**REVIEW**

**Origin, Microbiology, Nutrition, and Pharmacology of d-Amino Acids**

by Mendel Friedman

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Exposure of food proteins to certain processing conditions induces two major chemical changes: racemization of all l-amino acids (LAAs) to d-amino acids (DAAs) and concurrent formation of cross-linked amino acids such as lysinoalanine (LAL). The diet contains both processing-induced and naturally-formed DAA. The latter include those found in microorganisms, plants, and marine invertebrates. Racemization impairs digestibility and nutritional quality. Racemization rates of LAA residues in a protein vary, relative rates in different proteins are similar. The nutritional utilization of different DAAs varies widely in animals and humans. Some DAAs may exert both adverse and beneficial biological effects. Thus, although d-Phe is utilized as a nutritional source of l-Phe, high concentrations of d-Tyr in such diets inhibit the growth of mice. Both d-Ser and LAL induce histological changes in the rat kidney. The wide variation in the utilization of DAAs is illustrated by the fact that, whereas d-Meth is largely utilized as a nutritional source of the l-isomer, d-Lys is not. Similarly, although l-CysSH has a sparing effect on l-Meth when fed to mice, d-CysSH does not. Since DAAs are consumed as part of their normal diet, a need exists to develop a better understanding of their roles in foods, microbiology, nutrition, and medicine. To contribute to this effort, this overview surveys our present knowledge of the chemistry, nutrition, safety, microbiology, and pharmacology of DAAs. Also covered are the origin and distribution of DAAs in food and possible roles of DAAs in human physiology, aging, and the etiology and therapy of human diseases.

**1. Introduction.** – Microorganism-synthesized d-amino acids (DAAs) are estimated to constitute approximately one third of the human DAA burden [1]. During food processing, naturally occurring l-amino acids (LAAs) may be transformed to their mirror-image configuration d-isomers. A better understanding of the dietary significance of DAAs requires knowledge about the factors that induce racemization of LAA to DAA in food proteins during food processing. Processing-induced amino acid racemization includes those formed during exposure of food proteins to high pH, heat, and acids. The nutritional effectiveness of protein-bound essential DAAs depends on the amino acid composition, digestibility, and physiological utilization of released amino acids. Since an amino acid must be liberated by digestion before nutritional utilization can occur, the decreased susceptibility to digestion by proteolytic enzymes of D-D, D-L, and l-D peptide bonds in DAA-containing proteins is a major factor adversely affecting the bioavailability of protein-bound DAAs. In addition, some DAAs may be toxic. Some DAAs have been shown to impart beneficial effects *in vivo*. It is not known

Whether the biological effects of DAAs vary, depending on whether they are consumed in the free state or as part of protein that contains processing-induced DAAs. To cross-fertilize information among several disciplines and to stimulate further needed studies, this review attempts to integrate and correlate the widely scattered literature on the origin, formation, chemistry, microbiology, nutrition, pharmacology, and toxicology, and medical aspects of DAA. This review is intended to stimulate interest in further research to optimize beneficial effects of DAAs.

Origin of Chirality. Although the formation of L-amino acid (LAA) and D-sugars in the primordial environment during evolution of life is generally considered to be a result of chance [2], definitive proof of the origin of homochirality is still elusive, as illustrated by the following selected observations listed chronologically.

- A mathematical model was used to describe the parity-violating energy differences between enantiomeric molecules [3]. The model predicts that an enantiomeric excess (ee) is expected in chiral products resulting from reactions of achiral or racemic substrates. A non-equilibrium racemic reaction system such as the polymerization of D,L-amino acids to a protein spontaneously switches to a homochiral reaction channel, giving only the L-product. Calculations show that polypeptides of the L-series are preferentially stabilized by electroweak interactions. Such polypeptides have lower energies due to parity violation than the corresponding D-isomers. Since the formation of optically active biomolecules may be ‘energy-driven’, the creation of homochiral biochemistry may not be accidental.

- Based on crystallization studies, Cintas [4] suggests that homochirality may be the result of differences in energy and growth rates of crystal surfaces to which D- or L-amino acids bind. The autocatalytic cycles during crystallization and oligomerization may account for homochirality of living organisms.

- A review of the literature on cycles of solvation, crystallization, and adsorption processes in the primordial environments (geochromatography) suggests that very small differences in the physical properties of some enantiomers may account for the enhanced formation of pure L-enantiomers, as well as for the preferential incorporation of LAAs and D-carbohydrates into early life forms [5].

- Another plausible idea is that the asymmetric earth rotation caused (induced) at dawn and dusk circularly polarized UV light of opposite polarity and reversed temperature profiles in the oceans [6]. Destruction of the D-isomer by the polarized UV light in a sea surface hotter than at dawn created a daily L-isomer excess protected from radiation by nightfall. The LAAs are then preserved by mechanical diffusion into cold, darker regions of the sea leading to an LAA excess in early marine life. The authors suggest that these postulated events can be subjected to computer simulation.

- Although the selection of D-ribose in RNA and D-deoxyribose leads to the biosynthesis of proteins exclusively from L-amino acids [7], Bolik et al. [8] provide experimental evidence for the preferential stabilization of the natural D- over the nonnatural L-configuration in nucleic acids. The energy differences in electronic levels of D- and L-RNA may have led to the preferential stabilization of the naturally occurring D-RNA over the L-configuration.

- Nonenzymatic aminoacylation of an RNA minihelix occurred preferentially with L-amino acids [9]. This finding suggests that tRNA aminoacylation, a process by which amino acids first encounter RNA, may have been a critical step in determining LAA
homochirality. However, it should be noted that cells contain defenses against d-aminoacyl-tRNAs [10].

It is likely that more than one physical-chemical event in the primordial environment occurred simultaneously or consecutively that resulted in the creation of homochirality favoring LAAs. The creation of L-homochirality is not the end of creation, however, because special needs seem to have induced nature to also devise enzyme-catalyzed alternate biosynthetic pathways to DAAs [11][12]. Enzymes that are known to be involved in DAA synthesis and metabolism include DAA oxidases [13], deacylases [14], dehydrogenases [15], epimerases, [16][17], proteases [18], and racemases [15].

Because DAAs from natural sources are part of the human diet, we will first briefly review their formation and function in plants, microbes, and other natural sources, followed by a discussion of factors that favor racemization by conditions encountered during home and commercial processing of food.

2. Analysis. – Because all of the LAA residues (Fig. 1) in a protein and additional formed during food processing (Fig. 2) can undergo racemization simultaneously, but at differing rates, assessment of the extent of racemization in a protein is a difficult task that requires quantitative measurement of ca. 40 l- and d-optical isomers [19]. Reported methods include the use of chiral-phase gas chromatography [20], HPLC [21], HPLC with enzyme reactors [22], LC/MS [23][24], capillary electrophoresis [25][26], electrokinetic chromatography with detection by laser-induced fluorescence [27], and biosensors [28][29]. Detailed discussion of these and other methods for analysis of DAA in food and biological samples is beyond the scope of this review [30–40].

3. Natural Occurrence of DAAs. – Plants. Plants appear to be able to synthesize DAA derivatives and peptides de novo [41–43], as illustrated by the following examples. Germinating pea seedlings (Pisum sativum) contain high concentrations of N-malonyl-d-Ala and γ-L-glutamyl-d-Ala [44]. The concentration of the latter increased to 2.5 μm/seedling after 8 d of germination. d-Ala-d-Ala was found to be present in tobacco leaves [45]. The relative concentrations of three d-Ala peptides (d-Ala-Gly, d-Ala-d-Ala, and d-Ala-l-Ala) produced in rice plants (genus Oryzae) appears to be related to the nature of the rice strain [46]. Approximately 7% of the total alanine content in grass leaves consisted of d-Ala [47]. Exogenously supplied d-Ala, possibly of microbial origin, is required for the plant to synthesize the dipeptides. Ono et al. [48] demonstrated the presence of alanine racemase and of DAAs in alfalfa seedlings (Medicago sativa L.). Studies by Forsum et al. [49] indicate that transgenic plants can be engineered to utilize DAAs from the soil as a nitrogen source. The plant racemase resembled racemases of bacterial origin. Hydrolyzed proteins used as plant fertilizers also contain significant amounts of DAAs [25].

3.1. Microbes. Microbes provide a potential source of dietary DAAs. They produce, metabolize, and utilize DAAs [50][51]. Since microbial DAAs enter the food chain, I will briefly examine some aspects of the microbiology of DAAs. Brückner et al. [52] determined the DAA content produced by several classes of bacteria (Acetobacter, Bifidobacterium, Brevibacterium, Lactobacillus, Micrococcus,
Propionibacterium, and Streptococcus) used in starter cultures for the production of fermented foods and beverages. \(\text{d-Ala}\) and \(\text{d-Asp}\) were found at the highest concentrations in all bacteria. \(\text{d-Glu}\) was present in several classes of the microorganisms. Lower but significant amounts of \(\text{d-Leu}, \text{d-Lys}, \text{d-Met}, \text{d-Orn}, \text{d-Phe}, \text{d-Pro}, \text{d-Ser}, \text{d-Thr},\) and \(\text{d-Tyr}\) were also detected in some of the bacteria. DAAs in fermented foods may largely originate from bacteria. Bacteria from oral and intestinal floras and rumen microorganisms are also potential sources of dietary DAAs [53][54].

3.2. Peptidoglycans in Bacterial Cell Walls. Bacterial cell walls may constitute a major source of dietary DAAs. The thick cell wall of a Gram-positive bacterium consists of peptidoglycan (a sugar–amino acid polymer) and teichoic acid. Both polymers contain DAAs. The much thinner cell wall of Gram-negative bacteria consists entirely of peptidoglycan and associated proteins. The composition of the peptidoglycans and teichoic acids may vary among different classes of bacteria. The DAAs in the bacterial cell walls contribute to their resistance to digestion by proteolytic enzymes. Related studies showed that:

- \(\text{d-Ala}\) is required for the synthesis of the mucopeptide component of nearly all bacterial cell walls [55];
the peptidoglycan structure of *Lactobacillus casei* contained asparagine cross-links between d-Ala and l-Lys [56];

- glutamate racemase in *E. coli* provides d-glutamate, an indispensable component of peptidoglycans in bacteria [57];

- mutations leading to increased resistance of *Enterococcus faecalis* to antibiotics are governed by the amount of d-Ala-d-Ala and d-Ala-d-lactate incorporated into peptidoglycan precursors [58];

- d-Ala-d-Ala contributes to the resistance of antibiotics [59];

- the gene encoding d-Thr aldolase (an enzyme that catalyzes the inversion of d-Thr to d-allo-Thr in *E. coli*) is responsible for the incorporation of d-Ala into cell wall teichoic acid in *Bacillus subtilis* [60][61];

- d-Ser dehydratase from *E. coli* catalyzes the conversion of d-Ser, d-Thr, and allo-d-Thr to the corresponding α-keto acids and NH₃ [62];

- d-Ser inhibits the action of bacterial transaminase, whereas d-Ala partially protects against this inhibition [63];

- d-Asp and d-Glu can serve as indicators for proteins of bacterial origin [64];

- anaerobic bacteria are capable of using DAAs and l-sugars for growth [65];
- d-Asp and d-Glu levels correlated with growth of probiotic bacteria during fermentation in milk and whey powders [66].

3.3. Peptide Antibiotics. DAA-Containing synthetic and natural peptides possess strong antimicrobial properties. Synthetic peptides that inactivated *Clostridium botulinum* include glycyld-ð-Ala, myristoyld-ð-Asp, sorbyld-ð-Ala, sorbyld-ð-Trp [67]. DAAs in natural antibiotics include d-Asp and d-Glu (bacitracin, mycobacillin); d-Cys (malformin); d-Leu (circulin); d-Orn (bacitracin); d-Phe (funigsporin, polymixin, tyrocidine); d-Ala, d-Leu, d-Val (gramicidin); and d-Ala, d-Leu, d-Val (actinomycin, valinomycin) [68–70]. Substitution of DAAs in the peptides designed to reduce susceptibility to digestion enhances the activity of pleurocydin [71], bombinins H [72], lacticin [73], and other antibiotic peptides [74–76]. The mechanism of action of these peptide antibiotics involves the induction of ionic channels that lead to the disruption of bacterial cell membranes [77].

The cited studies suggest that an understanding of the biochemistry of genes and enzymes involved in the synthesis and metabolism of DAA-containing functional and structural microbial molecules that are targets of antibiotics may help efforts designed to discover improved bactericidal agents.

4. Food Processing-Induced Formation of DAAs. – Historical Perspective. Since the early part of the 20th century, alkali treatments have been known to racemize amino acids [78–80]. The early authors used changes in optical rotation to determine the alkali-induced racemization, and *Kjeldahl* N analysis of NH₂ groups of liberated amino acids and peptides to detect concurrent hydrolysis of peptide bonds. They reported that:

- l-Arg was racemized to the d-isomer, and that the protein clupein was concurrently partly hydrolyzed and racemized in Ba(OH)₂ and NaOH solution at 40 °C [79];
- optically active hydantoins prepared from α-amino acids underwent spontaneous racemization at room temperature, presumably as a result of keto–enol tautomerism involving the asymmetric C-atom [78][81];
- racemization of amino acids in gelatin occurred on treatment with 0.1 and 1.0 N NaOH, but not on treatment with 3.0 N alkali, presumably because in the latter case the rate of peptide-bond hydrolysis is higher than that of racemization [80][82–84];
- heating casein with 1.0 N NaOH at 125°C resulted in complete racemization of all amino acids, which occurred at a faster rate than analogous treatment of gelatin [83] or edestin [84].

The field of amino acid racemization in proteins lay largely dormant for approximately half century because of lack of suitable analytical methods to determine the formation of specific protein-bound DAAs. The development of GC and HPLC techniques along with chiral columns that can separate d- and l-isomers stimulated widespread interest in this subject. The observation by Masters and Friedman [85–89] that widely consumed processed foods contain DAAs stimulated worldwide interest in the distribution of DAAs in the human diet. They reported the following levels of d-Asp in widely consumed foods (d/l ratio; % as d-isomer): coffee-mate (0.208; 17); *Fritos* potato chips (0.164; 14); simulated bacon breakfast strips (0.134; 13); *Isomil* baby formula (0.108; 10); and textured soy protein meat analogue (0.095; 9). These
authors were also apparently the first to relate racemization rate constants of protein-bound DAAs to inductive parameters associated with free LAAs (linear free-energy relationships), and to describe the relationship between DAA content of racemized casein and in vitro digestibility by proteolytic enzymes [86]. First-time studies by Friedman and Gumbmann [90] on the biological utilization of DAA as source of the L-isomer using all amino acid diets and the predicted formation of cross-linked amino acids such as histidinoalanine that may accompany racemization [91] that were later realized are also of historical interest. Some of these aspects are further examined below.

**Racemization Mechanisms.** Fig. 3 offers a mathematical derivation of the first-order kinetic equations that govern reversible in vitro amino acid racemization. Fig. 4 depicts three different mechanisms that have been postulated to govern racemizations of amino acids during food processing. Fig. 5 depicts a postulated mechanism for the in vivo inversion of an LAA to its D-isomer catalyzed by pyridoxal phosphate. Figs. 6–9 illustrate the kinetics of racemization, and Fig. 10 (see below) shows susceptibilities of some amino acids at high pH that may accompany racemization. Tables 1–3 list the DAA contents of racemized proteins. The cited observations indicate that heat and high pH widely used in processing of food induce the racemization of all amino acid residues by the indicated pathways.

Alkali-induced peptide-bond cleavage of soy proteins determined by a ninhydrin assay [35] influenced the extent of racemization [92]. Racemization rates of the same amino acid in polyamino acids were much lower than in soy proteins. Related studies [93] showed that:

- free amino acids racemized approximately ten times slower than did protein-bound ones;
- alkali treatment of zein induced racemization of amino acids residues and caused nutritional damage without the presence of lysinoalanine (LAL) [94];
- serine residues in lactalbumin, leaf protein concentrate, and soy protein were highly susceptible to heat- and alkali-induced racemization [95];
- racemization of protein-bound amino acids, especially of lysine, induced by heat extrusion was greater in soy than in corn proteins [96];
- melanoidins formed during heating of glucose or fructose with LAA or DAA had similarly shaped but different absorption spectra [97].

5. Distribution of DAAs in Food. – As already mentioned, the observation by Masters and Friedman [88] that processed commercial foods contained DAAs was followed by numerous worldwide studies on the content of DAAs in a variety of foods. The aspect is briefly examined below for several food categories listed in alphabetical order.

5.1. Alfalfa Seeds. Alanine racemase catalyzes formation of d-Ala in alfalfa seeds (Medicago sativa L.) [48].

5.2. Animal Skins. Peptides and proteins isolated from animal skin are reported to contain DAAs [98][99].

5.3. Bread. Use of lactic acid bacteria and yeast in the fermentation of sourdough before baking results in the introduction of d-Ala and d-Glu into the dough [100]. Baking of the dough into bread induces a 44% decrease in the total free DAA content.
5.4. Coffee. Roasted coffee contained 10–40% of d-Asp, d-Glu, and d-Phe [101]. Higher amounts of DAAs were formed during roasting of Robusta than of Arabica coffees [102]. Liquid coffee contained ca. 20 mg of total DAA/100 ml [103].

5.5. Dairy Products. DAAs in cow’s milk originate from the digestion (autolysis) of peptides and proteins containing DAAs originating from microbial cell walls.
Raw milk from ruminants (cows, goats, and sheep), but not human milk, contains the following DAAs: $d$-Ala, $d$-Asp, $d$-Glu, $d$-Lys, and $d$-Ser [66][105][106]. Relatively high concentrations of these DAAs were also present in widely consumed ripened cheeses [21]. Emmental cheese contains as much as 70 mg of total DAAs per 100 g [103].

### Table 1. DAA Content \( [\frac{(d/d + d)}{C2} \times 100] = \% d \) of Eight Alkali-Treated Proteins. Adapted from [20][85][92][199][255].

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Casein</th>
<th>Lactalbumin</th>
<th>Wheat gluten</th>
<th>Corn protein</th>
<th>Fish protein</th>
<th>Soybean protein</th>
<th>Bovine albumin</th>
<th>Hemoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>15.2</td>
<td>14.4</td>
<td>18.6</td>
<td>22.2</td>
<td>19.3</td>
<td>15.8</td>
<td>22.1</td>
<td>17.1</td>
</tr>
<tr>
<td>Val</td>
<td>2.6</td>
<td>2.7</td>
<td>4.0</td>
<td>4.9</td>
<td>3.1</td>
<td>2.5</td>
<td>3.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Leu</td>
<td>7.4</td>
<td>5.0</td>
<td>2.7</td>
<td>7.2</td>
<td>7.8</td>
<td>6.8</td>
<td>6.3</td>
<td>8.2</td>
</tr>
<tr>
<td>Ile</td>
<td>3.3</td>
<td>3.1</td>
<td>4.0</td>
<td>5.5</td>
<td>3.6</td>
<td>3.9</td>
<td>5.7</td>
<td>5.0</td>
</tr>
<tr>
<td>Cys</td>
<td>–</td>
<td>32.1</td>
<td>32.0</td>
<td>43.7</td>
<td>22.8</td>
<td>21.0</td>
<td>23.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Met</td>
<td>24.7</td>
<td>32.3</td>
<td>33.1</td>
<td>29.8</td>
<td>29.2</td>
<td>24.3</td>
<td>30.0</td>
<td>26.2</td>
</tr>
<tr>
<td>Phe</td>
<td>24.4</td>
<td>24.3</td>
<td>24.4</td>
<td>32.4</td>
<td>28.0</td>
<td>25.5</td>
<td>28.1</td>
<td>30.0</td>
</tr>
<tr>
<td>Lys</td>
<td>8.1</td>
<td>7.2</td>
<td>9.4</td>
<td>8.0</td>
<td>11.5</td>
<td>11.3</td>
<td>13.3</td>
<td>9.9</td>
</tr>
<tr>
<td>Asp</td>
<td>29.2</td>
<td>22.6</td>
<td>25.6</td>
<td>41.6</td>
<td>25.0</td>
<td>30.8</td>
<td>27.0</td>
<td>18.9</td>
</tr>
<tr>
<td>Glu</td>
<td>19.7</td>
<td>19.5</td>
<td>32.3</td>
<td>35.0</td>
<td>18.9</td>
<td>21.1</td>
<td>18.4</td>
<td>19.8</td>
</tr>
<tr>
<td>Ser</td>
<td>41.0</td>
<td>47.1</td>
<td>42.2</td>
<td>44.0</td>
<td>42.1</td>
<td>44.2</td>
<td>43.0</td>
<td>44.5</td>
</tr>
<tr>
<td>Thr</td>
<td>29.3</td>
<td>29.1</td>
<td>30.0</td>
<td>36.3</td>
<td>32.8</td>
<td>27.8</td>
<td>28.3</td>
<td>31.2</td>
</tr>
<tr>
<td>Tyr</td>
<td>15.0</td>
<td>18.9</td>
<td>19.5</td>
<td>35.5</td>
<td>16.3</td>
<td>13.7</td>
<td>15.3</td>
<td>22.6</td>
</tr>
<tr>
<td>LAL$^a$</td>
<td>4.4</td>
<td>5.4</td>
<td>0.9</td>
<td>0.3</td>
<td>2.8</td>
<td>3.2</td>
<td>8.5</td>
<td>4.4</td>
</tr>
</tbody>
</table>

$^a$ Mixture of (LD + LL) LAL isomers in g/16 g N.

### Table 2. Variation in LAL Isomer Content of Alkali-Treated Proteins. Adapted from [20][199].

<table>
<thead>
<tr>
<th>Protein</th>
<th>LAL [g/16 g N]</th>
<th>[LL-LAL]/([LL-LAL] + [LD-LAL]), Isomer ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zein</td>
<td>0.32</td>
<td>Not detected</td>
</tr>
<tr>
<td>Wheat gluten</td>
<td>0.95</td>
<td>0.50</td>
</tr>
<tr>
<td>Fish protein</td>
<td>2.75</td>
<td>0.40</td>
</tr>
<tr>
<td>concentrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine hemoglobin</td>
<td>3.36</td>
<td>0.50</td>
</tr>
<tr>
<td>Casein</td>
<td>4.40</td>
<td>0.51</td>
</tr>
<tr>
<td>Lactalbumin</td>
<td>5.38</td>
<td>0.51</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>8.52</td>
<td>0.50</td>
</tr>
</tbody>
</table>

### Table 3. Aspartic Acid Racemization and LAL Content of Alkali-Treated Casein and Acetylated Casein. Adapted from [88].

<table>
<thead>
<tr>
<th>Protein</th>
<th>d/L Asp</th>
<th>LAL [mol-%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein, untreated</td>
<td>0.023</td>
<td>0.00</td>
</tr>
<tr>
<td>Casein + alkali</td>
<td>0.387</td>
<td>2.35</td>
</tr>
<tr>
<td>Acetylated casein + alkali$^a$</td>
<td>0.336</td>
<td>0.00</td>
</tr>
</tbody>
</table>

$^a$ Acetylation of $\epsilon$-NH$_2$ groups of lysine side chains prevents LAL but not D-AA formation.

(peptidoglycan) proteins in the rumen of the cows [104]. Raw milk from ruminants (cows, goats, and sheep), but not human milk, contains the following DAAs: $d$-Ala, $d$-Asp, $d$-Glu, $d$-Lys, and $d$-Ser [66][105][106]. Relatively high concentrations of these DAAs were also present in widely consumed ripened cheeses [21]. Emmental cheese contains as much as 70 mg of total DAAs per 100 g [103]. The DAA content of varied
Fig. 4. *Base-catalyzed racemization: Proton abstraction–addition mechanism. a*) An OH⁻ ion abstracts an H⁺ from the R-CH α-C-atom of an amino acid to form a negatively charged carbanion which has lost its original asymmetry [256][257]. The carbanion can then recombine with a proton from the solvent to regenerate the original amino acid which is now racemic (dl). Elimination–addition mechanism: The carbanion can also undergo an elimination reaction to form dehydroalanine side chain. The dehydroprotein can then react with H₂O to form racemized amino acid side chains (e.g., dl-Ser) [85][258]. The concurrent formation of lysinoalanine is also shown. b*) Acid-catalyzed racemization: Protonation of the carboxy group of an l-amino acid facilitates removal of a proton to form the dehydroalanine. Proton addition at two sides (re and si faces) of the double bond generates both d- and l-isomers. Adapted from [259]. c*) Racemization of a compound (fructose-l-phenylalanine) intermediate of dl-Phe. Adapted from [25].
among cheeses and changed during cheese production [107]. Peptide-bond hydrolysis of casein during storage of cheeses may also influence their DAA content [104].

Fig. 4 (cont.)

Fig. 5. Isomeric inversion in vivo: catalysis of inversion of an L-amino acid by the pyridoxal phosphate (PLP) coenzyme of a microbial racemase involving stereoselective shifts of H-atoms. Adapted from [260].
The cited authors also suggest that:

- because the d-Ala content of raw milk increased during storage at 4°C, d-Ala could serve as an indicator of contamination by d-Ala-producing psychotropic bacteria;
- because mastitis is an infection of the udder caused by bacteria, milk from infected cows should have a higher DAA content originating from the microorganisms;
- pasteurization of milk does not increase its d-amino content.

5.6. D-Glutamic Acid in Processed Foods. Monosodium glutamate (MSG), the sodium salt of the naturally occurring nonessential amino acid, is often added at levels of 0.2–0.9% to foods to improve flavor and palatability. A survey showed that a variety of processed foods contained significant amounts of d-Glu (which does not possess flavor-enhancing properties) [108]. The d-% ranged from 0.25 for pure MSG to 0.8 for soups (highest value), 2.9 for crackers, 7.9 for sauces, 18 for vinegars, 36 for sauerkraut juices, 1.6 for tomato products, and 6.2 for milk products.

The d-Glu could originate from microbial sources as well as from food-processing-induced racemization of free and protein-bound l-Glu. Table 2 shows that protein-bound l-Glu is one of the fastest racemizing amino acids. The possible impact of the d-isomer of MSG on the ‘Chinese Restaurant Syndrome’ has apparently not been evaluated.

5.7. Eggs. Alkali pickling of duck eggs in a 4.2% NaOH/5% NaCl solution for 20 d at room temperature used to prepare the traditional Chinese pidan resulted in extensive racemization of amino acid residues and concurrent formation of lysinoalanine (LAL) [109]. The relative racemization rates appear to be similar to those observed with egg albumin and other pure proteins.

5.8. Fish. Heating of laboratory-made herring meals at 125°C induced time-dependent formation of d-Asp [110]. The d-Asp content of twelve fishmeals from
various sources ranged from 0.7 to 3.7%. The D-Asp content may indicate the severity of the thermal treatment during cooking or drying of fish meals [111].

5.9. Food (Dietary) Supplements. Widely used dietary supplements are also reported to contain DAAs [112][113].

5.10. Fruits and Vegetables. Fruits (apples, grapes, oranges) and vegetables (cabbage, carrots, garlic, tomatoes) as well as the corresponding juices contain variable but measurable amounts of DAAs including D-Ala, D-Arg, D-Asp, and D-Glu [21][105]. These amino acids could originate from plant sources, from soil, and other microorganisms, and/or from heat treatments used to kill pathogens.

5.11. Fruit and Vegetable Juices. Brickner and Westhauser [114] reported the following total DAA amounts in fruit and vegetable juices (in µM): carrot juice, 8.5; orange and other fruit juices, 28–57; tomato juice, 14.5. Other investigators [22][27] confirmed that DAAs occur naturally in fruit juices, beers, and wines. Specific DAAs could permit differentiating juices from biologically dissimilar fruits, and could serve as an indicator for detecting bacterial activity and shelf-life of fruit juices.

5.12. Honey. The d/l ratios of Leu, Phe, and Pro could serve as indicators of age, processing, and storage histories of honeys [115].

5.13. Maize (corn grain). Significant differences were observed in the DAA content of some transgenic maize (corn) cultivars compared to standard varieties [36]. The authors suggest that this finding opens up new approaches in comparing the composition of transgenic plant foods to their conventional counterparts.

5.14. Meat Products. Low levels of DAAs (1.25–13.79 µg/g) were detected in both irradiated and non-irradiated cooked hams [116]. There was no correlation between radiation dose and DAA content.

5.15. Spices. Liquid spices contain very high amounts of DAAs, up to 600 mg/100 ml [103].

5.16. Vinegars. Several investigators reported that fermentation conditions used to prepare different vinegars (balsamic, cider, sherry, white wine) affect their DAA profiles [117–120].

5.17. Wines. Autolysis of yeast (Saccharomyces cervisiae) produces compounds that beneficially influence the quality of sparkling wines. Giuffrida et al. [121] found that the pattern of release of DAAs from conventional yeast strains differ from transgenic ones. The content of several DAAs of 26 wines did not correlate with storage times [122]. Wine marinades can be used to inactivate bacteria [123] that may produce DAAs. Other potential dietary sources of naturally occurring DAAs include fungi, insects, fish, and other marine organisms [13].

6. Nutrition, Pharmacology, and Toxicology of DAA. – Twenty naturally occurring amino acids and several hundred amino acid derivatives formed in vivo post-translationally in plants and animals, and in vitro during food processing play a fundamental role in nutrition and other aspects of animal and human health. The general requirements are adequate amounts of the essential amino acids with a reasonable balance among all [124]. Bioavailability and biological utilization of both LAAs and DAAs vary widely and depend on source, chemical and metabolic interactions, and on the diet and health of the consumer. To develop a better understanding of the nutritional significance of DAAs and alkali-treated food proteins,
Fig. 7. Relationship between racemization rates ($k$) relative to Ala, and the inductive constant ($\sigma^*$) of the amino acid side chain $R$ in $RCH(NH_2)COOH$. Adapted from [199] and [93].
we compared the weight gain in mice fed free amino acid diets in which the test LAA was replaced with the d-isomer. The results obtained reflect the ability of mice to utilize DAA in the complete absence of the l-form. In the case of essential amino acids, the mice must meet the entire metabolic demand from the d-isomeric forms.

Biological utilization by weanling male mice of DAAs was tested in a 14-day growth assay by using an all-free amino acid diet in which each l-amino acid was replaced by the d-isomer. The growth assay was standardized with six mice per group, with potency estimation being based on response of two to seven groups. Statistical evaluation of potencies of the DAA was calculated as the slopes of growth curves (weight gain after 14 d per unit of dietary concentration) where linearity was approximated. Mean body weight gains were compared by Duncan’s multiple range test using individual values. The results illustrated in Figs. 10 and 11, and Table 4 show wide variations in the utilization of DAAs as a nutritional source of the corresponding l-isomers.

Digestion and Nutrition. A number of studies described the adverse and beneficial effects of alkali treatment on protein nutrition [86][125–132]. Friedman et al. [86] attempted to quantitatively define the susceptibility of alkali-treated casein to in vitro digestion by trypsin and chymotrypsin (Fig. 11,a). Monitoring on a pH-stat revealed an approximately inverse relationship between DAA and LAL content on one hand and the extent of proteolysis measured on the other. The decreased susceptibility of alkali-treated casein to digestion could arise from loss of susceptible sites to enzyme cleavage. Sarwar and Paquet [133] reported that, although tripeptides composed of LAAs were completely hydrolyzed by intestinal mucosal peptidases, the following DAA-containing tripeptides largely resisted digestion: Ala-d-Met-Ala, Val-d-Met-Phe, Ala-d-Glu-Ala, Val-d-Asp-Ala, and Ala-d-Phe-Leu. Rat feeding studies also showed that d-Met in the tripeptides was less available for growth than l-Met. Alkali treatment by Jenkins et al.
induced formation of DAA and nutritional damage in corn proteins (zein) without formation of LAL [94].

To obtain additional information on this aspect, we evaluated nutritional and histopathological consequences of feeding-toasted and alkali-treated soy flours to baboons. Fig. 11, b, shows the growth curves for seven pre-adolescent male baboons each fed soy control and alkali-treated soy diets. There was no difference in growth rates over a 150-day period, since the slopes of the growth curves are not significantly different from each other. However, in absolute terms, weight gain of the baboons fed the treated soy diet (containing 370 mg of LAL/100 g air-dry diet) was ca. 20% lower than the gain observed with the control soy diet (containing 80 mg of LAL/100 of air-

Fig. 8. Arrhenius Plots for the racemization of protein amino acid residues in proteins: a) casein; b) soy protein. Adapted from [85][199].
dry diet). The decreased weight gains may be due to the decreased digestibility and utilization of the alkali-treated soy protein compared to the control.

Using a 15N-tracer technique, Heine et al. [134] investigated the utilization of DAAs by infants fed parenterally. Retention of 15N in the protein pool ranged from 23.2% for d-[15N]Val to 48.6% for d-[15N]Ala. DAAs were utilized by the infants for protein synthesis only to some and variable extent.

Possible causes for the reduction in digestibility–nutritional quality include destruction of proteolytic enzyme substrates such as Arg and Lys, isomerization of LAAs to less digestible d-forms, formation of inter- and intramolecular cross-links that hinder access of proteolytic enzymes, inhibition of proteolytic enzymes [135–139], and alkali-induced formation of trypsin- and chymotrypsin-inhibiting peptides [132].

7. Pharmacology, Toxicology, and Potential Medical Uses of Individual DAA. – To facilitate understanding the complex nature of biological activities of DAAs, we briefly summarize results of published studies on the following nutrition- and health-related aspects of DAA.

7.1. d-Ala. Because it is part of the structure of bacterial cell walls, d-Ala derived from microbial sources is present in many foods as well as in hydrolyzed protein fertilizers [25]. d-Ala is structural component of bacterial cell membranes [140][141] and is a possible indicator of bacterial contamination, heat treatment, and shelf life of fruit juices and other foods [22][105]. It can also be used in studies of molecular imaging [142], to induce cytotoxic oxidative stress in brain tumor cells [143], and to treat schizophrenia [144]. Changes in d-Ala content of the rat pancreas are related to their diurnal and nocturnal (circadian) habits [145]. The submandibular gland and oral epithelial cells, not ingested food or oral bacteria, appear to be the source of high levels of d-Ala and d-Asp in human saliva [146].
7.2. d-Arg. Both l- and d-Arg protected against oxygen-radical-induced injury of rat heart tissue [147] and against endotoxin shock in rabbits [148]. d-Arg and other DAAs inhibited cell proliferation and tumor growth in rats [149], acted as a central nervous system stimulant, and exhibited anticonvulsant activity in humans [150].

7.3. d-Asp. d-Asp aggravated the nephritis in rats induced by *Staphylococcus aureus* bacteria [151] and prevented K and Mg depletion in rats induced by diuretics [152].

7.4. d-Cysteine (d-CysSH). Although l-CysSH had a sparing effect of l-Met consumed by mice, d-CysSH did not [153]. In fact, d-CysSH imposed a metabolic burden as indicated by depressed growth when fed to mice with less than optimal levels of d-Met (Fig. 12, a). The 24% decrease in weight gain of the d-CysSH plus l-Met amino-acid diet compared to l-Met alone implies that d-Cys is nutritionally antagonistic or toxic.

d-CysSH but not N-acetyl-d-cysteine lowered rat blood cyanide levels derived from acrylonitrile [154]. d-CysSH is also reported to be involved in the detoxification and/or prevention of toxicities caused by cyanides [155], the drug paracetamol [156], and

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**Fig. 10. Effect of pH on the extent of degradation of susceptible amino acids.** Adapted from [89].
other drugs [157–159]. l-Cysteine-glutathione disulfide, but not the d-cysteine analog, protected mice against acetaminophen-induced liver damage [160]. d-Cysteine desulphydrase catalyzes the elimination of H₂S from d-cysteine, presumably to form dehydroalanine [161].

7.5. d-Cystine (d-Cys). Although l-Cys is not an essential amino acid for rodents, less l-Met is needed for growth if the diet contains l-Cys [153]. Our results also show that l-Cys is somewhat more efficient in sparing d-Met than in sparing l-Met in diets
containing low levels (0.29%) of the two isomers (Fig. 12b). Supplementation of d-Met with an equal sulfur equivalent of l-Cys doubled growth. Thus, the overall response was equal to that produced by l-Met in the presence of l-Cys. In contrast, supplementation of suboptimal levels of l-Met with increasing concentrations of d-Cys reduced the growth rate of mice. Excess d-Cys in the diet is toxic.

7.6. Dehydroalanine. Dehydroalanine is an intermediate in the racemization of protein-bound amino acids (Fig. 4). We found that its content (in g/16 g N) in alkali-treated casein was 0.33 and in alkali treated acetylated casein 1.39 [162]. Dehydroalanine can, in principle, act as a biological alkylating agent similar to that suggested for processing-induced acrylamide [163][164].

7.7. d-His. d-His enhanced Zn accumulation and reduced the fraction of Zn that was retained and absorbed by fish [165]. Both d- and l-His enhance DNA degradation by \( \text{H}_2\text{O}_2 \) and \( \text{Fe}^{2+} \) ions [166]. d-His-induced cell injury is mediated by an Fe-dependent formation of reactive oxygen species [167][168].


7.9. Lanthionine (LAN) Isomers. LAN Isomers are formed during the biosynthesis of microbially derived peptide antibiotics [171] and during exposure of proteins to alkali and heat. Reaction of the SH group of CysSH and the double bond of
dehydroalanine gives rise to one pair of optically active d- and l-isomers, and one diastereoisomeric (meso) form of LAN [33]. The mixture of \( \text{d}+\text{meso} \)-LAN has a sparing effect on L-Met, as evidenced by a 27% greater weight gain when the two amino acids were fed together, than when fed suboptimal L-Met [153][172]. Base-

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**Fig. 12. Effects of \( \alpha \)-amino acids added to mice diets.**

- **a**) Sparing effects of L- and D-Cys, and other sulfur amino acids in methionine-deficient diets.
- **b**) Effect of D-cystine in low methionine diets.
- **c**) Sparing effect of D-Tyr on growth of mice on L-Phe-deficient diets.
- **d**) Effect of D-Tyr on growth of mice on high- and low-protein diets. Adapted from [90][153].
catalyzed formation of LAN and LAL does not affect bioactivity in bovine somatotropin [173].

7.10. D-Lys. D-Lys is not utilized as a nutritional source of L-Lys by chicks, dogs, mice, rats, or humans, presumably because D-amino acid oxidase does not metabolize D-Lys [174]. The extent of nutritional damage of alkali treatment associated with loss of lysine may depend on the original lysine content of a protein. Decrease in lysine due to racemization and LAL formation in a high-lysine protein such as casein, high-lysine corn protein, or soy protein isolate may have a less adverse effect than in a low-lysine protein such as wheat gluten or corn protein, where lysine is a nutritionally limiting amino acid [175][176]. Because of its low toxicity, D-Lys may be a better candidate to reduce radioactivity uptake by the kidneys during cancer therapy with radionuclides than is L-Lys [177–179].

7.11. Lysinoalanine (LAL) Isomers. The cross-linked amino acid LAL, formed concurrently with DAA in alkali-treated proteins, has two asymmetric C-atoms, making possible four diastereoisomeric forms: DD, DL, LD, and LL. The nutritional value of the individual isomers as a source of L-Lys is not known. Table 2 shows that a mixture of LL- and LD-isomers has a value for the mouse equivalent on a molar basis to 3.8% of L-Lys.

We have examined the affinity of LAL towards a series of metal ions, of which Cu II was chelated the most strongly. The four LAL isomers differ in their ability to chelate metal ions such as Cu [180][181]. On this basis, we suggested a possible mechanism for kidney damage in the rat involving LAL’s interaction with Cu within the epithelial cells of the proximal tubules. The direct relationship between the observed affinities of the two LAL isomers for Cu II ions in vitro and their relative toxic manifestation in the rat kidney suggests that LAL exerts its biological effect through chelation of Cu in body fluids and tissues. The observed binding of LL- and LD-LAL to Co III, Zn II, and other metal ions imply that LAL could also influence Co utilization.

LAL, ornithinoalanine (OAL), and LAN are formed during alkaline treatment of wool during skin unhairing of sheep and other processes designed to enhance the strength of wool, and possibly also human hair, via cross-linking reactions [91][182]. Serine racemase contains a catalytically active LAL residue [183].

7.12. D-Met. The nutritional value of D-Met in mice approaches that of L-Met (Table 2). By contrast, D-Met appears to be poorly utilized by humans when consumed either orally or during total parenteral nutrition. One factor giving rise to inconsistencies in the utilization of D-Met is the dose dependency of the apparent potency of D-Met relative to its L-isomer, i.e., the dietary level of the D-form for any given growth response relative to that of the L-form that would produce the same growth response. This dose-dependency is a result of the non-linear nature of the dose–response curves (Fig. 13). This complicates attempts to compare results from mice with those of other animal species. The latter studies often provided data based on a single substitution of the D- for the L-isomer. The figures show that high levels of L-Met (but not of D-Met) are toxic since they inhibited growth of mice [184].

D-Met-Containing solutions inhibited tumor cell growth in vitro [185], and protected against cisplatin ototoxicity in humans [186–189] and against oral radiation [190]. A cascade of enzymes transformed D-Met to the L-form [191].

7.13. Orn Isomers. Martínez-Girón et al. [113][192] developed analytical methods for the separation of unnatural ornithine isomers that may be present in processed foods.
7.14. d-Phe. The relative growth rate of mice fed d-Phe replacing the l-isomer in a free-amino-acid diet is concentration-dependent, ranging from 28.3 to 81.3% when compared to control diets containing the same amounts of l-Phe (Figs. 12 and 13, and Table 4) [90]. The data suggest the absence of any antinutritional effects or toxicity from feeding either Phe isomer at twice the optimum dietary level. d-Phe and hydroxyproline were reported to define the native structure and presumably virulence of conotoxin, a toxic peptide obtained from the venom produced by cone snails [193].

7.15. d-Pro. Oral feeding of an aqueous solution of d-Pro for one month to rats induced fibrosis and necrosis of kidney liver cells and elevation of serum enzymes [194]. By contrast, orally fed d-Pro and d-Asp induced did not induce acute toxicity in rats [195].

7.16. Selenomethionine Isomers. Selenomethionine is a major source of Se in the diets of both animals and humans. Comparison of the effects of oral consumption of seleno-l-Met, seleno-dl-Met, and selenized yeast on the reproduction of mallard ducklings revealed that, although both seleno-Met preparations were of similar toxicity, their potency was greater than that of Se present in yeast [196]. Heinz et al. [196] found that the survival of day-old ducklings consuming l-seleno-Met after two weeks was significantly lower (36%) than that of ducklings consuming the dl-isomer (100%). d-seleno-Met protected against adverse effects induced by space radiation [197][198].

7.17. d-Ser. Protein-bound l-Ser racemizes faster to the d-isomer than the other amino acids (Table 1, and Figs. 6 and 7) [199]. d-Ser has been reported to enlarge rat kidney cells (cytomegaly) similar to that observed with LAL. Several approaches were used in an attempt to elucidate possible mechanisms of d-Ser renal toxicity [200–202]. Sodium benzoate [200][201], protein-deficient diets [203], and α-aminoisobutyric acid [204] attenuated d-Ser nephrotoxicity in rats.

d-Ser-induced kidney damage may be due to a lowering of the concentration of renal glutathione (GSH) that protects the kidneys against kidney-damaging reactive oxygen species [204]. The decrease in glutathione concentration takes place during the metabolism of d-Ser by DAA oxidase. Because nephrotoxicity induced by d-Ser is similar to that caused by LAL, the question arises as to whether effects of these two amino acids on the rat kidney are competitive, additive, or synergistic?

Because d-Ser also acts as an agonist of N-methyl-d-aspartate (NMDA) receptor-mediated neurotransmission in the brain, it may be useful as ‘innovative pharmacologic strategy in schizophrenia’ and pain [205]. A strain of protozoa (Drosophila melanogaster) has developed a transport mechanisms with adaptive specialization in the absorption and brain functions of d-Ser and other DAA that may be involved in neuronal functions [206]. d-Ser can be enzymatically transformed to l-Trp [207]. The d-configuration of Ser maintains the toxic conformation of the mushroom poison vioisin [208].

7.18. d-Thr. l-Thr is the second-limiting amino acid in maize (corn) proteins. The utilization of d-Thr by the chick, rat, mouse, or human as a nutritional source of the l-isomer is insignificant [174].

7.19. d-Trp. The relative nutritional potency of d-Trp compared to l-Trp in mice is strongly dose-dependent, being inversely related to the dietary concentration and ranging from 29 to 64% (Fig. 11) [209]. The maximum growth obtainable for l-Trp occurred at 0.174% in the diet. By increasing the dietary concentration of d-Trp up to
Nutritional utilization of D-amino acids as a source of L-isomers in mice

Fig. 13. Top four plots: weight gain in mice fed increasing dietary levels of L-Met, D-Met, and isomeric methionine derivatives and analogs for 14 d. Asterisk indicates significant difference from L-Met at the same dietary concentration (top four plots). Adapted from [153][172]. Lower two plots: relationships of weight gains to percent of L- and D-Phe, and L- and D-Trp isomers in amino acid diets fed to mice. Adapted from [90][209][216].
0.52%, growth passed through a maximum at 82% of that achieved with the l-isomer. This occurred at 0.44% of d-Trp.

Considerable species variation exists for the nutritive value of d-Trp [174]. In chicks’ diets, relative potency of the d- to the l-isomer has been reported to be 20%. d-Trp was well-utilized by growing pigs [210]. The value for humans is ca. 10%. Rats utilize d-Trp as efficiently as l-Trp. d-Trp can serve as a niacin precursor in rats to the same extent as does the l-form [211][212]. These studies also showed that d-Trp has one sixth of the activity of niacin. Both d-Phe and d-Trp taste sweet [209][213–215].

7.20. d-Tyr. Nutritionally, l-Tyr is classified as a semi-essential amino acid [7]. Combinations of l-Tyr and l-Phe are complementary in supporting growth of mice [90]. Thus, under conditions where l-Phe may be limiting, l-Tyr may supply half the requirement of l-Phe alone for chicks, mice, rats, and humans. Our mice-feeding studies showed that with d-Tyr in an amino acid diet, growth inhibition was severe at a d-Tyr:l-Tyr ratio of 2:1, but was less so when the ratio was 1:1 (Fig. 12,c). Similar results were obtained with a casein diet supplemented with d-Tyr (Fig. 12,d). The antimetabolic manifestation of d-Tyr may be ascribed to interference with the biosynthesis of vital neurotransmitters and proteins in vivo [90][216].

d-Tyr has no sparing effect for l-Phe in mice. Growth inhibition may become evident when d-Tyr is present in the diet at a level equal or greater than l-Phe. The potential for chronic toxicity following exposure to lower levels of d-Tyr remains unknown. Formation of d-tyrosyl-tRNA<sub>Tyr</sub> may be responsible for the toxicity of d-Tyr toward E. coli [217].

7.21. d-Val. Administration of total parenteral nutrition (TPN) containing d-Leu, d-Met, d-Phe, and d-Val to hepatoma-bearing rats showed that d-Val inhibited tumor growth without negative effects on the host. d-Leu and d-Met also improved the nutritional status of the sick rats [218]. These observations suggest that some DAA diets may benefit cancer patients.
In summary, mice provide a good animal model to study the biological utilization and biological effects of DAAs, both free and protein-bound. A major advantage of mouse bioassays is that they require approximately one-fifth of the test substance needed for rats and can be completed in 14 days [90][153][172][219–221].

8. Role of DAA in Humans. – Indicators of Aging. There appears to be a direct relationship between the age of the collagen proteins in rat teeth and their d,l-Asp or d,l-hydroxyproline ratios, presumably because of little or no metabolic activity in the molar teeth after their formation [222][223]. The extent of Asp racemization can also be used to estimate the age at death [224–226]. The use of spontaneous racemization of L- to D-Asp and of other amino acids to assess the age of tissues such as dentin, bone, and eye lens [227–231] has been questioned by Clarke and co-workers [232][233]. Their objection is based on their findings that red-cell protein aspartyl/asparagine residues racemize much more slowly in erythrocyte and other proteins than in proteins of the lens, tooth enamel, and dentin. The in vivo rates of D-Asp formation in erythrocyte proteins were similar to the in vitro rates. It is not known whether biochemical processes exist to degrade or repair deamidated and isomerized aspartyl and asparagine residues in tissue proteins. However, Chinese scientists [14] reported that human d-Tyr-tRNA (Tyr) deacylase protected cells and neurons against adverse effects of excess DAA by deacylating d-aminoacyl-tRNAs into free DAA and tRNA.

The in vivo formation of D-Asp may not be the result of a racemization process for which the d,l ratio cannot exceed 1.0, but rather of an inversion of configuration of L- to D-Asp, where the ratio can and does exceed 1.0 [234]. Age-related accumulation of both DAAs and advanced glycation end products, and the decreased functional capacities of structural and bioactive proteins contribute to the development of diabetic complications and eye diseases [235]. Moreover, because the accumulation of D-Asp during aging has been implicated in the pathogenesis of Alzheimer’s disease, arteriosclerosis, and cataracts, the question arises as to whether the enzyme d-aspartyl endopetidase reported to degrade D-Asp-containing peptides and proteins in mammalian mitochondria and nuclei [236] can slow down or reverse the aging of tissues.

Role of DAA in Disease. Changes in the concentrations of DAAs may be related to the pathogenesis and therapy of diseases [237]. The total content of D-Phe and D-Tyr was significantly greater in patients suffering from chronic renal failure than in normal humans [238]. The increase in DAAs in renal failure may arise from preferential retention of DAAs in the kidneys (low glomerular filtration compared to L-isomers), depletion, and inhibition of DAA oxidase activity, and the consumption of DAA-containing foods and antibiotics. High levels of D-Ser and D-Pro are also reported to accumulate during renal failure [239]. Down-regulation of energy metabolism after D-Ser treatment may be related to the mechanism of its nephrotoxicity [240].

The free D-Ser in normal human brain tissue (d,l ratio 0.086) was comparable to that in Alzheimer’s-affected human brain (d,l ratio 0.099). Because D-Ser (like the L-isomer) passes through the blood–brain barrier, D-Ser in the brain probably originates from the diet. D-Ser is metabolized by D-amino oxidase in the brain. In contrast, other studies suggested that synthesis of D-Ser in the brain occurs by in vivo racemization of L-Ser [241]. These conflicting suggestions need to be resolved in view of the fact that,
although at high pH protein-bound \textit{l}-Ser racemizes rapidly (Table 2), the amount of \textit{d}-Ser in food proteins that have not been exposed to alkaline conditions are too low to account for the relatively high amounts detectable in the brain.

An understanding of \textit{d}-Ser signaling in the human brain may facilitate development of therapies for brain disorders [242]. In contrast to the apparent beneficial role in neurotransmission [16][17], \textit{d}-Ser concentrations above physiological levels induced lipid and protein oxidative damage and decreased glutathione levels in brain cortex of rats [243]. \textit{d}-Ser availability in the nervous system may be altered in schizophrenia because of increased DAA degradation by DAA oxidase [244].

The involvement of \textit{d}-Ser in neurotransmission is supported by a double-blind, placebo-controlled trial with 31 schizophrenic patients. This study revealed that \textit{d}-Ser (30 mg/kg/day) added to an antipsychotic regimen had significant beneficial effects on cognitive function and performance [245]. The \textit{d}-Ser was well tolerated by the patients with no apparent side-effects. Will protein-bound \textit{d}-Ser induce similar improvements?

The following additional observations suggest that DAAs may benefit disease therapy:

- \textit{d}-Ser can contribute to the therapy of post-traumatic stress disorder [246];
- DAA oxidase oxidizes \textit{d}-DOPA in the brain, which may then be converted to dopamine into an alternate biosynthetic pathway for the treatment of Parkinson's disease [247];
- DAA inhibit tumor growth in rats, and may be useful in cancer gene therapy [143][248];
- Parenteral administration of \textit{d}-Val hepatoma-bearing rats induced significant improvement in nutritional status and tumor-inhibition compared to control diets [249];
- \textit{d}-Phe induces analgesia in mice and humans [250];
- The observed clinical relief in chronic-pain patients given oral doses of 750 to 1000 mg of \textit{d}-Phe is ascribed to inhibition of carboxypeptidase and the accompanying increase in the levels of brain enkephalin. Whether dietary levels of \textit{d}-Phe present in processed food proteins can induce analogous pain relief merits study.

Although healthy young adults can invert approximately one third of \textit{d}-Phe to its \textit{l}-isomer [251], and human plasma contained high concentrations of \textit{d}-Ser, \textit{d}-Ala, and \textit{d}-Pro (\(d/l = 0.24, 0.21, \) and 0.1, resp.), no DAAs were present in plasma proteins [155][252]. The total DAA content of the plasma correlated with the serum creatinine levels. The possible significance of high levels of DAAs in human saliva for oral health is not known [146]. The possible value of \textit{d}-Tyr in treating hypertension [253] and the possible significance for human reproduction of the observed high content of \textit{d}-Asp in rat testis fluids and tissues, and spermatozoa [254] are also not known. It appears that DAAs possess both adverse and health-promoting attributes. The challenge is to optimize the beneficial ones.

9. Conclusions and Outlook. – There is a need to standardize methods designed to ascertain the role of DAAs in nutrition. Because the organisms are forced to use the DAAs as the sole source of the \textit{l}-forms, the use of all-amino-acid diets in which the \textit{l}-isomer is completely replaced with different levels of the corresponding DAA may be preferable to supplementation of proteins with DAA. DAAs along a peptide chain may
be less utilized than the L-forms. The utilization of any DAA may be affected by the presence of other DAA in the diet. Largely unresolved are the following questions:

- Can analytical methods be developed for all four possible isomeric forms (L,L, L,D, D,L, and D,D) of cross-linked amino acids with two asymmetric C-atoms (Fig. 2)?
- How do biological effects of DAAs vary, depending on whether they are consumed in the free state or as part of a food protein?
- Can poorly digestible racemized food proteins serve as dietary fiber in the digestive tracts of humans?
- Do DAAs and d-peptides alter the normal microflora of the intestine?
- Investigate differences in the interactions of DAAs as compared to LAAs in the binding to the active sites of proteases, such as trypsin, chymotrypsin, and pepsin in the digestive tract.
- Do metabolic interactions, antagonisms, or synergisms among free and protein-bound DAAs occur in vivo?
- Do proteins and peptides acquire antibiotic competence upon racemization?
- Does racemization alter protein conformations and charge distributions (isoelectric points) resulting in beneficial protective effects against protein-induced allergy, celiac disease, and bacterial, plant, and venom toxic proteins?
- Does food processing-induced formation of DAAs adversely or beneficially affect the safety of concurrently formed potentially toxic acrylamide?
- Does pasteurization and other heat treatments widely used to kill pathogenic and spoilage bacteria and fungi in liquid (milk, fruit juices) and solid (meat) foods result in reduced levels of DAAs produced by the foodborne microorganisms?
- Will DAAs prevent biofilm formation by bacteria on or in contaminated food [263]?

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