administration of this compound improved nitrogen balance (Leander et al., 1985) and attenuated the loss of intracellular glutamine (Hammarqvist et al., 1990).

In patients with portal-systemic encephalopathy given ornithine orally (34 mmol/day, as salts of BCKA), plasma ornithine rose slightly, and no adverse effects were reported (Herlong et al., 1980). Infusion of ornithine α-ketoglutarate but not ornithine hydrochloride for 24 hours in cirrhotic patients improved the plasma amino acid pattern (Molimard et al., 1982). Cirrhotic patients given 60 g ornithine α-ketoglutarate orally showed no ill effects (Gay et al., 1979).

Intravenous infusion of ornithine α-ketoglutarate (28 mg/minute for 150 minutes providing a total dose of 2 g ornithine) in 7 fasting healthy nonobese adults produced a 6-fold increase in arterial ornithine and increased uptake by splanchic and leg tissues. Mean plasma concentration rose from 57 μmol/L (7.5 mg/L) initially to 354 μmol/L (47 mg/L). Few alterations were seen in concentrations of other amino acids and the authors concluded that intravenous infusion of ornithine α-ketoglutarate did not have a significant influence on hepatic or skeletal muscle protein metabolism (Eriksson et al., 1985). The report did not include mention of any adverse effects in these subjects.

Plasma concentrations of arginine and ornithine were reported in 3 subjects following separate oral loads of citrulline, arginine, and ornithine (0.5 mmol/kg body weight) given to 7 fasting healthy adults and 1 child 1.5 years of age. Six of the subjects were female. For a 60-kg individual, these doses would be 5.2 g arginine, 4.0 g ornithine, and 5.3 g citrulline. For an 11-kg child, the doses would be 1.0 g, 0.7 g, and 1.0 g, respectively. Plasma concentrations of arginine and ornithine peaked about 2 hours after loading and, in most cases, were within normal range after 4 hours (Rajantie et al., 1983). The report did not include mention of side effects from these single doses of amino acids. Consumption of 2 to 2.8 g/day citrulline for as long as 2 years by 17 patients with lysinuric protein intolerance was reported to be without adverse effects (Rajantie et al., 1980).

**Endocrine studies:** Intravenous infusion of ornithine hydrochloride (12 g/m² given over 30 minutes; isomer not specified) in 54 fasting children 4 to 14 years of age with constitutional short stature induced secretion of plasma growth hormone and cortisol, preceded by an increase in adrenocorticotropin hormone (Evain-Brion et al., 1982). The authors reported that the test was well tolerated and that vomiting in the older children was the only side effect observed. Vomiting was alleviated by maintaining a constant flow rate during the infusion. In a subsequent study of a subset of 13 of these children given ornithine by the same protocol, growth hormone secretion was not preceded by an increase in growth hormone releasing hormone, suggesting that ornithine does not induce growth hormone secretion by means of growth hormone releasing hormone (Donnadieu et al., 1985).

Ingestion of graded doses of L-ornithine hydrochloride (40, 100, and 170 mg/kg body weight) by 12 fasting weightlifters (9 male, 3 female) resulted in a significant, dose-dependent increase in plasma ornithine concentrations measured over a 90-minute period and in a significant elevation of serum growth hormone levels 90 minutes after ingestion with the highest dose (Bucci et al., 1990; 1991). Ornithine was administered in capsule form on 3 occasions, each 1 week apart. Mean total doses of ornithine on each occasion were 1.8, 4.6, and 7.8 g for females and 2.6, 6.6, and 11.1 g for males. At the highest dose, the plasma ornithine concentration was increased approximately 4-fold over basal level and all subjects experienced mild to severe stomach cramping and diarrhea. In the 2 papers, mean plasma ornithine concentrations were reported as 152 and 125 μmol/L (fasting) and 682 and 520 μmol/L 90 minutes after ingestion of the highest dose. The fasting concentration of ornithine was slightly above the normal range and the concentration after ingestion of the highest dose was well within the range reported in patients with inborn errors of ornithine metabolism reported by Valle and Simell (1983). (See p. 128) The authors reported that 7 of the 12 subjects already used supplements containing 500 or 750 mg ornithine but that the fasting values for these subjects were not higher than those of subjects who did not use the supplements.
In normal fasting subjects, oral administration of ornithine α-ketoglutarate (10 g ornithine α-ketoglutarate supplying 6.4 g ornithine) significantly increased plasma concentrations of insulin and glucagon and plasma concentrations of ornithine (approximately 10-fold) and citrulline. Although the increase in plasma insulin elicited by ornithine α-ketoglutarate was statistically significant, it was much smaller than a postprandial increase in normal subjects. Administration of ornithine hydrochloride also supplying 6.4 g ornithine resulted in a similar increase in the plasma ornithine concentration but did not increase the plasma concentrations of insulin, glucagon, or citrulline (Cynober et al., 1990). The report did not include mention of side effects with administration of either ornithine compound. The increases in plasma ornithine concentrations reported by Cynober et al. (1990) were very similar to those reported by Bucci et al. (1990, 1991).

**Functional assessments:** Ornithine, but not citrulline, significantly inhibited renal tubular protein reabsorption of β-microglobulin (Mogensen and Sølling, 1977). (See arginine section for a detailed description of this study.)

**Inborn errors of metabolism:** Increased plasma ornithine concentration is associated with two different syndromes. The first, gyrate atrophy of the choroid and retina, results in progressive visual deterioration beginning by late childhood. Gyrate atrophy is associated with plasma concentrations of ornithine of 400 to 1400 μmol/L (53 to 185 mg/L). Normal plasma ornithine levels usually range from 40 to 120 μmol/L (5 to 16 mg/L). Plasma ammonia is not increased but urinary excretion of ornithine is elevated.

With the second condition, hyperornithinemia–hyperammonemia–homocitrullinuria syndrome, plasma ornithine concentrations are elevated to a lesser extent than with gyrate atrophy, 380 to 630 μmol/L (50 to 83 mg/L). Signs and symptoms include growth retardation and developmental delays and, in some patients, seizures. Intellectual level was described as varying from low normal to severe retardation (Valle and Simell, 1983). Citrullinemia, an autosomal recessive disorder in which plasma, CSF, and urine concentrations of citrulline are elevated, results from a deficiency of arginosuccinate synthetase. Pathologic changes in brain and mental retardation in patients with this disorder were described by Brusilow and Horwich (1989) and Shih and Efron (1972).

**d. Summary and conclusions**

**Endpoints:** Intravitreous injection in rats of either D- or L-ornithine resulted in permanent retinal damage similar to that seen in gyrate atrophy resulting from hyperornithinemia. Studies on effects of ingestion of excess citrulline by animals were not found.

In humans, intravenous infusion of 12 g/m² of ornithine hydrochloride in children 4 to 14 years of age has been used to induce secretion of growth hormone and cortisol. Oral ingestion of capsules containing about 8 or 11 g of L-ornithine hydrochloride by adults (ingestion on 3 separate occasions each 1 week apart) induced significant elevations in plasma ornithine and growth hormone concentration. Gyrate atrophy of the choroid and retina causing progressive loss of vision and a hyperornithinemia–hyperammonemia–homocitrullinuria syndrome causing growth retardation, developmental delays, and seizures have been described in patients with extremely elevated levels of plasma ornithine resulting from inborn errors of metabolism. Mental retardation and brain pathology have been described in patients with citrullinemia.

**Safe levels of human intake:** Patients with chronic renal failure have been given ornithine as salts of branched-chain ketoacids at a level of 0.2 g/kg body weight (containing 3.6 g ornithine daily for a 70-kg patient) for extended periods of time without adverse effects. Ingestion of about 8 g and 11 g L-ornithine by healthy adult males and females, respectively, in capsule form as a single bolus has been reported to cause mild to severe stomach cramping and diarrhea. Plasma concentrations of ornithine following ingestion of this amount were within the range of levels reported in patients with ornithinemia.
with inborn errors of ornithine metabolism. Ingestion of about 2 and 5 g by females and 3 and 7 g by males in this manner did not result in such high plasma levels of ornithine and did not produce side effects. Although a single bolus of about 5 to 7 g of L-ornithine appears to be without side effects, safety of continued ingestion of this or other doses of L-ornithine cannot be determined from the available data.

Information was insufficient for the evaluation of the safety of oral ingestion of citrulline by healthy individuals.
3a. Asparagine

a. Background

Asparagine is generally considered a dispensable or nonessential amino acid in mammals. However, studies by Breuer et al. (1966) and Crosby and Cline (1973) indicate that a dietary source of asparagine may be required for maximal growth of infants.

Asparagine has distinct codons in the genetic code and is a distinct independent moiety in newly synthesized protein; however, because asparagine is either deaminated during food processing or converted to aspartate by mucosal cells, the physiological consequence of asparagine is generally included in discussion of aspartic acid.

b. Animal studies

Changes in food intake and body weight: As mentioned above, several groups have investigated the asparagine requirement for growth in rats (Breuer et al., 1966; Crosby and Cline, 1973; Newburg et al., 1975). The emphasis of these studies has been to assess the growth response to diets with and without asparagine. Breuer et al. (1966) noted a possible ceiling effect on growth in rats fed varying concentrations of asparagine. When compared with a 20 percent casein diet, young rats given asparagine at 0.4 percent along with casein hydrolysate had significantly increased growth when compared with rats fed 0.2 percent asparagine after 8 days. Addition of asparagine at 0.8 percent resulted in significantly decreased growth compared with rats fed 0.4 percent asparagine. All asparagine-supplemented diets resulted in greater growth than casein hydrolysate alone after the initial period. At the end of the continuation phase, 9 to 14 days, there were no significant differences between groups for growth. No subsequent reports were found that examined the impact of high doses of asparagine on growth or food intake in animals.

c. Human studies

No human studies were found that provided insights into the safety of asparagine as a single amino acid supplement.

d. Summary and conclusions

Endpoints and safe levels of human intake: Insufficient evidence exists to determine safe levels of asparagine intake as a dietary supplement; however, because of its role as a precursor of aspartic acid, all of the conditions described in the discussion of aspartic acid should be considered. It has been estimated that a person consuming 100 g protein per day would potentially consume about 7.4 g asparagine. (See Table 2.)
3b. Aspartic Acid

a. Background

Aspartic acid, a dispensable dicarboxylic acid, is produced endogenously by the vitamin B6-dependent transamination of oxaloacetic acid. This reversible reaction also converts aspartate in the presence of α-ketoglutarate to oxaloacetate and glutamate. Consequent to the inextricable relationship between aspartate and glutamate, these dicarboxylic amino acids are generally considered together in discussions of absorption, transport, and intermediary metabolism.

The role of aspartic acid in intermediary metabolism is via oxaloacetate, which enters either the TCA cycle directly or is converted to phosphoenolpyruvate for gluconeogenesis or conversion to pyruvate and acetyl CoA. Another important role for aspartate is in the malate shuttle system for the generation of reducing equivalents in mitochondria.

Aspartic acid also plays a role in the urea cycle where it combines with citrulline to form argininosuccinate. The argininosuccinate is subsequently converted to arginine and fumarate and eventually urea, ornithine (from arginine), and malate and oxaloacetate (from fumarate). The oxaloacetate may then be transaminated to regenerate aspartate; thereby replenishing the endogenous supply.

Baverel et al. (1990) recently investigated the role of aspartate in the production of glutamine in the kidney. These authors noted that aspartic acid is actively reabsorbed by the renal tubule. In investigating the fate of aspartic acid, Baverel et al. (1990) found that glutamine was the primary carbon and nitrogen product and that glutamate, ammonia, and alanine represented only minor products.

Absorption and transport: Both aspartate and glutamate, are absorbed via a sodium-dependent active transport system, designated system X\textsuperscript{−}_{AG} (Christensen, 1990). This is a saturable process subject to inhibition and influenced by sodium concentrations in the intestinal mucosa. Reichl (1989) found that lowering sodium concentrations reduced amino acid influx at any concentration; however, the maximal rate of amino acid influx (V\textsubscript{max}) can still be achieved by increasing extracellular amino acid concentrations to a point higher than that required when sodium is present. While sodium is necessary for transport of acidic amino acids such as aspartate and glutamate, the cation associated with these anionic amino acids in plasma or specific tissues is not known.

Erecinska et al. (1986) studied the transport of aspartic acid in neuronal cell cultures and reported that the movement of aspartic acid across the membrane occurred in association with two sodium ions/molecule of aspartic acid. This reinforces the role of the sodium-dependent active transport mechanism for transport of aspartate across biological membranes including the blood–brain barrier.

Aspartic acid may be absorbed as the free amino acid or as a constituent of a peptide; after a protein meal, it is primarily as the latter (Stegink, 1984). Aspartic acid, as the free amino acid, is absorbed via an active transport system whereas, the aspartic acid-containing peptides are directly absorbed and subsequently must be hydrolyzed by specific intracellular peptidases. A protein–bound aspartic acid load of 40 to 46 mg/kg body weight caused increased plasma aspartic acid concentrations (5.3 to 8.1 μmol/L over baseline) after 3 hours in humans (Stegink, 1984). Absorption of free aspartic acid has been found to be slower than other amino acids in humans (Adibi et al., 1967). Silk et al. (1973) reported that aspartic acid from peptides is absorbed more rapidly than the free form.

Metabolism: The fate of absorbed aspartic acid has been assumed to be related to its transamination to oxaloacetate. The amount of ingested aspartic acid or glutamate appearing in portal blood as alanine, glutamate, or aspartate is a function of the amount given and the availability of glucose as a
pyruvate precursor (Stegink, 1976). Neame and Wiseman (1957) reported that alanine was the major amino acid product of aspartic acid absorption and gut metabolism. This finding was supported by the work of Parsons and Volman-Mitchell (1974) who estimated that 85 percent of the intestinal metabolism of aspartate is through the transamination pathway and the eventual evolution of CO₂.

Another possible fate for ingested aspartic acid is related to the rapid interconversion of aspartate and glutamate. Blood concentrations of both aspartate and glutamate are significantly elevated following administration of a bolus of aspartic acid (Finkelstein et al., 1983).

Several other factors can influence the amount of aspartic acid that enters the circulation. It has been suggested that the composition of the meal, specifically carbohydrate content, can influence the mucosal intracellular metabolism of aspartic acid and subsequent release into the portal circulation through several possible mechanisms as outlined by Stegink (1984). The basis for these suggestions was a series of studies of glutamate absorption. Daabees et al. (1985a) found that, in contrast to glutamate, metabolizable carbohydrates had no significant effect on plasma aspartic acid concentrations.

Aspartic acid and the brain: Aspartic acid is ubiquitous in mammalian brain and is characterized, along with glutamate, by a strong excitatory effect (Palkovits et al., 1986). Despite the existence of a high-affinity selective transport system for the dicarboxylic amino acids, there had been a reluctance to definitively classify aspartate as a neurotransmitter for several reasons including the redundancy of response throughout the mammalian brain and a lack of a specific receptor antagonist (Bloom, 1990). The current presumption is that the dicarboxylic amino acids act as transmitters for fast synaptic excitation at various sites throughout the brain (Bloom, 1990).

Three receptor subtypes for excitatory amino acids have recently been recognized. The kainate, quisqualate, and NMDA receptors are named for their respective agonists. The NMDA receptor has been used extensively to study the role of glutamate in the nervous system (Willetts et al., 1990). Most of the receptor work using agonists such as NMDA involves the assumption that these receptors represent glutamate activity in the CNS and that this activity is synonymous with aspartate activity. Recent research has established that the majority of the effects of L-aspartate in the nervous system are mediated through NMDA receptors (Foster and Fagg, 1987).

There may be developmental differences in the transport and concentration of aspartate in the brain. Lefauconnier and Trouv (1983) noted that human studies have shown that whereas most amino acids are found at higher concentrations in immature than in mature brains, aspartate, glutamate, glutamine and GABA concentrations are lower. Seta et al. (1972) noted that the increases in brain content of amino acids in newborn mice as compared with adult mice were not the same for all amino acids after intraperitoneal injection. Specifically, in contrast to other amino acids studied, aspartate and glutamate injections resulted in little change in brain concentrations of the newborn animals. This finding is supported conceptually by Christensen (1990) who reviewed studies attesting to the lower efficiency of acidic amino acid transport across the blood-brain barrier in comparison to LNAA. While the exact functional significance of these findings is not known, it appears that aspartate and glutamate concentrations may be selectively restricted in the developing brain.

b. Animal studies

Changes in food intake and body weight: Schainker and Olney (1974) administered 4 injections (24 hours apart) of approximately 2 g/kg body weight L-aspartic acid subcutaneously to neonatal mice (24 hours postpartum) and compared them with mice given 4 injections of 850 mg/kg body weight and 6 injections of 2.4 g/kg body weight cysteic acid and DL-α-amino adipic acid (a
structural analogue lacking neuroexcitatory activity), respectively. Only animals that survived the entire seven-month experimental period (percentage not given) were included in the final analyses. Animals given L-cysteic acid and L-aspartic acid, but not DL-α-amino adipic acid, had hypothalamic lesions and consequent obesity, skeletal stunting, and reduced reproductive organ size. Neither blood nor brain concentrations of any of the challenge substances were reported. The control groups were sham-injected mice treated with sodium chloride.

The study by Schainker and Olney (1974) was replicated by Pizzi et al. (1978). In the latter study, mice were given gradually increasing daily doses of monosodium L-aspartate (MSA; 2.2 to 4.4 g/kg body weight) from 2 to 11 days postpartum and compared with saline-injected controls. Animals were followed for 150 days for growth and reproductive behavior studies and then sacrificed between 220 and 300 days of age. The MSA-treated animals were heavier and had stunted linear (bone) growth compared with controls. Reproductive dysfunction occurred in both males and females in the MSA group. Females had reduced litter size and fewer pregnancies; males had reduced fertility. As in the Schainker and Olney (1974) study, reproductive and endocrine organ weights were reduced. No neurochemical or blood measures were reported in this study.

As a result of the earlier studies demonstrating the characteristic changes in body morphology (Pizzi et al., 1978; Schainker and Olney, 1974), the L-aspartate–injected rat has been used as a model for obesity (Burbach et al., 1985). In the study by Burbach et al. (1985), neonatal rats injected subcutaneously with gradually increasing doses of MSA from days 2 to 11 postpartum were found to have significant differences in muscle size and histology. The MSA–treated animals had significant reductions in muscle mass (diaphragm, gastrocnemius, and soleus muscles) and fiber type when compared with saline-injected controls. No concentrations of aspartate or any other blood constituents were reported.

No studies were found that examined the impact of voluntary ingestion of L-aspartate alone. Olney et al. (1980) presented solutions of L-glutamate alone, L-glutamate plus L-aspartate, or L-glutamate plus L-aspartate plus aspartame to 21-day-old mice and found hypothalamic damage upon histological examination in all groups. As with most of the toxicological studies done, there were no other measures of functional changes besides the morphological anomalies found upon histological examination.

As mentioned previously, several factors may affect the absorption of aspartate. Paramount among these is the availability of glucose or gluconeogenic substrates. Paradoxically, Finkelstein et al. (1988) found that, in contrast to mice given 750 mg/kg body weight of L-aspartate without carbohydrate by gavage, mice given the same dose of L-aspartate with carbohydrate (Polycose®, 1 g/kg body weight) had no detectable histological changes in the arcuate nucleus of the hypothalamus (the only area examined). At 1000 mg/kg body weight of aspartate, significantly fewer lesions were found in mice given carbohydrate compared with those given the straight challenge (30 necrotic lesions/section versus 81 necrotic lesions, respectively). Similar reductions were noted in animals treated with subcutaneous injections of insulin four hours before gavage. No similar reduction in lesions was observed in animals given insulin followed by carbohydrate. The control group administered isotonic saline showed no evidence of histological changes.

In a parallel group of animals given the same treatments, Finkelstein et al. (1988) found no significant effect of insulin on mean peak plasma aspartate concentration after insulin pretreatment. Simultaneous administration of carbohydrate significantly reduced the mean peak aspartate concentration but had no significant effect on the plasma time concentration curve (area under the concentration curve over time, AUC). Likewise, there was no significant impact of insulin on the plasma aspartate AUC. These results support the findings of Daabees et al. (1985a) who found that, in contrast to glutamate, metabolizable carbohydrates had no significant effect on plasma aspartate concentrations.
The mechanism of aspartate–induced obesity in rodents is assumed to be mediated through lesions in the hypothalamus. The minimum single oral or intraperitoneally injected doses of potassium aspartate causing hypothalamic lesions in young rats were approximately 0.5 g/kg and 0.25 g/kg body weight, respectively (Okaniwa et al., 1979).

The blood–brain barrier and blood–CSF barrier exist in the choroid plexus and in essentially all areas of the brain parenchyma except the hypothalamus, where substances diffuse with ease into the tissue spaces. The necessity for passive diffusion is related to the role of the hypothalamus in feedback regulation of various physiologically important changes in body fluids, such as changes in osmolality and glucose concentrations (Guyton, 1986). In most of the studies of brain lesions associated with aspartate, the most vulnerable areas are the arcuate and periventricular nuclei in the hypothalamus, both of which have been shown to be involved in appetite regulation. Okaniwa et al. (1979) observed a significant age–dependent variation in the impact of aspartate challenge; the younger the animals the more susceptible to hypothalamic damage.

Agrawal et al. (1991) reported stunted growth, obesity, and reduced liver, kidney, adrenal, and pituitary size in newborn rats injected subcutaneously with 2 or 4 g/kg body weight MSA in a schedule similar to Pizzi et al. (1978). The effects were dose–dependent; the 4 g/kg dose produced more pronounced obesity and growth retardation than the lower dose of MSA. In addition to growth and obesity effects, Agrawal et al. (1991) reported that rats of both sexes given 4g/kg had no detectable plasma growth hormone in blood samples taken every 15 minutes over an 8–hour period. The mean concentration of circulating growth hormone of animals given 2 g/kg body weight MSA was reduced 70 to 90 percent compared with control rats. Hepatic cytochrome P450 activity was reduced in male but not female rats given 4 g/kg body weight of MSA. This enzyme system is normally induced to a greater extent in males than in females by growth hormone secretion patterns. The activity of these enzymes was not altered in the animals receiving 2 g/kg body weight of MSA.

None of the above studies included data on food intake of the animals receiving MSA. At this time, no studies measuring the impact of aspartic acid supplementation on actual food intake in rodents have been found. Moreover, there have been no studies that have evaluated the impact of acute or chronic aspartic acid challenge on appetite or growth in nonhuman primates. A long–term effect of chronic aspartate challenge has been demonstrated in rodents in which animals develop characteristic stunting, obesity, and anomalies in reproductive organ function and morphology. These effects have not been explored in nonhuman primates.

**Behavioral studies:** Although it is likely that aspartate is a ubiquitous excitatory neurotransmitter, few efforts, aside from those mentioned above, have been made to examine any functional consequences that might accompany a large challenge. In addition to the impact of L–aspartate on the hypothalamus, Wirtshafter et al. (1989) discussed a role for this amino acid in the raphe nucleus, a serotonin–rich area of the brain associated with arousal; however, limited information is available regarding the impact of elevated aspartate concentrations on the function of this area of the brain.

Pizzi et al. (1978), as part of the larger protocol discussed previously, performed behavioral testing. This included an open field test to measure activity and a test to measure exploratory behavior between 190 and 195 days postpartum in mice which had been injected with increasing doses of MSA (2.2 to 4.4 g/kg body weight daily) from 2 to 11 days postpartum. These behavioral paradigms demonstrated significant reductions in both activity and exploratory behaviors in the MSA–treated mice compared with the sham controls. An examination of the role of MSA in the raphe nucleus might advance the understanding of this effect. To date, there have been no published attempts to replicate these findings in rodents or nonhuman primates.

**Biochemical studies:** Itoh et al. (1979), in their study of the effect of intraperitoneal, intravenous, and oral routes of L–aspartate administration, measured changes in plasma concentrations of other amino acids in response to aspartate. As with the other results discussed above, the observed
changes were dependent on route of administration and age of the animals. Plasma alanine and proline concentrations increased significantly above pretreatment concentrations in response to oral dosage and alanine concentrations decreased with intraperitoneal and intravenous administration of aspartate. Both glutamate and glutamine concentrations increased regardless of route of administration; however, glutamate concentrations increased significantly over pretreatment in oral and intraperitoneal (18 \mu mol pretreatment compared with 106 \mu mol oral and 112 \mu mol intraperitoneal) but showed a much more attenuated response to intravenous aspartate challenge (31 \mu mol). The data presented represented the mean levels for the 35-day-old rats.

Various methods have been employed for administering challenges of aspartic acid. These include intraperitoneal injection, oral gavage, and intravenous injection. Based upon doses established by Okaniwa et al. (1979), Itoh et al. (1979) gave 7-, 21-, and 35-day-old rats doses of potassium-L-aspartate matching the maximum safe dose (that reported to produce no lesions) and the dose reported to produce lesions in 50 percent of the animals. The protocol consisted of two doses (maximum safe dose and lesion dose_{50}) for each route of administration (intraperitoneal and oral) for each age group. The dose for the intravenously infused group was the amount that induced the lesion in all the intraperitoneally treated animals.

These authors found that changes in plasma aspartate concentrations were affected by route of administration, and the trends were similar within age groups. The most significant increases were observed in animals given intraperitoneal challenges; peak plasma concentrations of aspartate were significantly higher than either of the other routes and the higher dose took longer to return to baseline levels. While the intravenous injections resulted in a rapid elevation of plasma aspartate concentrations, the peak was significantly lower than the peak following intraperitoneal injection and, despite the continued infusion of the intravenous challenge over a six-hour period, there was a lowering and leveling off of plasma aspartate concentrations. The oral dose was considerably higher than either intraperitoneal or intravenous challenges (e.g., 1.9 and 3.8 g/kg body weight oral, 0.5 and 1.0 g/kg intraperitoneal, and 1.3 g/kg intravenous in the 35-day-old group). However, the peak aspartate concentrations after the oral dose were significantly lower than after intraperitoneal administration. No histological evidence of hypothalamic neuronal damage was found in 21- or 35-day-old animals given L-aspartic acid intravenously.

Tanaka et al. (1983) compared the effects of three amino acid preparations given to newborn mice and kittens. The mice were given single intraperitoneal injections containing 18 dispensable plus indispensable amino acids, indispensable amino acids only, or dispensable amino acids plus aspartate and glutamate. Doses given to mice were 119, 238, 357, and 476 \mu g aspartic acid/g body weight and 36, 72, 108, and 144 \mu g L-glutamate/g body weight. Kittens were challenged with either constant infusion into the femoral vein or a single bolus given orally. No damage to the hypothalamus was found in mice given either the dispensable amino acids or saline control. All mice given 238, 357, and 476 \mu g/g challenges had damage upon histological examination. Kittens given dispensable plus indispensable amino acids had damage at 119, 238, and 357 \mu g/g. Plasma concentrations of all amino acids peaked within five minutes of infusion and increased linearly and significantly over control concentrations with increasing doses. These results, while confounded by the presence of the other amino acids, demonstrated neuronal damage at concentrations below the minimum concentrations of aspartic acid reported by Okaniwa et al. (1979). The hypothalamic changes reported by Tanaka et al. (1983) occurred at doses below those used by Daabees et al. (1985b) who documented neuronal damage when aspartate and glutamate were given together at doses of 250 mg/kg body weight of each amino acid.

**Vitamin B6 studies:** The relationship between vitamin B6 and the AST enzyme system has long been recognized. PLP is the coenzyme required for optimal activity of this enzyme; however, it is not clear whether the cofactor changes this activity through increased enzyme synthesis or decreased degradation. Sharma and Gehring (1987) documented differences in the effect of vitamin B6 on AST that were dependent on the location within the cell (cytosolic isozymes were more responsive than mitochondrial). These findings were supported in effect by Crouch and Solomon.
(1989) who found that B6 aldehyde vitaminers (PLP and pyridoxal) reversed the inhibition of cytosolic AST by acetaldehyde in rat liver.

Shibuya et al. (1982) studied the effects of vitamin B6 deficiency on the activities of AST in different rat tissues. These investigators found abnormally low activities of the enzymes in certain tissues (liver, heart, brain, and muscle) but not in others (kidney, intestine, spleen, and lung) when compared with vitamin B6–adequate control animals. These data suggest differences in susceptibility to vitamin B6 regulation and activity of this metabolically important enzyme. The functional ramifications of these findings remain to be determined.

The relevance of these findings to aspartic acid safety is not clear at this time; however, it is possible that excess aspartic acid fed to a vitamin B6–deficient subject might result in elevated plasma aspartate levels. If this were to occur in a susceptible individual, e.g., infant or fetus, it could expose a vulnerable nervous system to potentially harmful levels of aspartic acid. In light of the existence of population segments who are at higher risk for vitamin B6 deficiency (Life Sciences Research Office, 1989), this relationship warrants further investigation.

Gross pathology: In addition to the studies of hypothalamic damage, other types of morphological changes have been associated with L-aspartic acid (Pipalová and Pospíšil, 1980). Weanling male mice (24 days old) were given challenges that were incorporated into food pellets at levels of about 5 mg/g food (0.5 percent of diet). The average daily consumption of L-aspartate was about 10 to 25 mg per mouse. The diets were fed for 14 to 56 days with sacrifices at 14-day intervals.

Pipalová and Pospíšil (1980) reported that animals receiving either potassium or magnesium salts of L-aspartic acid had significantly greater thymus weights than matched (age and body weight) controls. The L-aspartic acid treatments had no effect on body weight or spleen size. The functional significance of these findings is unknown at this time.

Hugon et al. (1987) investigated the impact of L-aspartate and L-glutamate injection on function and morphology of sciatic nerves in rats. The exposed right sciatic nerve of anesthetized male and female rats (n = 24, body weight range 250 to 350 g) was injected with monosodium salts of either L-glutamate or L-aspartate (50 to 100 μmol; 6.6 to 13.3 mg); the left sciatic nerve was injected with sodium chloride. Animals were sacrificed 24 hours to 1 month post injection. Both right and left sciatic nerves were removed for histological examination. Changes in motor or muscle function were observed throughout the experimental period. A characteristic ipsilateral hind limb muscle spasm and contraction was noted in the right limbs of animals within 5 to 10 minutes of either aspartate or glutamate injection. In the days following challenge, clinical observation revealed "a moderate motor deficit of the right hind limb accompanied by a progressive muscle atrophy."

Postmortem lesions in rats sacrificed within 24 hours were described as acute axonal lesions characterized by axonal destruction with disruption of the myelin sheath. Long-term changes included endoneural edema with generalized axonal loss of myelinated fibers. L-aspartate and L-glutamate had similar effects. No changes were seen in the left or control limbs for any of the parameters studied.

c. Human studies

Few studies have specifically examined the safety of aspartic acid supplementation in humans. Carlson et al. (1989) studied the effects of a 10 g oral bolus of L-aspartic acid (in gel capsules given with 500 ml saline) on endocrine function in healthy male and female adults and found no changes in serum prolactin, growth hormone, or cortisol concentrations. Serum concentrations of aspartate increased only slightly after the load (40.0 μmol/L over baseline concentrations at 90 minutes). No side effects were reported. There was no analysis for possible sex differences.
There have been several clusters of investigations of the use of aspartic acid for the treatment of specific conditions. While discussions of efficacy of amino acid treatments are not within the Scope of Work for this report, studies of aspartic acid treatments may provide some useful data about safety.

Based on the hypothesis that opium addiction and the abstinence syndrome that develops upon removal is related to the role of asparaginase and asparagine synthetase in the addiction process, aspartic acid has been proposed as a treatment for abstinence symptoms of opiate withdrawal. As espoused by Koyuncuoglu (1983), the therapeutic effect of aspartic acid is due to its ability to reestablish an equilibrium between these two enzymes. The proposed mechanism is through aspartate inhibition of L-asparaginase followed first by the increased activity of asparagin synthetase to accommodate the increased aspartate concentrations and its eventual inhibition by the increase in asparagine concentrations. Because this reestablishment of metabolic equilibrium is a gradual process, it is less threatening than the acute effects of rapid opiate withdrawal.

The use of L-aspartic acid for opiate withdrawal has been studied by Koyuncuoglu (1983) who gave 8 g MSA/day (divided doses of 2 g/dose, given 4 times daily) for 4 to 5 days. There were no side effects reported, nor were there any blood concentrations of aspartate recorded. Similarly, Sener et al. (1986) performed an open trial in which they gave 8 g MSA/day to addicts. There were no side effects that could be distinguished from withdrawal symptoms and no blood concentrations of aspartate or any other metabolite were assessed. It is important to note that in all of the studies of L-aspartic acid use in opiate addiction, the parameters measured (e.g., changes in appetite, diarrhea, mental state, etc.) could potentially be associated with aspartic acid or any other treatment; however, the condition of the patients and the nature of the disease makes any direct association between aspartic acid and the symptomatology difficult.

L-aspartic acid has also been used as a potential ergogenic aid in exercise physiology experiments. Ahlborg et al. (1968) reported significant increases in the time to exhaustion from continuous work on a bicycle ergometer in 6 male subjects given 7g/day potassium–magnesium L-aspartate (KMA) during the 24 hours preceding exercise. The results were compared with baseline and with performance after a placebo. No side effects were noted, and blood concentrations of aspartic acid or its metabolites were not measured. Moreover, there was no consideration of the potential for independent effects of either magnesium, potassium, or the combination of the two. The dosage was equivalent to about 102 mg/kg of body weight KMA in subjects whose mean body weight was 68.6 kg. In a similar study, Sen Gupta and Srivastava (1973) also found a significant increase (22.6 percent over placebo) in duration of endurance effort on a bicycle ergometer. Dosage of KMA was the same as that used by Ahlborg et al. (1968). Again, no plasma constituents were measured and no side effects were reported.

A series of studies attempted to replicate these early positive findings. Only one attempted to reproduce the experimental conditions of the Ahlborg et al. (1968) study. Maugham and Sadler (1983) gave approximately 6 g/day KMA (about 75 mg/kg body weight) to 8 fasting subjects. These authors found no difference in endurance performance on a bicycle ergometer or in any biochemical parameter measured (i.e., free fatty acids, glucose, or lactose). There were no measures of aspartate levels. No side effects were reported. Other studies performed in this area have used KMA doses ranging from 2 g/day (Fallis et al., 1963) to 8.6 g/day (de Haan et al., 1985). No adverse effects were reported in any of these studies. However, it must be emphasized that these were not studies of safety but efficacy.

Aspartame studies: Data from studies on the artificial sweetener aspartame (L-aspartyl-L-phenylalanine methyl ester) represent a potential source of information on the safety of aspartic acid. A number of concerns about the safety of this compound have been reported, most of which are related to the phenylalanine moiety (see p. 78–82). Because aspartic acid makes up 40 percent by weight of aspartame, Olney (1984) has expressed concern about the possibility of adverse effects of elevated aspartate concentrations consequent to high intakes of aspartame.
Maher (1986) has addressed concerns about aspartic acid derived from dietary aspartame. In noting the report by Stegink (1984) who found no significant increases in plasma concentrations of aspartate in humans given 34 mg/kg body weight of aspartame (13.6 mg/kg body weight of L-aspartic acid), Maher (1986) concluded that there is insufficient evidence to support a toxicological effect of aspartame-derived aspartic acid.

In addition to questions about possible neurotoxic effects of elevated plasma aspartic acid concentrations resulting from aspartame ingestion, another area of uncertainty is the concern that aspartame has a paradoxical effect on weight control by increasing appetite (Blundell et al., 1988). Chen and Farham (1991) recently reported evidence to support the contention that aspartame sweetness does not reduce the intake of sugar; however, they did not find a decrease in appetite (food intake). Ryan-Harshman et al. (1987) similarly found no evidence to support any hypothesized changes in either energy or macronutrient intake consequent to aspartame intake. It should be noted that the focus of the latter study by Ryan-Harshman et al. (1987) was on the contribution of phenylalanine not aspartic acid to appetite changes.

d. Summary and conclusions

Endpoints: Aspartic acid has been found to cause characteristic lesions in areas of the brain not protected by a blood–brain barrier in young susceptible animals. Neither adult animals of the same species (i.e., rats, mice) nor nonhuman primates have demonstrated similar lesions upon acute exposure to aspartic acid. There have been no studies of the impact of chronic exposure to aspartic acid in nonhuman primates.

Aside from assessment of histological changes, few studies have reported potential long-term functional changes resulting from acute exposure to L-aspartic acid. Several studies have demonstrated endocrinological changes in aspartic acid–treated rodents. The aspartic acid–treated rat has been described as having a stunted growth pattern and a characteristic pattern of obesity. This treatment has been used as a model for obesity. Behavioral changes (decreased exploratory behavior and activity) have also been reported in aspartic acid–treated rats.

None of the studies reviewed included data on food intake of the animals receiving aspartic acid. At this time, no studies measuring the impact of aspartic acid supplementation on actual food intake in animals have been found. Moreover, no studies have evaluated the impact of acute or chronic aspartic acid challenge on appetite or growth in nonhuman primates. A long-term effect of systemic injections of aspartic acid (2.2 to 4.4 g/kg body weight of L-aspartic acid subcutaneously) to young animals has been demonstrated in rodents in which animals develop characteristic stunting, obesity, and anomalies in reproductive organ function and morphology. These effects have not been explored in nonhuman primates.

Doses of up to 8.6 g/day of the sodium salt of L-aspartic acid have been given to humans with no reported side effects. All of the human studies reviewed involved acute dosing protocols. There have been no long-term studies of the impact of either acute or chronic aspartic acid supplementation. Likewise, there have been no studies designed to look at any other functional changes such as appetite changes, endocrinological changes, or changes in behavior or cognition associated with aspartic acid supplementation.

Effects of ingestion of the artificial sweetener, aspartame, have been studied extensively. The available evidence suggests that in doses generally consumed as a sweetener, the aspartic acid moiety of aspartame is rapidly and readily absorbed and metabolized. Consequently, consumption of aspartame as a sweetener is unlikely to contribute to elevations in plasma aspartic acid concentrations that might be associated with any untoward effects.
Safe levels of human intake: Within the context of the studies reviewed, the use of aspartic acid as a supplement in doses up to 8.6 g/day has not been associated with documented detrimental effects in adult humans. For perspective, a person consuming 100 g protein per day would consume about 3.2 g aspartic acid. However, the potential for adverse effects in fetal or young developing animals or humans remains an open question. Until maximal safe levels of aspartic acid intake in fetal or young developing humans have been determined, the exposure of infants, children, adolescents, and pregnant women to supplemental aspartic acid should be discouraged.
4. Cysteine and cystine

a. Background

As detailed in the section on methionine, L-cysteine is categorized as a nutritionally dispensable amino acid, being formed from L-methionine and L-serine. However, it has been proposed that cysteine is an indispensable amino acid in the young infant (Pohlandt, 1974; Snyderman, 1971; Zlotkin and Anderson, 1982). L-Cysteine occurs in proteins as cysteine and in its oxidized form, cystine, in which the thiol groups of two molecules of cysteine have been oxidized to a disulfide group to form a covalent cross-linkage between them (Lehninger, 1975). The amino acid is reversibly interconverted between its oxidized and reduced forms. Both L-cysteine and L-cystine have a sparing effect on methionine as measured by weight gain in young rats (Friedman, 1991). Perfusion of normal adults and patients homozygous for cystinuria with L-cysteine and L-cystine suggests that the two forms are absorbed by separate transport mechanisms and that L-cysteine is absorbed more rapidly than L-cystine (Silk et al., 1974).

Cysteine can be metabolized to form taurine and CO$_2$ or sulfate, urea, and CO$_2$. The cysteinesulfinate pathway appears to be the major route of taurine formation in mammals, but other pathways represent possible alternative routes of taurine formation. Desulfuration pathways appear to be the primary cysteinesulfinate-independent routes of metabolism of cysteine via production of pyruvate and inorganic sulfur (Stipanuk, 1986).

The initial step in the cysteinesulfinate pathway, oxidation of cysteine to cysteinesulfinate, is catalyzed by cysteine dioxygenase. Cysteinesulfinate may then be decarboxylated to form taurine or, alternatively, it may be metabolized via the putative intermediate β-sulfinylpyruvate to pyruvate and sulfite and then to CO$_2$ and sulfate. In vitro studies on desulfuration pathways were described by Stipanuk (1986). These studies suggest that high cysteine concentrations may favor cysteine catabolism by desulfuration pathways; however, the roles of desulfuration pathways in intact animals remain to be evaluated.

Like tryptophan, a considerable portion of plasma cysteine (about 50 percent) is bound to plasma proteins (Malloy et al., 1981a,b). This binding is via the sulphydryl group and can be modified with reducing agents; it may regulate the structure of proteins such as immunoglobulins that are dependent upon disulfide bridges.

In discussions of cysteine metabolism, total cysteine is sometimes referred as cyst(e)ine, representing the sum of all forms of the compound. L-cystine is much less soluble than L-cysteine but is more stable. Studies must be interpreted with care as some investigators have used the disulfide and some the thiol form of cyst(e)ine, and the effects of the two compounds may be different in some respects. For example, cysteine may have some endogenous antioxidant activity.

L-Cysteine is a component of the endogenous antioxidant glutathione, a tripeptide consisting of glutamic acid, cysteine, and glycine. The sulphydryl group of cysteine is the active site of the molecule. Glutathione concentration in liver is ordinarily 4 to 10 times higher than concentrations of other hepatic coenzymes (Tateishi et al., 1974). Feeding of L-cysteine-supplemented diets following starvation has been shown to increase the hepatic concentration of glutathione in rats in a dose-related manner, and glutathione has been suggested as a possible reservoir for excess L-cysteine (Tateishi et al., 1977, 1981). Functions and metabolism of glutathione were reviewed by Meister and Anderson (1983).

D-Cysteine and D-cystine do not have a sparing effect on methionine in terms of supporting growth in young rats (Friedman, 1991; Friedman and Gumbmann, 1984). D-Cysteine is not metabolized to taurine (Cavallini et al., 1958; Ewetz et al., 1966; Krijgsheld et al., 1981).
b. Animal studies

Changes in food intake and body weight: Many investigators have reported reduced weight gain and food intake in rats fed low-protein diets supplemented with 0.5 to 10 percent L-cystine (Benevenga et al., 1968; Curtis and Newburgh, 1927; Daniel and Waisman, 1963; Earle and Victor, 1941; Graham et al., 1950; Sauberlich, 1961; Sullivan et al., 1982). As summarized by Harper et al. (1970), a high mortality rate which increased with increasing levels of the amino acid, was observed in many of these studies. Neither cysteic acid or taurine appear to be mediators of this aspect of cystine toxicity (Earle et al., 1942).

Muramatsu et al. (1971) reported that consumption of a 10 percent casein diet supplemented with 5 percent L-cystine resulted in reduced food intake and a 50 percent reduction in weight gain over a 3-week period in young male rats. Liver weight was not different from controls in the L-cystine supplemented rats, but protein and RNA concentrations were significantly decreased. All rats survived the experimental period.

Biochemical studies: Gastric intubation of a single dose of L-cysteine (8 mmol/kg body weight or 184 mg for a 200-g rat) in fasting rats resulted in an immediate increase in serum cystine from 5 μmol/L to 200 μmol/L with a subsequent doubling in serum inorganic sulfate and a significant increase in serum taurine which remained elevated for 24 hours (Krijgsheild et al., 1981). Over the 24-hour period following administration, urinary excretion of inorganic sulfate, increased 2.5-fold, accounting for 33 percent of the administered dose. Urinary taurine excretion was also increased, but cysteic acid was not detected. In contrast, feeding of the same amount of L-cysteine in the diet (dietary concentration 4.8 percent) produced a similar increase in the urinary excretion of inorganic sulfate, but the serum concentration of sulfate did not increase after 2 or 4 days of feeding. Urinary sulfate excretion accounted for about 30 percent of the administered dose of L-cysteine, whether given by gastric intubation or fed in the diet.

The cysteinesulfinate pathway appears to play a major role in the regulation of the metabolism of excess L-cysteine in rats. Feeding of diets supplemented with 2.6 percent L-cysteine to rats for 5 or 20 days was found to increase the activity of hepatic cysteine dioxygenase significantly compared with control animals, decrease the activity of cysteinesulfinate decarboxylase, and not affect the activities of cysteinesulfinate aminotransferase or cysteine desulhydratase significantly. These changes in enzyme activities suggest that excess cysteine may be catabolized in the liver by the cysteinesulfinate pathway. About 7 percent of the administered dose (a 9-fold increase) of labeled taurine was excreted in urine by rats fed the 2.6 percent L-cysteine diet as by pair-fed control rats following administration of a 5-g bolus of the diet labeled with L-[35S]cysteine. Inorganic sulfate excretion, which accounted for about 17 percent of the administered dose, was also increased but not to a significant extent (Daniels and Stipanuk, 1982).

In contrast to dietary supplements of other amino acids which either lowered or had no effect on plasma cholesterol concentrations, L-cystine was shown to increase plasma total cholesterol in low-cholesterol diets and to decrease plasma total cholesterol in a cholesterol-supplemented diet (Rukaj and Sérougne, 1983; Sérougne and Rukaj, 1983; Sérougne et al., 1987). Addition of 5 percent L-cystine to diets containing 0.05 percent cholesterol, 9 percent lard or tristearin, and 23 percent casein produced significant increases in plasma total cholesterol in male Wistar rats fed the diet for 2 months compared with unsupplemented animals. Chylomicron and VLDL cholesterol contents were reduced significantly, but cholesterol concentration in all other lipoprotein fractions was increased in the L-cystine-supplemented rats. Hepatic cholesterol synthesis, but not intestinal synthesis, was increased with ingestion of the L-cysteine-supplemented diet. Supplementation of the basal (lard) diet with 1 percent cholesterol also significantly increased plasma total cholesterol, but addition of 5 percent L-cystine to the cholesterol-supplemented diet reduced plasma total cholesterol to a level similar to that of the control group. Examination of plasma and liver cholesterol and the dynamics of cholesterol absorption, synthesis, excretion, and transformation into bile acids suggested that cholesterol supplementation reduced hepatic cholesterol synthesis in the L-cystine-supplemented group.
Baker and Czarnecki-Maulden (1987) reviewed the results of studies showing that diets supplemented with L-cysteine (about 0.4 to 1 percent) reduced cobalt, copper, arsenic, and selenium toxicities in chicks, rats, or pigs given excess amounts of these minerals. However, the toxicity of organic pentavalent arsenic was worsened by administration of L-cysteine. The results of these studies raise questions about chelation of dietary minerals by large doses of L-cysteine, possibly resulting in decreased absorption of essential minerals.

Behavioral studies: A high dose of L-cysteine (7.4 mmol/kg body weight or 313 mg for a 350-g rat) resulted in somnolent behavior and breathing difficulties for 4 to 8 hours after intraperitoneal injection. Convulsions did not occur. One of the 15 rats in this group died (Sprince et al., 1969).

Endocrine studies: Intravenous infusion of L-cysteine (1 mmol/kg body weight or 1.2 g for a 10-kg dog) over 15 minutes resulted in a significant increase in plasma glucagon and smaller increases in plasma insulin and glucose in 4 fasting dogs (Rocha et al., 1972). Infusion of this dose of L-cysteine also resulted in vomiting in all dogs.

Vitamin B6 studies: L-Cysteine is metabolized by PLP-dependent enzymes including cysteine desulphydrase, cysteinesulfinate decarboxylase, and cysteinesulfinate aminotransferase. Metabolism of L-cysteine to taurine was decreased and metabolism to pyruvate was increased in vitamin B6-deficient rats following a single load of L-cysteine (1 g/kg body weight or 150 mg for a 150-g rat) (Yamaguchi et al., 1975).

Functional assessments and gross pathology: Histopathologic changes in kidney, liver, brain, and retina of rats administered L-cysteine or L-cystine orally or subcutaneously have been described by many investigators. Curtis et al. (1927) reported pyknotic nuclei and tubular necrosis in kidneys of rats fed 8 percent casein diets supplemented with 2.5 to 20 percent cystine. Survival time was inversely related to cystine supplementation and ranged from less than 1 week to 10 months. Urinary excretion of casts, albumin, and blood occurred more rapidly as the level of added cystine increased. Although increasing the protein content of the diet has been shown to lessen the effects of excess levels of other amino acids, pathological effects in kidney also occurred when a high protein (18 percent casein) was supplemented with a low level of cystine (0.5 percent).

Progressively greater hemorrhagic necrosis in rat liver was also reported with increasing levels of cystine by Curtis and Newburgh (1927). Investigations of Griffith and Wade (1940) confirmed the incidence of enlarged hemorrhagic kidneys with addition of 0.3 or 0.5 percent cystine and described the occurrence of fatty liver, enlarged spleen, and small thymus as well. In contrast, Klavins (1963) found only mild kidney lesions (dilation of the convoluted tubules) with feeding of a diet containing 6 percent L-cystine for 4 weeks but found extensive pathological changes in liver. Earle and Victor (1941) also reported that feeding of diets containing 10 percent cystine and 5 percent casein resulted in hemorrhage and hepatic necrosis in about 4 days, with development of cirrhosis in rats surviving more than 2 weeks. With 5 percent cystine, liver lesions developed less rapidly, but portal hemorrhagic necrosis and fatty infiltration was found as early as 1 week.

Increased numbers of necrotic hypothalamic neurons and retinal lesions were found in mice 10 to 12 days of age given a single dose (3 g/kg body weight) of L-cysteine by gastric intubation (Olney and Ho, 1970). Hypothalamic and neuronal damage was similar to that reported with administration of glutamic acid. (See glutamic acid section.) Lower doses of L-cysteine resulted in more widespread damage in brain including cerebral cortex, hippocampus, caudate, and thalamus (Karlsen et al., 1981; Olney et al., 1972a). Misra (1989) reported that a concentration of at least 0.6 μmol/g of free cysteine in brain may be required to cause brain damage in neonatal rats. This concentration was reached in 6-day-old rats subcutaneously injected with 0.8 g/kg body weight L-cysteine; however, in 13-day-old rats given this dose, brain cysteine concentration did not exceed 0.25 μmol/g.
Recent investigations have provided some evidence to suggest that L-cysteine may be an endogenous excitotoxin that can severely damage the immature CNS in the rat, that the activity may be mediated primarily by the NMDA receptor-ionophore complex, and that bicarbonate is required to activate L-cysteine (Olney et al., 1990).

Immediate retinal degeneration, including pyknotic nuclei and swollen cytoplasms in many amacrine cells and about half of the ganglion cells, was found after a single subcutaneous injection of L-cysteine (1.2 mg/g body weight or 20.4 mg for a 17-g rat) in 9- or 10-day-old rats (Karlsen and Pedersen, 1982). Administration of the same dose to 4-day-old rats resulted in initial mortality of about 50 percent and brain atrophy and abnormal pathology in the inner layers of the retina and the optic nerve when the surviving animals were 4 to 6 weeks old (Karlsen et al., 1981; Pedersen and Karlsen, 1980). Two types of brain atrophy were identified. Brain weight was decreased by about 20 percent in type 1, which affected about 80 percent of the animals. Weights of cerebral cortex, hippocampus, and thalamus were reduced 30 to 40 percent, and the posterior section of the cortex showed severe atrophy. More severe type 2 atrophy occurred in about 10 percent of the animals. Weights of the hippocampus and cortex were reduced by 60 and 80 percent, respectively, and pathological changes were described in caudate-putamen, thalamus, pons, medulla oblongata, spinal cord, and cerebellum (Karlsen et al., 1981). Functional ability of the surviving animals was not described.

In the retina, ganglion cells were reduced by at least 50 percent and the thickness of the inner plexiform layer, the ganglion and nerve fiber layers, and the inner nuclear layer was markedly reduced. Areas of transversely sectioned optic nerves were reduced by about 60 percent. No differences in retinal lesions were seen with the two types of brain atrophy. These types of changes also occurred, albeit to a greater extent, in glutamate-injected animals (Pedersen and Karlsen, 1980).

Retinal abnormalities and pathologic changes in liver and spleen have also been described in cats. Neurotoxicity of L-cystine was more severe in adult female cats fed a diet containing 5 percent L-cystine without taurine than with a 5 percent L-cystine and 0.05 percent taurine supplement (Sturman et al., 1989). All 9 cats fed the L-cystine-supplemented diet without taurine exhibited extreme symptoms of nervous system involvement including lethargy, inability to stand, rigidity of the neck and lower limbs, absence, and epileptic seizures. Maximal survival time with this diet was 7 months. Of the cats fed the diet containing both L-cystine and taurine supplements, 4 died after showing only slight lethargy and unsteadiness, and the other 5 were sacrificed after 12 months, showing no outward symptoms. Severe pathologic changes in retinas in both groups including reduced numbers of cells in the inner nuclear and ganglion cell layers, swollen cells with degenerative features in these layers, and abnormalities in neurites and dendrites in the inner plexiform and nerve fiber layers. Ultrastructural changes in photoreceptor cells were observed and seemed to be more frequent and more severe in cats fed the taurine-free diet supplemented with L-cystine.

Livers of both groups showed prominent mottling with extensive hemosiderosis mainly in the hepatic acini but not in the portal areas. Spleens were small. Histology varied from essentially normal to marked atrophy in white pulp, red pulp, and ellipsoids. Lymph nodes showed nonspecific follicular hyperplasia. In both groups activities of LDH, AST, and ALT in serum were increased, and serum albumin concentration was decreased. A decrease in red cell count, hematocrit, and hemoglobin concentration and a significant eosinopenias were reported in both groups (Schuller-Levis et al., 1991; Sturman et al., 1989).
Biochemical studies: Tribble et al. (1989) measured plasma concentrations of free and protein-bound cyst(e)ine and urinary sulfur-containing compounds in 5 healthy subjects following a single oral load of L-cysteine (40.6 mg/kg ideal body weight or 2.8 g for an individual with ideal body weight of 70 kg). Plasma cyst(e)ine concentration (mainly in the form of cystine) increased significantly within 30 minutes and peaked at 1 hour after administration of the load. Plasma free cysteine and cystine remained elevated for about 4 hours. Over the 24-hour period after the bolus was given, about 56 percent of the sulfur from the load was excreted in the urine. In 4 patients with hepatic cirrhosis, the plasma concentration of cyst(e)ine peaked at a much higher level (665 vs 336 μmol/L) and remained elevated for about 8 hours; urinary excretion of sulfur-containing compounds was delayed, but about 50 percent of the sulfur in the load was excreted within 24 hours. No signs of clinical encephalopathy or changes in orientation or motor skills were noted after the L-cysteine load in any of the patients.

An oral load of L-cysteine (5 g L-cysteine monohydrochloride monohydrate supplying 3.4 g L-cysteine) given with breakfast to normal male subjects consuming a self-selected diet resulted in 1.1 to 3 percent of the dose being excreted as taurine and about 10 times that amount being excreted in the form of sulfate. Taurine excretion over the 24 hours following the L-cysteine load was about 1.5 times higher than the amount excreted during the 24 hours preceding the load (Swan et al., 1964). No mention of side effects following the L-cysteine load was included in the report.

Administration of oral loads of L-cysteine hydrochloride (100 g/kg body weight or 7 g for a 70-kg individual) resulted in increased plasma concentration and urinary excretion of cystine and a small increase in plasma concentration of methionine in a control subject, a possible heterozygote, and 2 obligate heterozygotes for homocystinuria due to cystathionine synthase deficiency. Neither homocysteine nor mixed disulfides were detected in plasma (Rassin et al., 1977b).

Behavioral studies: In 4 adults with schizophrenia, administration of L-cysteine (4, 8, 12, or 16 g daily totaling 216 g over a 21-day period) was associated with increased total amino acids in plasma; concentrations of most amino acids varied substantially among individuals. Behavioral deterioration based on clinical evaluation lagged behind the increase in total amino acid levels and was not highly correlated with the elevation in total amino acids (Spaide et al., 1971).

L-Cysteine was also administered to normal nonclinical subjects but only in combination with tranylcypromine (Davis et al., 1972; Narasimhachari et al., 1970). Doses of 5, 10, 15, or 20 g L-cysteine were given daily for 5 days at each dose level together with 10 mg tranylcypromine. In the nonclinical subjects, no psychopathological symptoms such as hallucinations, illusions, delusions, obsessions, or psychotic behavior were observed with the combined treatment. However, side effects such as fatigue, dizziness, nausea, and insomnia occurred. The side effects were milder during the early part of the experimental period when the lower doses were given but became more severe as the L-cysteine doses were increased. With the highest dose of L-cysteine, occasional severe gastric cramps and diarrhea and insomnia occurred in most of the nonclinical subjects. Urinary excretion of tryptamine was increased with the combined treatment. In schizophrenic patients given the same L-cysteine and tranylcypromine treatment, psychotic symptoms were exacerbated and were most severe toward the end and immediately after the combined loading; however, the side effects noted in the nonclinical subjects were much less prominent than in the schizophrenic patients (Davis et al., 1972; Narasimhachari et al., 1970).

Endocrine studies: Oral administration of a single dose of 5 or 10 g cysteine (isomer not specified) given in capsules about 3 hours postprandially resulted in an inconsistent elevation of serum prolactin or cortisol in 4 normal subjects (Carlson et al., 1989). Serum taurine was not increased after cysteine administration. At both dose levels cysteine produced nausea and an ill-defined feeling of light-headedness or dissociation in all subjects, resulting in termination of cysteine administration in this study.
Vitamin B6 studies: The percentage of an oral load of L-cysteine excreted as taurine decreased from prededication values in normal male subjects fed a diet low in vitamin B6 for 31 or 41 days. Before depletion 1.1 to 3 percent and after depletion 0 to 0.8 percent of a 3.4 mg load of L-cysteine was excreted as taurine. The basal amount of taurine excreted in the urine also decreased. Overall, little change was observed in urinary sulfate excretion before or after the load of L-cysteine in vitamin B6-depleted subjects (Swan et al., 1964).

Inborn errors of metabolism: Cystinuria is an inherited disorder characterized by the excessive urinary excretion of cystine, arginine, lysine, ornithine, and homocysteine-cysteine disulfide. The only clinical manifestation of the disease is production of urinary calculi, a consequence of the limited solubility of cystine. Cystinuria results from a malfunction of a specific membrane transport system located in the brush-border membrane of the renal proximal straight tubule and the small intestine, and increased urinary excretion occurs with low or normal plasma levels of cysteine and cystine (Milliner, 1990).

d. Summary and conclusions

Endpoints: Depression of food intake and weight gain and, in some studies, a high mortality rate have been reported in rats fed diets supplemented with 0.5 to 10 percent L-cystine. Activity of hepatic cysteine dioxygenase and urinary excretion of taurine were significantly increased in rats fed a diet supplemented with 2.6 percent L-cysteine. The activity of cysteinesulfinate decarboxylase was significantly decreased, but activities of other cysteine-metabolizing enzymes were not affected by excess dietary L-cysteine. Metabolism of a load of L-cysteine to taurine was decreased in vitamin B6-deficient rats. Plasma cholesterol was significantly increased, apparently by increased hepatic synthesis, in rats fed a low-cholesterol diet supplemented with 5 percent L-cystine; however, plasma cholesterol was decreased in rats fed a high-cholesterol diet supplemented with the same level of the amino acid. Infusion of L-cysteine increased the plasma glucagon concentration in dogs in one study.

Abnormal pathology in kidney, liver, brain, and retina of rats has been reported when animals were fed low-protein diets supplemented with 2.5 to 20 percent cystine. Some evidence suggested that L-cysteine administered by intubation may be an endogenous excitotoxin that may damage the CNS when given to immature rats; however, questions have been raised regarding the use of this model for assessing neurotoxicity. (See glutamic acid section.) Histopathologic changes in brain, liver, and spleen and some signs of neurotoxicity (lethargy and unsteadiness) have been reported in cats fed diets containing 0.5 percent taurine and supplemented with 5 percent L-cystine. Four of the 9 cats given this treatment died during the 1-year trial.

Toxicity resulting from ingestion of excess levels of minerals including cobalt, copper, selenium, or arsenic has been reduced in experimental animals by dietary addition of 0.4 to 1.0 percent L-cysteine. The effects of chelation of usual levels of dietary minerals by large doses of L-cysteine have not been examined.

Administration of single oral loads of about 2.5 to 3.5 g L-cysteine resulted in increased plasma cyst(e)line concentrations and increased urinary excretion of sulfur-containing compounds and taurine in healthy human subjects. Metabolism of the L-cysteine load was much slower in patients with hepatic cirrhosis. Administration of 5 or 10 g loads orally resulted in an inconsistent increase in plasma prolactin and cortisol in healthy subjects but both dose levels produced nausea and vague feelings of light-headedness and dissociation. Urinary excretion of taurine was less following an oral load of L-cysteine in subjects depleted of vitamin B6.

Reports of effects of repeated doses of L-cysteine or L-cystine administered alone to normal subjects were not found. However, fatigue, dizziness, and insomnia were reported in nonclinical
subjects given 5 to 20-g doses of L-cysteine in combination with a monoamine oxidase inhibitor. Side effects were reported to be more severe with the higher doses of L-cysteine. Psychotic symptoms were exacerbated in schizophrenic patients given the same treatment.

Safe levels of human intake: Single oral doses of 5 and 10 g L-cysteine have produced nausea, light-headedness, and dissociation in normal subjects. For comparison, a person consuming 100 g protein daily would consume about 2.2 g cysteine. Repeated doses of 5, 10, 15, or 20 g L-cysteine in combination with a monoamine oxidase inhibitor resulted in fatigue, dizziness, and insomnia; these effects were more severe as the dose of the amino acid increased. Administration of L-cysteine resulted in slower metabolism of the amino acid in patients with hepatic cirrhosis and in behavioral deterioration in patients with schizophrenia.

No information was found on effects of chronic intake of lower doses of L-cysteine or L-cystine in humans. However, the side effects of larger doses given once or several times raises concern about the safety of continued use of lower doses of L-cysteine or L-cystine. The available information is not sufficient to determine a safe level for ingestion of L-cysteine or L-cystine as dietary supplements.
5a.  Glutamine

a.  Background

Glutamine is the most abundant amino acid in the body accounting for more than 60 percent of the entire free amino acid pool in skeletal muscle and 20 percent of the total circulating free amino acid pool. The daily intake of glutamine for a person consuming 100 g of protein would be approximately 9.6 g (see Table 2).

Functions: Glutamine has several general metabolic functions including its roles: 1) as a vehicle for the transfer of nitrogen between tissues; 2) as a regulator of acid–base homeostasis in the kidney; 3) as an essential component for normal immune system function; 4) as a regulator of skeletal muscle catabolism; 5) the primary fuel source for enterocytes and colonocytes; 6) in times of stress, as a substrate for gluconeogenesis and the production of acute phase reactants; and, 7) as a structural amino acid constituent of protein (Smith and Wilmore, 1990). An additional role for glutamine is in the regulation of pancreatic function. Opara et al. (1990) found that, in their isolated pancreatic cell culture system, glutamine inhibited insulin production and stimulated glucagon release under basal glucose conditions.

Absorption and transport: Oral glutamine is readily absorbed in the intestine; however, little reaches the blood as it is primarily metabolized in situ (Windmueller and Spaeth, 1974). According to Souba (1991), glutamine is absorbed predominantly by the sodium–dependent ASC transport system in the small intestine. Kilberg et al. (1980) described the characteristics of the glutamine transport system in rat liver that differs from the ASC transporter and referred to it as sodium–dependent system N. Salloum et al. (1990), using a rat cell culture system, provided evidence of a similar brush border system N transporter that is particularly sensitive to dietary glutamine.

The metabolic fate of absorbed glutamine in the enterocyte has been described (Windmueller and Spaeth, 1974, 1975; Windmueller, 1982). The carbon skeleton goes primarily to CO$_2$ (64 percent of glutamine carbons and 40 percent of total CO$_2$ produced in postabsorptive rat jejunum) and other substances (lactate, citrulline, proline, and other compounds). Nitrogenous compounds resulting from glutamine metabolism in the gastrointestinal tract include ammonia, alanine, citrulline, and proline, all of which are released into the portal circulation and eventually utilized in hepatic metabolic processes (e.g., ureagenesis from ammonia and citrulline or gluconeogenesis from alanine) (Souba, 1991).

Metabolism: Two primary enzymes regulate glutamine metabolism. Glutaminase catalyzes the hydrolysis of glutamine to glutamate and glutamine synthetase synthesizes glutamine from glutamate and ammonia (Souba, 1991). Because it is produced endogenously from glutamate in the presence of ammonia, phosphate–dependent glutamine synthetase, a divalent cation such as Mg$^{2+}$ and energy from ATP, glutamine is generally regarded as a dispensable amino acid. Glutamine synthesis occurs primarily in kidney, skeletal muscle, brain, and, to some extent, in the liver.

Catabolism of glutamine occurs predominantly in mitotically active cells such as enterocytes, lymphocytes, tumor cells, and those in the kidney by the action of the enzyme glutaminase and results in ammonia and an α–amino group that can serve as the precursor for several amino acids including aspartate and alanine via transamination reactions (Souba, 1991). Other products include α–ketoglutarate which can serve as a source of energy through oxidative metabolism (Lacey and Wilmore, 1990). In addition, glutamine has been shown to be the primary substrate for renal ammoniagenesis (Welbourne et al., 1986).

Under certain circumstances, metabolic needs surpass the capacity for endogenous production of glutamine. The mobilization of protein from skeletal muscle after injury or surgery is largely due to glutamine efflux. The primary target site for the glutamine is the intestinal tract. Glutamine stores can be depleted during metabolic stress (e.g., injury, surgery, infection, or starvation) as a result of
an increased demand by the gastrointestinal tract, immunologic cells, inflammatory tissue, and the kidney (Soubia et al., 1985). This depletion is characterized by a reduced muscle glutamine pool followed by decreased plasma levels, muscle wasting, a negative nitrogen balance, and diminished gastrointestinal function. Baskerville et al. (1990) demonstrated edema and ulcerations of the intestinal mucosa associated with glutamine depletion consequent to the administration of glutaminase. Roth et al. (1982) reported that decreases of more than 50 percent of the skeletal muscle glutamine concentration were associated with increased mortality.

Smith et al. (1984) investigated factors that may regulate glutamine synthesis in skeletal muscle and reported that 3 key factors controlled glutamine synthetase activity in their cell culture system: product (glutamine) and substrate (glutamate) availability which increased activity 3-fold and 4-fold, respectively, and glucocorticoids which caused a 3-fold increase. These investigators found no substance that affected glutaminase activity in a similar manner.

Newsholme and Parry-Billings (1990) observed that under normal conditions glutamine synthesis is not linked to the rate of release and that the transport, rather than synthesis, is the key step in glutamine release from skeletal muscle, the primary glutamine reservoir. During stress there may be increased synthesis (reflected in increased glutamine synthetase activity), but this controls the availability of glutamine for transport rather than the rate of release (Newsholme and Parry-Billings, 1990).

Parry-Billings et al. (1990) have demonstrated that glucocorticoid (dexamethasone) administration increased the concentration and the rate of release of glutamine from isolated rat muscle. Smith et al. (1984) demonstrated glucocorticoid induction of glutamine synthetase in rat skeletal muscle. A logical corollary of this muscle synthesis and release was described by Ardawi et al. (1988) who demonstrated increased rates of glutamine utilization in isolated rat enterocytes. Consistent with this finding was a 35 percent increase in glutaminase activity in intestinal mucosal scrapings of dexamethasone-treated rats (Ardawi et al., 1988). These investigators concluded that, in stress, the role of glucocorticoids is to conserve glucose in part through the increase in synthesis (muscle) and utilization (gastrointestinal tract) of glutamine (Ardawi et al., 1988).

Glutamine and the liver: Aside from providing energy substrates, the liver through the balance of glutamine catabolism in the periportal cell and biosynthesis in perivenous cells can also regulate ureagenesis and ammonia detoxification, respectively (Häussinger, 1990). Soubia (1991) noted that the liver consumes glutamine in the postabsorptive state and releases it during stress.

Soubia (1991) discussed the possibility that the metabolism of glutamine is dependent on transport and availability rather than metabolic signals. Such a hepatic transport system has been described by Bode et al. (1990) as a sodium-dependent active transport system designated as system N. The adaptability of hepatic glutamine transport to differing metabolic states is explained in part by Gebhardt and Kleemann (1987) who showed that this transport system is equally induced in both periportal and perivenous cells by glucocorticoids and insulin.

Glutamine and the kidney: Glutamine is also involved with the maintenance of acid-base balance through an interaction between the kidneys and the liver (see review by Welbourne and Phrompetchcharat, 1984). One of the kidneys' primary tools in the regulation of acid-base balance is through ammonia production which is increased during acidosis and decreased in alkalosis. The ammonia is primarily derived from the catalysis of glutamine via the activity of glutaminase; while glutamine synthetase, which is found in highest quantities in renal tissue, causes the production of glutamine used to detoxify and transport ammonia between kidney and liver (Rodwell, 1990d).

Welbourne and Phrompetchcharat (1984) demonstrated that in chronic acidosis, skeletal muscle production of glutamine increases and the direction of the glutamine flux changes from liver (for gluconeogenesis and ureagenesis) to the kidneys where it is utilized for ammoniagenesis, gluconeogenesis, and bicarbonate generation (Welbourne and Phrompetchcharat 1984). Welbourne
et al. (1986) showed that as arterial ammonia concentrations increase, arterial glutamine concentrations and splanchnic bed uptake decrease thereby regulating glutamine flow and nitrogen metabolism in metabolic acidosis.

Several investigators have noted that, except to the very limited extent that ammonium concentrations change coincidently with shifts in urea or glutamine levels, the liver cannot regulate plasma bicarbonate concentration by altering glutamine or urea production (Marsh and Knepper, 1992; Walser, 1986). Since the liver does not excrete fixed ions, hepatic glutamine release can neither aggravate nor ameliorate hyperchloremic acidosis. Furthermore, ammonium excretion by the kidney rids the body of acid only if it occurs in exchange for sodium or is accompanied by chloride.

Glutamine and the immune system: Newsholme and Parry–Billings (1990) have reviewed those mechanisms that may control the availability of glutamine to the immune system where it acts as an important fuel for both macrophages and lymphocytes. The fate of glutamine in these cells, where it is used at a rate similar to or greater than glucose, is its partial oxidation to alanine, lactate, and aspartate (Brand et al., 1989). As in the enteroctyes, glutamine is transported into lymphocytes primarily via the ASC transport system (Ardawi and Newsholme, 1986).

The primary source of glutamine for the immune system appears to be skeletal muscle (Ardawi and Newsholme, 1990). During sepsis, skeletal muscle is catabolized to make BCAA available for transamination, thereby allowing for eventual production of glutamine to be used by the rapidly proliferating cells of the immune system. Concomitantly, there is a decreased utilization of glutamine by the small intestine.

Salloum et al. (1991) described a decrease in brush border glutamine carriers and glutaminase activity that, in conjunction with diminished consumption of circulating glutamine, may result in inadequate supplies of this nutrient to the gut during infection. Moreover, independent of the need for glutamine to maintain the structural integrity of the intestinal tract, there are numerous lymphoid cells in the small intestine that would presumably need to increase their utilization of glutamine in a similar manner, which could lead to further depletion of glutamine stores during infection (Sobba, 1991).

Alverdy (1990) pointed out that after stress the gut must maintain its roles in nutrient absorption, as a barrier against bacterial translocation, and as the major source of secretory IgA. Alverdy et al. (1992) have recently confirmed the importance of glutamine in the maintenance of gut structural and immune functions after surgical stress.

While the peripheral cells of the immune response require and use more glutamine (supplied by the skeletal muscle) during infection, there is a concomitant decrease in gut uptake, catabolism, and utilization (Salloum et al., 1991). Ardawi and Newsholme (1990) suggested that while the overall rate of glutamine utilization as a metabolic fuel for enterocytes is decreased, the immune cells of the intestine increase their utilization so that what appears as a net decrease is perhaps a shift in the compartmental use of glutamine.

The use of glutamine for the remediation of the deleterious outcomes of infection has been controversial. Rombeau (1990) concluded that glutamine improved nutritional status, decreased intestinal injury, decreased bacterial translocation, reduced endotoxemia, and improved survival in animals with a lethal form of enterocolitis. Barber et al. (1990) could reproduce only the nutritional and structural effects but found that glutamine was unable to protect against spontaneous or endotoxin-induced bacterial translocation. This issue remains an active area of clinical research.

Glutamine and the brain: Aside from acting as a precursor for the neurotransmitters glutamate and GABA (Bradford et al., 1983), glutamine is essential for detoxification of brain ammonia (Cooper and Lei, 1987). The presumption that this latter detoxification process is beneficial in protection
against hyperammonemia has been recently questioned by Hawkins and Jessy (1991), who suggested that the deleterious effect of chronic hyperammonemia is highly correlated with and perhaps begins with the synthesis of glutamine.

Many of the details of brain glutamine metabolism have been elucidated. Norenberg and Martinez-Hernandez (1979) described the localization of glutamine synthetase as occurring primarily in the astrocytes. Aside from the astrocytes, no glutamine synthetase activity was found in neurons, synapses, oligodendrocytes, microglial cells, endothelial cells, and pericytes, thereby establishing the astrocytes as key sites for brain ammonia detoxification and metabolism of glutamic acid and GABA. Since the major location of glutamate catabolism is in the high-affinity uptake by astrocytes (Schousboe et al., 1983), these cells assume a critical role in the protection of the CNS against the potential toxic effects of ammonia and glutamate.

Ferenci et al. (1989) observed that, in contrast to hepatic glutaminase which is stimulated by ammonia (Häussinger et al., 1983), glutaminase in GABA–ergic or glutamatergic neurons is inhibited by increased glutamate and ammonia. While Ferenci et al. (1989) reported that glutamine was not a modulator of GABA neurotransmission, glutamine is an important source of glutamate released during neuronal depolarization.

This latter finding supports the hypothesized "glutamate–glutamine cycle" whereby glutamine is produced in the astrocytes by the activity of glutamine synthetase and subsequently catabolized by neuronal glutaminase reaction to produce glutamate. Yudkoff et al. (1989) confirmed a tightly regulated interrelationship between glutamine and glutamate at the neuronal level. The importance of this relationship was demonstrated by Simantov (1989) who found that in the media containing reduced amounts of glutamine, glutamate (both monosodium and monopotassium forms) was not toxic to cultured neuronal cells. Simantov (1989) suggested that the ratio of glutamate:glutamine is regulated by glial cells, astrocytes, and neuronal activation and is the key factor in potential glutamate neurotoxicity.

b. Animal studies

Due to the historical view of glutamine as a dispensable amino acid, no reports could be found that addressed specific aspects of safety of use of oral dietary glutamine supplements in animals. No studies were found on food intake, growth, clinical and behavioral changes, hematology, vitamin B6, functional changes and gross pathology, and teratology and developmental changes. The animal studies that were available were performed within the context of glutamine's impact on intermediary metabolism and its clinical utility in the prevention of some of the deleterious effects of metabolic stress.

Biochemical studies: Specific problems for which glutamine supplementation has been tested include: radiation injury (Klimberg et al., 1990a,b), gastrointestinal growth in parenterally fed infants (Burrin et al., 1991), gastrointestinal atrophy (McCauley et al., 1991; Platell et al., 1991; Shizuka et al., 1990) and decreased organ function associated with parenteral feeding (Helton et al., 1990a,b; Li et al., 1990), nutritional repletion versus tumor growth in malignant disease (Austgen et al., 1992; Klimberg et al., 1990c), gastrointestinal immune function (Alverdy et al., 1992), and drug toxicity (Okabe et al., 1975).

Animals used in these reports included infant pigs and rats. All of the studies were short term averaging about 6 or 7 days for the active feeding phase. Dose ranges were also similar among the studies with the highest intake of 28 mg/ml diet. None of these studies reported any side effects; however, no attempts were made to study any parameters other than the primary outcome measures. Observations about the efficacy of parenteral or enteral glutamine in the treatment of surgical or tumor–bearing animals bears little relevance to the question of safety of oral glutamine supplementation.
c. Human studies

**Biochemical studies:** Interest has arisen recently about the potential of supplemental glutamine for reducing the catabolic effects of chronic disease. Several studies about enteral (oral and intragastric tube feeding) and parenteral use of glutamine (Déchelotte et al., 1991; Hammarqvist et al., 1989; Lowe et al., 1990; Welbourne et al., 1972; Ziegler et al., 1990) have employed normal healthy subjects and thereby provide some useful information about glutamine safety.

Welbourne et al. (1972) gave 260 mmol (approximately 35 g) of glutamine to 6 normal men under 3 different states of acid-base balance: normal, metabolic acidosis, and metabolic alkalosis. The latter 2 conditions were precipitated by oral challenge for 4 days with ammonium chloride (12 g) and sodium bicarbonate (8 g), respectively. The glutamine challenge was given on the morning of the fifth day followed by five days of recovery after the acidosis and alkalosis stages. Data from these normal subjects were compared with five patients with "intrinsic renal disease," none of whom had undergone dialysis of any kind prior to the study.

In the normal subjects, the glutamine challenge caused an increase in urinary ammonium excretion and a rise in urine pH under control and acidicotic conditions. No rise in urine ammonium excretion was seen in the alkalosis stage despite similar rises in plasma glutamine concentrations. Urine ammonium excretion did not change in the renal patients. Only one patient was challenged in the alkalosis condition. Other than a discussion of the outcome measures, there were no comments on any aspect of safety or adverse effects of the glutamine challenge.

Déchelotte et al. (1991) investigated the impact of jejunal infusion (via nasojejunal tube) of L-glutamine at graded infusion rates (ranging from 0 to 18 g/hour) in 10 healthy adults (4 male, 6 female). A total of seven infusion rates (0, 15.6, 46.8, 72, 90, 108, and 126 mmol/hour) were used and each subject received 3 (n = 8) or 4 (n = 2) randomly assigned rates, consecutively, for periods of 2.5 or 1 hour. All challenges were given within one day for each subject, and no dose was repeated within a subject. The time of perfusion ranged from 3 to 7.5 hours; total dose ranged from 22.5 g to 47 g. The highest dose was 47 g. In addition, eight subjects received continuous infusions of stable isotopes of glucose, leucine, and alanine simultaneously with glutamine challenge.

Enteral glutamine infusion produced a dose-dependent rise in plasma glutamine, alanine, glutamate, citrulline, aspartate, and urea; a decrease in plasma free fatty acid and glycerol concentrations that was not associated with changes in insulin; and no change in either leucine or glucose. Déchelotte et al. (1991) postulated that the rise in alanine was from de novo synthesis probably in the intestine and not from muscle, since there was no change in leucine concentrations. Since the glutamine infusions did not cause any elevations in circulating insulin levels, the decrease in free fatty acids and glycerol may be indicative of a glutamine-dependent decrease in lipolysis. There was no mention of how the infusions were tolerated nor was there any attempt at assessing any other endpoint related to the safety of glutamine.

Ziegler et al. (1990) reported on several trials undertaken to assess the safety of glutamine given orally and intravenously to healthy subjects. In the first trial, Ziegler et al. (1990) studied the effects of glutamine administration to healthy adult male subjects. After an overnight fast, subjects blinded to dose only received either 0, 0.1, or 0.3 g glutamine/kg body weight dissolved in distilled water; the average doses of glutamine administered were 0, 7.4, and 22.2 g, respectively. Blood and urine were collected over a 4-hour period after the challenge. Subjects were also queried about subjective mental or physical symptoms.

In a second study, 9 healthy volunteers (5 male, 4 female) were infused intravenously with glutamine at 3 concentrations on 3 separate occasions (Ziegler et al., 1990). The infusion rates were 0, 0.0125, and 0.025 g glutamine/kg body weight/hour; the average doses over the 4-hour trial were 0, 3.4 and 6.8 g, respectively. In the third trial, Ziegler et al. (1990) gave isonitrogenous, isocaloric parenteral solutions differing in glutamine content to 7 normal volunteers (sex unspecified) for
5 consecutive days. The test diets contained 1.5 g protein/day, nonprotein calories were supplied by lipid (62 percent) and glucose (38 percent). Glutamine content was 0, 0.285, and 0.570 g glutamine/kg body weight/day for an average daily dose of 0, 21.9, and 43.9 g.

Blood glutamine rose significantly, peaking at 30 to 45 minutes after oral challenge; concentrations returned to baseline within 90 to 120 minutes of the low dose (peak 1028 μmol/L) and 180 to 240 minutes after the high dose (peak 1328 μmol/L). The peak concentrations were approximately 50 and 100 percent above normal ranges. Significant dose-related increases were found in the concentrations of other amino acids associated with glutamine catabolism, e.g., alanine, citrulline, and arginine. Other amino acid shifts included significant increases in blood histidine concentrations and decreases in total BCAA (predominantly decreased leucine), glycine, methionine, and phenylalanine concentrations. No changes were seen in any of the other amino acids measured compared with baseline levels.

Urinary excretion of creatinine or ammonia did not change; however, excretion of urea and total nitrogen increased significantly with glutamine dose. Glutamine dose (oral and parenteral) was positively associated with glucagon response in normal subjects (Ziegler et al., 1990). None of the subjects reported adverse effects of the glutamine supplementation.

Hammarqvist et al. (1990) studied the effects of parenteral glutamine supplementation in postoperative catabolic patients. Twenty-two patients undergoing elective cholecystectomy were randomly divided into two groups: isocaloric intravenous diets containing either amino acid solution alone or the same amino acid solution with addition of 0.285 g/kg body weight/day of glutamine (20 g/day). The glutamine group was spared the catabolic depletion of muscle mass as reflected by a significantly lower cumulative nitrogen loss than patients not receiving glutamine. As compared with the glutamine subjects, who were in nitrogen balance throughout the trial, patients in the non-glutamine group were in negative nitrogen balance on each postoperative day and had a significant postoperative decrease in skeletal protein synthesis as reflected by ribosomal numbers computed from biopsy samples. No deleterious side effects were reported.

This same group expanded its subject pool to study the differences between glutamine and its metabolites (ornithine α-ketoglutarate, α-ketoglutarate, or alanyl-glutamine) in preventing postoperative muscle wasting in 30 patients similar to those described by Hammarqvist et al. (1989). Glutamine or its α-ketoglutarate skeleton fed at 0.285 g/kg body weight/day was again found to prevent muscle catabolism without negative side effects (Vinnars et al., 1990).

In reporting on the effects of glutamine administration on nitrogen balance, the investigators in the preceding studies appear to have overlooked the potential contribution to total nitrogen balance by changes in the free glutamine nitrogen pool (Walser, 1991). Consequently, under the conditions reported in these studies, nitrogen balance may only reflect glutamine balance rather than protein homeostasis.

Behavioral studies: Because of the potential neurotoxicity of glutamine and its by-products, ammonia and glutamate, Lowe et al. (1990), in an expansion of the study reported in Ziegler et al. (1990), investigated potential neurophysiological, cognitive, and behavioral changes in seven healthy male subjects non-randomly assigned parenteral diets containing 0, 20, and 40 g/day of glutamine, in that order. Blood analyses as well as psychological and mental performance testing were performed at 8 a.m. and 3 p.m. of the first, third, and fifth days of each of three 5-day study periods. Plasma glutamine concentrations increased significantly over control values, but ammonia and glutamate levels, as well as nitrogen balance and hormonal levels were unchanged during the three dietary periods. No changes were found in mental status, mood (as established by an uncharacterized "standardized mental-status exam"), or performance on the Continuous Performance Test (CPT).
To date no controlled long-term studies have been found of the effects of oral/enteral glutamine supplementation. Numerous reports exist of glutamine supplementation for various psychological disorders including alcoholism (Rogers and Pelton, 1957) and affective disorders (Cocchi, 1976). These trials were anecdotal in nature and varied in duration (several months to a year) and dosages (250 to 1000 mg/day). Neither paper evaluated the safety of glutamine supplementation.

d. Summary and conclusions

Endpoints: The majority of studies that have utilized glutamine have been investigations of its impact on aspects of intermediary metabolism; consequently, the endpoints have generally been production of catabolic products such as alanine and glutamate, changes in concentration and or flux of amino acids, and physiological parameters such as kidney function and related aspects of acid–base regulation. The studies by Ziegler et al. (1990) are the only ones that have addressed safety by specifically looking at the same types of metabolic endpoints in healthy subjects. The Expert Panel found no long-term studies of the safety of oral glutamine supplementation.

Safe levels of human intake: Doses infused in the several human studies employing healthy subjects ranged from 3.4 g (given over a 4-hour observation period) to 47 g/day. These doses may be compared with an intake of 9.6 g glutamine by a person consuming 100 g protein (see Table 2). No adverse side effects have been reported. It should be noted that all the challenges given resulted in some changes in metabolism differing from baseline levels that were dependent on route of administration and study conditions. Changes included alterations in metabolic fuels (e.g., glucose, alanine), hormone levels, lipolysis, blood concentrations of dispensable and indispensable amino acids, and renal function.

No studies were found of possible developmental or teratogenic effects of short or long-term oral glutamine supplementation in animals or humans. While glutamine may have benefits under certain clinical conditions, the lack of data on specific endpoints of safety dictates the need for further investigation.
5b. Glutamic acid

Glutamic acid presents a special issue in regard to evaluation of safety of amino acids as dietary supplements. There are numerous studies and extensive data available on the sodium salt, monosodium glutamate (MSG), a regulated substance added to various foods. This salt is not sold as a dietary supplement, per se; however, studies on administration of MSG are included because the data were judged useful in the evaluation of the safety of glutamic acid. The Expert Panel is well aware that these data have been cited in considerations of the safety of glutamic acid salts as food ingredients. It must be emphasized that this review provides an assessment of the adequacy of data for the evaluation of the safety of glutamic acid and its salts from the perspective of use as dietary supplements.

a. Background

Glutamic acid is a dispensable dicarboxylic amino acid involved in many aspects of intermediary metabolism including roles in gluconeogenesis, protein synthesis, neurochemistry (both as an excitatory neurotransmitter and as the precursor for the major inhibitory neurotransmitter, GABA), and as the precursor for both glutamine and glutathione. Because of the interrelationship among glutamate, glutamine, and aspartic acid, the reader is referred to sections covering those related amino acids for further discussions of these associations.

Glutamate constitutes approximately 8 g/100 g protein consumed/day (Table 2). Giacometti (1979) observed that glutamate makes up 13.5 percent of the free amino acid pool in muscle. Due to regulatory mechanisms predominantly at the intestinal sites of absorption and metabolism (Munro, 1979), glutamate is found in low concentrations in the plasma relative to amounts found in various other tissues.

Metabolism: The key enzymes in both glutamate and glutamine metabolism are located in the mitochondria of cells (Kovacevic and McGivan, 1983). A main source of endogenous glutamate is the catabolism of glutamine by glutaminase. As opposed to glutaminase in the liver, glutamate is the most important inhibitor of glutaminase activity in the kidney. Other endogenous sources of glutamate include arginine, ornithine, proline, and histidine. Glutamate is catabolized to α-ketoglutarate via the action of glutamate dehydrogenase (GDH), an enzyme that is activated by leucine (McGivan et al., 1973). In addition to leucine other substances that influence GDH activity include ADP (activation), and ATP, GTP, and GDP all of which inhibit its activity (Kovacevic and McGivan, 1983).

Glutamate acts as an amino donor for many transamination reactions. Vitamin B6-dependent AST plays an important role in glutamate metabolism intracellularly along with vitamin B6-dependent ALT which catalyzes the transfer of an ammonium ion from glutamate to pyruvate to form alanine (see aspartic acid section). This latter reaction is the predominant fate of ingested glutamate. Both of these enzymes have received considerable attention with regard to their use as status indicators for both liver function (serum levels of activity) and vitamin B6 (serum and erythrocyte–derived forms) (Sauberlich et al., 1973).

Other metabolic roles for glutamate include acting as a precursor for proline, an ammonium ion donor for urea production (via the activity of GDH) in the liver and an ammonium ion acceptor in the brain (see below), and along with aspartate (again via transamination reactions) as a transporter of reducing equivalents intracellularly via the "malate shuttle" system (Rodwell, 1990b).

Absorption and transport: Along with aspartic acid, free glutamate is absorbed slowly relative to other amino acids from the gastrointestinal tract via a sodium–dependent transporter (see aspartic acid section); however, it is more rapidly absorbed as a component of peptides derived from protein,
i.e., casein. Much of the absorbed glutamate is transaminated in the intestine to alanine which then appears in increased amounts in the portal circulation. Windmueller and Spaeth (1975) demonstrated that the majority of absorbed dietary glutamate is metabolized in the intestine.

This latter effect may reflect the importance of glutamate as a gluconeogenic precursor and effector of intermediary metabolism. In the gastrointestinal tract, absorption and subsequent metabolism of glutamate is dependent on the presence or absence of other macronutrients, in particular, carbohydrates and probably glucose or starch but not fructose. Stegink et al. (1979c) have shown that free glutamate fed in water results in significant increases in plasma glutamate whereas there are no significant changes in plasma glutamate concentrations when fed with formula or feed.

Glutamate and glutamine are inextricably linked metabolically (see glutamine section). More of an enterally fed load of glutamate will appear as glutamine in the blood rather than glutamate. Aside from gut metabolism of glutamate, this may be due to the fact that glutamine crosses membranes more readily than glutamate and thereby serves as the transport form of exogenous glutamate and ammonia (Meister, 1979). Because of the efficient interconversion between glutamate and glutamine, evaluation of glutamate status in the blood must also include glutamine. Moreover, the sum of glutamate and glutamine concentrations in the blood may be a better reflection of glutamate status than glutamate alone alone.

**Glutamate and the liver:** Because exogenous glutamate does not readily cross liver cell membranes, it is not a primary exogenous substrate for liver metabolism (Kovacevic and McGivan, 1983). The main sources of liver glutamate are from the transamination of α-ketoglutarate and the amination reaction resulting in glutamate formed from ammonia and α-ketoglutarate via the action of GDH.

**Glutamate and the brain:** Glutamate plays an important role in the brain and most of the safety issues about glutamate supplementation are focused on potential neurological effects. The various roles for glutamate in the nervous system have been reviewed extensively (Erecinska and Silver, 1990; Kovacevic and McGivan, 1983).

The characterization of the molecular basis of the actions of the excitatory amino acid neurotransmitter receptors has received much attention (Gasic and Hollman, 1992; Monaghan et al., 1989). There are three types of receptors currently being studied named according to their respective agonists, kainic, quisqualate, and NMDA. The latter has been studied most extensively (Willetts, 1990).

Numerous mechanisms related to receptor activity have been advanced to explain the excitatory and neurotoxic effects of glutamate including anomalies in glutamate receptor function (Freese et al., 1990), changes in neuronal ionic flux and ion:receptor interactions (Choi, 1987; Choi et al., 1988; Duca et al., 1988; Hahn et al., 1988; Manev et al., 1989; Michaels and Rothman, 1990; Nicholls and Attwell, 1990), and oxidative stress (Chan et al., 1990; Miyamoto et al., 1989; Murphy et al., 1989, 1990).

While the molecular mechanisms by which glutamate and related compounds exert their influence on the functional integrity of the CNS are important topics for study, it is the metabolism, including transport and compartmentalization in the nervous system, that perhaps is more relevant to the evaluation of safety of L-glutamic acid used as a dietary supplement.

Peripherally administered glutamate does not cross the blood–brain barrier to any great extent. When plasma levels increase, brain content does not change appreciably because the glutamate transport system across the blood–brain barrier is saturated at normal physiological concentrations (Pardridge, 1979). Pardridge (1979) observed that glutamate leaves the brain and the rate of efflux is greater than any other amino acid. He noted that the rate of glutamate efflux is seven times greater than the influx and, because of this disparity at the blood–brain barrier, the glutamate transport system may be defined as an active efflux system actively transporting glutamate out of
the brain against a concentration gradient. These observations were supported by the studies of Berl et al. (1961) who found that after intracerebral injection, labelled glutamate left the brain to be metabolized in other organs. Partridge (1979) speculated that total brain glutamate concentrations did not increase significantly after a large oral bolus because of this efflux system.

The primary mechanism for inactivation of both glutamate and aspartate is their removal from the extracellular space by the sodium-dependent transporter located in the astrocytes and neurons. The high-affinity glutamate transport system cannot distinguish between aspartate and glutamate and may be responsible for clearance of the excitatory neurotransmitters from the synaptic space (Nicholls and Attwell, 1990).

The transport of glutamine differs from that of glutamate in that the former is transported into brain cells primarily by a low-affinity, sodium-independent system, while the latter is predominantly transported via a high-affinity, sodium-dependent transporter (Erecinska and Silver, 1990; Weiler et al., 1979). The sodium dependency is important as glutamate is an anion at physiological pH, necessitating the presence of the sodium cation as a driving force of the transporter across the concentration gradient (Erecinska and Silver, 1990).

Two other glutamate transport systems have been identified in mammalian brain, a chloride-dependent, high-affinity system and a low-affinity glutamate transporter. The former may mediate the exchange of external for internal glutamate in both neurons and glial cells; the latter may be involved in glutamate uptake by GABA-ergic cells (Erecinska and Silver, 1990).

The relative importance of each of these transport systems may be associated with their localization and involvement in regulation of the glutamate:glutamine compartmentalization (see glutamine section). Kovacevic and McGivan (1983) described two distinct glutamate pools. The "small pool" is largely associated with metabolism of exogenous glutamate in glial cells and the "large pool" is associated with neuronal catabolism of endogenous glutamate. The equilibrium between these pools may be the controlling factor in the neurotoxicity of glutamate. The metabolic compartmentalization concept has also been reviewed by Berl and Clarke (1983).

Berl et al. (1961) observed that when isotopically labeled glutamate was injected into the brain, the specific radioactivity of glutamine was significantly higher than that of glutamate after a short time period. Benjamin and Quastel (1974) studied the fate of endogenous versus exogenous glutamate and found that the former is initially catabolized by GDH with the release of ammonia, while the latter is converted via transamination to aspartate. Using cortical brain slices, Benjamin and Quastel (1974) found that 49 percent of the glutamate dose was converted to aspartate, 37 percent to glutamine, and the rest was oxidized via GDH. These authors suggested that endogenous glutamate is associated with the "large pool" affiliated with neurons while exogenous glutamate is metabolized as part of the "small pool" and represents glutamate released by neurons and taken up by glial cells (astrocytes).

The results of the study by Benjamin and Quastel (1974) were consistent with the findings of Noreenbarg and Martinez–Hernandez (1979) who noted that the "small pool" of glutamate is associated with astrocytes containing glutamine synthetase for the rapid conversion of glutamate to glutamine while the "large pool," probably located at nerve endings, is associated with glutaminase activity. Since the major method of glutamate catabolism is in the high-affinity uptake by astrocytes (Schousboe et al., 1983), these cells may have a critical role in the protection of the CNS against the potential toxic effects of ammonia and glutamate.

Clearly, the mechanisms controlling the endogenous production of glutamate and related compounds involves a tight regulation of transport and metabolism both peripherally and in the brain. Similarly, the health and function of the nervous system requires the ability to control the entry of these powerful neuroactive substances into the brain. How and under what conditions these controls might break down allowing the nervous system to be overexposed to these neuroactive substances is an area of active research, concern, and debate.
b. Animal studies

The majority of studies of the mechanisms of glutamate neurotoxicity are based on theories supported primarily by data generated from in vitro studies and discussion of the relative strengths of each of these theories is outside the scope of this presentation. The material presented for the evaluation of the safety of L-glutamate as a dietary supplement is restricted to in vivo studies. Moreover, while studies involving systemic challenge either as intraperitoneal or subcutaneous injection will be noted, the extended discussions will be limited to studies employing oral (dietary or gastric intubation) challenges.

Changes in food intake and body weight: Systemic challenge in neonatal rodents with MSG has been shown to be associated with changes in body morphology characterized by obesity and stunted linear bone growth similar to that described for aspartic acid (Schainker and Olney, 1974). (See aspartic acid section.)

Takasaki et al. (1979) reported a series of studies designed to assess the impact of MSG, given orally and parenterally, on the arcuate nucleus and somatic growth in rats. In the first study the least effective dose (LED) was determined in neonatal (2-day-old) and infant (10-day-old) rats given either subcutaneous or oral intubations of 3 doses of MSG (0.3, 0.4, or 0.5 g/kg body weight). All animals were killed 8 hours after challenge and arcuate nuclei were examined histologically.

In a long-term study, 2- and 10-day-old rats were subcutaneously injected with 4 g/kg body weight MSG for 10 consecutive days while other 2-day-old rats were injected with 0.2 g/kg (1/2 the LED) for 10 days. Littermate controls were treated with subcutaneous injections of physiologic saline solution. In the other long-term study, 10-day-old rats were given oral intubations of 0.5 g/kg MSG (about 1/3 LED) for 10 days while another group of 10-day-old rats were given the same oral challenge for 10 days plus a diet containing 5 percent MSG ad libitum for 10 days after weaning. The mean intake of MSG was 7.2 g/kg body weight/day. Controls for the oral challenge groups were given intubations of saline.

The only significant findings for the orally challenged groups were reduced intake (mean over 10 days post-weaning) in females, increased pituitary gland weight at 11 months in male rats, increased serum glucose in both sexes of the rats given 0.5 mg/kg MSG and in males of the high MSG group, and increased serum triglycerides in males of the high MSG intake group. While males and females in the injected groups had damaged arcuate nuclei, no histological changes were reported for orally treated animals. Significant changes were seen in intake (greater than controls in the post-weaning stage), linear bone growth (stunted bone length and tail length), and adiposity (increased Lee index in both sexes) in the injected animals. The younger animals (2 days old at injection) had more significant changes. Numerous differences in organ weights and blood parameters were also noted in the parenterally challenged rats throughout the study.

The deleterious effects of systemic (either intraperitoneal or subcutaneous) administration of MSG on food intake in rodents have been documented in numerous reports (Badillo-Martinez et al., 1984; Dawson and Wallace, 1989; Kanarek and Marks-Kaufman, 1981; Kanarek et al., 1979; Ninomiya and Funakoshi, 1989a,b; Racotta and Hernandez-Garcia, 1989; Reddy et al., 1986; Rietveld et al., 1986; Utsumi et al., 1980). No other reports have been found, in rodents or any other animal model, of changes in intake or growth associated with oral administration of MSG.

Behavioral studies: Numerous reports have documented behavioral changes associated with systemic administration of MSG (Araujo and Mayer, 1973; Dawson and Annau, 1983; Dawson and Lorden, 1981; Katz, 1983; Lorden and Caudle, 1986; Saari et al., 1990). Pradhan and Lynch (1972) examined the effects of neonatal intragastric infusion of MSG on subsequent adult behavior of rats. Eighty pups (8 each from 10 litters) were divided into 4 groups which were given 1.25, 2.5, and 5 g/kg body weight of MSG in water from the fifth to the tenth day postpartum. On day 21 animals were separated by sex. Body weights were recorded from day 5 and intake measured daily for 6 days.
per week from day 31 until animals were 3 months old. An unknown number of animals died and complete diet records were available for only 30 animals. Only 6 rats from each treatment group (except 4 in the 2.5 mg/kg group) were given behavioral tests.

During the first two weeks, spontaneous motor activity was measured in an actophotometer. Animals were also trained to run in a T-maze for food reinforcement. Fifteen trials were run every day for 5 days in each of 2 weeks. During the following 3 weeks rats were trained on a fixed-ratio 24-hour schedule (number of bar presses required for food reward) for food reinforcement. Animals were deprived of food for 23 hours before the food-reinforced behavioral sessions.

A dose-dependent decrease was seen in both food intake and body weight in rats of both sexes compared with controls. Spontaneous activity (the average of 5 days during the second week of the trial) was also reduced in a dose-dependent manner (significantly in the 5 g/kg group). Maze running data from the tenth session indicated significant decreases in latency or time (seconds) required to run the maze, in all challenge groups compared to controls and a significant decrease in number of correct runs between 5 g/kg group and controls (percent score 47 versus 92). No other differences were reported.

Iwata et al. (1979) in a MSG challenge design identical to that described above in Takasaki et al. (1979), administered several behavioral paradigms to rats. The orally challenged 10-day-old rats received either 0.5 g/kg body weight of MSG by forced intubation for 10 days or ad libitum exposure to 10 percent MSG in their diet for 10 days following the intubation. Behavioral measures included activity measurements (disturbances in a radio field and recorded on a digital counter) in males at 1, 3, and 9 months of age and recorded over 24-hour periods, and open field tests recorded during 3-minute free exploration periods at 1, 3, and 7 months of age.

Lashley III maze learning (errors and running time to the goal box) was evaluated in 3-month-old males only. Six-month-old females were tested for swimming or learning ability to escape from a water-filled multiple T-maze; errors or escape times were recorded. Other measures included grip strength, time on a rotating rod, corneal and pinna reflexes, and an inclined plane test, administered to equal numbers of males and females at 1, 3, and 7 months, except for grip strength which was only done at 7 months. No significant differences were found between either of the enterally challenged MSG groups and controls for any of the behavioral or neurological tests reported. As in the Takasaki et al. (1979) report, neonatal and, to a lesser extent, infant animals receiving systemic MSG challenges exhibited deficits in all parameters studied.

Biochemical and neurochemical studies: Many investigators have documented significant changes in brain and peripheral chemistry in neonatal, infant, adult, and aged rats exposed to subcutaneous and intraperitoneal challenges with MSG (Ahluwalia and Malik, 1989; Dawson, 1983; Dawson et al., 1989; Meister et al., 1989; Walaas and Fonnum, 1978; Wallace and Dawson, 1990).

Frieder and Grimm (1987) compared the offspring of 10 female rats given MSG (10 g/kg body weight orally) on 14 consecutive days with offspring of 10 control dams. Litter size was adjusted to 10 pups per dam. At 28 days postpartum, the pups were separated from their dams and housed in same gender groups (5/cage). At 15, 21, 28, and 60 days, 6 male and 6 female rats from treatment and control groups were decapitated and brains were extracted for neurochemical studies.

Frieder and Grimm (1987) found neurochemical changes at all time periods assessed. In 15-day-old rats, changes included an 80 percent decrease in frontal cortex choline uptake along with a 25 percent reduction in choline acyl-transferase activity and a concurrent increase in norepinephrine uptake. Choline uptake increased during the follow-up periods to a point were it was significantly greater than controls at adulthood. By contrast, norepinephrine uptake showed a reverse pattern especially in males such that by 60 days MSG animals had a significant reduction in norepinephrine uptake. No significant neurochemical differences were found at 60 days in the hypothalamus of male or female rats given MSG. GABA uptake did not differ in any animals in any
of the areas assayed at 60 days. Glutamate, glutamine, and aspartate were not measured at any time during this trial.

Aside from those studies in the background section which describe extensive gastrointestinal tract and peripheral tissue metabolism of oral L-glutamate loads, no other studies were found that have examined the impact of oral or enteral L-glutamate or MSG challenge on chemical changes in the nervous system or peripheral metabolism, e.g., hepatic or endocrine responses. While the above cited studies of brain chemistry provide evidence of biochemical changes associated with systemic administration of MSG, few reported on the concentrations of glutamate-related metabolites, specifically glutamine. Moreover, there were no assessments of peripheral metabolic adaptations, i.e., liver metabolism, in response to the parenteral infusion of MSG. The study by Ahluwalia and Malik (1989) did provide evidence of significant metabolic changes associated with large parenteral infusions of MSG, e.g., increases in serum glucose, that could independently cause changes in neurotransmitter and/or brain amino acid concentrations. Consequently, in the absence of supporting evidence about peripheral glutamate metabolism, the mechanisms by which large parenteral boluses of MSG per se may influence brain chemistry are unknown. Furthermore, the relevance of these studies to the safety of oral dietary supplements of L-glutamate is unclear.

**Vitamin B6 studies:** The active coenzymatic form of vitamin B6, PLP, is the cofactor for numerous enzymes in glutamate metabolism including glutamate decarboxylase (GAD) which converts glutamate to GABA, GABA transaminase which degrades GABA, and AST. However, the most important functional role for vitamin B6 is in brain glutamate metabolism.

Vitamin B6-dependent seizures are a well recognized disorder of glutamate metabolism. As the name implies, the seizures in this disorder are associated with a presumed increased dependency of the enzyme GAD for the cofactor, PLP. Another genetic disorder of GABA metabolism is associated with decreased vitamin B6-dependent GABA transaminase activity. While the former disorder is responsive to supplementation with vitamin B6, the latter is not (Scriber and Perry, 1989).

Study of the relationship of vitamin B6 to glutamate metabolism in the brain has focused primarily on the activity of GAD. In addition, there have been reports of an interaction between vitamin B6 and various receptors in the glutamate/GABA system. Tunnicliff (1979) observed reversible in vitro competitive inhibition by PLP of GABA receptors. Guixart (1991a,b) has studied the impact of vitamin B6 restriction on glutamate and GABA metabolism and receptor activity in infant rats and found changes in both glutamate receptor binding and efflux of both glutamate and GABA in various brain areas of vitamin B6-deficient infant rats. Wasyczuk et al. (1983a,b) also documented reductions in GABA concentrations in discrete brain areas of neonatal mice born of vitamin B6-deficient dams.

Wen and Gershoff (1972) demonstrated increased peak concentrations and decreased clearance of plasma glutamate in vitamin B6-deficient rats given intraduodenally injected boluses of MSG (1 mg MSG/g body weight). These authors attributed the rise in plasma glutamate concentrations to decreased intestinal utilization due to reduced activity of the vitamin B6-dependent enzymes, ALT and AST.

Vitamin B6 status is a critical component of glutamate metabolism both peripherally and in the nervous system. Studies examining the potential toxicity of oral supplements of glutamate in any form should carefully consider this important factor.

**Functional assessment and gross pathology:** The lesions associated with MSG are primarily localized in the neurons of the arcuate nucleus of the mediobasal hypothalamus. Morphological and neuroendocrinological correlates of this phenomenon have been reproduced repeatedly in systemically challenged rodents (Arbogast and Voogt, 1990; Bakke et al., 1978; Bloch et al., 1984; Dada and Blake, 1985; Magarinos et al., 1988; Millard et al., 1982; Olney, 1971; Pampori et al., 1991; Rodriguez-Sierra et al., 1990; Seress, 1982; Terry et al., 1981; Utsumi et al., 1980; Waxman et al., 1990).
Other areas of the brain that have been examined for lesions consequent to MSG injection include optic nerve and optic chiasma (Brankack and Klingberg, 1990), cochlear nuclear complex (Schweitzer et al., 1991), and the area postrema, an area lacking in blood–brain barrier and contiguous with the base of the fourth ventricle (Phelix and Hartle, 1990).

Lemkey–Johnston and Reynolds (1974) described the sequence of events associated with lesion formation in 7 to 10 day–old mice, as progressing from cells and neurons close to the median eminence (within 15 to 20 minutes of a single oral bolus of 4 mg/g body weight MSG) and appearing first on the cell surface and moving inward, suggesting influx of glutamate. Perez and Olney (1972) observed that glial cells and axons passing through the nucleus were not affected and the neighboring ventromedial nucleus was completely intact. While there is no question that these lesions occur in rodents, there have been conflicting reports about the extent of the damage and the reproducibility of these lesions in other animals. This section will review the histological studies across species that have employed oral or enteral challenges.

Olney et al. (1972b), in a follow–up to an earlier report of MSG–induced lesions in a premature rhesus monkey (Olney and Sharpe, 1969), investigated the impact of oral MSG challenge in neonatal rhesus monkeys and responded to allegations that attributed their earlier findings to poor tissue fixation. Three infant monkeys (all age 7 days) were given MSG by oral gavage as single boluses of 1, 2, and 4 g/kg body weight, respectively. Two other monkeys were given 2.7 g/kg body weight MSG and one additional monkey was given 4.0 g/kg body weight MSG by subcutaneous injection. Controls for both routes of administration were given saline, calculated to provide molar equivalents of the MSG doses.

Olney et al. (1972b) described small focal lesions associated with the oral challenges and evidence of rapid necrosis of neurons occurring within five hours of challenge. There were no changes seen in the hypothalami of sodium chloride–treated controls. These authors also responded to critiques of their earlier findings of damage in nonhuman primates, i.e., that the lesions were artifacts of tissue preparation, with an extended discussion of their methodologies.

Several studies have reported no effects from oral MSG challenges of infant monkeys. Reynolds et al. (1971) gave doses of 1, 2, and 4 g/kg body weight to 4 infant monkeys (2 different species) of differing ages (range <1 day to 8 days). The 3 control (distilled water) monkeys were 9, 21, and 36 days old. All animals were intubated after a 4–hour fast and sacrificed 6 hours after challenge. Animals were perfused under anesthesia with fixative.

Reynolds et al. (1971) reported "no significant differences" between treatments upon histological examination by both light and electron microscopy. They did note swollen dendrites and a spectrum of "degenerative changes" which they attributed to "poor fixation." At one point in the paper it was not clear whether these observations occurred only in one treatment group while elsewhere there were descriptions of damage in both control and MSG animals similar to that described originally by Olney et al. (1969) in a premature monkey given 2.7 g/kg body weight MSG. Reynolds et al. (1971) attributed all abnormalities to poor fixation; they did not provide a detailed explanation for how this poor fixation occurred or the criteria for its determination.

Newman et al. (1973) performed four studies in which oral challenges of MSG were given by intragastric intubation. In the first study, animals ranging in age from 3 to 99 days in the challenge group and one 108–day–old control, were given 2 mg/kg body weight MSG and sacrificed after 4 hours. In a second study, comparisons were made between controls (n = 5) and experimental (n = 11) infant monkeys. The experimental groups received a single bolus of either 2 (n = 8) or 4 (n = 3) g/kg body weight MSG and all animals were sacrificed 4 hours after intubation. The third study examined the effects of prenatal administration of MSG (voluntary consumption by dams of 4 mg/kg body weight/day MSG for the last trimester of pregnancy, n = 6); neonatal monkeys were sacrificed within 4 hours of birth. In the final study, 2–day–old infant monkeys were sacrificed after receiving either 2 g/kg body weight MSG (n = 2) or no treatment (n = 2). This last protocol
differed only in the perfusion procedure used to prepare the tissue samples (glutaraldehyde versus formalin as the fixative).

Upon histological examination, the three-day-old monkey in the first study had "hypochromatic nuclei and a minimal degree of vacuolation in the ventral hypothalamus but these findings were not regarded as significant." Similar changes were noted in the second study in both challenged and control animals. The mean birth weights of controls versus MSG–exposed infants in the third study were 471 and 429 g, respectively. No evidence of macroscopic or histological changes was reported. In the final study, changes were noted in both control and challenged animals that were attributed to poor tissue fixation.

This study was unable to replicate the original findings of Olney and Sharpe (1969); however, it should be noted that Newman et al. (1973) did not reproduce the conditions of the earlier study in terms of the age of the animals (the monkeys given the larger dose were 80 days old). The three-day-old monkey in the first study did have evidence of MSG–induced changes similar to that described by Olney et al. (1972) but these changes were dismissed as "not significant" without further explanation. The interpretation of these studies is compromised by the small sample size and the use of older animals, especially at the higher doses. The criteria for drawing conclusions about the nature and extent of the changes reported were not elucidated. Baseline pre–dose plasma glutamate concentrations were not measured in any groups and only terminal values were reported. Several comments were made about behavior of the infants; however, the study did not include a systematic protocol for the behavioral evaluation of these infant monkeys.

Abraham et al. (1975) orally challenged (method not given) 5 infant rhesus monkeys with either 4 g/kg body weight MSG (n = 2, ages 5 days old) in either single bolus (sacrificed at 3 or 24 hours post–dose) or daily doses of either 1 (n = 2 for 8 or 30 days) or 0.25 (n = 1 for 15 days) g/kg body weight MSG to 1–day–old infants. A single control three–day–old infant was given water. In contrast to the studies of Olney et al. (1972), Newman et al. (1973), and Reynolds et al. (1971) in which animals were perfused while under anesthesia, Abraham et al. (1975) euthanized first with lethal injections, removed the brains, and then prepared the specimens for histological examination.

Abraham et al. (1975) observed no signs of convulsions, vomiting, or cyanosis in any of these orally challenged animals. While they described "shrinkage of the ependymal area" (cells lining the third ventricle), they attributed this observation to "the effects of immersion fixation." They did not note similar findings in the control subject nor did they say whether this phenomenon occurred across doses of MSG. They also reported necrotic neurons in the area of the arcuate nucleus in a monkey receiving 1 g/kg body weight/day MSG for 30 days. Abraham et al. (1975) then described similar changes in a saline–treated monkey, but there was no saline–treated group included in the methods section. Other changes that were not fully described, were attributed to immersion fixation. Abraham et al. (1975) stated that while there were necrotic or damaged neurons and oligodendrocytes in the arcuate nucleus of the hypothalamus, they occurred in both treatment and control animals. These authors attributed the changes to "the normal process of attrition in morphogenesis" and concluded that they were not MSG–induced.

Other animals that have been shown to be responsive to acute oral MSG challenge include guinea pigs and rabbits (Heywood and Worden, 1979). The dog has been used as a model for acute and chronic glutamate toxicity in several studies with inconclusive results. In both acute (Oser et al., 1975) and chronic (Owen et al., 1978) feeding studies, vomiting has been the most prevalent effect of MSG in dogs over a dose range of 1.0 g to 4.4 g/kg body weight. No other changes have been consistently associated with MSG challenge in dogs.

Studies in various animal species exposed to acute systemic MSG challenges have attempted to replicate histological findings of focal lesions in the arcuate nucleus of the neonatal hypothalamus. Rodent species have been consistently vulnerable to such changes; however, monogastric species including nonhuman primates have been inconsistent in demonstrating histological changes. The
differences in response to acute oral challenge in monkeys have been reported to be due to methods in tissue preparation. To date, there have been no studies that have examined potential behavioral or other functional changes associated with either short- or long-term exposure to glutamate in monkeys. Because of inconsistencies in reporting data and methodologies used, it is not possible to draw any definitive conclusion about the impact of either short- or long-term exposure to oral supplements of glutamate in nonhuman primates.

Studies of other organ systems: Other organ systems that have been studied with regard to toxicity of systemic MSG include alveolar macrophages (Liu et al., 1989) and response to myocardial infarction (Singh et al., 1989). There were no studies uncovered of the impact of oral challenge of L-glutamate on the functions of different organs or systems.

Teratology and developmental studies: In a multigenerational study, Anantharman (1979) examined the potential for developmental changes that might result from prenatal exposure to MSG. Four generations of mice were included in a complex design involving free feeding trials, reproduction studies, voluntary intake of food and MSG, and histopathological evaluation of brain tissue.

Data were presented predominantly as histograms, tables, and line graphs; no statistical analyses were reported. The median daily intakes of MSG on a g/kg body weight basis were 1.5 and 6.0 g for males and 1.8 and 7.2 g for females on 1 percent and 4 percent diets, respectively. Intakes of dams increased progressively during lactation, averaging 18 to 20 g/day for the 3-week lactation period with a peak of 25 g/kg body weight/day during the last week. No differences were found for any of the parameters studied. Tests of behavior, blood concentrations of glutamate or related metabolites, or examination of organ size were not performed.

Frieder and Grimm (1984) examined the potential for damage to pups born of 3 dams orally challenged with MSG (10 g/kg body weight) for 14 consecutive days (starting on day 7 through day 20 of gestation). At birth, litter size was adjusted to 10 pups and comparisons were made between 30 MSG pups and 20 controls.

At 20 days of age, pups were tested for activity in an open field apparatus. The number of squares crossed and rearings were counted over 3-minute periods. Pups were separated from dams and weighed at day 28. At day 35, activity was measured again in a large open field with a start box; measures included latency of entrance from start box to open field, number of squares crossed, and number of rearings in 3-minute trials. At 60 days, the animals were given three different learning tests: discrimination learning (a successive discrimination learning task and a 6-unit simultaneous choice black–white discrimination maze) and an avoidance learning task (using foot-shock as the aversive stimulus). The discrimination task used water as the reward after 23-hour water deprivation.

Body weight of dams in different treatment groups did not differ; however, MSG offspring had significantly lower birth weights than controls. At day 28, MSG pups were significantly heavier than controls of both sexes. The weight difference disappeared by six months. At 35 days, MSG animals had significantly lower latency to enter the open field, squares crossed, and rearing than the controls. While no statistical differences were found in the no-choice successive discrimination task, in the 6-unit choice discrimination test, MSG-treated rats made significantly more errors than controls especially on the last 4 (out of 8) trials. There were no measures of organ weight or blood or brain concentrations of glutamate or related compounds.

No studies were found of the potential effect of oral challenges of L-glutamate on behavioral or cognitive development in prenatally exposed nonhuman primates. Likewise, the Expert Panel was unable to find any reports on the potential teratogenic effects of L-glutamate supplementation. The studies covered indicate a need for further investigation in these areas.
c. Human studies

There have been several studies that have used L-glutamate in specific clinical populations (Baertl et al., 1959; Davey, 1964; Eiseman et al., 1956; Iber et al., 1957; McDermott et al., 1955; and Thomassen et al., 1990). These studies will not be reviewed in detail as the focus is primarily on efficacy and they provide little information regarding the safety of oral supplementation with L-glutamate.

As a general caveat to the examination of studies using MSG as a glutamate challenge, the 2 most commonly used doses are 150 mg/kg body weight and 34 mg/kg body weight. According to Stegink et al. (1979c), the former represents the Acceptable Daily Intake (ADI) as established by the WHO/FAO, while the latter represents the concentration calculated to be above the 90th percentile of daily intake of MSG added to the diet.

Carlson et al. (1989) gave 10 g (about 150 mg/kg body weight) of L-glutamic acid (in gel capsules consumed with saline solution) to a group of healthy adult volunteers (age range 23 to 32 years) of both sexes. All subjects were medication-free, women were tested within first ten days of menstrual period, and none had a history of reactions to MSG. Measures included serum prolactin, growth hormone, and cortisol; serum samples from four subjects given glutamate were assayed for amino acid levels.

Oral supplementation with 10 g L-glutamate resulted in significant increases in serum concentrations of both prolactin (6.6 ng/ml at baseline versus peak at 1 hour of 12.9 ng/ml; n = 11) and cortisol (6.6 ng/ml at baseline versus peak of 12.6 ng/ml at 1 hour; n = 11). Serum growth hormone concentrations did not differ. Serum glutamate concentrations rose significantly over baseline (486 μmol/L over baseline concentrations peaking at 105 minutes post-challenge). No side effects or measures of any functional changes associated with the increases of prolactin or cortisol were reported nor was the possibility of a sex-dependent effect tested.

Factors affecting the fate of a large oral bolus of MSG in humans have been studied by Stegink et al. (1979c). They listed several factors that may affect the biological response to glutamate challenge including: 1) biological variation (studies with relatively few subjects have reported large ranges in plasma response to challenge); 2) challenge vehicle (plasma glutamate concentrations of 580 and 160 μmol/L were reported after a dose of 100 mg/kg body weight MSG in water versus gel capsules; consumption of tomato juice resulted in concentrations roughly between the two); 3) meal composition (increases in plasma glutamate were attenuated in subjects given MSG with a meal; peak plasma glutamate level of 105 μmol/L after 150 mg/kg body weight dose of MSG plus an enteral formula); and, 4) carbohydrate content of the challenge vehicle (addition of sucrose to a 100 mg/kg body weight challenge resulted in significantly lower plasma glutamate concentrations compared with challenge plus water in a single individual). The focus of these reports was on the metabolic fate of oral MSG. Stegink et al. (1979c) did not discuss safety, functional changes, or any side effects in these studies.

In the early 1970s, a series of studies examined the effects of oral doses of MSG. Bazzano et al. (1970) fed a chemically defined diet that included an amino acid formulation with L-glutamate as the sole source of nonessential nitrogen to 11 adult male subjects for periods ranging from 14 to 42 days. There was no placebo group; each subject's baseline values were used for comparisons. Actual intakes were not reported. No changes in neurologic or hepatic function were found, nor were any data or indications of measures used for these determinations provided. They did find a significant reduction in serum cholesterol and β-lipoproteins (25 to 40 percent of control concentrations). In the same paper, Bazzano et al. (1970) reported that supplements of glutamate of up to 100 g/day to "ordinary" diets (number or description of subjects not given) were ineffective in lowering the serum lipids, but were not associated with changes in weight, irritability, appetite, or mentation.
Rosenblum et al. (1971) orally challenged 4 groups of adult males after a 15-hour fast with 5 g of MSG dissolved in either water or chicken stock. Subjects were observed for one hour after the challenge and administered a structured questionnaire about somatic complaints. This trial was followed by another in which comparisons were made between subjects receiving either chicken stock with (1.7 g designed to be osmotically matched with 5 g MSG; n = 24) or without sodium chloride (n = 25). Subjects were observed and questioned in a similar manner as in the first study. In the next phase, 11 subjects were randomly selected from the same pool and given either 8 g MSG (n = 6) or 2.8 g sodium chloride (n = 5) in chicken stock, or chicken stock alone. In this latter study, pre- and post-challenge blood pressure and radial pulse were taken along with blood samples. This protocol was repeated two weeks later in 10 of the 11 subjects at a dose of 12 g MSG (n = 5) versus 4.2 g sodium chloride (n = 5). One subject dropped out because of perceived ill effects from the 8-g MSG dose.

While there was overlap in reported effects, there was a significantly greater incidence of complaints in subjects receiving MSG than controls. Light-headedness and tightness in the face appeared with a significantly greater frequency in MSG than controls. Comparisons between the group receiving 8 or 12 g MSG and those receiving 2.8 or 4.2 g sodium chloride revealed no differences in the clinical parameters measured.

Kenney and Tidball (1972) reported that out of 77 subjects challenged with 5 g of MSG given in tomato juice, 25 (32 percent) reported somatic complaints including warmth or burning, stiffness or tightness, weakness in the limbs, pressure, tingling, headache, light-headedness, heartburn, or gastric discomfort. A significant difference in the frequency of occurrence of symptoms was seen between males and females (females > males). Based on the self-selection of the pre-challenge meal, subjects were divided into four groups: no breakfast, liquid breakfast (coffee, tea, milk, or juice only), largely carbohydrate breakfast (toast, muffin, etc.), or largely protein breakfast (eggs, breakfast meats, etc.). Analysis based on breakfast type (eaten 2 hours prior to challenge) revealed no differences in blood glutamate concentrations (based on samples taken 30 to 40 minutes post-challenge) between placebo and MSG challenge.

In a follow-up to the first phase, 22 of the 25 identified responders were recruited for a double-blind 3-day protocol during which they were given, in random order, either 150 ml of tomato juice with sodium chloride (0.7 or 0.8 g sodium chloride) or MSG (1, 2, 3, or 4 g). Approximately 80 percent of these "responders" responded again to the MSG challenge.

Kenney and Tidball (1972) observed that the nature of these responses could be categorized into three types differing in dose dependency. Type 1 was associated with heartburn-gastric discomfort, weakness in limbs, and light-headedness (no dose dependency). Type 2 symptoms included stiffness-tightness with a high degree of dose dependency. Type 3 was associated with pressure sensations, warmth and burning, tingling, and headache. All of these symptoms did not occur at low levels, but occurred beyond an unspecified threshold in a dose-dependent manner.

Kenney (1979) conducted two additional clinical trials. In the first trial, a double-blind crossover study, 51 subjects were randomly assigned to schedules of 3 challenges, placebo and 2 tomato juice drinks with 5 g MSG. All subjects ate the same breakfast at 7 a.m. followed by the challenge at 10 a.m. and a standardized questionnaire. Subjects were asked to classify reactions according to three categories representing: no reaction (O), headache, thirst, light-headedness, or gastric discomfort (A), and warmth or burning, stiffness or tightness, weakness in the limbs, or tingling (B).

Of the original 51 subjects, 47 (24 men, 23 women) completed the study. There were 11 confirmed responders and 11 nonresponders. Of these, 9 nonreactors and 7 reactors participated in a second phase in which subjects were randomly assigned sequences of placebo (tomato juice plus 0.8 g sodium chloride) or MSG challenges (1 to 5 g MSG) over the course of 9 visits, each preceded by the same breakfast as above. In addition to the questionnaire, subjects were tested for fine tremor of the hand and visual acuity. This study confirmed the classification of the two groups and a dose-
response relationship in the reactor group. There were no significant changes in hand tremor, visual acuity, or blood chemistry in response to the challenges.

In a separate study, Kenney (1979) recruited 57 (22 male, 35 female) new subjects and used a different challenge vehicle, a specially designed soft drink, taste-tested to conceal the MSG/sodium chloride challenge/placebo. On two 2-day sessions, subjects received either placebo or 6 g MSG without the breakfast used in the previous studies. Subjects then returned to normal work and were asked at one hour post-challenge to respond to questions about the drink.

Forty-eight subjects completed the full protocol. There were two groups of responders, those that reacted to both placebo and MSG, and those that reacted only to MSG; the groups corresponded to the type A and B in the earlier report. Sixteen (15 females and 1 male) subjects were designated responders (type B) and recruited for a follow-up study in which similar dose-related responses were observed. In closer studies of responders, no observable objective clinical signs, e.g., changes in blood pressure or electrocardiogram, could be associated with the subjects' reported sensations.

In a double-blind trial, Zanda et al. (1973) gave a lunch of 150 ml beef bouillon with or without 3 g MSG on consecutive days to 73 healthy adult volunteers (38 males, 35 females). Subjects were queried about the presence of symptoms; blood pressure and pulse rate were measured at designated times by physicians blinded to treatment.

Overlap was seen in reported responses between control and MSG challenges. No significant differences between control and MSG challenges were found in any clinical parameter measured, except that females given MSG experienced significantly more headaches. Further, there were more females than males reporting at least one symptom (irrespective of challenge condition). Some symptoms were reported more frequently; for example, the symptom "fainting feeling" was reported by 2 females during the placebo period while 8 subjects (6 females, 2 males) reported it during the MSG challenge. Seven subjects (4 females, 3 males) registered complaints at both sessions, compared with 3 males and 13 females making complaints during the placebo challenge. During the MSG session, a total of 30 complaints from 6 males and 24 females were documented. The combined total of subject complaints during placebo and combined sessions for all subjects was 23 compared with 30 (24 females, 6 males) subject complaints during the MSG challenge session. No analysis of variance was performed to detect potential interactions, nor was there any control or documentation of pre-challenge intakes.

Ghezzi et al. (1980), in studies similar to those reported by Stegink et al. (1979c), compared response of blood glutamate to boluses of MSG given in different doses (30, 60, 120 mg/kg body weight; total dose of 1.9, 3.7, and 7.4 g) and in different vehicles (bouillon versus tomato juice). Subjects were 109 healthy volunteers (49 female, 60 male). In addition, glutamate concentrations were measured in 5 subjects after a normal meal compared with 5 subjects challenged with 3.7 g MSG in the same meal.

The AUC of plasma glutamate was found to be lower in females than in males and lower when MSG was given with tomato juice compared with bouillon. No differences were found in plasma glutamate when MSG was given with a meal versus meal alone. Thirty-three percent of the subjects experienced side effects (not broken down by dose or route of administration). Symptoms included "headache, gastric acidity, nausea, etc." While there were differences in peak concentrations or AUC between subjects with or without symptoms, multiple regression analyses revealed no association between plasma glutamate or AUC and age, weight, cigarette, coffee, or tea consumption.

There have been several reports of an association between asthma and MSG that employed normal control subjects, thereby supplying useful information about glutamate safety. Moneret-Vautrin (1987) gave 2.5 g of MSG (in capsules) to 6 controls (3 males, 3 females) and 30 asthmatic patients (14 males, 16 females) in a single-blind challenge protocol (day 1 placebo followed by MSG on
day 2). All subjects were drug free for at least 3 days prior to the protocol. The challenge was given at 8 a.m. without food after an overnight fast. Midday and evening meals were not controlled. Peak flow rate (a measure of respiratory capacity) was followed hourly from 8 a.m. to 10 p.m. No side effects or variations in peak flow rates were reported for the controls. Two asthmatic patients were reported to have presented with mild bronchospasm, occurring 6 to 10 hours after the MSG challenge. No other side effects were reported. The results were confounded by the presence of concurrent allergies in many patients. There was no crossover period for comparisons. These authors note in their discussion that daily consumption of MSG in European diets does not typically exceed 350 mg.

d. Summary and conclusions:

Endpoints: Animal studies have primarily focused on the impact of the sodium salt of L-glutamate on neurological function and neuronal histology. There continues to be controversy regarding the species specificity of susceptibility to toxic effects of glutamate. Rodents have been shown consistently to be susceptible to short-term and long-term effects of oral glutamate. Documented problems include developmental changes both in terms of brain lesions and adverse behavioral outcomes. Brain areas that have received the most attention have been the hypothalamus, specifically the arcuate nucleus and other areas generally referred to as circumventricular organs that lack the protection of a blood–brain barrier. Functional parameters that have been studied have generally been related to the hypothalamic–pituitary adrenal axis and include hormone secretion, growth, reproductive performance, and appetite regulation. There has been an inability to consistently replicate similar functional and histological changes in nonhuman primates exposed to high concentrations of oral glutamate.

There have been few studies of the safety of oral supplements of L-glutamic acid in humans. Early trials with large doses of MSG have consistently identified subgroups of responders; however, questions about challenge protocol and experimental methods and analysis continue to cloud the issue about safety of glutamate as a food additive. The studies demonstrating a response used large amounts in bolus form, thereby providing useful data about L-glutamic acid as a dietary supplement. A single 10-g bolus of glutamic acid given to normal healthy adult subjects resulted in significant elevations (twice baseline) in prolactin and cortisol. In another placebo controlled trial with variable doses of MSG given in tomato juice or novel carbonated beverage, somatic complaints were consistently documented in 33 percent of males and 50 percent of females in a dose-dependent manner. There were no biochemical correlates observed. There have been no studies on the impact of oral dietary supplements of L-glutamic acid on appetite, growth, or development in humans.

Safe levels of human intake: There is insufficient evidence to determine safe concentrations of dietary supplements of L-glutamic acid in the diet of normal healthy humans. Persons consuming 100 g protein per day consume about 8 g glutamic acid. Factors that should be considered in the evaluation of studies of glutamic acid safety in humans include challenge vehicle, fed versus fasted state, sex, and vitamin B6 status. The continuing controversy over the potential effects of glutamate on growth and development of neonatal animal models suggests that it is prudent to avoid the use of dietary supplements of L-glutamic acid by pregnant women, infants, and children. The existence of evidence of potential endocrine responses, i.e., elevated cortisol and prolactin, and differential responses between males and females, would also suggest a neuroendocrine link and that supplemental L-glutamic acid should be avoided by women of childbearing age and individuals with affective disorders.
6. Glycine

a. Background

Glycine is the only amino acid that does not have an asymmetric carbon atom and, thus, the only one that does not exhibit optical activity, i.e., no D- and L-isomers. The metabolism of glycine is closely linked to that of L-serine. Both amino acids are sources of methyl groups for formation of N5, N10-methylenetetrahydrofolate which plays a central role in one-carbon metabolism. The major pathway for glycine cleavage involves its conversion to CO2, NH4+, and N5, N10-methylenetetrahydrofolate, a reaction catalyzed by the PLP-dependent glycine cleavage complex in liver mitochondria. Glycine is also readily converted to L-serine by a freely reversible reaction catalyzed by serine hydroxymethyltransferase, an enzyme also requiring PLP. Methylene-tetrahydrofolate acts as the one-carbon donor for this conversion. The L-serine generated by this reaction may then form pyruvate (Rodwell, 1990a).

Glycine participates in the biosynthesis of heme, purines, creatine, and glycine conjugates such as glycocholic acid and hippuric acid (Rodwell, 1990c). It is an inhibitory neurotransmitter in many parts of the CNS including the retina and spinal cord (Pycock and Kerwin, 1981). Both ammonia and formate which may be generated in the glycine cleavage system are also known to produce ocular toxicity (Martin-Amat et al., 1978).

b. Animal studies

Changes in food intake and body weight: Growth depression in rats and chicks has been reported in many studies with feeding of diets containing as much as 10 percent glycine, with the degree of growth depression dependent on the amino acid composition and protein content of the diet. See review of Harper et al. (1970). A dose-response relationship between level of glycine addition and growth depression in rats was reported with incremental additions of glycine to a low-protein diet (Benevenga and Harper, 1967). Deficiencies of the B vitamins increased the severity of growth depression; for example, rats given diets deficient in the B vitamins, but not those given adequate amounts of the vitamins, showed deleterious effects when given as much as 400 mg glycine daily by stomach tube (Pagé and Gingras, 1946).

Addition of graded levels of glycine (up to 10 percent) to a 10 percent casein diet resulted in progressively decreased growth rates above the 3 percent level of addition; with 10 percent glycine, no weight gain occurred in 3 weeks. Addition of 5 percent glycine reduced food intake by 25 percent and weight gain by 47 percent. The amount of protein per liver (but not liver weight, DNA, or RNA content) decreased significantly (Muramatsu et al., 1971). Although adverse effects were reported in rats given other amino acids, none were described in rats given glycine supplements.

Food intake, liver weight, and body weight gain were decreased in male Donryu rats fed a 10 percent casein diet supplemented with 7 percent glycine for 14 days (Takeuchi et al., 1973). Supplementation of a 25 percent casein diet with 1 percent glycine reduced nitrogen retention but not body weight gain in male ARS Sprague-Dawley rats traumatized by hind leg fracture compared with traumatized rats fed the 25 percent casein diet without an amino acid supplement (Pui and Fisher, 1979).

Evidence for and against a role of oxalate produced from excess glycine in the growth depression has been reported; a role of labile methyl groups in the metabolism of excess glycine has also been suggested (Harper et al., 1970).

Biochemical studies: Plasma and urinary amino acids and liver enzyme activities were measured 2 hours postprandially in male weanling rats of the Donryu strain fed diets containing 10 percent casein or 10 percent casein plus 7 percent glycine for 14 days (Takeuchi et al., 1973). Serum glycine
in the glycine-fed animals was about 9 times that of controls; differences in concentrations of other amino acids were much smaller, 0.5 to 2 times the concentrations in the control animals. Urea excretion was significantly elevated and urinary free glycine (μg/g glycine consumed) was approximately doubled in the glycine-supplemented group. Activities of serine dehydratase and AST but not ALT were significantly higher in the glycine-supplemented group. Additional work by these investigators indicated that concentrations of plasma, liver, and muscle glycine were elevated above control levels for as long as 24 hours without food. Serine concentrations were also elevated in both groups 24 hours postprandially (Takeuchi et al., 1981).

**Endocrine studies:** Intraperitoneal injection of 200 mg glycine elevated serum LH and estradiol concentrations in adult female Wistar rats during diestrus. Lower doses (50 and 100 mg) did not have this effect. Serum concentration of FSH was not affected by glycine administration at any of the 3 doses (Morishita et al., 1981).

**Carcinogenicity studies:** Chronic administration of glycine (2.5 mmoles glycine in 0.9 percent sodium chloride/100 g body weight/day given intraperitoneally 5 days per week for 20 weeks to 9 male Fischer 344 rats) resulted in a 44 percent increased incidence of hyperplastic, preneoplastic, and neoplastic bladder lesions (cancer 1/9; papillomas 3/9; hyperplasia 1/9) (Vasudevan et al., 1987). Description of a control group and incidence of lesions in the control was not included in this article. Within 6 hours of administration of this dose of glycine, orotic acid excretion increased 100-fold and was considered by the authors to be responsible for the increase in bladder lesions. The Expert Panel was not aware of this observation being confirmed with a larger number of animals or appropriate control groups.

**Functional assessments and gross pathology:** While CNS effects were not reported in feeding studies, such effects have been reported consistently in studies in which glycine was injected by various routes. Microinjection of 3 to 30 ng of glycine directly into the rat nucleus tractus solitarius resulted in a dose-dependent increase in blood pressure and heart rate (Kubo and Kihara, 1990). A single intracisternal injection of 10 μmol (750 μg) glycine decreased arterial blood pressure and heart rate transiently in conscious normotensive control male Wistar rats; larger decreases were observed in renovascular hypertensive rats (Takemoto, 1991).

Intrathecal injection of glycine produced dose-related abnormalities in movements in mice. Injection of less than 5 μg did not elicit motor activity; doses larger than 55 μg produced hindlimb weakness, and doses between 100 and 500 μg resulted in hindlimb paralysis (Larson, 1989). Little experimental detail was given in this study. The mode of administration may not be relevant to oral administration.

Intravenous infusion of glycine in amounts comparable to amounts absorbed in humans during transurethral resection of the prostate (1 g/kg body weight given over 20 minutes) resulted in alterations in amplitude and latency in the principal waveform components of the visual evoked potential in 10 mongrel dogs (Wang et al., 1985). The animals in this study also demonstrated hemodynamic changes (increased cardiac output and decreased systemic vascular resistance and mean arterial pressure) immediately after glycine infusion. The responses were associated with increased blood levels of glycine and ammonia, but not of formate (Wang et al., 1985).

Inhibition of ganglion cells in the retina and suppression of the waveforms of the electroretinogram and visual evoked potential have also been demonstrated following injection of 2 or 5 mg glycine into the eyes of rabbits (Korol, 1973).
c. Human studies

Changes in food intake and body weight: In studies of the amino acid requirements of humans, Rose et al. (1954) fed diets constant in nitrogen content (10 g/day) and containing varying amounts of the indispensable amino acid under study plus 9 to 31 g/day of glycine to supply additional nitrogen. For comparison, daily glycine intake would be about 7 or 8 g for an adult consuming 100 g protein (see Table 2). Diets were fed to healthy young men for 1 or more 6-day periods. Over the course of 12 years, about 231 men participated in the experiments. The only adverse effects reported were decreased appetite and increased irritability in the presence of strongly negative nitrogen balance, which was attributed to a deficiency of the amino acid under study (Rose et al., 1955d).

ORS containing 60 mmol sodium/L, a glycine supplement (60 or 110 mmol glycine/L, [4.5 or 8.25 g/L]), and a total of 140 mmol glucose equivalents/L (glucose or glucose plus glycine) were tested for treatment of diarrheal dehydration in children (Vesikari and Isolauri, 1986). With use of the ORS containing the higher level of glycine, patients had poor weight gain and increased stool and urine volumes after rehydration. The authors concluded that excess glycine in relation to sodium in an ORS may lead to osmotic diarrhea and a high amount of absorbed glycine may result in osmotic diuresis.

Biochemical studies: Oral ingestion of 25 g glycine by 2 healthy adult men resulted in a 71 percent increase in average uric acid output and a 47 percent increase in average uric acid clearance; only small increases (about 15 percent) were observed in blood uric acid concentrations (Friedman, 1947). The subjects had been on a "purine-free" diet for 48 hours before ingesting glycine but it was not clear whether they were fasting on the morning of ingestion. Similarly, uric acid excretion was increased in 1 healthy male subject who ingested 10 g glycine following an overnight fast (Christman and Mosier, 1929). The authors made no mention of occurrence of adverse effects following glycine ingestion in either study.

Plasma glycine concentrations increased less in 24 fasting OCA users than in 14 fasting nonuser control subjects following an oral glycine load (140 mg/kg body weight or 7 g for a 50-kg female). Blood pyruvate concentrations were not affected by the glycine load in either group (Rose et al., 1976). Using the same loading dose, Craft et al. (1970) reported that increases in total α-amino nitrogen were less in OCA users than in nonusers.

Lithium treatment of patients with bipolar affective disorders has been associated with elevated glycine concentrations in erythrocytes in the presence of normal plasma concentrations (Rosenblatt et al., 1979, 1982). Possible effects of large intakes of glycine in patients treated with lithium have not been examined.

Endocrine studies: Glycine administered orally (250 ml of a 0.3 M solution [5.6 g glycine]) to 19 normal adults resulted in a significant increase in serum concentrations of growth hormone but did not affect prolactin, immunoreactive insulin, or blood sugar concentrations. Intraduodenal administration resulted in a much greater increase in serum growth hormone than oral administration (Kasai et al., 1978). Intravenous infusion of 4, 8, or 12 g of glycine over a 30-minute period in 25 fasting normal subjects (13 males and 12 females) 18 to 46 years of age resulted in a dose-dependent increase in serum concentrations of growth hormone. Blood glucose concentration was transiently increased after intravenous infusion of 12 g glycine (Kasai et al., 1980). The investigators reported that no adverse effects of glycine administration occurred during these experiments.

Intravenous infusion of 250 mg glycine/kg body weight (e.g., 7.5 g glycine in a 30-kg child) resulted in release of growth hormone in 22 normal children 6 to 15 years of age but not in 22 children with impaired pituitary function (Florea et al., 1976). Apparently no untoward effects occurred as the investigators proposed the use of a glycine load as an innocuous procedure for the detection of growth hormone deficiency in children.
**Functional assessments:** Barbeau (1974) reported some diminution of spasticity and increased mobility with no toxicity symptoms when 1 g/day glycine was orally administered in 4 divided doses for at least 6 months to 10 patients with spasticity. Similarly, Stern and Bokonjic (1974) reported an improvement in spasticity and mobility with no deleterious side effects in a study of oral administration of 3 to 4 g glycine daily in 3 divided doses to 7 patients with spasticity for 5 to 10 weeks. These studies did not include a control group or placebo treatment.

CNS symptoms such as nausea, transient blindness or other visual impairment, and disturbed consciousness have been reported with use of 1.5 or 2.2 percent glycine solutions as irrigating fluids in patients after transurethral resection of the prostate (Creel et al., 1987; Hahn et al., 1988; Mizutani et al., 1990; Wang et al., 1989), in 1 patient after percutaneous ultrasonic lithotripsy (Sinclair et al., 1985), and in 1 patient undergoing knee arthroscopy (Burkhart et al., 1990).

Adverse effects of use of glycine irrigant solution have been investigated in patients undergoing transurethral resection. Data of Mizutani et al. (1990) indicated that patients with and without changes in visual acuity had elevated concentrations of serum glycine; however, differences between the groups were not significant, possibly because of the small number of patients studied and the great variability in serum glycine concentrations observed. Creel et al. (1987) and Wang et al. (1989) reported that only patients with serum glycine concentrations greater than 4000 µmol/L (300 mg/L) complained of visual impairment and demonstrated loss of oscillatory potentials from electroretinograms and cessation of 30–hertz flicker—following.

Both glycine and serine concentrations were increased in plasma and muscle of patients following transurethral resection of the prostate (Hahn, 1988; Norlén et al., 1990). Changes in plasma and muscle free amino acid and electrolyte concentrations and in total, intracellular, and extracellular water were measured (Hahn, 1988; Mizutani et al., 1990; Norlén et al., 1990); however, the contributions of metabolic effects of glycine infusion and catabolic effects of the surgical trauma on these biochemical changes have not been separated.

Hyponatremia and increased urinary excretion of oxalate and glycolate in 3 of 34 patients receiving glycine irrigant solutions during transurethral prostate resection was reported by Fitzpatrick et al. (1981). Increased oxalate excretion was also reported in 2 of 15 patients by Malone et al. (1986), but it was not associated with hyponatremia. Decreased serum sodium concentration also occurred in the patients studied by Wang et al. (1989). Low sodium concentrations were not thought to be associated with visual disturbances as this effect appeared to be independent of low serum sodium in experimental animals (Wang et al., 1989). Plasma concentrations of formate in dogs were not affected by glycine infusion (Wang et al., 1985), and concentrations of formate were not measured in the human study.

Hahn (1988) reported that patients with CNS symptoms following transurethral resection absorbed significantly larger volumes of the irrigant fluid and had a greater decrease in serum sodium concentration than patients with no symptoms; however, symptoms did not coincide with peak concentrations of glycine and serine but rather with increased concentrations of the other dispensable amino acids. This investigator calculated that CNS symptoms developed in cases where the amount of glycine transferred exceeded 0.5 g/kg body weight.

In a subsequent study, intravenous infusion of 1 L of 2.2 percent glycine solution (22 g glycine) over a 20–minute period did not result in greater excretion of oxalate than infusion of a 5 percent mannitol solution in 4 healthy fasting male volunteers (Hahn, 1989). The total volume infused was not as great as the volume of irrigant that may be absorbed during surgery but, for ethical reasons, was the largest volume that could be given. In 9 patients in which 2.2 or 1.5 percent glycine irrigating solutions were used, urinary excretion of oxalate was not correlated with volume of irrigant absorbed. In the 1 patient in this study who developed a severe reaction (hypotension, severe confusion, and stupor), urinary oxalate concentration was very low, but the excretion rate was higher in the first 2 hours after surgery than in the other patients (Hahn, 1989). These data would suggest that the pattern of oxalate excretion was not indicative of hyperoxaluria.
Complement conversion was found, but antibodies and circulating immune complexes were not detected in 1 patient who had an anaphylactic reaction after 23 cystoscopic procedures with a glycine irrigating fluid for bladder carcinoma. This finding led to investigation of immunologic reactions in 6 patients who had also had cystoscopic procedures with a glycine irrigating fluid (Moskovits et al., 1987). In 1 of the 6 patients, complement levels were increased and complement conversion decreased in response to glycine and to normal saline. The significance of the complement conversion in these patients was not clear.

**Inborn errors of metabolism:** The investigation of nonketotic hyperglycinemia, a rare autosomal recessive disorder, may provide some insight into the consequences of high concentrations of glycine in plasma and particularly in CSF during fetal development, infancy, and childhood. Most infants born with this disorder die in early infancy. Neurologic abnormalities including spastic cerebral palsy, opisthotonos, exaggerated deep tendon reflexes, seizures, and mental retardation occur in those who survive beyond the neonatal period. Glycine concentrations in patients with this condition have ranged from about 800 to 1200 μmol/L (normal 243 μmol/L or 18.2 mg/L) in plasma and about 90 to 360 μmol/L (normal <13 μmol/L or 1 mg/L) in CSF; the ratio of CSF to plasma concentration of glycine is very high (Nyhan, 1989).

**d. Summary and conclusions**

**Endpoints:** Various clinical, biochemical, neurological, and organ system endpoints have been monitored in several studies with administration of glycine. However, there has been no systematic evaluation of dose–related responses to orally administered glycine. It should be noted that glycine loading via intravenous infusion (250 mg/kg body weight) has been investigated as a clinical procedure for detection of growth hormone deficiency in children. In addition, several clinical investigators have reported adverse effects of glycine solutions (up to 2.2 percent glycine) used as surgical irrigating solutions. These effects included transient visual disturbances and disturbed consciousness.

**Safe levels of human intake:** While doses of up to 250 mg/kg body weight given intravenously to children and oral doses up to 30 g/day in adults are reported to be without adverse effects in some studies, other studies have reported that oral administration of 14 to 30 g of glycine resulted in nausea and loose stools. Several studies in which glycine containing irrigant solutions were used during surgery reported functional visual disturbances and other central nervous system effects at levels of absorption (calculated) above 0.5 g/kg body weight. The significance of these observations to oral ingestion of glycine is unclear, although animal studies have indicated that glycine inhibits neurotransmission in the central nervous system, including the retina and spinal cord.

A person consuming 100 g protein daily would ingest about 7.6 g glycine (see Table 2). While there are few data that suggest intakes of glycine to be unsafe at levels equivalent to those that might occur from ingestion of dietary protein, data from animal and human studies suggest the possibility of central nervous system effects from ingestion of glycine. In the absence of a systematic evaluation of the effects of administration of glycine, it is not possible to estimate a maximal safe level of oral intake of glycine taken in addition to amounts consumed in foods.
7. **Proline and hydroxyproline**

   a. **Background**

   Proline and hydroxyproline are nutritionally dispensable imino acids found in large amounts in collagen. Although hydroxyproline occurs in the preformed state in food proteins, it is not incorporated into tissue proteins. Instead, dietary proline is incorporated into nascent proteins and is subsequently hydroxylated to form hydroxyproline (Rodwell, 1990d).

   Proline can be formed from glutamic acid. Reversal of the pathway results in its conversion to glutamic acid with subsequent catabolism identical to that of glutamic acid (Rodwell, 1990d). (See section on glutamic acid.)

   A neurotransmitter function has been postulated for L-proline (Hauptmann et al., 1983; Johnson and Roberts, 1984). This hypothesis was based on the observation that mechanisms of high affinity uptake and release of L-proline in rat brain slices and synaptosomes are similar to those for amino acid neurotransmitters (GABA, aspartate, and glutamate).

   b. **Animal studies**

   **Changes in food intake and body weight:** Addition of 5 percent L-proline to a low–protein diet (Sauberlich, 1961) or to an adequate protein diet (Hier et al., 1944) resulted in slight growth depression in rats. However, addition of 3 percent proline or 4 percent hydroxyproline to a 14 percent casein diet did not depress growth in rats (Abernathy and Miller, 1965). Similarly, addition of 3 percent proline to an amino acid diet devoid of arginine, proline, hydroxyproline, and glutamic acid resulted in normal growth in rats, but addition of 3.4 percent proline to this amino acid diet inhibited growth (Womack and Rose, 1947). Addition of 4 percent proline did not depress growth of chicks (Harper et al., 1970). Data on other parameters that could be considered adverse effects were not reported in these studies.

   Both isomers of proline have been shown to support growth in rats (Kampel et al., 1990).

   **Biochemical studies:** Kampel et al. (1990) administered D– or L-proline in drinking water (mean dose 50 mg/kg body weight/day) to female Sprague Dawley rats for 1 month. In rats given L-proline, activities of enzymes indicative of liver damage (ALT, AST, γ–GT, LDH, HBDH [hydroxybutyrate dehydrogenase], and alkaline phosphatase) were not significantly different from control animals. The concentration of serum creatinine was increased significantly but no difference was found in concentration of serum protein. In rats given D–proline, no D–proline was detected in serum or tissue homogenates. However, activities of all of the serum enzymes listed above and serum creatinine were significantly increased. Serum protein concentration was not significantly different from controls.

   **Behavioral studies:** Proline concentrations in brain and plasma of genetically hyperprolinemic mice are 6 to 7 times and 10 times higher, respectively, than the proline levels in these tissues of control mice. Hyperprolinemic mice took a longer time to make an initial avoidance response to foot shock in a T-maze and required more trials to reach criterion (learning) of the avoidance response than control animals; however they learned shuttlebox avoidance more quickly than the controls (Baxter et al., 1985).

   Intraventricular injection of L-proline (10 μl of 300 or 600 mM solution in distilled water or saline solution, total dose 34 and 69 μg, respectively) produced an amnestic response in chicks; however, administration of the same concentrations of D-proline produced convulsions and death but only when the amino acid was injected in saline solution (Cherkin et al., 1978).
Functional assessments and gross pathology: Kampel et al. (1990) examined histological changes in female Sprague Dawley rats given D- or L-proline in drinking water for 1 month (mean dose 50 mg body weight/kg body weight/day). No histological changes were found in liver and kidney of animals given L-proline. In rats given D-proline, periportal fibrosis and hepatocellular necrosis of liver cells and severe tubular lesions in kidney were found.

Intraperitoneal administration of L-proline for 3 weeks (145 μmol, or about 17 mg, per 24 hours) resulted in hyperplasia, but not inflammation, of the bile duct epithelium of male rats (Modavi and Isseroff, 1984). Specifically, L-proline caused a significant increase in the area of the bile duct containing collagen but not in the wall area of the bile duct in comparison with control animals; however, these differences were significantly less compared with bile duct enlargement caused by implantation of the parasite Fasciola hepatica.

c. Human studies

Functional assessments: Based on the finding of hyperornithinemia and the presumed impaired de novo formation of proline associated with gyrate atrophy of the choroid and retina, Hayasaka et al. (1985) treated 4 patients having normal serum proline levels and ranging in age from 4 to 32 years with proline (isomer not specified). Daily doses ranged from 3 to 10 g (mode 3 g) and were given for 2 to 4 years. In 3 of the 4 patients proline was reported to minimize or halt the gyrate atrophy progression while producing, on average, a doubling of serum proline concentrations. No overt adverse effects were noted.

Inborn errors of metabolism: Two forms of inborn errors of proline metabolism in which plasma, CSF, and urinary concentrations of proline are elevated were described by Phang and Scriver (1989). Type I, in which the plasma proline value is often below 2000 μmol/L (normal value, 100 to 450 μmol/L), is associated with deficient proline dehydrogenase activity but is not necessarily linked with clinical manifestations. Type II, in which the plasma proline value is usually greater than 2000 μmol, is associated with deficient δ1-pyrroline-5-carboxylic acid dehydrogenase activity and with higher levels of proline in plasma, CSF, and urine; it remains uncertain whether this form is a benign metabolic disorder or whether it may predispose patients to neurologic manifestations (seizures and mental retardation) (Phang and Scriver, 1989).

Hyperhydroxyprolinemia, in which hydroxyproline catabolism is impaired, was considered a benign condition by Phang and Scriver (1989). Plasma hydroxyproline concentrations are elevated at least 10-fold (normal values <10 μmol/L), and urinary excretion of the free amino acid is increased. However, CSF concentrations of hydroxyproline are not elevated. Clinical manifestations of the conditions are heterogeneous, with retarded mental development reported in some patients and normal development in others (Phang and Scriver, 1989).

d. Summary and conclusions

Endpoints: In rats, D-proline administration has been associated with biochemical changes indicative of liver damage and with histopathological changes in liver and kidney. Administration of D-proline (50 mg/kg body weight) to rats for 1 month resulted in histopathological changes in liver and kidney and increased activities of enzymes indicative of liver damage. However L-proline did not produce these changes. With the exception of animal studies in which L-proline was added to the diet and one study of behavioral effects in hyperprolinemic hybrid mice fed a protein as the source of proline, there has been no further systematic investigation of the safety of L-proline or hydroxyproline. Proline, but not hydroxyproline, may be a neurotransmitter, suggesting that the two imino acids may not act in an identical manner. The safety of each of these compounds should be assessed separately.
Safe levels of human intake: Animal studies provide only limited data on the safety of orally ingested D-proline, L-proline, or hydroxyproline. In the only study found on administration of L-proline to humans (3 to 10 g daily for 2 to 4 years in 4 patients), no evidence was found for overt adverse effects. In comparison, a person consuming 100 g protein daily would consume about 5.6 g proline. The absence of data on the effects of administration of proline or hydroxyproline does not permit an informed estimate of the maximal safe level of oral intake of dietary supplements of proline or hydroxyproline taken in addition to amounts consumed in foods.
8. Serine

a. Background

L-Serine is a nutritionally dispensable amino acid that is synthesized from D-3-phosphoglycerate, an intermediate in the glycolytic pathway, or from glycine via the freely reversible serine hydroxymethyltransferase reaction. Methylene tetrahydrofolate provides the one-carbon unit for this reaction. L-Serine is the major source of methyl groups for formation of N⁶, N¹⁰-methylene tetrahydrofolate, which plays a key role in one-carbon metabolism (Rodwell, 1990a).

Because L-serine and glycine are so readily interconverted, the pathways for glycine metabolism apply to serine as well and the glycine cleavage system (with the potential production of ammonia and formate) should also be considered a possible pathway for metabolism of excess amounts of L-serine. (See section on glycine.) It is possible that adverse effects of consumption of large amounts of L- or DL-serine might result from conversion to glycine but this remains to be investigated.

b. Animal studies

Changes in food intake and body weight: Harper et al. (1970) summarized results of studies in which L-serine or the racemate were fed to experimental animals. Rats given 100 mg/day of L-serine by stomach tube for as long as 14 days showed a decrease in appetite but no adverse effects on body weight gain, urine composition, or mortality. Rats given as little as 10 mg/day of the racemate for 2 to 3 days showed adverse effects including decreased appetite, marked weight loss, reversible renal necrosis, polyuria, altered urine composition (free serine, sugar, protein, and ketoacids), and increased mortality (Artom et al., 1945; Morehead et al., 1945; Wachstein, 1947). Subcutaneous injection of a single dose of D-serine (50 mg/100 g body weight or 150 mg for rats weighing 300 g) or DL-serine (100 mg/100 g body weight or 300 mg for a 300-g rat) resulted in anorexia, weight loss, and hyperaminoaciduria in rats; injection of L-serine (50 mg/100 g body weight) did not produce adverse effects (Wise and Elwyn, 1966). More severe results were observed in rats fed diets low in protein and the B vitamins.

Consumption of DL-serine in the diet (2 percent in diets of rats and 5 percent in diets of chicks) did not result in reduced weight gain or death even though the amount consumed was greater than amounts administered by stomach tube in the experiments described above. However, addition of 4 to 6 percent DL-serine to low-protein diets did result in reduced weight gain in rats (Harper et al., 1970).

Addition of 5 percent L-serine to a 10 percent casein diet reduced food intake by 30 percent and weight gain by 50 percent in male weanling rats of the Donryu strain fed the diet for 3 weeks. Liver weight and DNA and RNA contents of the L-serine-supplemented rats were not decreased significantly from control animals fed the 10 percent casein diet but liver protein content (mg/liver) was significantly reduced.

Endocrine studies: Intravenous infusion of L-serine (1 mmol/kg body weight or about 1 g for a 10-kg dog) was reported to stimulate secretion of glucagon in dogs (Rocha, 1972).

Functional assessments and gross pathology: With intraperitoneal doses as little as 5 mg/100 g body weight, D-serine (given as 10 mg of the racemic mixture) caused a reversible selective diffuse necrosis of the proximal straight tubules of the rat kidney (Ganote et al., 1974; Morehead et al., 1946; Wachstein, 1947; Wachstein and Besen, 1964) and urinary excretion of protein, amino acids, and glucose (Carone and Ganote, 1975; Wise and Elwyn, 1966). These changes were not induced by D-serine in other laboratory animals (Kaltenbach et al., 1979; Morehead et al., 1946) and have not been associated with administration of L-serine.
A single intracisternal injection of 10 μmol (1 mg) of serine (isomer not specified) decreased arterial blood pressure and heart rate significantly in conscious normotensive control male Wistar rats (Takemoto, 1991). Larger decreases in these parameters were observed in renovascular hypertensive rats.

Injection of 1, 2, or 4 mg serine (isomer not specified) into the lateral cerebral ventricle of 16 rabbits resulted in a dose-related hypothermogenic effect when the animals were housed at cold temperatures but not at warmer temperatures. Development of pyrogen-induced fever was inhibited by the serine injection (Glyn and Lipton, 1980).

c. Human studies

Behavioral studies: Pepplinkhuizen et al. (1980) reported the recurrence of psychotic symptoms following administration of a single oral dose of serine (2 mmol/kg body weight or 210 mg/kg, about 15 g for a 70-kg person; isomer not specified) in 4 psychotic patients who had recovered from acute psychoses. No effects were reported in 4 healthy control subjects given the same dose of serine. The investigators reported that oral administration of 2 mmol glycine/kg body weight (150 mg/kg body weight or about 10.5 g for a 70-kg person) had no effect on the patients or controls. Only urinary excretion data for the amino acids were reported in this study and the criteria for behavioral assessment were not specified. In contrast, changes in symptoms of psychoses by self-assessment or a psychiatric rating scale were not observed following administration of L-serine (4 mmol/kg body weight or 420 mg/kg, 30 g for a 70-kg person) dissolved in milk to 12 psychotic patients and 10 control subjects (4 with major depression, 2 with hypomania, and 4 normal) (Wilcox et al., 1985). None of the subjects reported perceptual or cognitive psychiatric symptomatology following administration of the L-serine load. No changes were found in the Brief Psychiatric Rating Scale scores and no changes in psychiatric symptoms of the patients were reported by the clinical staff. In this study, baseline plasma levels of serine were significantly higher (203 and 131 mg/L in patients and controls, respectively) and the increase in plasma serine concentration following serine administration was reported to be significantly greater in patients (11 percent increase for patients and 8 percent for controls 2 hours after administration). Elimination of serine from plasma followed a bimodal pattern in the patients and was significantly different from the controls.

Concentrations of serine and glycine in plasma and CSF were similar in patients with schizophrenia and healthy control subjects (Perry and Hansen, 1985). Lithium treatment has been associated with increased glycine concentrations in erythrocytes but not in plasma of patients with bipolar affective disorders (Rosenblatt et al., 1979, 1982). Possible effects of large intakes of serine on glycine metabolism in patients receiving lithium treatment have not been examined.

d. Summary and conclusions

Endpoints: Animal studies suggest that D-serine and DL-serine administered by several routes affect growth and produce renal necrosis. L-Serine given to animals by routes other than oral administration has been associated with changes in blood pressure, thermal regulation, and glucagon secretion. However, human studies reported to date provide few data on possible adverse effects of orally administered L-serine. Available data are limited, and the range of possible endpoints has not been examined systematically to evaluate effects of chronic consumption of L-serine as a supplement to the typical human diet.

Safe levels of human intake: Information is lacking on the nephrotoxicity of D-serine in humans; however, demonstration of nephrotoxicity in rats gives cause for concern. No data have been reported that suggest intakes of L-serine at levels equivalent to those occurring in the proteins of a
typical adult diet would be unsafe. Data in 4 subjects suggest the possibility that L-serine can produce psychotic symptoms in recovering psychotic patients but confirming data are lacking. However, control subjects (including 4 patients with depression and 4 non-psychotic patients) did not demonstrate adverse effects with administration of a single 30-g dose of L-serine. No data on intakes of L-serine from a typical diet containing protein sources of L-serine plus supplements of L-serine have been found. An adult consuming 100 g protein daily would consume about 7.2 g serine. A single bolus of up to 30 g of L-serine appears to be tolerated but safety of ingestion of high doses of L-serine cannot be determined from the available data. Studies of possible behavioral and neurochemical effects of L-serine should be considered.
9. Tyrosine

a. Background

L-Tyrosine is the least soluble of the α-amino acids (0.453 g/L in water at 25°C) (Merck Index, 1983). Formation of L-tyrosine from L-phenylalanine occurs in liver by the action of phenylalanine hydroxylase and, to a very limited extent, in brain by a secondary action of tyrosine hydroxylase on L-phenylalanine (Abita et al., 1974; Katz et al., 1976). Because L-tyrosine can be synthesized from L-phenylalanine, it is considered a nutritionally dispensable amino acid except in cases where phenylalanine intake must be severely restricted (e.g., patients with hyperphenylalaninemia).

L-Tyrosine is classified as an LNAA. As such, it shares a transplanumal sodium-dependent active transport system in the gut with other neutral amino acids (Alpers, 1987; Hopfer, 1987). For uptake into other tissues including brain, L-tyrosine shares a carrier system (system L) with leucine, isoleucine, valine, phenylalanine, tryptophan, and methionine (Pardridge and Oldendorf, 1977; Skeie et al., 1990). The affinity of L-tyrosine for the transport system is higher than the affinities of some of the other LNAA (Pardridge and Oldendorf, 1977).

The concentrations of all of the LNAA in relation to the concentration of L-tyrosine determine its uptake into peripheral tissues. The plasma ratio of tyrosine:LNAA has been considered predictive of brain tyrosine concentration (Pardridge, 1979). At levels up to 150 g/day, the amount of protein in the diet has been shown to influence the levels of tyrosine and other neutral amino acids in plasma and the amplitudes of their diurnal variation (Fernstrom et al., 1979). High protein and high carbohydrate meals also influence the plasma ratios among the LNAA. For example, the tyrosine:LNAA ratio is low following a meal high in protein because plasma levels of the BCAA (leucine, isoleucine, and valine) are increased with high protein intake. In this situation, tyrosine is at a comparative disadvantage for uptake into brain. Conversely, after a meal high in carbohydrates, the tyrosine:LNAA ratio is high because the insulin secreted in response to the carbohydrates also promotes entry of the BCAA into peripheral tissues (Fernstrom and Faller, 1978). Thus, less competition for tyrosine uptake into brain from LNAA exists after a meal high in carbohydrate.

L-Tyrosine is a precursor for several biologically active substances including catecholamine neurotransmitters (epinephrine, norepinephrine, and dopamine), hormones (thyroxine, triiodothyronine, epinephrine, and norepinephrine), and melanin skin pigments (Rodwell, 1990a). Urinary excretion products of the catecholamines include homovanillic acid (HVA), methoxyhydroxy-phenylethyleneglycol (MOPEG), and vanillylmandelic acid (VMA). Although synthesis of the catecholamine neurotransmitters is dependent in part on plasma or tissue concentrations of tyrosine, the Expert Panel was not aware of studies showing whether synthesis of other biologically active compounds from L-tyrosine is dependent on tyrosine concentration.

L-Tyrosine may also be metabolized for energy and can enter both glucogenic and ketogenic pathways. The rate-limiting step in its degradation in the liver is conversion to p-hydroxyphenylpyruvate, catalyzed by tyrosine aminotransferase, which requires vitamin B6 as a cofactor (Benevenga and Steele, 1984). In a series of reactions including formation of homogentisic acid, L-tyrosine is further degraded to fumarate and acetoacetate before being metabolized for energy or used for fat or glycogen synthesis (Rodwell, 1990a).

L-Tyrosine can also be decarboxylated to form tyramine in liver microsomes (David et al., 1974; Lemberger et al., 1965) and by the bacterial flora in the gut (Fell et al., 1978). A possible role of tyramine and tyramine-containing foods has been investigated as a cause of migraine headaches but methodological differences among the studies make evaluation of the findings difficult. See review by Kohlenberg (1982).
D-Tyrosine has no known nutritional function and has been shown to inhibit growth of mice (Friedman and Gumbman, 1984).

b. Animal studies

Changes in food intake and body weight: Reviews of Harper et al. (1970) and Benevenga and Steele (1984) provide extensive documentation of the effects of feeding low-protein diets supplemented with L-tyrosine on food intake and growth depression in rats as well as on development of cataracts, skin lesions, and histopathological changes.

Weight loss occurred in rats fed 5 percent added L-tyrosine in a 10 percent casein diet and weight gain was only about 20 to 30 percent of control values in rats fed 3 percent added L-tyrosine in a 6 percent casein diet for 2 to 4 weeks (Boctor and Harper, 1968; Muramatsu et al., 1971; Sauberlich, 1961). A lessening in the severity of growth depression with lower levels of supplemental L-tyrosine in low-protein diets suggested that rats may adapt to these diets. Increasing the dietary protein content from 10 percent to 25 or 50 percent lessened or prevented growth depression even with high levels of tyrosine (Muramatsu et al., 1971, 1975) as did addition of certain limiting amino acids such as L-cysteine or L-threonine singly or together with L-methionine or L-tryptophan in the case of casein-based diets (Alam et al., 1966a; Datta and Ghosh 1977; Godin 1967; Muramatsu et al., 1976; Yamamoto et al., 1976).

Johnston et al. (1986) fed diets containing 20 percent casein supplemented with 4 percent L-tyrosine to young female lean and obese mice for up to 1 month. In the tyrosine-supplemented lean and obese animals, the plasma tyrosine and tyrosine:LNAA ratio remained elevated above the controls for the entire experimental period. Oxygen consumption, food intake, and final carcass energy density were not affected by consumption of the L-tyrosine-supplemented diet.

Feeding of a diet containing 20 percent casein plus 12 percent L-tyrosine added in replacement of carbohydrate did not suppress food intake or weight gain in male Wistar rats fed the diet for as long as 21 days (Nagaoka et al., 1986, 1990). No mention of development of cataracts or skin lesions was included in these reports.

Addition of 0.83 or 1.55 percent D-tyrosine to amino acid diets inhibited weight gain in mice fed these diets for 14 days. Growth inhibition of D-tyrosine was much more marked with diets containing a low, but ordinarily adequate, level of L-phenylalanine (Friedman and Gumbman, 1984).

Biochemical studies: Feeding of low-protein diets containing 3 or 5 percent L-tyrosine to rats resulted in elevated plasma tyrosine concentrations (1.5 to 3.0 mmol/L compared with a normal level of about 0.04 mmol/L) (Alam et al., 1966a; Rich et al., 1973; Yamamoto et al., 1977). Tyrosine concentrations in the eye were elevated to a greater extent (see p. 183) and levels in liver, muscle, and brain were elevated about 20 times higher than normal (Ip and Harper, 1975).

As summarized by Benevenga and Steele (1984) and Harper et al. (1970), administration of hormones that increased the activity of tyrosine aminotransferase have not shown consistent effects on levels of plasma tyrosine or on growth depression in rats fed an excess of L-tyrosine. Furthermore, feeding of diets containing 5 percent p-hydroxyphenylpyruvic acid, the product of this enzymatic reaction, did not result in development of eye and paw lesions (Boctor and Harper, 1968; Yamamoto and Muramatsu, 1982). Taken together, these observations suggest that intermediates generated in the main pathway of tyrosine catabolism may not be responsible for development of toxicity in rats when diets containing excess tyrosine are fed (Benevenga and Steele, 1984).
Harper et al. (1970) also reviewed work of several investigators which has shown that administration of thyroxine increases the activity of tyrosine transaminase but also produces greatly elevated levels of tyrosine in blood and increased severity of the pathologic eye and paw lesions in rats. L-Tyrosine administration has also been shown to increase thyroid uptake of iodine in rats fed a low-protein diet (Simon et al., 1962). Adrenalectomized and hyperthyroid rats were very sensitive to the pathologic effects of excess L-tyrosine. Administration of cortisol, which decreases plasma tyrosine concentration by increasing hepatic tyrosine transaminase activity (Rosen et al., 1963), prevented development of pathologic lesions in rats fed low-protein diets containing 3 percent L-tyrosine (Alam et al., 1967). Interrelationships between adrenal and thyroid hormones appear to be important in the regulation of tyrosine metabolism in humans as well as rats (Rivlin and Asper, 1966; Rivlin and Levine, 1963; Rivlin and Melmon, 1965) and were suggested as a factor in the development of tyrosine toxicity in rats (Harper et al., 1970).

Because of the limited solubility of L-tyrosine, it may be incompletely absorbed from the small intestine, leaving a portion of the dose to be decarboxylated to tyramine by the bacterial flora (Fell et al., 1978). Results of an early investigation suggested that effects of administration of excess L-tyrosine were not caused by its conversion to tyramine in that feeding of a diet containing 1 percent added tyramine did not result in pathologic lesions resembling tyrosine toxicity in rats (Harper et al., 1970). However, later work has suggested that conversion of L-tyrosine to tyramine may become a more important pathway with high doses of L-tyrosine. Quantitatively, most tyrosine decarboxylase activity is located in liver and kidney although a smaller amount is found in catecholaminergic neurons. It should be noted that tyramine has also been investigated as a causative agent for migraine headaches but results are not conclusive.

David et al. (1974) reported evidence suggesting that decarboxylation of L-tyrosine to tyramine became the predominant route of metabolism when plasma and tissue levels were elevated in MF1 mice. In these experiments the animals were pretreated with an antibiotic to minimize production of tyramine by the gut flora, an MAO inhibitor to prevent oxidative deamination of tyramine, and a decarboxylase inhibitor (for half of the animals only). A dose-related increase in the amount of tyramine produced in the total carcass after 6 hours was observed following oral administration of 0.5, 1.0, and 1.5 g/kg body weight L-tyrosine. At these doses, 25, 32, and 42 percent of the administered dose was metabolized to tyramine and 11.5 percent was metabolized by the tyrosine aminotransferase pathway in the absence of the decarboxylase inhibitor.

In further studies on hepatic metabolism of L-tyrosine given orally in lethal (LD50) doses to MF1 mice, David (1976a,b) provided evidence of involvement of the cytochrome P450-containing microsomal drug metabolizing system in production of a toxic metabolite of L-tyrosine. A marked reduction in 24-hour mortality (as shown by an increase in the LD50 from about 1.4 g/kg body weight to more than 3.5 g/kg given twice daily) was produced by pretreatment with inhibitors of the cytochrome P450 system (SKF525A or cobaltalox chloride) and potentiation of tyrosine lethality (a decrease in LD50 from 1.4 to 0.6 g/kg body weight given twice daily) was produced by pretreatment with substances that induce the cytochrome P450 system (3-methylcholanthrene or phenobarbital). Evidence for the production of a tyrosine epoxide or other electrophilic metabolite of tyrosine capable of reacting with glutathione was discussed (David 1976a,b). No additional studies on this metabolic pathway were found.

Diets containing 20 percent casein plus 12 percent L-tyrosine by weight were reported to lead to significantly increased plasma concentrations of total cholesterol and all cholesterol subfractions and hepatomegaly in male Wistar rats fed the diet for 7, 14, or 21 days (Nagaoa et al., 1986, 1990). These changes were amplified if animals were meal-fed and were reversible if animals were fed the basal diet for 7 days after discontinuation of the high L-tyrosine diet. Activity of hepatic 3-hydroxy-3-methylglutaryl CoA reductase and incorporation of [3H]0 into liver cholesterol were significantly increased in rats meal-fed the diet containing excess L-tyrosine for 7 days. Although the enzyme activity was 2 to 3 times higher in rats fed the diet containing excess L-tyrosine, liver microsomal cholesterol was not significantly different from control levels (Nagaoa et al., 1986).
Cytochrome P450 and \( b_c \) contents in liver were significantly increased by excess tyrosine feeding. Bile flow and biliary excretion of total bile acids, tyrosine, and taurocholic acid were significantly increased and the glycine:taurine conjugation ratio of cholic acid was significantly decreased. Fecal excretion of neutral steroids was not significantly altered by tyrosine feeding (Nagaoka et al., 1990). Taken together, these findings suggest that L-tyrosine stimulates hepatic synthesis of cholesterol. Whether this effect of L-tyrosine is mediated by catecholamines remains to be investigated.

**Neurotransmitter studies:** Tyrosine concentrations in brain have been shown to influence the amounts of catecholamines synthesized. Because tyrosine is not synthesized to any great extent in brain, tyrosine must be supplied by the systemic circulation (Gibson and Gelenberg, 1983). As shown by Wurtman et al. (1974) and Gibson and Wurtman (1977, 1978), the level of tyrosine in plasma influences tyrosine uptake by brain.

Relatively small increases in catecholamine concentrations were first demonstrated in whole rat brain following intraperitoneal injections of L-tyrosine (50 or 100 mg/kg body weight) (Gibson and Wurtman, 1977; Wurtman et al., 1974). Tyrosine levels were increased in all areas of rat brain but highest in cortex and hippocampus after intraperitoneal injection of 100 mg/kg body weight of L-tyrosine (Morre et al., 1980). Effects of other amino acids on synaptosomal uptake of tyrosine in several regions of brain have also been characterized (Morre and Wurtman, 1981).

Further studies investigated effects of L-tyrosine administration on catecholamine levels in conjunction with treatments or procedures increasing the firing rate of particular groups of catecholamine-containing neurons (Carlsson and Lindqvist, 1978; Fuller and Snoddy, 1980; Gibson and Wurtman 1978; Melamed et al., 1980a; Reinstein et al., 1984; Scally et al., 1977; Sved and Fernstrom, 1981; Sved et al., 1979a,b; Westerink and Wirix, 1983; Yamori et al., 1980). These included pretreatment with reserpine or haloperidol, procedures causing lesions of dopaminergic nigrostriatal neurons, or stresses such as an electric shock or a cold environment. Increased availability of tyrosine (doses of 25 to 50 mg/kg body weight L-tyrosine in rats) has been shown to stimulate synthesis and release of catecholamines in rapidly firing neurons but not in quiescent neurons. Light exposure, a physiological stimulus, has also been used to demonstrate tyrosine dependence of catecholamine synthesis in dopaminergic neurons in rat retina (Gibson et al., 1988).

Studies utilizing microdialysis techniques to measure changes in catecholamine concentrations in very small and well defined areas of the brain have shown that intraperitoneal administration of 50 to 200 mg/kg body weight of L-tyrosine induces a 50 to 100 percent increase in catecholamines in striatum and smaller increases in other defined areas of the rat brain. These findings have been interpreted as suggesting that the level of activity of a particular catecholaminergic neuron might influence the degree of responsiveness of that neuron to added L-tyrosine (Acworth et al., 1988; During et al., 1988; Milner and Wurtman, 1986).

Feeding of a diet containing 20 percent casein plus 4 percent L-tyrosine to young female lean and obese mice for 4 days resulted in a 2-fold increase in brain tyrosine concentration but had no effect on norepinephrine synthesis in whole brain or efflux of MOPEG from brain (Johnston et al., 1986). Tyrosine supplementation of this diet or a 40 percent casein diet had no effect on norepinephrine turnover rates in interscapular brown adipose tissue, heart, kidney, and brain despite a 2-fold increase in plasma and tissue tyrosine concentrations (Johnston and Balachandran, 1987).

**Behavioral studies:** Intraperitoneal injections of L-tyrosine (200 mg/kg body weight) in male Sprague-Dawley rats prevented reductions in norepinephrine induced by shock and the deficits in open-field locomotion and exploratory behavior (e.g., standing up on hind legs and hole-poking) usually associated with this type of acute, uncontrollable stress (Reinstein et al., 1984). Tyrosine concentrations in all brain regions studied increased by 60 to 100 percent in stressed and unstressed animals. Pretreatment with L-tyrosine prevented the stress-induced reductions in norepinephrine in the locus coerulceus and in hypothalamus and significantly elevated norepinephrine levels in hippocampus. Concentrations of MOPEG-SO\(_4\) were increased by stress in all brain regions studied.
Norepinephrine and MOPEG-SO₄ levels and behavioral measures were not affected by L-tyrosine treatment in unstressed animals. Essentially identical results were obtained in male Sprague-Dawley rats subjected to the same tests and treatments when the L-tyrosine was fed as a component of the diet for 4 days prior to testing (Lehnert et al., 1984). Total dietary L-tyrosine in this investigation was 3.68 percent (0.92 percent in protein and 2.76 percent added).

Intraperitoneal injection of 400 mg/kg body weight of L-tyrosine completely reversed the behavioral depression observed in control rats exposed to cold temperatures to accelerate firing frequency of catecholaminergic neurons (Rauch and Lieberman, 1990). Administration of L-tyrosine did not significantly affect the rate of deep body cooling during the cold exposure, a finding also reported in mice by Thurmond and Brown (1984).

Gibson et al. (1982a) reported that a single intraperitoneal injection of L-tyrosine (50, 100, 200, or 400 mg/kg) significantly decreased swim test immobility of young male Charles River CD-1 mice at the highest dose and markedly increased their activity in an open field–test at all doses, suggesting that L-tyrosine may have an activating effect on behavior. Whole brain catecholamine concentrations were not significantly increased by these treatments.

Thurmond and Brown (1984) demonstrated that L-tyrosine induced differences in stress–induced behavior and neurochemical changes in male "very old" CF–1 mice (30 months of age) compared with "young" or "aged" mice (3 and 22 months of age, respectively). When a 12 percent casein diet with 4 percent added L-tyrosine was fed for 2 weeks, the age–related decline in locomotor activity in unstressed mice but not in cold–swim stressed mice was alleviated. Although no significant age–related differences in inter–male aggression were observed in nonstressed mice fed the L-tyrosine–supplemented diet compared with animals fed the control diet, supplementation with L-tyrosine did not alleviate the age–related decline in aggressive activity in cold–swim stressed mice.

L-Tyrosine supplementation increased brain tyrosine concentrations markedly in young mice but only moderately in older animals. Brain tyrosine was reduced by stress in young animals but increased by stress in older animals, particularly in animals on L-tyrosine–supplemented diets. Brain norepinephrine and HVA concentrations in older mice were significantly less than in younger mice. L-Tyrosine supplementation decreased norepinephrine levels and increased HVA levels significantly. Brain norepinephrine concentration was reduced by stress but only to a significant extent in the young mice given L-tyrosine supplements. L-Tyrosine supplementation had no effect on the age–related decline in brain dopamine concentration or on DOPAC concentrations. Brain tryptophan concentrations were not affected by L-tyrosine supplementation but serotonin concentrations were decreased in young animals and increased in the very old animals given the supplement (Thurmond and Brown, 1984).

Endocrine studies: Intraperitoneal administration of L-tyrosine (200 mg/kg body weight) rapidly suppressed serum prolactin concentrations and raised levels of the dopamine metabolites HVA and DOPAC in the striatum and hypothalamus of rats made hyperprolactinemic by reserpine injection (Sved et al., 1979a). Hypothalamic dopamine has been shown to exert a powerful inhibitory effect on prolactin synthesis and secretion (Melmed, 1984).

Functional assessments and gross pathology: Feeding of low–protein diets containing 3 to 5 percent L-tyrosine to rats typically resulted in the formation of cataracts and lesions on the bottoms of the paws within 5 to 8 days (Benevenga and Steele, 1984). In one experiment describing development of these lesions, Martin (1943) also reported that blood pressure was elevated in rats fed an 18 percent casein diet supplemented with 5 or 10 percent L-tyrosine for 2 or 3 weeks.

Corneal changes in rats were apparent within 24 hours of the time a low–protein diet containing 5 percent tyrosine was begun. The initial changes were described by Beard et al. (1974) as dots which developed into "snow flake" opacity. At the cellular level, the "cellular architecture becomes distorted, the integrity of the tissue lessens, and the tissue is invaded by polymorphonuclear
leukocytes. The cornea thickens and becomes opaque" (Benevenga and Steele, 1984). Needle-shaped crystals similar to those of L-tyrosine have been observed moving among cells and disrupting membranes in lesioned areas of corneas of rats fed diets containing 5 percent L-tyrosine. However, the narrow width of the crystals prevented their positive identification as tyrosine. Crystalline tyrosine was also implicated as the mediator of corneal damage by elevated levels of tyrosine of 2.8 to 5.0 mmol/L in the aqueous humor and 4.7 to 6.0 mmol/L in the whole rat eye (Alam et al., 1966b; Rich et al., 1973), concentrations greater than 3.5 mmol/L, the maximum solubility of tyrosine in water at 35°C. Crystals were localized in damaged areas of the corneal epithelium and passed through cellular and nuclear membranes (Gipson et al., 1975). The corneal changes regressed and the cornea appeared almost normal when the tyrosine-supplemented diet was continued for 3 or 4 weeks (Alam et al., 1966a), suggesting an adaptation to excess tyrosine that results in reduced localized tyrosine concentrations in corneal epithelium. Administration of adrenal corticosteroids prevented development of signs of tyrosine toxicity including corneal lesions and crystal formation (Burns et al., 1974).

Increased adrenal weights and marked depletion of cholesterol in cells of the adrenal cortex were reported upon histochemical examination of male Wistar rats fed a diet containing 16 percent casein and supplemented with 5 percent L-tyrosine (Biswa and Deb, 1966). The investigators also reported decreased weights and histological abnormalities, including inhibition of spermatogenesis, in testes and accessory sex organs of these animals.

Administration of L-tyrosine has been associated with changes in catecholamine-mediated function such as blood pressure, regulation of heart rate, and restoration of estrous cycles (Milner and Wurtman, 1986). Decreased blood pressure stimulates catecholaminergic neurons in the adrenal medulla leading to a compensatory response to increase blood pressure, whereas increased blood pressure activates catecholaminergic neurons in the brainstem leading to a compensatory reflex to decrease blood pressure. Administration of L-tyrosine can either increase or decrease blood pressure, depending on initial blood pressure status.

In male Sprague-Dawley rats made hypertensive by blood loss, intraarterial administration of 50 or 100 mg/kg body weight of L-tyrosine increased blood pressure significantly (Conlay et al., 1981). In a group of hypertensive adrenalectomized animals, L-tyrosine administration failed to cause a significant increase in blood pressure, suggesting that the adrenal is the major target of tyrosine-induced catecholamine synthesis in this model. The effect of tyrosine did not appear to be mediated by tyramine (Conlay et al., 1984). Administration of selected dipeptides containing L-tyrosine that are more soluble than L-tyrosine itself has also been shown to be effective in raising blood pressure in hemorrhaged hypertensive rats and in lowering blood pressure in spontaneously hypertensive rats (SHR) in lower doses (12.5 to 50 mg/kg body weight, intraperitoneally) than L-tyrosine alone (Maher et al., 1990).

Intraperitoneal injection of graded doses ranging from 25 to 400 mg/kg body weight of L-tyrosine in male SHR and normotensive Sprague-Dawley rats was shown to produce a dose-related fall in blood pressure up to a dose of 200 mg/kg body weight (Sved et al., 1979b). The effect was about five times greater in the SHR than in the normotensive animals. Whole brain MOPEG-SO₄ levels were increased in SHR given 200 mg/kg body weight L-tyrosine but were not measured in normotensive animals (Sved et al., 1979b). Intravenous infusion of about 400 mg L-tyrosine as the ethyl ester in male hypertensive rats and normotensive rats resulted in decreased blood pressure and heart rate; a lower dose of about 80 mg L-tyrosine did not have these effects (Bresnahan et al., 1980). In these experiments, plasma dopamine was elevated significantly in all groups and epinephrine in the hypertensive groups; however, norepinephrine did not rise significantly and plasma renin remained in the expected range for each animal model. Intraventricular injection of only 15 µg L-tyrosine produced a significant fall in blood pressure and an increased turnover of norepinephrine in brain of SHR (Yamori et al., 1980).
Bossy et al. (1983) fed a diet supplemented with 0.6, 1.2, or 2.4 percent L-tyrosine to SHR for 15 weeks after weaning. Blood pressure increased more slowly in rats fed all of the L-tyrosine-supplemented diets. Although serum tyrosine concentrations increased with increasing dietary levels of L-tyrosine, hypotensive effects were equal at all levels of addition. Feeding a diet containing 2.4 percent L-tyrosine to SHR with established hypertension resulted in blood pressure lowering in a few days similar to that in rats given the L-tyrosine-supplemented diet over the 15-week period. The effect was rapidly reversed when SHR were fed a diet without added L-tyrosine.

Intravenous infusion of 1 to 4 mg/kg body weight L-tyrosine (a range of about 9 to 88 mg for 9- to 22-kg animals) in anesthetized healthy mongrel dogs for 20 to 30 minutes raised the threshold current required to produce ventricular fibrillation (Scott et al., 1981). Protection against the ventricular fibrillation increased with increasing doses in this range but a dose of 6 mg/kg body weight of L-tyrosine was less effective than doses of 2 or 4 mg/kg body weight. Infusion with equimolar amounts of L-valine did not affect the ventricular fibrillation threshold significantly but when valine was given with tyrosine, the effect of L-tyrosine on ventricular fibrillation was lessened. These findings suggest that L-tyrosine acted at a central locus rather than at peripheral loci (Scott et al., 1981).

In one study, subcutaneous injection of either L-tyrosine (200 mg/kg body weight) or L-DOPA (200 mg/kg body weight) daily for 2 weeks induced vaginal cycling in constant estrous female rats 20 months of age and did not change cycling in animals 2 months of age. The L-tyrosine-induced cycling was irregular and fewer cycles were observed than when L-DOPA was administered (Linnoila and Cooper, 1976).

**Teratology and developmental studies:** A diet containing 2 percent added L-tyrosine was fed to male and female Wistar rats beginning 2 weeks before initial mating and continued for 3 consecutive generations (F1, F2, and F3) (Thoemke and Huether, 1984). Brain weights, concentrations of amino acids in plasma, and concentrations of putative amino acid neurotransmitters in the brainstem were measured at 5, 10, 15, and 20 days postpartum and compared with those of rats fed a control diet over the 3 generations.

Brain weights were significantly decreased (to 92 and 95 percent of control values) only in the F2 generation. Serum concentrations of tyrosine were increased significantly only in the F3 generation. In the F1 generation, the concentration of taurine was significantly increased to 125 percent of control values while the concentrations of glycine and aspartate were significantly decreased to about 75 percent of control values at day 5 postpartum. Significantly increased concentrations of aspartate and GABA and significantly decreased concentrations of glycine were observed at days 15 and 10, respectively. Significantly decreased concentrations of glutamic acid and taurine were observed at day 20. In the F2 generation, aspartate concentration was increased to about 125 percent of control values at days 15 and 20. In the F3 generation, brainstem concentration of aspartate was increased throughout the 20-day postpartum period and the concentrations of other amino acids which were initially decreased rose as age increased (Thoemke and Huether, 1984).

Feeding of a 6 percent casein diet containing 5 percent added L-tyrosine to pregnant Sprague-Dawley rats from day 1 to day 14 or 21 of pregnancy resulted in significantly lower fetal weight than in ad libitum or pair-fed controls but no difference in litter size (Matsueda and Niiyama, 1982). Excess L-tryptophan produced a further significant reduction in brain weight, DNA, RNA, and protein than food restriction alone. No changes were found in concentrations of amino acids in fetal brain.

Behavioral effects were studied in male offspring of Sprague-Dawley dams given L-tyrosine during gestation (Arevalo et al., 1987). Dams received either 20 or 500 mg/kg body weight of L-tyrosine once a day administered by gastric intubation on days 11 through 20 of gestation. Spontaneous
locomotor activity, an open-field test, a swim test, and a motor ability test were administered to the offspring at 70 days of age. Offspring of dams treated with the higher level of L-tyrosine showed marked increases in spontaneous locomotor activity and open-field activity but decreases in swimming activity and motor ability in the rotating rod test and in number of defection boluses produced in the open-field test. Behavior of offspring of dams treated with the lower level of L-tyrosine was not different from that of control animals treated with saline. Feeding of a 6 percent casein diet containing 5 percent added L-tyrosine to pregnant Sprague-Dawley rats from day 1 to day 14 or 21 of pregnancy resulted in significantly lower fetal weight than in ad libitum or pair-fed controls but no change in litter size (Matsueda and Niyama, 1982). Excess L-tyrosine produced a significant reduction in brain weight, DNA, RNA, and protein that was not produced by food restriction. No appreciable changes in amino acid concentrations were observed in maternal plasma or fetal brain.

In rat pups injected subcutaneously with tyrosine (5 g/kg body weight daily in 3 divided doses) from birth to 18 days of age incorporation of $^{35}$SO$_4$ into cerebroside sulfate of myelin lipids was diminished by 50 percent. Body weight, brain weight, and total brain lipids were also significantly reduced in tyrosine-treated pups at 18 days of age (Chase and O'Brien, 1970). However, the changes were similar to the results reported for a group of 18 malnourished pups raised as a single large litter and were also quantitatively similar for each of 8 individual amino acids fed in the study.

c. Human studies

Biochemical studies: Glaeser et al. (1979) administered single oral doses of 100 or 150 mg/kg body weight of L-tyrosine suspended in water to 12 normal fasting human subjects (6 subjects per dose level). Plasma tyrosine rose from an initial fasting level of 69 µmol/L to a peak concentration of 154 µmol/L 2 hours after ingestion of the lower dose and to 203 µmol/L within 2 hours after the higher dose. Plasma concentrations remained significantly elevated at 8 hours following both doses. Over the 8-hour time period, concentrations of other LNAA decreased slightly and the tyrosine:LNAA ratios increased significantly in a dose-related manner 2 hours after tyrosine administration. In these experiments, blood pressure or pulse rate did not change significantly and no subjects complained of side effects after oral ingestion of L-tyrosine (Glaeser et al., 1979). Similar results in the magnitude of increases in plasma tyrosine concentrations and in the time course for the plasma tyrosine elevations were reported with similar loads of L-tyrosine in 2 normal subjects by Tocci et al. (1972), in 1 normal adult by Leeming et al. (1976), in 15 normal control subjects by Takahashi et al. (1968), and in 2 normal control subjects (1 infant and 1 adult) by Gaull et al. (1970). The similarity in results among these studies suggested that oral administration of L-tyrosine in a dose of 100 mg/kg body weight results in a doubling of plasma tyrosine concentration and doses of L-tyrosine in many subsequent studies were based on these findings.

In the investigation of Takahashi et al. (1968), plasma tyrosine concentrations were reported to peak at a significantly higher level in patients with mania or depression (about 150 percent above baseline 2 hours after ingestion) than in control subjects (about 69 percent above baseline) and to remain at the peak level for 3 and 4 hours after ingestion in the patients. Neither parahydroxyphenylpyruvic acid nor homogentisic acid were detected in urine of either group after the L-tyrosine load. Administration of daily subcutaneous injections of cofactors required for tyrosine metabolism (50 mg vitamin B6, 100 mg vitamin C, and 15 mg folic acid) to 1 patient with depression for 1 week before a 100 mg/kg body weight load of L-tyrosine did not alter the plasma tyrosine response (Takahashi et al., 1968).

Effects of oral administration of L-tyrosine on levels of circulating and excreted catecholamines have been investigated in a number of studies. Changes in plasma tyrosine and tyrosine:LNAA ratios were compared in 11 healthy male subjects following consumption of 3 standardized meals each containing 38 g protein on 1 day and consumption of the same meals plus a dose of L-tyrosine
(33 mg/kg body weight in water, total dose 100 mg/kg body weight or 7 g for a 70-kg person) 1 hour before each meal on the following day (Melamed et al., 1980b). Plasma levels of tyrosine increased significantly and were highest (94 to 96 μmol/L) between 1 p.m. and 9 p.m. on the day when subjects consumed the diet alone. Plasma levels returned to baseline overnight. On the following day after supplemental L-tyrosine was given, all plasma levels were significantly higher than the respective levels on the preceding day. Peak levels on the second day were 178 μmol/L at 1 p.m. and 5 p.m. The increase in tyrosine:L NAA ratio was significantly greater on the second day than the first although increases in plasma LNAA concentrations were similar on both days, indicating that the increased ratio resulted from the increased plasma tyrosine concentration (Melamed et al., 1980b). The investigators reported that they observed no significant changes in blood pressure, pulse rate, urinary volume, and no abnormal neurologic or psychologic phenomena during the study. Likewise, they reported that the subjects had no gastrointestinal complaints after administration of 100 mg/kg body weight L-tyrosine given in 3 divided doses 1 hour before meals.

Administration of 14 g of L-tyrosine in capsules induced a significant increase in plasma norepinephrine, dopamine, and epinephrine concentrations in 5 of 6 fasting men compared with either baseline levels or control subjects receiving a lactose placebo (Rasmussen et al., 1983). The increase occurred about 45 minutes after ingestion and lasted about 30 minutes. Catecholamine concentrations in plasma of the sixth subject did not increase after ingestion of L-tyrosine. Plasma concentrations of DOPAC decreased over this time period. Because peripheral tissues are the source of plasma catecholamines and both brain and peripheral tissues may be sources for DOPAC, the authors concluded that oral ingestion of a very large dose of L-tyrosine might exert acute effects on catecholaminergic systems in both brain and peripheral tissues (Rasmussen et al., 1983). The investigators reported that there were no discernible physical or psychological effects following the L-tyrosine load.

An increase in urinary excretion of catecholamines (norepinephrine, epinephrine, and dopamine) and catecholamine metabolites (HVA, MOPEG, and VMA) similar to the increase in plasma catecholamine concentrations observed by Glasser et al. (1979) was reported in 8 fasting men given single oral doses of 100 mg/kg body weight (n = 2) or 150 mg/kg (n = 6) L-tyrosine (Alonso et al., 1982). In addition, elevations in plasma tyrosine and the tyrosine:L NAA ratio were associated with increased concentrations of tyrosine and the dopamine metabolite, HVA, in CSF of 14 patients with Parkinson’s disease given 100 mg/kg body weight of L-tyrosine daily for 4 to 7 days (Growdon et al., 1982).

Cuche et al. (1985) gave 9 fasting normotensive healthy men single loads of 100 mg/kg body weight of L-tyrosine in capsules. Although the plasma tyrosine concentration was doubled by this treatment, neither free nor sulfon conjugated catecholamine concentrations were changed at 1, 2, or 4 hours after the L-tyrosine load.

Benedict et al. (1983) gave 6 normal men 7.5 g/day of L-tyrosine in capsules in 3 divided doses with meals in a placebo-controlled, double-blind trial. Plasma and urine samples were collected after the subjects had taken the supplement with a small breakfast on the morning of the fourth day of supplementation. Significant decreases occurred in both free and conjugated plasma norepinephrine concentrations (27 and 20 percent decreases, respectively). Changes in free and conjugated epinephrine in plasma and urine, free and conjugated dopamine in plasma and urine, plasma DOPA, or plasma renin activity were relatively small and not statistically significant. The plasma tyrosine concentration in these subjects increased 153 percent at 1 and 1.5 hours after taking the supplement and the increase was reported to be greater in subjects with lower body weights. There were no significant changes in heart rate, blood pressure, plasma sodium level, or 24-hour urinary sodium excretion. The authors speculated that oral ingestion of L-tyrosine with meals may cause increased synthesis of brain catecholamines, thereby resulting in the stimulation of central catecholaminergic receptors with a consequent decrease in the release of norepinephrine by peripheral sympathetic neurons (Benedict et al., 1983).
Agharanya et al. (1981) had reported earlier on changes in catecholamine excretion in 11 healthy men who ingested 100 mg/kg body weight of L-tyrosine in 3 divided doses with meals (38 g protein, 112 g carbohydrate, and 44 g fat per meal). Total daily intake of supplemental L-tyrosine ranged from 6.0 to 8.5 g. In 10 of the 11 subjects, ingestion of the L-tyrosine supplement increased the 24-hour catecholamine (norepinephrine, epinephrine, and dopamine) excretion by 25 percent and tyrosine excretion increased by 138 percent. Diurnal variation was noted, with at least 60 percent of total catecholamine excretion occurring during the day. In the eleventh subject, no changes in tyrosine or catecholamine excretion occurred. The increase in catecholamine excretion was regarded as evidence that the administration of L-tyrosine stimulated catecholamine synthesis in the sympathoadrenal system (Agharanya et al., 1981).

Differences in catecholamine metabolite production in 9 obese and 12 normal–weight women 22 to 38 years of age were shown with administration of a formula diet supplemented with L-tyrosine for 2 days (Johnston et al., 1983). L-Tyrosine supplements were added to the formula diet in amounts supplying 260 mg/kg fat–free mass daily (a mean of 10.4 g for normal–weight women with a mean fat–free mass of 40 kg and a mean of 14.3 g for obese women with a mean fat–free mass of 55 kg). Plasma concentrations of tyrosine:LNAA and MOPEG and urinary excretion of VMA and HVA were elevated in normal subjects. In contrast, although the tyrosine:LNAA ratio was also significantly increased in the obese subjects, only excretion of VMA and HVA were increased. The report did not include mention of side effects in either group (Johnston et al., 1983).

Behavioral studies: In a randomized, double-blind, placebo–controlled crossover study, Lieberman et al. (1985) gave single oral doses of L-tyrosine of 100 mg/kg body weight (7 g for a 70-kg individual) in capsules to 20 healthy male subjects 18 to 45 years of age. Two self–reported mood questionnaires (VAMS and POMS) and 4 tests of performance (Simple Auditory Reaction Time, Two–Choice Visual Reaction Time, Grooved Pegboard Test, and Thurstone Tapping Test) were administered. Tyrosine did not result in behavioral changes compared with placebo. The report did not include mention of side effects.

In a subsequent study, 23 healthy male U.S. Army personnel 18 to 28 years of age exposed to high altitude (hypobaric hypoxia) or cold temperatures were given 100 mg/kg body weight of L-tyrosine in capsules in 2 50–mg/kg does 40 minutes apart (Banderet et al., 1987; Banderet and Lieberman, 1989). Symptoms, mood states, cognitive performance, reaction time, and vigilance were evaluated. Instruments used for these assessments were the Environmental Symptoms Questionnaire, Clyde Mood Scale, Multiple Affect Adjective Check List, POMS, Stanford Sleepiness Scale, and Catecholaminergic Effects Scale. Additional performance and cognitive tasks were employed. Because exposure to the stressors affected individuals differently, analyses were limited to the subjects substantially impaired by exposure to the environmental conditions. Overall, administration of L-tyrosine significantly decreased many adverse behavioral and emotional effects resulting from exposure to cold and hypoxia, improved performance (accuracy and completion of tasks), increased vigilance, and significantly decreased latencies on the choice reaction time task in these subjects. Plasma L-tyrosine concentrations were significantly increased during the testing period (43 µmol/L at baseline, 109 µmol/L at 150 minutes, and 99 µmol/L after 265 minutes). Administration of L-tyrosine did not affect heart rate or blood pressure and the investigators reported that tyrosine did not produce any apparent side effects in these subjects (Banderet et al., 1987; Banderet and Lieberman, 1989).

Gelenberg et al. (1990) reported results of a 4–week prospective, randomized, double–blind trial of the effects of L-tyrosine (n = 21), imipramine (a tricyclic antidepressant) as a positive control (n = 22), or lactose placebo (n = 22) on depression in 65 outpatients 18 to 75 years of age with major depression as determined by Research Diagnostic Criteria. About 75 percent of the subjects were male because the research protocol excluded women with childbearing potential. Subjects were given sufficient capsules to supply daily doses of 100 mg/kg body weight of L-tyrosine (7 g for a 70–kg subject), 2.5 mg/kg imipramine, or an unspecified amount of placebo. Capsules were taken orally in 3 divided doses each day. Comparison of mean Hamilton Depression Scale scores, Beck
Depression Rating Scale scores, Clinical Global Impression scores, and dichotomous outcome analyses did not show significant differences between patients receiving L-tyrosine and those receiving lactose placebo. Patients were given a low-monoamine diet for 3 days before treatment and at the end of the study when psychological testing was done. Administration of L-tyrosine was associated with significant elevations of plasma tyrosine (an increase of about 20 μmol/L above baseline, normal plasma levels < 180 μmol/L) and urinary MOPEG excretion (an increase of about 0.2 mg/day above baseline). Four subjects in the group receiving L-tyrosine withdrew during the study. One dropped out because of side effects (palpitations), 1 because of clinical worsening, and the other 2 for administrative reasons. In an earlier pilot study (Gelenberg et al., 1983), the only side effect reported was mild epigastric distress which was temporally associated with taking the L-tyrosine tablets and alleviated by taking the medication with food. These investigators also reported that no laboratory abnormalities occurred but did not specify the measurements supporting this conclusion. Overall, these investigators concluded that their series of studies of L-tyrosine in depressed patients (Gelenberg et al., 1990, 1983, 1980) suggested no harmful effects from L-tyrosine therapy (100 mg/kg body weight/day in divided doses) for as long as 4 weeks.

In a study of 8 patients with narcolepsy, Mouret et al. (1988) reported that oral administration of L-tyrosine in daily doses ranging from 64 to 120 mg/kg body weight (4.5 to 8.4 g in divided doses) for 6 months reversed symptoms of narcolepsy. Side effects included irritability, headache, and difficulty in falling asleep. Exposure to physical or mental stress required increased doses for effectiveness. The authors reported that concurrent treatment of two patients with vitamin B6 (amount not specified) caused a rapid reversal to pretreatment status but no further information was provided.

Treatment of 10 patients 48 to 82 years of age having severe dementia with L-tyrosine (4 g/day) in combination with 5-hydroxytryptophan (800 mg/day) and carbidopa (an aromatic amino acid decarboxylase inhibitor in amounts of 100 mg/day) was associated with immediate development of side effects consisting of diarrhea in all patients, frequent nausea, drowsiness in 7 patients (attributed to the administration of 5-hydroxytryptophan), restlessness and agitation in 5 patients, and hyperventilation in 2 patients (Meyers et al., 1977). Doses were then lowered by 50 or 75 percent and continued for 6 to 12 weeks without these effects. Use of L-tyrosine in combination with 5-hydroxytryptophan and carbidopa was associated with a depression in dopamine turnover in CSF, attributed to a competitive inhibition of tyrosine uptake by 5-hydroxytryptophan at the blood–brain barrier (Meyers et al., 1977).

L-Tyrosine was given orally to patients with attention deficit disorder in three studies. The first was a placebo-controlled, double-blind study in which 140 mg/kg body weight of L-tyrosine plus 50 mg pyridoxine hydrochloride were given daily in divided doses to 14 children 7 to 12 years of age for 1 week (Nemzer et al., 1986), the second a single-blind study in which 3 g (about 100 mg/kg body weight) tyrosine (isomer not specified) was given daily in divided doses for 3 weeks to 7 children 8 to 14 years of age (Eisenberg et al., 1988), and the third a placebo-controlled open trial in which 50 to 150 mg/kg body weight was given daily in divided doses to 12 adults for 8 weeks (Reimherr et al., 1987). No side effects were reported in any of the studies.

High circulating levels of tyrosine but not other amino acids during infancy (transient neonatal tyrosinemia [TNT]) have been reported in association with the feeding of high protein formulas (e.g., inappropriately diluted evaporated milk formulas). Although some investigators have reported normal development of children with TNT (Light et al., 1973; Martin et al., 1974; Partington et al., 1968), others have reported deficits in intellectual performance (Mamunes et al., 1976; Menkes et al., 1966, 1972).

In a sample of 71 premature infants, Menkes et al. (1966, 1972) reported a significant correlation of maximal blood tyrosine concentration with gestational age but not with birth weight. Blood tyrosine concentrations were abnormally high in 89 percent of these infants and the maximal level reported was 150 mg/L (830 μmol/L) or higher in 38 percent (normal in infants <220 μmol/L).
Duration of the tyrosinemia was not specified. In a follow-up of 62 of these children at 2.5 years of age, differences in neurological or psychometric evaluation were not found (Menkes et al., 1966). In a later follow-up at 7 to 8 years of age, an association was found between elevated blood tyrosine levels during the early postnatal period in larger (but not smaller) premature infants and impaired perceptual function and reduced achievement test scores. The association persisted after controlling for physical and socioeconomic factors suspected to influence intellectual development. However, the investigators did not consider this finding sufficient to implicate tyrosinemia during early infancy with subsequent impairments in intellectual performance (Menkes et al., 1972).

TNT attributed to a combination of a high protein formula plus a lack of supplemental vitamin C was also reported in 15 term infants by Mámunes (1976). The mean maximum detected tyrosine level was 258 mg/L (1425 μmol/L) and the mean duration of the elevation was 50 days. The intellectual abilities of these children at about 4.5 years of age were compared with an age, sex, and socioeconomic-matched control group without TNT. Children in the TNT group whose tyrosinemia had lasted more than 45 days had significantly lower mean scores than the controls on the McCarthy Scale of Children’s Abilities and the Illinois Test of Psycholinguistic Ability. Scores on the Peabody Picture Vocabulary Test were not significantly different. No gross motor deficits were evident although formal neurological examinations were not conducted.

Endocrine studies: Oral administration of 50, 250, or 500 mg/kg body weight of L-tyrosine given as a slurry in 150 ml water to 8 normal subjects (4 male, 4 female) in a randomized, double-blind, placebo-controlled study resulted in a dose-related increase of prolactin secretion and plasma tyrosine concentrations (Al-Damluji et al., 1988). The effect on serum prolactin concentrations was maximal at the 250 mg/kg-dose, whereas plasma tyrosine concentrations increased significantly with each dose increment. This reported effect on prolactin secretion differed from the prolactin response in rats following L-tyrosine administration. (See p. 182.)

All three doses of L-tyrosine had a dampening effect on the circadian fall in plasma thyrotropin concentrations observed after administration of the placebo (calcium carbonate) but concentrations of triiodothyronine and thyroxine were not changed significantly (Al-Damluji et al., 1988). Administration of L-tyrosine did not have an effect on plasma adrenocorticotrophic hormone, cortisol, LH, FSH, or growth hormone concentrations. Administration of cortisol has been reported to depress plasma tyrosine in normal subjects given an oral load of L-tyrosine but not in hyperthyroid subjects (Rivlin and Melmon, 1965). Additionally, L-tyrosine in the 50, 250, and 500-mg/kg body weight doses given by Al-Damluji et al. (1988) did not have a significant effect on heart rate or blood pressure or on visual analogue scales indicative of behavioral arousal. These investigators indicated that their subjects did not report side effects or unpleasant sensations with ingestion of L-tyrosine.

Inborn errors of metabolism: Tyrosinemia I, an autosomal recessive disorder, is not a primary disorder of tyrosine metabolism but is accompanied by elevated levels of tyrosine and its metabolites (Goldsmith and Leberge, 1989). The activity of fumarylacetoacetate hydrolase is decreased, with resultant accumulation of succinylacetoacetone and succinylacetone which inhibit renal tubular function, hepatic enzymes of tyrosine catabolism, and porphobilinogen synthetase (δ-aminolevulinic acid dehydratase). Anemia, abnormal liver function, increased plasma α-fetoprotein, moderate increases in plasma tyrosine and its metabolites, and increased plasma methionine are laboratory findings associated with the disease. In its acute form, the disorder is associated with failure to thrive, vomiting, diarrhea, and a cabbagelike odor probably caused by methanethiol and other volatile sulfur compounds. Liver failure develops and infants rarely live more than 1 year. Abnormalities occurring with the chronic form of the disease include renal tubular dysfunction, vitamin D-resistant rickets, and acute, intermittent porphyria–like symptoms. Hepatoma may be a late complication. Serum tyrosine may be normalized by a diet restricted in tyrosine and phenylalanine (and often methionine) but the elevated serum methionine is less amenable to dietary interventions (Goldsmith and Leberge, 1989).
Tyrosinemia II, also known as Richner–Hanhart syndrome, is an autosomal recessive disorder associated with a deficiency of hepatic tyrosine aminotransferase (Goldsmith and Laberge, 1989). Renal and hepatic functions and metabolism of other amino acids appear to be normal.

Corneal erosions and/or skin lesions on the palms and soles analogous to those described in the animal model of this disease (see p. 182–183) usually develop early in life; mild to moderate mental retardation has been reported in some cases but it is not certain whether it is directly associated with elevated tyrosine levels. The mean plasma concentrations of tyrosine in this disorder are 2390 μmol/L in children up to 7 years of age and 1270 μmol/L in patients 8 to 55 years of age (normal <180 μmol/L). Tyrosinuria and increases in urinary phenolic acids, N–acetyltirosine, and tyramine also occur. Many younger patients are asymptomatic at plasma tyrosine concentrations at which older patients have skin and eye lesions. Clinical signs and symptoms rapidly disappear with a diet low in tyrosine and phenylalanine (Goldsmith and Laberge, 1989). An autosomal recessive disease in ranch mink has been associated with many of the signs and symptoms of tyrosinemia II in humans. A deficiency of 4–hydroxyphenylpyruvic–acid oxidase has also been associated with development of tyrosinemia and CNS symptoms, but not with eye or skin lesions in humans (Goldsmith and Laberge, 1989).

Clinical studies: Elevated fasting concentrations of plasma tyrosine and abnormal tyrosine metabolism have been described in patients with hepatic cirrhosis. Nordlinger et al. (1979) found significantly increased plasma concentrations of tyrosine and impaired tolerance to intermediates of tyrosine catabolism (p–hydroxyphenylpyruvate and homogentisic acid) in cirrhotic subjects following an oral load of 50 mg/kg body weight of tyrosine (3.5 g for a 70–kg individual). However, tyrosine transaminase activity was similar in biopsy samples of cirrhotic and noncirrhotic livers, indicating that decreased activity of this enzyme was not the mechanism of abnormal tyrosine metabolism as previously thought (Henderson et al., 1981). Production of false neurotransmitters derived from tyrosine (tyramine and octopamine) has also been hypothesized as the mechanism responsible for hepatic encephalopathy, hyperdynamic circulation, and hepatorenal syndrome (Fischer et al., 1975b).

d. Summary and conclusions

Endpoints: Excess L–tyrosine in low–protein diets produces a distinct syndrome of cataracts, skin lesions, and histopathological changes in rats. These changes have resulted from feeding of L–tyrosine at levels of 3 to 5 percent in low–protein diets and the eye lesions appear to be related, at least in part, to the low solubility of the amino acid. These adverse effects of excess L–tyrosine appear to be exacerbated by adrenalectomy and hyperthyroidism. This syndrome has not been described in rats fed high–protein diets, even when as much as 12 percent L–tyrosine was added.

Weight gain is depressed in rats fed low–protein diets containing 3 to 5 percent L–tyrosine but not in rats fed high–protein diets containing 12 percent L–tyrosine. Addition of 12 percent L–tyrosine to a high–protein diet has also been associated with increased hepatic content of cytochrome P450 and b5 and with increased hepatic synthesis of cholesterol in rats.

Administration of L–tyrosine has been shown to prevent changes in stress–induced behavior in rats. Increased tyrosine concentrations in brain were associated with changes in the turnover of norepinephrine. Tyrosine uptake into brain increases with elevated plasma tyrosine concentrations, in particular when the tyrosine:LNAAA ratio is increased. Increased concentrations of tyrosine have been shown to stimulate synthesis and release of catecholamines in rapidly firing but less so in quiescent neurons in discrete areas of rat brain. Addition of 2.76 percent L–tyrosine to the diet of rats has been associated with increased concentrations of tyrosine in brain and with stress–induced turnover of norepinephrine evidenced by an accumulation of methoxyhydroxyphenylethleneglycol in brain, and reversal of stress–associated behavioral depression in rats.
Administration of L-tyrosine to rats has produced increases or decreases in blood pressure, depending on whether catecholaminergic neurons are stimulated in the brain or in the adrenal medulla. In addition, feeding of high protein diets containing 12 percent added L-tyrosine was associated with increased synthesis of cholesterol in livers of rats.

Feeding diets containing 2 percent L-tyrosine to rats over 3 generations resulted in a significant increase in plasma tyrosine concentration in the third generation and some changes in concentrations of putative neurotransmitters. Developmental changes (e.g., changes in activity and exploratory behavior) have also been observed in offspring of rats given 500 mg/kg body weight L-tyrosine daily during gestation.

Administration of large doses of L-tyrosine has been studied extensively in humans as a therapeutic agent because of the demonstration that precursor availability can influence the synthesis and release of catecholamines in brain. Administration of 100 mg/kg body weight of L-tyrosine (7 g for a 70-kg individual) with or without food and as single or divided doses has been associated with a doubling of plasma tyrosine concentrations and with an increase in the tyrosine:LNAA ratio in humans. Administration of this amount of L-tyrosine on a single day has not been associated with significant changes in blood pressure, pulse rate, urinary volume, or abnormal neurologic or psychologic phenomena. Some studies have reported gastrointestinal side effects in some subjects receiving 100 mg/kg body weight of L-tyrosine given apart from food but not with food.

It is not yet known whether peripheral catecholamine concentrations reflect concentrations in the central nervous system. Significant changes in concentrations of plasma catecholamines following administration of L-tyrosine have not been observed in humans despite significant increases in plasma tyrosine concentrations. This lack of change in plasma concentrations may reflect overall status resulting from stimulation of the central catecholaminergic neurons and a consequent decrease in release of norepinephrine by peripheral sympathetic neurons. Additionally, this may result from near saturation of tyrosine hydroxylase in peripheral tissues not subjected to enhanced synthesis demands (i.e., at rest). Direct evidence for this hypothesis has not been demonstrated in humans. Some studies of urinary excretion of catecholamines and their metabolites following L-tyrosine administration in humans have reported increased excretion of catecholamines while others have reported no change.

The only effect observed when a single dose of 500 mg/kg body weight of L-tyrosine (about 35 g) was given orally was an increase in plasma prolactin concentration in the subjects. Administration of L-tyrosine in doses of 100 mg/kg body weight on one occasion did not result in behavioral effects in unstressed individuals but significantly decreased many behavioral and emotional effects resulting from exposure to cold and hypoxia, improved performance, increased vigilance, and significantly decreased latencies on choice reaction time tasks in subjects whose behavior had previously been shown to be substantially impaired by exposure to cold and hypoxic conditions. Administration of 100 mg/kg in divided doses for 4 weeks was associated with palpitations in 1 of 21 patients given L-tyrosine as a treatment for major depression.

High plasma concentrations of tyrosine are associated with hepatic and renal failure in patients with tyrosinemia I and with development of eye and skin lesions in patients with tyrosinemia II. Transitory neonatal tyrosinemia in premature and term infants has been associated with impaired intellectual abilities in childhood in some studies.

Safe levels of human intake: Daily L-tyrosine intake is about 2.2 g for an individual consuming 100 g protein per day. In studies of the pharmacologic actions of L-tyrosine, administration of 100 mg/kg body weight of the amino acid (7 g for a 70-kg individual) as a single dose or in divided doses on 1 day has not been associated with significant changes in blood pressure, pulse rate, urinary volume, or abnormal neurologic or psychologic phenomena. In some studies, gastrointestinal side effects have been reported in some subjects receiving 100 mg/kg given apart from food but not with food. No side effects were reported in one study in which 500 mg/kg body weight (35 g
for a 70-kg individual) was given in a single dose. With daily administration of 100 mg/kg body weight of L-tyrosine in divided doses for 4 weeks, palpitations were reported in 1 of 21 patients given L-tyrosine therapy for major depression.

The occurrence of more severe effects of high doses of L-tyrosine with low-protein diets in animals suggests that persons with low intakes of protein may be more susceptible to possible adverse effects of chronic consumption of L-tyrosine as a dietary supplement. Evidence of biochemical and behavioral effects in offspring of female rats given L-tyrosine during gestation suggests that women should not take L-tyrosine during pregnancy or during lactation. A possible association of impaired intellectual performance with transient neonatal tyrosinemia resulting from high protein intake indicates that L-tyrosine should not be given to infants or children as a dietary supplement. Evidence that L-tyrosine increases the cytochrome P450 content in liver of rats raises the possibility of interactions of this compound with some drugs. Likewise, evidence that compounds which stimulate the cytochrome P450-containing drug metabolizing system also increase the acute toxicity of L-tyrosine in rats also suggests that L-tyrosine should not be used by persons taking pharmaceutical preparations which stimulate this system. While most studies utilizing L-tyrosine have suggested toxic responses as a result of hepatic interactions, no studies to date have evaluated L-tyrosine-induced effects on hepatic function.

The safety of continued ingestion of L-tyrosine as a dietary supplement by normal adults cannot be determined from the extant data. The relative insolvability of L-tyrosine raises concern about the possibility of localized adverse effects in the small intestine or in the lens with chronic exposure to L-tyrosine. Knowledge that greatly elevated plasma concentrations of tyrosine are associated with eye and skin lesions in persons with tyrosinemia II and that these lesions can be reversed by lowering plasma concentrations of L-tyrosine gives cause for concern about chronic high concentrations of plasma tyrosine. Likewise, demonstrations of pharmacologic effects on catecholamine synthesis and release in stimulated catecholaminergic neurons in rats and on behavior of humans subjected to stressful situations by L-tyrosine given orally in single doses of 100 mg/kg body weight do not provide evidence of the safety of L-tyrosine at this level of intake. Studies demonstrating evidence of safety or lack thereof with lower oral doses of L-tyrosine are not available.
VI. GROUPS POTENTIALLY AT HIGHER RISK FOR ADVERSE HEALTH EFFECTS RESULTING FROM USE OF AMINO ACID SUPPLEMENTS

Based upon their review of the available information pertaining to safety of individual amino acids, the Expert Panel members found reason for particular concern about the use of dietary supplements of amino acids by several subgroups of the general healthy population and by patients with certain diseases. The groups considered to be at higher risk for possible adverse effects and the rationale for concern about use of amino acid supplements by these groups are detailed below.

A. SUBGROUPS OF THE GENERAL HEALTHY POPULATION

1. Infants and pregnant and lactating women

The prenatal and early postnatal periods are the times of most rapid brain development and growth in animals and humans. As detailed in Chapter V, high doses of individual amino acids given to rats during pregnancy resulted in lower birth weight, decreased brain weight, and altered behavior of offspring. Administration of some amino acids directly to neonatal rats resulted in decreased body weight, brain weight, and altered brain lipid composition.

Administration of oral loads of amino acids results in elevations of plasma concentrations of amino acids for extended periods of time. Use of amino acid supplements by women during pregnancy would be expected to result in increased concentrations of amino acids in the fetal as well as the maternal circulation. For ethical reasons, it is not possible to investigate effects of amino acid supplements on the developing human fetus. However, the occurrence of adverse effects in fetuses exposed to high levels of amino acids during gestation can be inferred from studies of the pregnancies of women with PKU or hyperphenylalaninemia. Higher blood concentrations of phenylalanine during pregnancy in women with PKU have been associated with smaller head circumference and lower IQ levels in the offspring. Lowering the plasma phenylalanine concentration in PKU patients during pregnancy is associated with improvements in these parameters (Levy and Waisbren, 1983; Matalon et al., 1991).

High phenylalanine concentrations have been reported in milk of lactating mothers with PKU. Amino acid concentrations have not been measured in milk of lactating women taking amino acid supplements; however, the higher plasma concentrations of amino acids following oral loads of amino acids may likely result in increased concentrations in milk.

Adverse effects of accumulation of individual amino acids in brain and other organs occur in infants with inborn errors of amino acid metabolism. Prevention of this accumulation is associated with improved growth and development in these children. In some studies (Menkes et al., 1966, 1972; Mamunes, 1976), transient elevations in plasma concentrations of tyrosine which occurred in infants without inborn errors of metabolism who were fed formulas containing inappropriately high levels of protein have been associated with lower IQ levels later in childhood.

Because of the potential for adverse effects on the growth and development of infants, the Expert Panel considered the use of amino acids as dietary supplements for infants and pregnant and lactating women ill advised unless taken under responsible medical supervision. In addition, the Expert Panel considered it prudent for all women of childbearing age to refrain from use of amino acids as dietary supplements, unless taken with the same supervision.
2. **Children and adolescents**

Children and adolescents are another group for whom the Expert Panel had concern about the use of supplemental amino acids. Experimental studies have shown repeatedly that growth and food intake are depressed in young animals fed diets supplemented with many single amino acids including methionine, tyrosine, and tryptophan. The degree of growth depression varied considerably among amino acids but was almost always more severe in those animals consuming diets low in protein.

Several expert groups have identified factors which may influence health risks resulting from exposure to exogenous substances (Guzelian et al., 1992; Hunt et al., 1982; World Health Organization, 1986). These factors include differences in gastrointestinal permeability, pH, transit time, and enzymatic activity (Roberts, 1992; World Health Organization, 1986); the chemical species and rate of distribution to organ systems (Roberts, 1992; World Health Organization, 1986); increased circulatory flow rates and reduced plasma protein binding (Plunkett et al., 1992; Snodgrass, 1992); developmental changes in hepatic enzyme systems that may result in increased or decreased rates of metabolism of particular compounds (Abel and Kleiman, 1990; Kauffman, 1992; Plunkett et al., 1992); and biliary and renal excretion which is often less efficient in children than in adults (Besunder et al., 1988; Snodgrass, 1992). In addition, some receptor–mediated biological processes differ significantly between adults and children. For example, some drugs such as phenobarbital and diphenhydramine, which have neurological effects that depend on receptor–mediated processes, have opposite effects on the CNS of children than of adults (Kacew, 1992).

The Expert Panel considered that such physiological and metabolic differences may affect utilization and, thus, the safety of supplemental amino acids for children and adolescents. Potential neurological effects of amino acids are a particular concern in children because of possible differences in receptor–mediated processes in the CNS.

3. **Elderly individuals**

Age–related changes in physiological functions, biochemical processes, and increased or decreased sensitivity of responses have been cited as conditions which affect the intensity of actions of pharmacologic agents in elderly individuals (Tumer et al., 1992) and may also modify the effects of supplemental amino acids in this group. Although little or no change in absorption of nutrients or drugs has been identified in elderly persons with intact gastric mucosa (Bender, 1968), increased bioavailability of several drugs including propanolol, metoprolol, and labetalol has been shown in the elderly as a result of reduction in first–pass extraction of the compounds entering the liver from the portal circulation (Tumer et al., 1992). Alterations in body composition including decreases in lean body mass and total body water and an increase in body fat affect drug distribution (Park, 1986), generally resulting in a smaller volume of distribution for water–soluble compounds including amino acids. Renal function is also decreased in the elderly. Beginning around age 30, there is a 6 to 10 percent decrease in GFR and RPF per decade (Davies and Shock, 1950) and, by age 70, an individual may have as much as a 40 percent decrease in renal function even in the absence of kidney disease. In addition, the responsiveness of receptor systems changes with age; however, there is no uniformity in the magnitude and direction of changes in receptor systems, signal transduction, cellular responses, or homeostatic regulation (Tumer et al., 1992). Overall, these age-related changes may result in higher plasma concentrations (Rudman et al., 1991) and enhanced and prolonged pharmacologic activity of amino acids.

As well as the age–related changes described in the preceding paragraph, nutritional status and drug–nutrient interactions are also of concern with regard to use of supplemental amino acids by the elderly population. Total protein and energy intake generally decrease in parallel as age increases (Munro et al., 1987). Impaired protein–energy status, as indicated by subnormal
nutritional indicators, has been identified in only about 3 percent of healthy elderly but has been estimated to occur in as many as 60 percent of institutionalized elderly (Rudman and Feller, 1989). In addition, extensive use of prescription and OTC drugs (polypharmacy) by many members of the elderly population (Jick, 1978), increases the likelihood of interactions of supplemental amino acids with drugs, particularly when multiple drugs or amino acids are being taken.

Characteristics of drugs having priority for study in the elderly because of the potential for adverse effects are well recognized. The drugs include substances that: 1) affect biologic homeostatic mechanisms which may be deficient in the elderly; 2) act in the central nervous system; 3) have a low therapeutic-to-safety ratio; 4) are excreted largely by the kidney; 5) are subject to large first-pass effect; 6) are metabolized by oxidative mechanisms; or 7) generate significant metabolites (Abrams and Reidenberg, 1984). The characteristics of these drugs and the properties of amino acids are strikingly similar.

In light of the age-related physiological and metabolic changes and the use of multiple pharmaceutical preparations by elderly individuals, as well as the increased likelihood of impaired protein-energy status in the institutionalized elderly, the Expert Panel regarded the potential for adverse health effects of supplemental amino acids to be increased in this group.

4. Persons homozygous or heterozygous for inborn errors of amino acid metabolism

Because of demonstrated anomalies in metabolism of certain amino acids, dietary supplements of amino acids should not be used by persons who are homozygous or heterozygous for inherited disorders of amino acid metabolism. The question of PKU was discussed above but similar concerns exist for individuals with a whole spectrum of other metabolic disorders including branched-chain amino aciduria, homocysteinemia, tyrosinemia, and hyperglycinemia. In many cases, the presence of the heterozygous state is not known to the individual and, therefore, the added risk will not be recognized.

5. Individuals with low intakes of protein

Studies in experimental animals have shown repeatedly that excesses of individual amino acids produce more severe depressions in growth and food intake when low-protein diets are fed. The majority of the general U.S. population has adequate protein intake; however, groups that may be likely to have lower protein intakes include the elderly, some vegetarians, persons consuming diets very low in calories, alcoholic individuals, and some patient populations with chronic diseases, particularly the institutionalized elderly. Some social conditions associated with a greater likelihood of poor dietary quality which may include low protein intakes are poverty, homelessness, and social isolation.

6. Smokers

While there is no direct evidence from experimental studies, smokers may represent a population deserving special consideration when using amino acid supplements. The premise that smokers may be at higher risk is inferred from evidence suggesting that vitamin B6 status (plasma PLP concentration) may be adversely affected by smoking (Serfontein et al., 1986; Vermaak et al., 1990). Thus, their amino acid metabolism may be compromised if a key vitamin B6-dependent enzyme is affected. Urinary excretion of tryptophan metabolites has been studied in smokers. In one study of tryptophan metabolism in 12 smokers, urinary excretion of tryptophan metabolites following a 2-g
oral load of L-tryptophan was normal compared with nonsmokers (Brown et al., 1970). However, in 6 subjects who had smoked for 5 weeks, urinary excretion of 3-hydroxykynurenine and 3-hydroxyanthranilic acid was elevated after a 50 mg/kg load of L-tryptophan (Kerr et al., 1965b).

B. PATIENT POPULATIONS

The use of amino acids by patients with various diseases in the absence of responsible medical supervision presents myriad potential problems with regard to the safety of consuming amino acid supplements. These range from effects of the amino acid on the disease condition to interactions of amino acids with medications or treatment regimens. For example, administration of methionine has been shown to exacerbate symptom expression in psychotic patients (Cohen et al., 1974). Tyrosine metabolism has been shown to be abnormal in patients with hepatic cirrhosis (Nordlinger et al., 1979; Henderson et al., 1981) and methionine has been implicated in precipitating episodes of hepatic encephalopathy (Phear et al., 1956). In addition, several interactions of amino acids with drugs have been identified, including interactions of tyrosine or tryptophan with MAO inhibitors. The potential gamut of consequences of unrestricted use of amino acid supplements by both healthy and patient population subgroups has not been investigated. The Expert Panel did not find sufficient data to be comfortable in establishing safe levels of intake in healthy individuals and concluded that amino acid supplement use by these special groups requires responsible medical advice and supervision.
VII. PROPOSED STRATEGY FOR EVALUATION OF SAFETY OF AMINO ACIDS AS DIETARY SUPPLEMENTS

Because the fragmentary nature of the available data provided only a limited basis for evaluation of the safety of amino acids as dietary supplements, the Expert Panel concluded that a systematic approach to safety testing is needed. Evaluation of safety of amino acids used as dietary supplements either singly or in mixtures should be considered within the larger context of the assessment of safety of food ingredients and nutrients. As outlined below, several organizations have provided guidelines and perspective on safety testing of substances used in foods.

A. SAFETY TESTING OF FOOD INGREDIENTS

The FDA has published a compilation of guidelines for toxicological testing of direct food additives (Food and Drug Administration, 1982). Those guidelines are currently being revised and expanded to include neurotoxicity testing and updated information on other types of toxicological testing. At the time that FDA published its guidelines for toxicity testing in 1982, the Food Safety Council also proposed a decision-tree approach for evaluation of the safety of food additives (Food Safety Council, 1982).

From 1972 to 1982, the Select Committee on GRAS Substances (SCOGS) evaluated health aspects of use of substances as GRAS food ingredients. The Select Committee commented on their experiences and reviewed in great detail the range of factors to be taken into consideration in the safety assessment of a given food ingredient (Siu et al., 1977). Their report provided commentary on the state of the art and the nature of the technical dilemmas that were encountered in rendering scientific judgments on safety of food ingredients; suggestions concerning the philosophical, procedural, and scientific ramifications of such an evaluation process; and suggestions for research needed to improve the validity and meaningfulness of the associated data. More recently, the adequacy of existing guidelines for toxicity testing of food ingredients was evaluated in a symposium of the International Life Sciences Institute, Europe (Kroes, 1990; Kroes and Hicks, 1990).

In general, components for evaluation of the safety of food ingredients include estimation of human exposure, acute and chronic toxicity in animals, and assessment of human responses to exposures at proposed usage levels or higher doses. These latter studies are typically predicated on results of the animal feeding trials. While studies of chronic toxicity in animals usually include reproductive or multigenerational studies, the extent to which mutagenicity, carcinogenicity, and allergenicity studies are conducted depends in part on the chemical nature of the substance.

In addition to the above general components for evaluation of safety of food ingredients, special considerations have been identified that are unique to the assessment of the safety of nutrients (vitamins and minerals). Campbell et al. (1980) addressed issues related to the adequacy of analytical methodology, the availability of appropriate populations for study, and the ethical considerations in such studies. Allison et al. (1980) concluded that safety evaluation would require a nutrient–by–nutrient approach and that, when possible, consideration should be given to the identification of predictive indicators rather than diagnostic endpoints of toxicity in humans. The points made in these reports are equally relevant to the evaluation of amino acids used as dietary supplements.
B. APPROACH FOR SAFETY TESTING OF AMINO ACIDS USED AS DIETARY SUPPLEMENTS

1. Test materials

Amino acids used as test materials in safety evaluations should meet specifications for food-grade or pharmaceutical-grade products and be produced in accordance with Good Manufacturing Practices (121 CFR 110) (Office of the Federal Register, 1991a). These specifications are listed, respectively, in the Food Chemicals Codex (National Research Council, 1980) and the U.S. Pharmacopeia – National Formulary (United States Pharmacopeial Convention, 1989). The Food Chemicals Codex specifications for food-grade amino acids are included as Appendix B of this report. In general, standards for food-grade amino acids are slightly less stringent than standards for pharmaceutical-grade amino acids in terms of percent amino acid by weight and arsenic and heavy metal impurities. Standards for pharmaceutical-grade amino acids also set upper limits for organic volatile impurities. A comparison of standards for food-grade and pharmaceutical-grade L-arginine is given in Table 6.

The amino acids that are to be used as test materials in safety studies represent a special case. These substances should come from a single lot and the stability over time should be verified. Because 1.5 to 2.0 percent of the composition of amino acids meeting food- or pharmaceutical-grade standards may be compounds other than the amino acid, the chemical composition should be characterized as completely as analytically possible. Complete chemical characterization is essential in light of the experience with the L-tryptophan association with EMS. For example, if adverse health effects are reported in the future for amino acids used as supplements, comparison of the composition and chemical specifications of a standard test material with the composition and chemical specifications of suspect products will be needed.

2. Types of testing needed

Types of testing needed for evaluation of the safety of nutrients, including amino acids, differ little from safety testing for other substances in foods. Both animal and human studies are needed to determine safe levels of intake for amino acids used as dietary supplements. The Expert Panel concluded, as had Campbell et al. (1980), that "... adverse long-term effects of large quantities of nutrients [i.e., amino acids] may be difficult to correlate with their intake because, in addition to being delayed, they may be related to genetic and environmental factors: race, endocrine and kidney function, degree of obesity, lifestyle, and other factors difficult to correlate with nutrient intake over a lifetime." Because a great number of factors can influence and be influenced by amino acid metabolism, a comprehensive database is needed on absorption, transport, distribution, metabolic interconversions and transformations, biochemical actions, interactions with drugs, nutrients, and other food components, and excretion of each amino acid in the dose ranges actually taken as supplements.

The Expert Panel concluded that adverse effects of amino acids on organ function or metabolic pathways are dependent on the particular structures and metabolic interrelationships of the individual amino acids. Excesses of each of the amino acids result in depressed food intake and growth in animals to varying degrees although there does not appear to be a common underlying mechanism. Each of the amino acids produces unique effects ranging from relatively mild side effects at comparatively large doses for alanine to severe effects at much lower doses for methionine. These individual differences require that safety of each of the amino acids be evaluated separately. In addition, because of potential interactions between and among combinations of amino acids, any mixtures of amino acids sold as dietary supplements should be tested together in the proportions that they are combined.
Table 6. Food-Grade and Pharmaceutical-Grade Specifications for L-Arginine

<table>
<thead>
<tr>
<th></th>
<th>Food-grade specifications</th>
<th>Pharmaceutical-grade specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content of amino acid</td>
<td>98.0 to 102.0%</td>
<td>98.5 to 101.5%</td>
</tr>
<tr>
<td>Specific rotation</td>
<td>+25.0° to 27.9°</td>
<td>+26.2° to +27.6°</td>
</tr>
<tr>
<td>Residue on ignition</td>
<td>not more than 0.2%</td>
<td>not more than 0.3%</td>
</tr>
<tr>
<td>Arsenic</td>
<td>not more than 3 ppm</td>
<td>1.5 ppm</td>
</tr>
<tr>
<td>Lead</td>
<td>not more than 10 ppm</td>
<td>NS</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>not more than 0.002%</td>
<td>0.0015%</td>
</tr>
<tr>
<td>Loss in drying</td>
<td>not more than 1%</td>
<td>not more than 0.5%</td>
</tr>
<tr>
<td>Organic volatile impurities</td>
<td>NS</td>
<td>meets requirements in Method 1 (467) of U. S. Pharmacopeia</td>
</tr>
<tr>
<td>Reference standard</td>
<td>NS</td>
<td>USP L-Arginine Reference Standard</td>
</tr>
</tbody>
</table>

1 From National Research Council (1980) and United States Pharmacopeial Convention (1989)

2 Calculated on a dry weight basis

3 Not specified
There are several categories of endpoints including biochemical, functional, and behavioral changes that may provide predictive indicators in screening for adverse effects of large doses of amino acids.

3. Approach for safety testing in animals

The Expert Panel proposed a two-tiered approach for safety testing of individual or incomplete mixtures of amino acids in animals. A generalized schema for the proposed approach is shown in Figure 2. Implementation of the components of the schema would follow evaluation of information in the extant scientific literature.

Studies of acute and chronic effects of ingestion of amino acids and studies of teratologic and developmental effects comprised the first-tier components of the schema. The components of the first tier were viewed as a screening mechanism for identifying adverse effects and as an indicator of the need for further testing. The specialized studies comprising the second tier included more extensive functional assessments and gross pathological examinations of organs and systems reflecting adverse effects in the first-tier screening phase of the investigation.

a. Endpoints for the first-tier screening studies

Endpoints for the components of the first-tier screening studies are listed in Table 7. These included such general effects as reduced weight gain and food intake shifts, nitrogen balance, and occurrence of diarrhea. More specific effects considered valuable for screening purposes included clinical and behavioral changes, routine biochemical and hematologic studies, and plasma concentrations of amino acids and selected metabolites, plasma concentrations of selected hormones, vitamin B6 status, and acute pharmacokinetic studies.

Effects of amino acids on neurologic processes were a primary concern. In the first tier of testing, evaluation of clinical and behavioral changes would provide an indication of possible functional changes elicited by amino acids and determine which amino acids would require further testing in the second tier of specialized tests. At present there is no complete scientific consensus on the most appropriate set of tests to screen for neurotoxicity. However, suggested approaches including those employed by some Federal agencies have been discussed (National Academy of Sciences, 1992; U.S. Congress, Office of Technology, 1990). An example of an FOB is provided in Table 8. In neurotoxicological screening programs functional changes are often assessed by a Functional Observational Battery (FOB), a collection of noninvasive nervous system tests to evaluate manifestations of neurologic dysfunctions (Moser, 1989; National Academy of Sciences, 1991; U.S. Congress, Office of Technology, 1990). Use of an FOB is proposed here in the first-tier evaluations.

Routine biochemical and hematological analyses were included as endpoints to monitor functional changes in metabolism and to suggest both the necessity and possible focus of the more in-depth investigations in the second tier of testing. General biochemical indicators should include, at a minimum, concentrations of blood glucose, plasma electrolytes, and activities of at least two enzymes indicative of liver function (e.g., ALT and AST). Hematologic indices should include number of red cells, red cell distribution width, hemoglobin concentration and hemoglobin/red cell, total white cell count, and a differential count.

Plasma amino acid concentrations were included, not as an endpoint in themselves, but rather as an indication of whether ingestion of a bolus of an amino acid results in elevated plasma concentrations of the administered amino acid and/or changes in concentrations of other amino acids and as an indirect measure of the hepatic and renal clearance of the amino acid. Studies such as those of Bialik et al. (1989) and Ng and Anderson (1992) suggest that it should not be assumed that plasma
Figure 2. Generalized Schema for Evaluation of Safety of Amino Acids -- Animal Studies
Table 7. Components of First-Tier Screening Studies for Evaluation of Safety of Amino Acids in Animals

<table>
<thead>
<tr>
<th>Acute and Chronic Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both sexes with multiple observation points throughout studies</td>
</tr>
<tr>
<td>Oral administration of amino acid with and without food (several dose levels given as bolus)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Teratology and Developmental Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral administration of amino acid with and without food (multiple doses given as bolus) to males prior to mating and to females during pregnancy and lactation</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Table 8. Suggested Measures for a Functional Observational Battery

<table>
<thead>
<tr>
<th>Home Cage and Open Field</th>
<th>Manipulative</th>
<th>Physiologic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posture ² (D) ³</td>
<td>Ease of removal (R)</td>
<td>Body temperature (I)</td>
</tr>
<tr>
<td>Convulsions and tremors ² (D)</td>
<td>Ease of handling (R)</td>
<td>Body weight (I)</td>
</tr>
<tr>
<td>Palpebral closure ² (D)</td>
<td>Palpebral closure ² (R)</td>
<td></td>
</tr>
<tr>
<td>Lacrimation ² (R)</td>
<td>Approach response ² (R)</td>
<td></td>
</tr>
<tr>
<td>Piloerection ² (Q)</td>
<td>Click response ² (R)</td>
<td></td>
</tr>
<tr>
<td>Salivation ² (R)</td>
<td>Tail pinch response ² (R)</td>
<td></td>
</tr>
<tr>
<td>Vocalizations ² (Q)</td>
<td>Righting reflex (R)</td>
<td></td>
</tr>
<tr>
<td>Rearing (C)</td>
<td>Landing foot splay (I)</td>
<td></td>
</tr>
<tr>
<td>Urination ² (C)</td>
<td>Forelimb grip strength ² (I)</td>
<td></td>
</tr>
<tr>
<td>Defecation ² (C)</td>
<td>Hindlimb grip strength ² (I)</td>
<td></td>
</tr>
<tr>
<td>Gait ² (R)</td>
<td>Pupil response (Q)</td>
<td></td>
</tr>
<tr>
<td>Arousal ² (R)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mobility (R)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stereotypy (D)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bizarre behavior (D)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ From Moser (1989). Used with permission.
² Measures specified by U.S. Environmental Protection Agency (1985).
³ D=descriptive data; R=rank order, scalar data; Q=quantal data; I=interval or continuous data; C=count data
concentrations are necessarily predictive of functional changes occurring after administration of amino acids.

Administration of individual amino acids has been shown to elicit secretion of hormones from endocrine glands including the pituitary, adrenal, and pancreas, as well as gastrointestinal hormones such as cholecystokinin. Monitoring of selected hormones during amino acid safety studies may be instrumental in identification of functional changes and suggest directions for the more in-depth investigations in the second tier of testing.

Because of the central role of vitamin B6 in amino acid metabolism (Merrill and Burnham, 1990), assessment of vitamin B6 status was considered essential for interpretation of effects of amino acid supplements on metabolic and functional changes observed. The requirement for the vitamin is known to increase as protein intake increases (National Research Council, 1989) and consumption of excess amounts of amino acids may increase the dietary requirement for vitamin B6. Measurement of plasma pyridoxal and pyridoxal phosphate and urinary 4-pyridoxic acid was recommended for this assessment.

Acute pharmacokinetic studies including investigations of absorption, distribution, biotransformation, and excretion of the administered amino acid were recommended to trace the fate of the amino acid after ingestion. In conjunction with the dosage administered, these factors determine the concentration of a chemical compound at its sites of action and, therefore, the intensity of its effects as a function of time after dosing (Benet and Sheiner, 1985).

Teratology and developmental studies were included as a part of the schema because studies of inborn errors of amino acid metabolism have shown that high circulating levels of certain amino acids during fetal and infant development may result in growth retardation and abnormalities of nervous system function including mental retardation and seizures. The Expert Panel recommended inclusion of studies of spermatogenesis in males and studies of teratogenic and developmental effects in offspring of female animals given high doses of amino acids during pregnancy and lactation. To evaluate effects of amino acids during early development, changes in neurological parameters (i.e., behavior and/or cognition) and growth effects as assessed by a variety of methods were considered appropriate parameters for the assessment of possible deviations from normal during later phases of development.

b. Endpoints for second-tier studies in animals

In some instances, endpoints for second-tier investigations may be suggested by previous studies. For the most part, necessity and endpoints for second-tier studies will be indicated by results of the first-tier screening studies.

c. Experimental protocols

Available information suggests that amino acids may be taken as dietary supplements for prolonged periods of time. As outlined above, assessment of pharmacokinetic effects, effects with delayed onset, and metabolic adaptations that might occur with higher intakes over a long term were considered most useful in evaluations of amino acid safety. To investigate these types of effects, the Expert Panel recommended the use of acute and chronic studies (at least three months duration for chronic studies in rats) with appropriate controls and multiple observation points.

Male and female laboratory rats were considered the most appropriate experimental model for evaluation of amino acid safety. Amino acid requirements for rats have been established and metabolic pathways for amino acids have been studied extensively in this species. Metabolic and neurological effects of amino acids have been tested extensively in rodent models.
Administration of test substances intragastrically as a bolus both apart from and with food would most closely approximate the manner of human ingestion of amino acid supplements. Effects initiated in the gastrointestinal tract such as release of cholecystokinin may be important mediators of functional changes occurring after ingestion of an amino acid bolus. Route of administration was also shown to be an important variable influencing the concentrations of certain amino acids (e.g., phenylalanine, tyrosine, and tryptophan) in plasma and brain and their effects on short-term food intake in rats (Bialik et al., 1989; Ng and Anderson, 1992). Paired-feeding techniques may be required to differentiate between effects caused by decreased food intake and those caused by administration of an amino acid.

Amino acids should be given in addition to basal diets of known composition which contain adequate amounts of all essential nutrients. Sources and composition of the dietary components should be identified. The amino acid should be administered in different amounts in order to evaluate dose-response relationships of amino acids and endpoints and to determine safe levels of intake. For titration of endpoint responses, the Expert Panel suggested doses of 3, 10, 30, and 100 times the nutritional requirements for indispensable amino acids and 3, 10, 30, and 100 times the levels permitted for addition for protein fortification (21 CFR 172.320) for dispensable amino acids.

4. Approach for safety testing in humans

Figure 3 and Table 9 show components of a generalized schema for humans, again consisting of a two-tiered approach. Epidemiological studies may provide useful information on exposure and populations potentially at greater risk of adverse effects, i.e., populations using specific products, doses taken, and patterns and duration of usage. Information derived from the literature on effects of large doses of amino acids in humans plus data from safety testing in animals form the basis for acute and chronic screening studies included in the first tier. Endpoints for the screening studies include the same categories as the animal testing. With the exception of behavioral and clinical changes, endpoints within each category would be the same or very similar to those described for the animal studies. Test batteries for behavioral testing in neurotoxicology studies recommended by the World Health Organization (Johnson, 1987) and newer computer–implemented test systems such as the widely used Neurobehavioral Evaluation System are recommended for the first-tier evaluations (Anger, 1990; National Research Council, 1992; U.S. Congress, Office of Technology Assessment, 1990). The animal behavioral test batteries suggested above assess functions related to those found in the human testing batteries and can thus be used as a guide to the general functional areas requiring further testing with the human batteries.

Amino acid supplements eligible for testing would be those for which no serious adverse effects were observed in the animal studies. Outcomes of these screening studies will, in turn, determine the need for specialized functional studies. In general, this approach is similar to clinical protocols required for Phase 1 testing of investigational new drugs. (See 21 CFR 312.22. [Office of the Federal Register, 1991u].) The general principles for the conduct of clinical trials for drugs (Food and Drug Administration, 1977) apply to the study of the safety of high doses of amino acids. These principles are listed as follows:

- State clearly the objective(s) of the study.
- Define the selection criteria (including diagnostic criteria and reasons for exclusion) and show comparability of the population studied with the population likely to receive the test substance.
Figure 3. Generalized Schema for Evaluation of Safety of Amino Acids -- Human Studies
Table 9. Components of Acute and Chronic Studies for Evaluation of Amino Acids in Humans

**Acute and Chronic Studies**

Ingestion of amino acid with and without food (multiple doses given as bolus)
- Weight change, food intake, gastrointestinal problems (e.g., diarrhea, malabsorption)
- Neurological signs (e.g., seizures, movement disorders)
- Behavioral changes (e.g., attentional problems, incoordination, sensory losses)
- Routine biochemical studies including blood glucose, electrolytes, and at least 2 liver-function enzymes (AST, ALT)
- Routine hematologic studies including hemoglobin, hematocrit, and complete blood count as a minimum
- Blood levels of all amino acids and selected metabolites
- Blood levels of selected hormones
- Vitamin B6 status (plasma pyridoxal and pyridoxal phosphate, urine 4-pyridoxic acid)
- Acute pharmacokinetic studies
Document the method of randomization and the analysis performed to verify how well the randomization procedure worked.

Plan the suitable size of a clinical experiment. This will also depend upon the following appropriate decisions concerning the precision desired:

(a) the degree of response one can or wishes to detect;
(b) the desired assurance against a false positive finding; and,
(c) the acceptable risk of failure to demonstrate the response when it is, in fact, present in the population.

Include, when appropriate, comparison group(s), usually simultaneously.

Perform double-blind studies whenever feasible, as a means of avoiding patient and physician response bias and selection bias.

Use objective methods of observation where possible and appropriate.

Define response variables (parameters) rigorously, including description of methods of observation and quantification.

Maintain strict adherence to the protocol, or document any modifications that may be necessary or desirable.

Specify limitations imposed upon the study by failure to comply with the written protocol (withdrawals, failure of randomization to produce similar groups, etc.) with some idea of the effect the limitation might have on the result.

The Expert Panel viewed clinical studies in metabolic wards as the experimental model required to provide an adequately controlled situation to screen for safety of amino acid supplements in humans. Because chronic effects are of greatest concern, such studies should be carried out over an extended time period; two months was regarded as the minimal experimental period for these investigations.

Initial studies should be carried out in normal, healthy adult volunteers and consideration should be given to inclusion of members of special populations who are expected to take the amino acids. This suggestion is based on the premise that such individuals would be at least risk if adverse health effects were to result from safety testing. Data from the animal studies should provide information for estimation of the appropriate dose range for the screening studies. Again, several dose levels of the amino acid(s) taken with and apart from meals should be used with multiple observation points over the experimental period. The form in which the amino acid is given (capsules or solution) (Stegink et al., 1987b) may influence its disposition. The approach also suggests inclusion of adults who might be consumers of amino acid supplements such as body builders and athletes. This group of subjects might be at greater risk than other groups of healthy adults if amino acid supplements were used in combination with other drugs (e.g., anabolic steroids or sympathomimetic amines). Nevertheless, this would represent a group that may have had previous exposure to amino acid supplements.

Certain subsets of the population were considered to be more susceptible to adverse effects and thereby excluded by the Expert Panel as participants in safety studies. These include infants, children, adolescents, pregnant and lactating women, and the elderly. Other population groups might be at even greater risk and should be avoided in studies assessing the safety of amino acids as dietary supplements. These include persons with specific diseases or conditions, e.g., hepatic disease, endocrine disorders, diabetes mellitus, and other conditions. Undiagnosed carriers of inborn errors of metabolism represent an additional risk group.
Discussions in the preceding paragraphs are directed to considerations associated with the suggested approach to safety testing in humans. The Expert Panel recognized that the recommended approach does not address the need to understand the consequences of consumption of amino acid supplements by individuals in these several population groups. The Expert Panel considered this issue beyond the Scope of Work of this report.

5. Other endpoints of safety testing

The proposed schemata for safety evaluation of individual amino acids, (Figures 2 and 3) do not include direct study of mutagenicity and carcinogenicity, endpoints of toxicity of universal significance, nor do they include hypersensitivity testing. The rationale for treatment of these specific endpoints is noted in the following paragraphs.

a. Mutagenicity of amino acids

Ishidate et al. (1984) screened a number of food additives, including amino acids for mutagenic activity using two systems: a *Salmonella typhimurium* reverse mutation system (Ames test) and an in vitro chromosomal aberration test with Chinese hamster fibroblast cells. In regard to amino acids, they observed the following results.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Ames Test (one or more strains)</th>
<th>Chromosomal Aberration</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL-alanine</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>L-cysteine hydrochloride</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>glycine</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>L-isoleucine</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>monosodium L-aspartate</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>L-phenylalanine</td>
<td>--</td>
<td>+</td>
</tr>
<tr>
<td>sodium L-glutamate</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>L-threonine</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>L-tryptophan</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>L-valine</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

No other amino acids were reported by Ishidate et al. (1984) as being tested in these studies. The mutagenic activity of L-cysteine had been reported earlier by Yamaguchi and Yamashita (1981) and Glatt et al. (1983). Glatt and Oesch (1985) have reported that L-cysteine is mutagenic in the Ames test following exposure to the postmitochondrial supernatant (S9 fraction) from rat liver and kidney cells. In subsequent studies, Glatt et al. (1990) observed L-cysteine did induce mutations in *S. typhimurium* strains, but that mammalian cells appeared to have protective mechanisms against genotoxic physiological substances such as L-cysteine.

Other investigators have reported chromosomal aberrations when cell systems are exposed to various amino acids. These include L-arginine and L-histidine in grasshopper spermatocytes (Bhattacharya et al., 1986) and L-histidine in human fibroblasts (Oya and Yamamoto, 1988).

Glatt (1990) also noted that amino acids are precursors of metabolically reactive intermediates. For example the L-methionine metabolite, ado-met, is capable of nonenzymatic methylation of deoxyribonucleic acids; similarly, L-arginine is a precursor of nitric oxide which may act through formation of nitroso compounds.
The significance of these results in relation to potential mutagenicity of orally ingested amino acid dietary supplements or to amino acids produced in the gastrointestinal tract following protein degradation is unclear. Glatt (1990) has pointed out that most investigators consider physiologically active intermediates as both nongenotoxic and nonmutagenic. Because of this preconceived opinion, few investigations on genotoxicity of amino acids have been undertaken. Nevertheless, the studies conducted to date indicate that L-cysteine is a bacterial mutagen, and that several amino acids (L-arginine, L-histidine, L-isoleucine, L-Leucine, glycine) and certain metabolites of L-arginine and L-methionine can, in certain in vitro systems, induce chromosomal changes and alter cell division. As Glatt (1990) concluded:

"If specific genotoxic agents are regularly present in the organism, one might assume that protective systems have evolved, e.g., by the coupling of sequential enzymatic reactions, compartmentalization, detoxification and repair. . . . In perturbed systems, such as mutagenicity test systems with bacteria and cultured cells, protective systems may be lost or overflown. It is conceivable that in vivo this may hold, for example, when a physiological compound is administered in excessive doses or in a non-physiological manner; . . . and under extreme physiological conditions."

The Expert Panel concurred with this view. While the evidence for mutagenicity of amino acids is quite limited, the potential for certain amino acids to induce or promote genotoxic responses in mammalian systems or organisms remains to be investigated more fully.

b. Carcinogenicity of amino acids

During the past several decades, numerous foods and food ingredients as well as many industrial chemicals have been evaluated for carcinogenic potential. The most extensive compilations of data are the monographs of the International Agency for Research on Cancer (1971–1991) such as International Agency for Research on Cancer (1991), and the Survey of Compounds Which Have Been Tested for Carcinogenicity ("PHS 149") prepared by the National Cancer Institute (1989). This annotated series has been published more or less annually since 1961 and covers the scientific literature from 1854 to 1986.

Examination of the 52 IARC monographs identified no specific references to any of the amino acids being reviewed in this report. The National Cancer Institute compendia makes reference to studies of carcinogenicity in which arginine, cysteine, glutamic acid, histidine, methionine, proline, tryptophan, and tyrosine are present in the diet or administered with the test substance. In one case, an amino acid (tryptophan) was the test substance; in other cases, the amino acid affected the incidence of tumors occurring after administration of a suspected or known carcinogen. These studies, if pertinent to safety considerations regarding amino acids, were discussed in Chapter V.

Neither Harper et al. (1970) nor Benavenga and Steele (1984), in their comprehensive reviews of adverse effects of amino acids, make reference to or provide analyses of investigations on the carcinogenicity of amino acids. On the basis of the foregoing, it seems reasonable to conclude that, while carcinogenic potential should always be considered in safety evaluations, available information does not support a conclusion that carcinogenicity of orally ingested amino acids taken in addition to dietary protein is of primary importance in evaluation of the safety of most amino acids used as dietary supplements.
c. Hypersensitivity responses to amino acids

The immunologic properties of proteins and large molecular weight peptides are well known. Immunological recognition of foreign proteinaceous substances leads to antibody–antigen reactions, resulting in destruction of the antigenic substances. The recognition phenomenon is both physical and chemical in that structural and surface properties of the antigen elicit the recognition of the antigen by the antibody or related substances.

The Expert Panel was not aware of experimental studies on hypersensitivity to individual amino acids. There are clinical reports of idiosyncratic and adverse reactions to amino acids; however, there are no data to suggest these have an immunologic origin. Based on the molecular size of the amino acids and their ubiquity in intermediary metabolism, there is little scientific rationale to predict that hypersensitivity would be expected.
VIII. CONCLUSIONS AND RECOMMENDATIONS

The Expert Panel was charged in the contractual scope of work with the development of guidelines for the evaluation of the safety of amino acids marketed as dietary supplements. However, this charge included the caveat that the Panel should not consider whether the amino acids were consumed for purposes consistent with the definition of dietary supplement (see page 13). That is, amino acids are marketed and used as dietary supplements, as nutrients for the fortification of proteins, as components of medical foods for nutritive and technical effects, as food ingredients for their nutritive and technical effects, and as drugs for their pharmacological effects. These uses are based, in part, on studies that address efficacy for intended effects and, occasionally, safety for the intended purpose.

In developing its approach to evaluation of the safety of amino acids as dietary supplements, the Expert Panel recognized that the manner of use created a dilemma in safety evaluation because amino acid dietary supplements are, in fact, used primarily for pharmacological purposes or enhancement of physiological function rather than for nutritional purposes. This dilemma also encompasses a regulatory incongruity which is beyond the contractual scope of work. That is, dietary supplements are currently regulated as foods. As foods, they are evaluated only for safety, because foods are considered efficacious as sources of nutrients. In contrast, evaluation of drugs requires consideration of pharmacological efficacy and potential for adverse effects. If evaluation of amino acids were to include consideration of pharmacological efficacy, regulatory considerations would require that supplemental amino acids be considered drugs, not foods. The manner of current use is more consistent with the definition of drugs rather than foods, except for uses in protein fortification, medical foods, and food ingredients added for technical effects.

Because of these issues, the Expert Panel has focused solely on safety of use of amino acids as dietary supplements. In evaluating safety, they examined published scientific literature related to the several purposes (nutritional, physiological, and pharmacological) of consuming or administering amino acids. However, the conclusions of the Expert Panel pertain only to the use of amino acids as dietary supplements.

A. CONCLUSIONS

- An examination of products currently available in the marketplace revealed a wide diversity and/or the absence of standardization of label information. Data on chemical composition, isomeric identification, purity, shelf-life, suggested doses, and contraindications for use were not generally available.

- Data on exposure to and actual consumption patterns of amino acid supplements are extremely limited for all subsets of the U.S. population. Usage data (poundage figures) were available from some manufacturers and distributors of amino acid supplements and provided an indication, albeit incomplete, of the amounts of amino acids marketed as dietary supplements.

- Much of the advertising and product label information suggests that amino acid supplements are used for pharmacological reasons rather than for the nutritional properties of these substances. In this regard, there are many reports (often anecdotal in nature) reporting or refuting the efficacy of amino acids, taken singly or as nutritionally incomplete mixtures, as therapeutic agents or ergogenic aids. The Expert Panel regarded these types of uses as pharmacologic applications intended to prevent or ameliorate specific physiological or psychological conditions. As such, efficacy becomes a criterion for regulatory approval.
However, few if any of these reports are supported by data from carefully controlled, double-blind, investigational protocols.

- There is a paucity of data on the safety of consumption of dietary supplements of amino acids, ingested singly or as mixtures. A review of studies in the published scientific literature indicated that a systematic evaluation of safety of amino acids, regardless of intended use, has not been done.

- In developing conclusions on the adequacy of information on safety of individual amino acids, the Expert Panel relied on published scientific literature, much of which was found to be limited in scope and quality with regard to considerations of safety. Much of the available information used by the Expert Panel was extrapolated from animal studies related to improvement of protein quality, development of enteral products for specific uses, and investigations of pharmacologic properties of amino acids.

- The Expert Panel, in relying on data from animal studies which frequently investigated acute and short-term effects of amino acid administration, was constrained in its ability to extrapolate data from animal studies to effects of chronic consumption by humans.

- For the most part, the Expert Panel considered the available data with respect to safety of amino acids used as dietary supplements by normal, healthy adult males, not taking drugs such as monoamine oxidase inhibitors, using alcohol, or having metabolic disorders or diagnosed diseases.

- The Expert Panel was not able to identify a safe upper level of intake for dietary supplements of amino acids for any of the amino acids reviewed in this report.

- Use of D-amino acids as dietary supplements is clearly inappropriate as they have not been shown to have nutritional functions in humans. In addition, certain D-amino acids, e.g., D-serine and D-proline are potentially quite toxic.

- There are sufficient data to recommend that dietary supplement use of amino acids by women of childbearing age, in particular those who are pregnant or lactating, is inappropriate unless administered under responsible medical supervision. Other ostensibly healthy populations such as infants, children, and the elderly must be considered separately as they may be more liable to the possible deleterious effects of the intake of supplemental amino acids. These groups of individuals may represent population groups potentially at risk or harm from chronic consumption of amino acids, ingested either singly or as incomplete mixtures, in addition to amino acids consumed as constituents of dietary protein. With regard to the elderly, the increased frequency and number of therapeutic agents consumed, coupled with a decreased intake of protein and energy, raise additional concerns with respect to consumption of amino acid supplements.

- As a corollary, persons with various disorders or diseases (e.g., chronic liver disease or diabetes mellitus), persons using over-the-counter or prescription drugs, and persons with inborn errors of metabolism represent unique populations for which the Expert Panel has special concerns regarding the safety of use of supplemental amino acids. The Expert Panel specifically recommended against the use of amino acid supplements by individuals in these categories unless administered under responsible medical supervision.

- The available scientific literature provided an inadequate basis to predict reliably the extent to which amino acids may interact with drugs or other nutrients. These interactions may be important in the consideration of safety of use of amino acids as dietary supplements.
The Expert Panel recognized that, because of differences in metabolism of amino acids among species, there is no single animal model that would be universally acceptable for screening and special studies of all amino acids. The Expert Panel is aware that, for various reasons, the laboratory rat will probably be the animal model selected. If so used for experimental purposes, investigators should identify similarities and differences in metabolism of the particular amino acid between the rat and the human.

B. RECOMMENDATIONS

- There is an immediate need to label products currently in the marketplace to provide accurate information on chemical composition and purity of ingredients, isomeric identity of the amino acid(s), shelf-life, suggested doses, and contraindications for use. As a further safeguard for use of amino acid supplements, a mechanism should be provided for consumer comment on the products marketed. A toll-free number should be included on products for this purpose. Consumer comments to manufacturers, which should be available to the Food and Drug Administration, would provide an additional measure of safety.

- There is need for additional information on consumption of amino acids as dietary supplements. Epidemiological data on exposure including types and amounts of supplements taken and characteristics of consumers are extremely limited. Data and information from national surveys would serve as baseline data in assessing trends of amino acid supplement usage and, more importantly, could serve as the source of estimates of dose levels to be used in screening studies. Particular attention should be paid to obtaining data on consumption of amino acid supplements by certain population subgroups, such as women of childbearing age, infants, children and adolescents, the elderly, and patients who are on chronic drug therapy.

- Evaluation of limited information on patterns of use and occurrence of adverse health effects indicates that there is reason to question the safety of unrestricted use of particular amino acids as dietary supplements. For this reason, systematic evaluation of the safety of all amino acids should be undertaken.

- The paucity of data on safety of amino acids used singly (and in combination) as dietary supplements is adequate reason to recommend consideration of a systematic evaluation of certain biological effects of these substances. There is a need for acute and chronic time course studies in both animals and humans of the consequences of supplemental amino acid ingestion. The Expert Panel has developed a model for systematic approaches for such investigations that includes both screening and specialized studies.
IX. LITERATURE CITED


Abrams, W.B.; Reidenberg, M.J. 1984. Workshop on proposed FDA guidelines for clinical evaluation of drugs being developed for use in the elderly, September 13–14, Rockville, MD. Sponsored by the American Society for Clinical Pharmacology and Therapeutics and the Food and Drug Administration.


231


Food and Drug Administration. 1991. Food labeling; general provisions; nutrition labeling; nutrient content claims; health claims; ingredient labeling; state and local requirements; and exemptions; proposed rules. Fed. Reg. 56:60366–60878.


242


Haskell Laboratory for Toxicology and Industrial Medicine, Stine Laboratory, Grasselli Chemicals Department. 1957. Lysine for dietary supplementation.


Murphy, T.H.; Miyamoto, M.; Sastre, A.; Schnaar, R.L.; Coyle, J.T. 1989. Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. Neuron 2:1547–1558.

Murphy, T.H.; Schnaar, R.L.; Coyle, J.T. 1990. Immature cortical neurons are uniquely sensitive to glutamate toxicity by inhibition of cystine uptake. FASEB J. 4:1624–1633.


X. STUDY PARTICIPANTS

Safety of Amino Acids Used as Dietary Supplements

A. AD HOC EXPERT PANEL

Alvito P. Alvares, Ph.D.
Professor
Department of Pharmacology
Uniformed Services University
of the Health Sciences
Bethesda, Maryland

Ronald L. Prior, Ph.D.
Scientific Program Officer
USDA – ARS
USDA Human Nutrition Research Center
on Aging at Tufts University
Boston, Massachusetts

W. Kent Anger, Ph.D.
Associate Director for Occupational
Research and Health Promotion
Center for Research on Occupational
and Environmental Toxicology
Oregon Health Sciences University
Portland, Oregon

David K. Rassin, Ph.D.
Professor
Department of Pediatrics
Director, Division of
Medical Education and
Research Facilitation
The University of Texas
Medical Branch at Galveston
Galveston, Texas

James E. Leklem, Ph.D.
Professor
Department of Nutrition and
Food Management
Oregon State University
Corvallis, Oregon

Daniel Rudman, M.D.
Professor
Department of Medicine
and Geriatrics
V.A. Medical Center
Milwaukee, Wisconsin

Timothy J. Maher, Ph.D.
Professor and Chairman
Department of Pharmacology
Massachusetts College of Pharmacy
and Allied Health Sciences
Boston, Massachusetts

Robert D. Steele, Ph.D.
Professor
Department of Nutritional
Sciences
University of Wisconsin
Madison, Wisconsin

Mackenzie Walser, M.D.
Professor
Departments of Pharmacology,
Molecular Science, and Medicine
The Johns Hopkins University
School of Medicine
Baltimore, Maryland
B. FOOD AND DRUG ADMINISTRATION

Mona Calvo, Ph.D.
Nutritionist
Experimental Nutrition Section
Clinical Nutrition Branch

Jeanne Rader, Ph.D.
Experimental Nutritionist
Research Surveillance Branch

C. William Cooper
Assistant Director
CFSAN

Elizabeth Yetley, Ph.D.
Chief
Clinical Nutrition Branch

C. LIFE SCIENCES RESEARCH OFFICE STAFF

Sue Ann Anderson, R.D., Ph.D.
Senior Staff Scientist

Carol Rilley
Administrative Assistant

Kenneth D. Fisher, Ph.D. ¹
Director

Donald G. Smith
Literature Retrieval Specialist

J. Elaine Huey
Technical Literature Specialist

E. Theresa Smith
Systems Manager

Robin D. Johnson
Secretary IV

Rosemarie V. Soulen
Administrative Secretary

Daniel J. Raiten, Ph.D.
Senior Staff Scientist

John M. Talbot, M.D.
Senior Medical Consultant

Elwood O. Titus, Ph.D.
Senior Scientific Consultant

¹ Dr. Walser is the inventor of several therapeutically useful preparations containing α-keto acids and certain amino acids discussed in this report. Even though the patents are assigned to Johns Hopkins University, Dr. Walser abstained from development of the ad hoc Expert Panel's conclusions on arginine, lysine, ornithine, serine, threonine, and tyrosine. Dr. Fisher abstained from the final LSRO review of portions of this report that relate to glutamate and glutamine because of pending contractual obligations of the LSRO regarding these substances.
XI. INDIVIDUALS AND ORGANIZATIONS SUBMITTING INFORMATION ON AMINO ACIDS

A. OPEN MEETING PARTICIPANTS

The Open Meeting on the Evaluation of the Safety of Amino Acids and Related Products was held February 4, 1991. Oral presentations were presented by eight individuals and organizations. The following eight individuals participated in the presentations:

Stanley Brassington, Esq., Thomas and Brassington, Schuylkill Haven, PA
Burton Kallman, Ph.D., National Nutritional Foods Association Costa Mesa, CA
Terrence J. Marlowe, Esq., Downingtown, PA
C. Kenneth Mehrling, Sigma–Tau Pharmaceuticals, Inc., Gaithersburg, MD
Abbey S. Meyers, National Organization for Rare Disorders, Inc., New Fairfield, CT
Robert L. Pollack, Ph.D., Philadelphia, PA
James B. Roufs, M.S., R.D., Tyson and Associates, Inc., Hawthorne, CA
Jack L. Samuels, Northfield, IL

B. WRITTEN COMMENTS

The following organizations and individuals submitted written materials for consideration by the ad hoc Expert Panel:

Stanley Brassington, Esq., Thomas and Brassington, Schuylkill Haven, PA
Catherine A. Brownholtz, Philadelphia, PA
John R. Carlson, President, J. R. Carlson Laboratories, Inc., Arlington Heights, IL
Memorandum on Status of Glutamates and Hydrolyzed Protein Products, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Department of Health and Human Services, Washington, DC
Robert H. Cox, Ph.D., Robert H. Cox & Company, Ltd. Scarsdale, NY
Richard E. Cristol, The Glutamate Association, Atlanta, GA

1 Copies of the Open Meeting transcript are available from:
Ace Federal Reporters
444 North Capitol Street, NW
Washington, DC 20001
Andrew G. Ebert, Ph.D., International Glutamate Technical Committee, Atlanta, GA

Lloyd J. Filer, M.D., Ph.D., The University of Iowa, Iowa City, IA

N. W. Flodin, Ph.D., University of South Alabama, Mobile, AL

Expert Advisory Committee on Amino Acids, Health and Welfare Canada, received from Center for Food Safety and Applied Nutrition, Food and Drug Administration

Burton Kallman, Ph.D., National Nutritional Foods Association Costa Mesa, CA

Terrence J. Marlowe, Esq., Downingtown, PA

C. Kenneth Mehring, Sigma-Tau Pharmaceuticals, Inc., Gaithersburg, MD

Abbey S. Meyers, National Organization for Rare Disorders, Inc., New Fairfield, CT

Neil S. Orenstein, Ph.D., Nutritional Biochemistry, Lenox, MA

Jon B. Pangborn, Ph.D., Bionotics, Inc., Lisle, IL

Robert L. Pollack, Ph.D., Philadelphia, PA

W. Ann Reynolds, City University of New York, New York, NY

Joseph V. Rodriggs, Ph.D., ENVIRON Corporation, Arlington, VA

Sandra Rosenthal, Del Mar, CA

James B. Roufs, M.S., R.D., Tyson and Associates, Inc., Hawthorne, CA

Adrienne Samuels, Ph.D., Northfield IL

Jack L. Samuels, Northfield, IL

G. Sarwar, Ph.D., Banting Research Centre, Ottawa, Ontario, Canada

William J. Sauber, Rose City, MI

Karen P. Slifkin, Degussa Corporation, Ridgefield Park, NJ

Lewis D. Stegink, Ph.D., The University of Iowa, Iowa City, IA

Yoshi-Hisa Sugita, Ph.D., Ajinomoto Co., Inc., Chuo-Ku, Tokyo, Japan

Michael C. Trachtenberg, Ph.D., Neuro Genesis, Inc., Houston, TX
APPENDIX A

Regulations for Use of Amino Acids as Food Additives

§ 172.320 Amino acids.

The food additive amino acids may be safely used as nutrients added to foods in accordance with the following conditions:

(a) The food additive consists of one or more of the following individual amino acids in the free, hydrated or anhydrous form or as the hydrochloride, sodium or potassium salts:

L-Alanine
L-Arginine
L-Asparagine
L-Aspartic acid
L-Cysteine
L-Cystine
L-Glutamic acid
L-Glutamine
Aminoacetic acid (glycine)
L-Histidine
L-Isoleucine
L-Leucine
L-Lysine
DL-Methionine (not for infant foods)
L-Methionine
L-Phenylalanine
L-Proline
L-Serine
L-Threonine
L-Tryptophan
L-Tyrosine
L-Valine

(b) The food additive meets the following specifications:

(1) As found in "Food Chemicals Codex," National Academy of Sciences/National Research Council (NAS/NRC), 3d Ed. (1981), which is incorporated by reference (copies may be obtained from the National Academy Press, 2101 Constitution Ave., N.W., Washington, DC 20418, or may be examined at the Office of the Federal Register, 1100 L St., N.W., Washington, DC 20408) for the following:

L-Alanine
L-Arginine
L-Arginine Monohydrochloride
L-Cysteine Monohydrochloride
L-Cystine
Aminoacetic acid (glycine)

1 (21 CFR 172.320) (Office of the Federal Register, 1991g)
L-Leucine
L-Methionine
L-Methionine
L-Tryptophan
L-Phenylalanine
L-Proline
L-Serine
L-Threonine
Glutamic Acid Hydrochloride
L-Isoleucine
L-Lysine Monohydrochloride
Monopotassium L-glutamate
L-Tyrosine
L-Valine

(2) As found in "Specifications and Criteria for Biochemical Compounds," NAS/NRC Publication, 3rd Ed. (1972), which is incorporated by reference (copies are available from the Division of Food and Color Additives, Center for Food Safety and Applied Nutrition (HFF–330), Food and Drug Administration, 200 C St., SW, Washington, DC 20204, or available for inspection at the Office of the Federal Register, 1100 L St., NW, Washington, DC 20408) for the following:

L-Asparagine
L-Aspartic acid
L-Glutamine
L-Histidine

(c) The additive(s) is used or intended for use to significantly improve the biological quality of the total protein in a food containing naturally occurring primarily–intact protein that is considered a significant dietary protein source, provided that:

(1) A reasonable daily adult intake of the finished food furnishes at least 6.5 grams of naturally occurring primarily intact protein (based upon 10 percent of the daily allowance for the "reference" adult male recommended by the National Academy of Sciences in "Recommended Dietary Allowances," NAS Publication No. 1694, 7th Ed. (1968), which is incorporated by reference. Copies are available from the Division of Food and Color Additives, Center for Food Safety and Applied Nutrition (HFF–330), Food and Drug Administration, 200 C St., SW, Washington, DC 20204, or available for inspection at the Office of the Federal Register, 1100 L St., NW, Washington, DC 20408.

(2) The additive(s) results in a protein efficiency ratio (PER) of protein in the finished ready-to-eat food equivalent to casein as determined by the method specified in paragraph (d) of this section.

(3) Each amino acid (or combination of the minimum number necessary to achieve a statistically significant increase) added results in a statistically significant increase in the PER as determined by the method described in paragraph (d) of this section. The minimum amount of the amino acid(s) to achieve the desired effect must be used and the increase in PER over the primarily–intact naturally occurring protein in the food must be substantiated as a statistically significant difference with at least a probability (P) value of less than 0.05.

(4) The amount of the additive added for nutritive purposes plus the amount naturally present in free and combined (as protein) form does not exceed the following levels of amino acids expressed as percent by weight of the total protein of the finished food:
Percent by weight of total protein (expressed as free amino acid)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arginine</td>
<td>6.6</td>
</tr>
<tr>
<td>L-Asparagine (including L-asparagine)</td>
<td>7.0</td>
</tr>
<tr>
<td>L-Cystine (including L-cysteine)</td>
<td>2.3</td>
</tr>
<tr>
<td>L-Glutamic acid (including L-glutamine)</td>
<td>12.4</td>
</tr>
<tr>
<td>Aminoacetic acid (glycine)</td>
<td>3.5</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>2.4</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>6.6</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>8.8</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>6.4</td>
</tr>
<tr>
<td>L-and DL-Methionine</td>
<td>3.1</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>5.8</td>
</tr>
<tr>
<td>L-Proline</td>
<td>4.2</td>
</tr>
<tr>
<td>L-Serine</td>
<td>8.4</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>5.0</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>1.6</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>4.3</td>
</tr>
<tr>
<td>L-Valine</td>
<td>7.4</td>
</tr>
</tbody>
</table>

(d) Compliance with the limitations concerning PER under paragraph (c) of this section shall be determined by the method described in sections 43.212–43.216, "Official Methods of Analysis of the Association of Official Analytical Chemists," 13th Ed. (1980), which is incorporated by reference. Copies may be obtained from the Association of Official Analytical Chemists, 2200 Wilson Blvd., Suite 400, Arlington, VA 22201–3301, or may be examined at the Office of the Federal Register, 1100 L St., N.W., Washington, DC 20408. Each manufacturer or person employing the additive(s) under the provisions of this section shall keep and maintain throughout the period of his use of the additive(s) and for a minimum of 3 years thereafter, records of the tests required by this paragraph and other records required to assure effectiveness and compliance with this regulation and shall make such records available upon request at all reasonable hours by any officer or employee of the Food and Drug Administration, or any other officer or employee acting on behalf of the Secretary of Health and Human Services and shall permit such officer or employee to conduct such inventories of raw and finished materials on hand as he deems necessary and otherwise to check the correctness of such records.

(e) To assure safe use of the additive, the label and labeling of the additive and any premix thereof shall bear, in addition to the other information required by the Act, the following:

1. The name of the amino acid(s) contained therein including the specific optical and chemical form.

2. The amounts of each amino acid contained in any mixture.

3. Adequate directions for use to provide a finished food meeting the limitations prescribed by paragraph (c) of this section.

(f) The food additive amino acids added as nutrients to special dietary foods that are intended for use solely under medical supervision to meet nutritional requirements in specific medical conditions and comply with the requirements of part 105 of this chapter are exempt from the limitations in paragraphs (c) and (d) of this section and may be used in such foods at levels not to exceed good manufacturing practices.

APPENDIX B

Specifications for Food-Grade Amino Acids


L-Alanine

Chemical formula: \( \text{CH}_3\text{CH(NH}_2\text{)COOH} \)

Molecular weight: 89.09

L-Alanine (L-2-aminopropanoic acid) is a white, odorless, crystalline powder having a sweetish taste. It is freely soluble in water, sparingly soluble in alcohol, and insoluble in ether.

The compound must contain not less than 98.5% and not more than 102.0% of L-alanine by weight, not more than 3 ppm arsenic (as As), not more than 0.002% heavy metals (as Pb) and not more than 10 ppm lead. Its specific rotation \([\alpha]_D^{20}\) is between +13.5° and +15.5°.

L-Arginine

Chemical formula:
\[
\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CHCOOH} \\
\text{C} = \text{NH} \\
\text{NH}_2
\]

Molecular weight: 174.20

L-Arginine (L-1-aminoo-4-guanidovaleric acid) is white crystals or a white crystalline powder. It is soluble in water, but insoluble in ether and sparingly soluble in alcohol. It is strongly alkaline, and its water solutions absorb carbon dioxide from the air.

The compound must contain not less than 98.0% and not more than the equivalent of 102.0% of L-arginine by weight, not more than 3 ppm arsenic (as As), not more than 0.002% heavy metals (as Pb) and not more than 10 ppm lead. Its specific rotation \([\alpha]_D^{20}\) is between +25.0° and +27.9°.
L-Asparagine

Chemical formula: \( \text{H}_2\text{NOCCH}_2\text{CH(NH}_2\text{)COOH} \)

Molecular weight: 150.13

L-Asparagine (L- \( \alpha \)-aminosuccinamic acid) is white crystals or crystalline powder having a slightly sweet taste. It is soluble in water and practically insoluble in alcohol and in ether.

The compound must contain not less than 98.0% and not more than 101.0% of L-asparagine, not more than 3 ppm arsenic (as As) and not more than 10 ppm heavy metals (as Pb). Its specific rotation \( [\alpha]^{20}_D \) is between +33.0° and +36.5°.

L-Aspartic Acid

Chemical formula: \( \text{HOOCCH}_2\text{CH(NH}_2\text{)COOH} \)

Molecular weight: 133.10

L-Aspartic acid (L-aminosuccinic acid) is white, odorless crystals or crystalline powder having a slightly acid taste. It is slightly soluble in water but insoluble in alcohol and ether.

The compound must contain not less than 98.5% of L-aspartic acid by weight, not more than 3 ppm arsenic (as As), not more than 10 ppm heavy metals (as Pb). Its specific rotation \( [\alpha]^{20}_D \) is between +24.5° and +26.0°.

L-Cysteine Monohydrochloride

Chemical formula: \( \text{HSCH}_2\text{CHCOOH} \cdot \text{H}_2\text{O} \)

Molecular weight: 175.63

L-Cysteine monohydrochloride (L-2-amino-3-mercaptopropanoic acid monohydrochloride) is a white, odorless, crystalline powder having a characteristic acidic taste. It is freely soluble in water and in alcohol.

The compound must contain not less than 98.0% and not more than 102.0% of L-cysteine monohydrochloride by weight, not more than 3 ppm arsenic (as As), not more than 0.002% heavy metals (as Pb) and not more than 10 ppm lead. Its specific rotation \( [\alpha]^{20}_D \) is between +5.0° and +8.0°.
L–Cystine

Chemical formula: \( \text{HOOCCH(NH}_2\text{)CH}_2\text{SSCH}_2\text{CH(NH}_2\text{)COOH} \)

Molecular weight: 240.29

L–Cystine \([3,3'\text{–dithiobis(2–aminopropanoic acid)}]\) is colorless, practically odorless, white crystals. It is soluble in dilute mineral acids and in alkaline solutions. It is very slightly soluble in water and in alcohol.

The compound must contain not less than 98.0\% and not more than the equivalent of 102.0\% of L–cystine, not more than 3 ppm arsenic (as As), not more than 0.004\% heavy metals (as Pb), not more than 0.005\% iron and not more than 10 ppm lead. Nitrogen (total) must be between 11.5\% and 11.9\%. Its specific rotation \( [\alpha]_D^{20} \) is between \(-215^\circ\) and \(-225^\circ\).

L–Glutamic Acid

Chemical formula: \( \text{HOOCCH}_2\text{CH}_2\text{CH(NH}_2\text{)COOH} \)

Molecular weight: 147.13

L–Glutamic acid (L–2–aminopentanedioic acid) is a white, practically odorless, free–flowing, crystalline powder. It is slightly soluble in water, forming acidic solutions.

The compound must contain not less than 99.0\% L–glutamic acid, not more than 3 ppm arsenic (as As), not more than 0.2\% chloride, not more than 0.002\% heavy metals (as Pb), not more than 10 ppm lead. Its specific rotation \( [\alpha]_{546.1 \text{ nm}}^{25} \) is between \(+37.7^\circ\) and \(+38.5^\circ\); \( [\alpha]_D^{20} \) is between \(+31.5^\circ\) and \(+32.2^\circ\).

L–Glutamine

Chemical formula: \( \text{H}_2\text{NOCCH}_2\text{CH}_2\text{CH(NH}_2\text{)COOH} \)

Molecular weight: 146.15

L–Glutamine (L–2–aminoglutaric acid) is white, odorless crystals or crystalline powder having a slightly sweet taste. It is soluble in water and practically insoluble in alcohol and in ether.

The compound must contain not less than 98.0\% and not more than 101.0\% of L–glutamine, not more than 3 ppm arsenic (as As), not more than 10 ppm heavy metals (as Pb). Its specific rotation \( [\alpha]_D^{20} \) is between \(+6.3^\circ\) and \(+7.3^\circ\).
Glycine

Chemical formula: \( \text{H}_2\text{NCH}_2\text{COOH} \)

Molecular weight: 75.07

Glycine (aminoacetic acid; glycocoll) is a white odorless, crystalline powder having a sweetish taste. One g dissolves in about 4 ml of water. It is very slightly soluble in alcohol and in ether.

The compound must contain not less than 98.5% and not more than the equivalent of 101.5% of glycine by weight, not more than 3 ppm arsenic (as As), not more than 0.002% heavy metals (as Pb), not more than 5 ppm lead.

L-Histidine

Chemical formula:

\[
\begin{array}{c}
\text{CH}_2\text{CHC} \\
\text{N}  \\
\text{NH} \\
\text{NH}_2
\end{array}
\]

Molecular weight: 155.16

Histidine [L- \( \alpha \)-amino-4(or 5)-imidazolepropionic acid] is white, odorless crystals or crystalline powder having a slightly bitter taste. It is soluble in water, very slightly soluble in alcohol, and insoluble in ether.

The compound must contain not less than 98.0% and not more than 101.0% of histidine by weight, not more than 3 ppm arsenic (as As), not more than 10 ppm heavy metals (as Pb). Its specific rotation \( [\alpha]_D^{20} \) is between +11.5° and +13.0°.

L-Isoleucine

Chemical formula:

\[
\begin{array}{c}
\text{CH}_3\text{CH}_2\text{CHCHC} \\
\text{H}_2\text{C}  \\
\text{NH}_2
\end{array}
\]

Molecular weight: 131.18

L-Isoleucine (L-2-amino-3-methylvaleric acid) is crystalline leaflets or a white crystalline powder having a bitter taste. It is soluble in 25 parts of water, slightly soluble in hot alcohol, and soluble in dilute mineral acids and in alkaline solutions.

The compound must contain not less than 98.0% and not more than the equivalent of 102.0% of L-isoleucine by weight, not more than 3 ppm arsenic (as As), not more than 0.002% heavy metals (as Pb), not more than 10 ppm lead. Its specific rotation \( [\alpha]_D^{20} \) is between +38.0° and +41.5°.
L-Leucine

Chemical formula: \[ \text{CH}_3-\text{CHCH}_2\text{CH}-\text{COOH} \]
\[ \text{CH}_3 \quad \text{NH}_2 \]

Molecular weight: 131.18

L-Leucine (L-2-amino-4-methylvaleric acid) is small, white, lustrous plates, or a white crystalline powder. One g dissolves in about 40 ml of water and in about 100 ml of acetic acid. It is sparingly soluble in alcohol, but is soluble in dilute hydrochloric acid and in solutions of alkali hydroxides and carbonates.

The compound must contain not less than 98.5\% of L-leucine by weight, not more than 3 ppm arsenic (as As), not more than 0.003\% heavy metals (as Pb), not more than 10 ppm lead. Its specific rotation \([\alpha]^{25}_D\) is between +15.0° and +16.0°; \([\alpha]^{20}_D\) is between +14.9° and +16.5°.

L-Lysine Monohydrochloride

Chemical formula: \[ \text{NH}_2(\text{CH}_2)_4\text{CH(NH}_2\text{)COOH.HCL} \]

Molecular weight: 182.65

L-Lysine monohydrochloride (2,6-diaminohexanoic acid hydrochloride) is a white or nearly white, practically odorless, free-flowing, crystalline powder. It is freely soluble in water, but is almost insoluble in alcohol and in ether.

The compound must contain not less than 98.0\% of L-lysine monohydrochloride by weight, not more than 3 ppm arsenic (as As) and not more than 10 ppm heavy metals (as Pb). Its specific rotation \([\alpha]^{25}_D\) is between +19.6° and +21.6°.

L-Methionine

Chemical formula: \[ \text{CH}_3\text{SCH}_2\text{CH}_2\text{CHCOOH} \]
\[ \text{NH}_2 \]

Molecular weight: 149.21

L-Methionine (L-2-amino-4-(methylthio)butyric acid) is colorless or white lustrous plates, or a white crystalline powder. It has a slight, characteristic odor. It is soluble in water, in alkali solutions, and in dilute mineral acids. It is slightly soluble in alcohol and practically insoluble in ether.

The compound must contain not less than 99.0\% of L-methionine by weight, not more than 3 ppm arsenic (as As), not more than 0.002\% heavy metals (as Pb), not more than 10 ppm lead. Its specific rotation \([\alpha]^{25}_D\) is between −6.8° and −8.2°; \([\alpha]^{20}_D\) is between +21.0° and +25.0°.
DL-Phenylalanine

Chemical formula: $C_6H_5CH_2CH(NH_2)COOH$

Molecular weight: 165.19

DL-Phenylalanine (DL-α-amino-β-phenylpropionic acid) is white, odorless, crystalline platelets. It is soluble in water, in dilute mineral acids, and in solutions of alkali hydroxides. It is very slightly soluble in alcohol.

The compound must contain not less than 98.0% and not more than 102.0% of DL-phenylalanine, not more than 0.03% ammonium salts (as NH$_3$), not more than 3 ppm arsenic (as As), not more than 0.02% chloride, not more than 0.002% heavy metals (as Pb), not more than 0.005% iron, not more than 10 ppm lead.

L-Phenylalanine

Chemical formula: $C_6H_5CH_2CH(NH_2)COOH$

Molecular weight: 165.19

L-Phenylalanine (L-α-amino-β-phenylpropionic acid) is colorless or white platelike crystals or a white crystalline powder having a slight characteristic odor and a slightly bitter taste. One g is soluble in about 35 ml of water. It is slightly soluble in alcohol, in dilute mineral acids, and in alkali hydroxide solutions.

The compound must contain not less than 98.5% and not more than the equivalent of 102.0% of L-phenylalanine, not more than 3 ppm arsenic (as As), not more than 0.002% heavy metals (as Pb), not more than 10 ppm lead. Its specific rotation $[\alpha]_{20}^D$ is between $-33.0^\circ$ and $-35.2^\circ$.

L-Proline

Chemical formula: \[ \begin{array}{c}
\text{N} \\
\text{H} \\
\text{COOH}
\end{array} \]

Molecular weight: 115.13

L-Proline (L-2-pyrrolidinecarboxylic acid) is white crystals or a crystalline powder. It is odorless and has a slightly sweet taste. It is very soluble in water and in alcohol, but insoluble in ether.

The compound must contain not less than 98.5% and not more than equivalent of 101.0% of L-proline by weight, not more than 3 ppm arsenic (as As), not more than 0.002% heavy metals (as Pb), not more than 10 ppm lead. Its specific rotation $[\alpha]_{20}^D$ is between $-84.0^\circ$ and $-86.0^\circ$.
L-Serine

Chemical formula: \[ \text{H}_2\text{C} \rightarrow \text{CH} \rightarrow \text{COOH} \]
\[ \text{HO} \quad \text{NH}_2 \]

Molecular weight: 105.10

L-Serine (L-2-amino-3-hydroxypropanoic acid) is a white crystalline powder without odor and having a sweet taste. It is soluble in water, but is insoluble in alcohol and in ether.

The compound must contain not less than 98.0% and not more than the equivalent of 102.0% of L-serine by weight, not more than 3 ppm arsenic (as As), not more than 0.002% heavy metals (as Pb), not more than 10 ppm lead. Its specific rotation \([\alpha]^{20}_D\) is between +13.5° and +16°.

L-Threonine

Chemical formula: \[ \text{CH}_3\text{CHCHCOOH} \]
\[ \text{HO} \quad \text{NH}_2 \]

Molecular weight: 119.12

L-Threonine (L-2-amino-3-hydroxybutyric acid) is a white, odorless, crystalline powder having a slightly sweet taste. It is freely soluble in water, but insoluble in alcohol, ether, and in chloroform.

The compound must contain not less than 98.0% and not more than the equivalent of 102.0% of L-threonine by weight, not more than 3 ppm arsenic (as As), not more than 0.002% heavy metals (as Pb), and not more than 10 ppm lead. Its specific rotation \([\alpha]^{20}_D\) is between −26.0° and −29.0°.

L-Tryptophan

Chemical formula:

\[
\begin{array}{c}
\text{CH}_3\text{CHCOOH} \\
\text{H} \\
\text{NH}_2
\end{array}
\]

Molecular weight: 204.23

L-Tryptophan (L-α-amino-3-indolepropionic acid) is white to yellowish white crystals or a crystalline powder. It is odorless and has a slightly bitter taste. One g dissolves in about 100 ml of water. It is soluble in hot alcohol, in dilute hydrochloric acid, and in alkali hydroxide solutions.

The compound must contain not less than 98.0% and not more than the equivalent of 102.0% of L-tryptophan by weight, not more than 3 ppm arsenic (as As), not more than 0.002% heavy metals (as Pb), and not more than 10 ppm lead. Its specific rotation \([\alpha]^{20}_D\) is between −30.0° and −33.0°.
L-Tyrosine

Chemical formula: \[ \text{HO-} \overset{\text{CH}_{2}}{\text{CH}} \text{(NH}_{2}\text{)} \text{COOH} \]

Molecular weight: 181.19

L-Tyrosine (L-β-(p-hydroxyphenyl)alanine) is colorless, silky needles, or a white crystalline powder. One g is soluble in about 230 ml of water. It is soluble in dilute mineral acids and in alkaline solutions. It is very slightly soluble in alcohol.

The compound must contain not less than 98.5% of L-tyrosine by weight, not more than 3 ppm arsenic (as As), not more than 0.003% heavy metals (as Pb), and not more than 10 ppm lead. Its specific rotation \( [\alpha]_{D}^{20} \) is between -9.8 and -11.2 \( [\alpha]_{D}^{20} \) between -11.3 and -12.3.

L-Valine

Chemical formula: \[ \text{CH}_{2}\text{CHCHCOOH} \]

\[ \overset{\text{H}_{3}\text{C}}{\text{H}_{2}\text{C}} \text{ NH}_{2} \]

Molecular weight: 117.15

L-Valine (L-2-amino-3-methylbutyric acid) is a white, odorless, crystalline powder having a characteristic taste. It is freely soluble in water, but is practically insoluble in alcohol and in ether.

The compound must contain not less than 98.0% and not more than the equivalent of 102.0% of L-valine by weight, not more than 3 ppm arsenic (as As), not more than 0.002% heavy metals (as Pb), and not more than 10 ppm lead. Its specific rotation is \( [\alpha]_{D}^{20} \) is between +26.5° and +29.0°.