Recent estimates of human requirement for indispensable amino acids have shown that the requirement for key amino acids such as lysine is twofold–threefold higher than previously thought. As a consequence recommended intakes for protein, particularly for vulnerable groups such as the elderly, need to be revised. Indispensable amino acids can also be used directly to improve amino acid supply in diets. A range of nitrogen compounds such as creatine and branched chain amino acids are currently used by groups such as athletes, although their efficacy is open to question. Peptides are natural components of the diet and some of these have been shown to have beneficial effects. The emergence of methods for genetic modification of food proteins raises possibilities of the development of novel foods for a variety of benefits including improved supply of indispensable amino acids, reduced allergenicity, or preformed antibodies to reduce risk of disease.

Key words: dietary protein, genetic modification, indispensable amino acids, novel foods, peptides.

Introduction
With the rapid development of biological technologies affecting plant and animal reproduction and growth, as well as food technologies creating new methods for processing food components, there exist many avenues for the development of novel foods. But there is also the possibility of altering foods in such a way that there is either loss of nutritional quality or even the introduction into processed foods of components that have adverse effects on health. It is essential that agricultural and food technologists understand the implications of innovations in food production, processing and formulation. Foods contain a very large number of different proteins as well as peptides and amino acids, and there are many ways in which alterations in these components can alter the nutritional value or safety of a food.

Protein metabolism and nutrition
Metabolism of protein and amino acids is now well understood. All body proteins are in a continuous cycle of breakdown and resynthesis (turnover), with widely varying half-lives from a few minutes in the case of some enzymes to approximately a year for connective tissues. Cells contain a pool of amino acids that are drawn on for protein synthesis and added to by protein breakdown. Whole cells also die and the constituent proteins are broken down to contribute to the extracellular and intracellular amino acid pools. Protein synthesis is dependent on the availability of all 20 ‘standard’ amino acids in the cytoplasm. Of the 20 amino acids, 10 are essential or indispensable and must be supplied in the diet. The remaining 10 are able to be synthesized by transfer of an amino group to the appropriate metabolic intermediate. Total absence of protein (or indispensable amino acids, IAA) from the diet does not block protein synthesis because amino acids are continually supplied into the intracellular pool by breakdown (turnover) of body proteins. Total protein synthesis approximates 250–350 g per day in the adult, while dietary protein intake is usually around 80–110 g per day. Dietary protein is therefore ‘topping up’ the pool of amino acids, which feeds into protein synthesis.

Many of the proteins and peptides in food could have untoward pharmacological or toxic effects in the body if they entered the blood stream but the body is mostly protected by the cell membranes of the gut wall and the digestive enzymes, which break down protein and peptides in the gut. The end-products of protein digestion by the pancreatic enzymes include a proportion of free amino acids, with the major part left as short chain peptides having up to six or so amino acid residues. These amino acids and short chain peptides diffuse through the mucous layer overlying the villi, and come into contact with the brush border membrane that carries transport proteins for the uptake of amino acids. There are two general routes of uptake of amino acids from the gut lumen into the epithelial cells: one for free amino acids and a second for di- and tripeptides. The brush border membrane holds peptidase enzymes on its external membrane. A proportion of the short chain peptides is hydrolysed to single amino acids. Active transport proteins for the amino acids in close proximity to the peptidase enzymes. A

Correspondence address: Dr Richard Read, 32 Liardet Street, Port Melbourne, Vic. 3207, Australia.
Tel: + 61 3 9645 2303; Fax: + 61 3 9645 2303
Email: rsdread@netlink.com.au
second system for the uptake of amino acids involves the transport of di- and tripeptides. Once absorbed, most of the di- and tripeptides are hydrolysed inside the epithelial cells and diffuse into the portal blood as single amino acids. Amino acid transport is specific for the L stereoisomers of the amino acids, but the diet also contains small amounts of D-amino acids, mostly from digested bacteria and derived from fermented foods such as cheese and yoghurt. These D-amino acids are taken up relatively slowly by diffusion. They cannot be used in protein synthesis, with the exception of D-methionine, which can be converted to L-methionine. The D-amino acids are mostly degraded by a D-amino acid oxidase in the kidney.

Although the activities of the pancreatic proteolytic enzymes result in most of the dietary protein being digested, a small fraction escapes digestion and very small amounts of peptides and whole proteins are absorbed into the body. Absorption probably occurs by diffusion through the paracellular pathway. Neonates take up significant amounts of protein, particularly antibodies, from colostrum, probably by pinocytosis.

Protein requirement and protein quality.
Adequate protein nutrition depends on two factors: (i) sufficient protein in the diet, including sufficient metabolisable nitrogen; and (ii) satisfactory protein quality in terms of the amount of IAA in the protein. Protein requirement has been determined predominantly from nitrogen balance studies. Nitrogen balance studies, usually carried out on young healthy adults, have indicated an average requirement of approximately 0.625 g/kg per day for high-quality protein. The recommended intake is derived from this figure by adding 25% (two times the estimated standard deviation) to cover the requirement of almost all of the population, giving a recommended intake of 0.75 g/kg per day for high-quality protein. The US RDI is set at 0.8 g/kg per day, allowing for the average quality of dietary protein being a little less than that of the highest nutritional quality proteins (eggs, milk and meat). There could be a further adjustment upwards if the RDI was to be applicable to diets of lower quality and digestibility, as for vegetarians or for populations consuming relatively high intakes of cereal staples, as in some developing countries.

There are two sources of uncertainty in relation to adult protein and amino acid requirement and recommended intake; the first is that the definition of protein requirement may not be optimum; and second, there is uncertainty regarding the requirement for individual IAA. The estimation of protein requirement is based on determining the amount of dietary protein that will just maintain nitrogen balance. However, there is no firm basis for the assumption that maintenance of nitrogen balance is satisfactory in terms of maintaining optimum human health. In fact adaptation to low protein intake certainly occurs. Chronically low intakes of protein (and energy) bring about a reduction in bodyweight and cell mass. The rates of protein synthesis and breakdown both decline. The IAA are more efficiently recycled and the N-balance can be re-established after some time at a lower protein intake. The reduction in body protein (muscle and internal organ mass) is almost certainly not desirable, and it is likely that there are other tests of physiological function, for example, maintenance of optimum immune function that would result in a higher estimate of requirement than that given by N-balance studies.

For infants and children, recommended intakes are based on estimates of normal intakes compatible with optimum growth rate. In recent years research using stable isotopes has allowed more accurate measurement of intakes of breast milk by healthy infants, and it appears that earlier estimates of breast milk intake were high and it is now considered that the estimated requirement for protein can be reduced from 120 mg N/kg per day to 90 mg N/kg per day.

In the case of the elderly, it has been suggested that the current RDI is too low. Moreover, as people age, energy expenditure is reduced and there is a loss of lean body mass. With the reduction in energy expenditure there is a consequent lower energy intake, and assuming that protein makes up the usual 12–16% of energy, protein intake is also likely to be low. In the USA it was found that 10–25% of women over 55 years of age had protein intakes less than 30 g per day. It is recognized that elderly people can adapt to a low protein intake but this may involve (i) further loss of lean mass; and (ii) reduction in functional capacities such as immune competence. Campbell and Evans reviewed studies on protein requirements of the elderly and calculated a requirement of 0.9 g/kg per day, which, with an appropriate safety margin, would lead to a recommended safe and adequate intake for high-quality protein of 1.0 g/kg per day. Estimated protein requirement and recommended intake are specified in relation of kilograms of bodyweight. Yet body composition varies widely in elderly people and there is a lack of information on the effect of differences in body composition on protein requirement. The United Nations Food and Agriculture Organization (FAO) is initiating a review of current estimates of protein requirement and RDI.

There has been much discussion regarding the protein requirement of sports players in a variety of sports, and there is a commonly held view that high intakes of protein are beneficial for athletes endeavouring to increase muscle mass and strength. In fact the high energy expenditure involved in training and playing sport generally results in a higher intake of food energy, with the consequence that intake of protein is usually high in relation to bodyweight anyway. High intakes of protein do not increase muscle mass if not accompanied by appropriate physical training but relatively high intakes, approximately 1.5 g/kg per day, in combination with appropriate strength training does assist gain of muscle mass. The current consensus is that intakes in the range of 1.2–1.6 g/kg per day should be adequate for all sports players.

It is important to note that adequacy of protein supply depends on adequacy of energy supply, and under circumstances where energy intake is inadequate, both the amino acids from dietary protein and amino acids liberated by body
protein turnover are substantially diverted to energy production through gluconeogenesis. There is little point therefore in taking action to improve protein input if total energy intake is inadequate. Protein–energy malnutrition in children is a major problem in developing countries. The aetiology of this condition is complex, usually involving early cessation of breast-feeding, frequent infections and chronic intestinal infestation, use of inappropriate weaning foods that may have a low energy and protein density, lack of knowledge on the part of parents and overall family and community poverty. Although most of the possible remedial actions are outside the scope of this paper, two related factors should be mentioned: first, education of parents; and second, use of appropriate weaning foods. Young children have high metabolic rates per kg bodyweight compared to adults, and coupled with this is the generally small volume of food that such children can consume at a meal. As a consequence it is important to educate parents about the needs of young children for richer foods that deliver the required energy and protein in a small volume. There are many programmes around the world working to develop and supply appropriate weaning foods. There are, as well, many opportunities for the further development of novel food products in this area: foods that are energy- and protein-dense, readily digestible, affordable, with good keeping qualities, and culturally acceptable.

Determinants of protein quality

The nutritional value of dietary protein depends on adequacy of supply of each of the indispensable amino acids in relation to the specific requirement. Thus any food treatment process that affects any of the 10 indispensable amino acids has the potential to reduce nutritional value. In practice a relatively small number of amino acids are of practical importance in determining protein quality in overall diets. The amino acids that are most often limiting in staple foods, and therefore effectively determine the nutritional quality of food proteins, are lysine (in cereal protein), methionine (in legumes), tryptophan (in corn) and threonine, together with lysine (in rice). Any process that affects the level of any of these amino acids in foods has the potential to adversely affect protein quality in general diets.

Lysine is of greatest importance because it is the most limiting amino acid in cereal diets that are staples in most parts of the world. There is still some doubt, however, regarding human requirement for lysine. The studies of Rose and others in determining requirements for individual amino acids using maintenance of nitrogen balance in adults as the criterion of requirement, provided the basis of the FAO/WHO summary of requirements for specific amino acids. But more recent studies have led to a reassessment of amino acid requirements, as summarized in Table 1.

Young et al. at the Massachusetts Institute of Technology (MIT) drew attention to the marked difference between the estimated IAA requirements of adults, based on N-balance, and those of children, based on intakes that support maximum growth rate. Suspecting that the estimated requirements of adults were low, they developed a new method for determining IAA requirement based on rates of oxidation of isotopically labelled IAA in experimental subjects (tracer balance method). The requirement for several key IAA determined by this method were found to be two to three times higher than those previously reported, and were closer to, though still less than, the requirements estimated for infants and children. Comparison of estimated lysine supply in usual third world diets with the requirement determined by Young et al. suggested that lysine intake would commonly be deficient. However, as with protein, there is adaptation to low intakes of IAA. Millward et al. have shown that adaptation to low intakes of lysine does occur and that this is probably important in third world environments with major dependence on cereal proteins. The research of Millward et al. also using rates of oxidation of labelled IAA indicates a lysine requirement for adults of 23.2 ± 2 mg/kg per day for adults adapted to a low lysine diet. This is approximately twice the earlier estimate and approximately 20% below that of Young and El-Khoury. Although the question of requirement for lysine is not fully resolved, there is now a shift towards acceptance of the MIT values as being the best estimate of adult IAA requirement.

The question of IAA requirement and supply is important because it is the basis for estimating protein quality. Lysine is particularly important because on a worldwide basis it is likely to be in shortest supply in relation to requirement and,

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>WHO1 Child 2–5 years</th>
<th>Adult</th>
<th>Young &amp; el-Khoury14 Adult</th>
<th>Millward et al.13 Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>?</td>
<td>8–12†</td>
<td>–</td>
<td>23.2 (adapted)</td>
</tr>
<tr>
<td>Leucine</td>
<td>73</td>
<td>14</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>64</td>
<td>12</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Methionine + Cys</td>
<td>27</td>
<td>13</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine + Tyr</td>
<td>69</td>
<td>14</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>37</td>
<td>7</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>12</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>38</td>
<td>10</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

1 Recommendation for histidine; although not based on definitive data.
in addition, it is the IAA most susceptible to destruction in food processing.

**Amino acids**

A number of amino acids are now produced in quantity by fermentation. Lysine, for example, is produced relatively cheaply in large quantities for improving the protein quality of animal diets. There exist broad possibilities for the addition of lysine as the free amino acid or in a peptide form to novel food products in areas where lysine is commonly deficient. Although the same logic applies to the other indispensable amino acids, there is not the same wide-ranging occurrence of low intakes as there is with regard to lysine. When single amino acids are used in foods problems of taste may have to be overcome but technologies are available to handle this. There have been trials of the use of lysine-fortified wheat flour in some areas. Zhao et al. report increased serum transferrin and improved immunological parameters in subjects consuming lysine-fortified wheat flour over a 3-month period in a region where wheat flour provides nearly two-thirds of the protein consumed.\(^\text{15}\)

High intakes of single or several amino acids already occur in various situations. For example, soy sauce made by fermentation of soy and wheat protein has a long history as a flavour enhancer and contains high levels of glutamate as the monosodium salt (MSG). High intakes of particular amino acids can distort the normal amino acid profile in blood and tissues and produce unwanted effects, at least in some individuals. Some are susceptible to MSG and develop a ‘Chinese restaurant syndrome’ characterized by headache, flushing and dizziness. The systemic circulation is protected to a large extent from exposure to high levels of glutamate because of the high extraction rate of glutamate and glutamine in the first pass through the splanchnic bed (gut and liver), close to 90% being removed, that being the highest extraction rate of all of the amino acids. However, the key role of glutamate and glutamine in amino acid metabolism and in neurotransmitter function is not surprising that excessive dietary intake could evoke a reaction in some people. Similarly tyramine, decarboxylated tyrosine, formed in fermented foods can provoke neurological reactions such as migraine in susceptible individuals.

Athletes, particularly weightlifters and body builders, have a reputation for trying almost any substance they believe may give them an advantage in their sport. Liquid and powder protein formulations are widely used despite the fact that high biological value food proteins (meat and dairy products) are as effective and much less expensive. Various amino acid preparations are also used. Glutamine and branched chain amino acids (BCAA) are popular at the present time. The BCAA (leucine, isoleucine and valine) are the only indispensable amino acids metabolized in peripheral tissues rather than the liver. The BCAA breakdown occurs in muscle, and the enzymes for the first two steps in the breakdown pathway are common to the three amino acids. There is a general view that the BCAA are ‘anabolic’, stimulating muscle hypertrophy, hence their attraction for athletes wanting to add muscle bulk. But there has been mixed evidence as to the efficacy of BCAA in aiding muscle gain or work output. The BCAA do not seem to be an important fuel for muscles, and even in prolonged exercise BCAA concentrations in muscle do not change to a significant degree. The BCAA have also been claimed to reduce fatigue in athletes on the basis that BCAA reduce the uptake of tryptophan into the brain by competition for a common transport mechanism, thus reducing the rate of formation of serotonin, which is suggested to be the mediator of central fatigue. But taking BCAA prior to exercise has shown no beneficial effect in reducing fatigue.\(^\text{9,16}\)

Various combinations of free amino acids have been used by athletes to try to enhance endogenous production of growth hormone, including arginine, ornithine, lysine and tyrosine, but experimental studies have not been able to show any stimulatory effect.\(^\text{17}\) Tryptophan has been used by athletes and in alternative medicine for a range of conditions including sleep disturbance, anxiety and Tourette’s syndrome. Tryptophan is a precursor of serotonin (5-OH-tryptamine), a neurotransmitter that is involved in a variety of functions including sleep rhythm, pain perception etc. α-lactalbumin in milk is relatively rich in tryptophan, which may explain the anecdotal association of a milk drink before bed with better sleep. The role of tryptophan in nutrition has been reviewed by Heine et al.\(^\text{18}\) Following an outbreak of a condition called eosinophilia–myalgia syndrome (EMS) associated with tryptophan usage in the USA in 1989, the availability of tryptophan was restricted to prescription only. Eosinophilia–myalgia syndrome involves accumulation of eosinophils in tissues, muscle and joint pains, neurological manifestations, and a number of deaths were recorded. It appears that the condition may have been caused by a tryptophan dimer in the tryptophan preparations used but this is still uncertain. This experience argues for considerable caution in experimentation with new preparations of what may be thought to be normal food molecules.\(^\text{19}\)

Creatine is an amino acid for which the function is to maintain an energy reserve in muscle as creatine phosphate, recharging adenosine triphosphate (ATP) as energy is used in muscle contraction. The amount of creatine phosphate in muscle provides sufficient energy reserve to support vigorous muscle contraction for only 10–20 s. It has a key role, however, in short duration sports events where explosive muscle power is required as in sprints, jumps, and throws. Creatine is synthesized in the body and is obtained naturally in the diet as a component of meat. In recent years athletes have taken to using supplements of creatine and these have been shown to be effective in raising the concentration of muscle creatine phosphate. Because of the natural variation in individual sports performance it is difficult to show statistically significant improvements in performance in response to treatments such as the taking of supplements. However a number of studies have shown improvement in performance for short events, and it appears likely that it is effective, but to what degree and for which events is less
certain. Creatine intake causes an increase in weight, which is mainly a result of retention of water in muscle, but may be due in part to a gain in muscle protein. Creatine is now one of the most popular dietary supplements used by athletes and body builders, some taking more than 50 g per day. There appear to be no adverse affects as a result of consuming high intakes of creatine in the short term but the long-term safety is less certain. Questions have been raised regarding possible deterioration in renal function but there is little firm evidence as yet.

**Peptides**

Many peptides and peptide derivatives have key roles in regulating internal physiological processes, functioning as hormones, neurotransmitters etc. It might be assumed that peptides in food or derived by digestion of protein in food would not have biological effects within the body because the internal environment is protected by the gastrointestinal membrane barrier and the powerful protein digestive enzymes of the stomach and pancreas. Although this is largely so, it has been shown that small amounts of protein and peptides are able to enter the circulation intact. Protein digestion in the gut produces a very large number of peptides and many of these could have potential biological effects. It has been found that at least some peptides, taken orally or formed within the gut, can have physiological effects. Whether this is a response to absorbed peptide or a response to a peptide activating a receptor in the luminal membrane is not always clear. Peptides and proteins do cross the gut wall to enter the circulation but only in very small amounts, detectable by sensitive radioimmunosorbent assays. Entry is apparently by diffusion through the intercellular junctions of the villous epithelium, entering the blood stream via the lymphatics. Disturbance of the integrity of the intestinal wall as in inflammatory disease (e.g. coeliac disease) or following the taking of alcohol or drugs such as indomethacin, increases the permeability of the intestine to larger molecules and is likely to increase the possibility of systemic response to peptides formed in the lumen.

As an example, it was demonstrated that luteinizing hormone-releasing hormone given to rats orally resulted in a dose-dependent release of luteinizing hormone into the blood, showing that administration into the gut provoked a systemic response. Peptides that are active in the gut are commonly modified (blocked) at one or both ends with groups such as a pyrrolidine group at the N-terminal end and/or amidation at the C-terminal end. Blocking of the end groups may influence biological activity and/or increase resistance to hydrolysis by peptidases. Brantl et al. fractionated active peptides from casein peptone, a tryptic digest of casein, and identified a casomorphin thought to be an agonist for the µ-type opiate receptors, which appear to predominate in the gut, raising the possibility that such casomorphins (exorphins) could modulate gut function.

A number of bioactive peptides have now been identified in mammalian and microbial enzyme digests of milk proteins. Peptides formed by digestion of milk protein that exert an antihypertensive action by inhibition of angiotensin-converting enzyme (ACE inhibitors) have been studied in some detail. Following the feeding of caseinomacropeptide to rats, fragments of the protein could be detected in plasma by an immunosorbent assay. The immunoreactive material peaked in the plasma at approximately 60 min after feeding. There has been extensive study of ACE-inhibitory peptides derived from bovine casein, and the major whey fractions have also been shown to yield ACE inhibitors both by hydrolysis with commercial enzymes and by fermentation with cultures of lactic acid bacteria, which release extracellular proteinases and endopeptidases by lysis.

A cholesterol-reducing soy peptide (CSPHP) has been manufactured which significantly decreases both low-density lipoprotein (LDL)-cholesterol and total cholesterol in plasma. The peptide was made by binding ‘enzymatically decomposed’ lecithin to hydrolysed isolated soy protein in the ratio of 80:20. Consumption of CSPHP at 3 g per day decreased LDL-cholesterol by up to 27% in hypercholesterolaemic patients after 3 months. The product is said to have a suitable taste and texture for incorporation into a wide range of food products.

Several peptides and peptide derivatives are used as sweeteners in foods. Aspartame, for example, is an L-aspartyl-L-phenylalanine methyl ester. Hydrolysis yields aspartate and phenylalanine. The phenylalanine is of significance to those with phenylketonuria and it is essential that appropriate labelling is used so that consumers are informed. Alitame is a related sweetener made up of an L-aspartate–D-alanine tetramethylthietane yielding aspartate and D-alanine.

Glutathione (GSH), a tripeptide made up of glutamate, cysteine, and glycine, offers considerable potential as a dietary constituent. Glutathione is a modulator of immune function, an antimicrobial and possibly an anticancer agent. It is a key reducing agent within cells, and is maintained in the reduced state by the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) and in turn maintains ascorbic acid and vitamin E in their reduced forms. It functions as a redox buffer and removes toxic peroxides through the action of the enzyme glutathione peroxidase, which contains selenocysteine. Benefits that have been attributed to dietary proteins such as whey, which are relatively high in methionine and cysteine, may have their action by boosting the intracellular concentration of GSH. Glutathione can now be produced in bulk in yeast cultures, making it available as a food additive.

**Adverse reactions, allergy**

Adverse reactions to particular foods may be experienced by susceptible individuals and include food intolerance and food allergy. Food allergy is mediated through the immune system and involves initial exposure to an allergenic component in the food, usually a glycoprotein, which elicits formation of an allergen-specific IgE. The allergic response occurs on subsequent exposure when absorbed allergen binds to the allergen-specific IgE bound to the cell membranes of mast cells and basophils, thereby provoking
eliminate allergenicity. The key control for problems of tent, suggesting that breeding and selection could reduce or varieties has shown considerable variation in allergen con-

with two known peanut allergens. Screening of peanut trypsin inhibitor revealed 93% and 95% homology all allergenic variety. Interestingly, parallel work to sequence a removing the specific allergen, thereby creating a non-

for major allergens to ensure that ingredients that are com-

Coeliac disease is of particular significance because it is a potently severe condition that is relatively common in Caucasian populations, occurring with a frequency of approximately 1 in 2000. Coeliac disease is an autoimmune response that arises from the formation of an antigenic complex of a subfraction of the gluten protein of wheat and related cereals with tissue transglutaminase. The result is a chronic inflammatory response leading to atrophy of the small intestinal mucosa. The villi become clubbed and normal absorptive function is severely reduced, resulting in malabsorption and diarrhoea. There is frequently loss of weight accompanied by various vitamin and mineral defi-

cencies. Coeliac disease is not curable but is managed by strict exclusion of gluten from the diet. Sensitivity to gluten varies widely; some individuals are extremely sensitive and others much less so. In developed countries more and more commercially manufactured gluten-free products are becoming available in supermarkets. It is important that there is adequate surveillance to ensure their gluten-free status. Coeliac disease is rare in Asian and African populations. Unfortunately there is some tendency to confuse coeliac disease with allergy to wheat. Coeliac disease requires careful diagnosis and strict exclusion of gluten from all sources, whereas in allergy to wheat (which should also be correctly diagnosed), wheat and wheat products can be consumed up to the level of tolerance.

Cooking, processing and preservation
The cooking of food generally improves nutritional value by increasing digestibility, as well as the attractiveness of taste and texture. Moist food cooking softens coarse textures, aids chewing and swallowing and increases access by digestive enzymes. Simple food processing such as milling of grains also assists digestion. Cooking with dry heat above 100°C causes browning, which is the result of many complex reactions in which compounds with pleasant flavours are created. One of the more simple of these chemical reactions is known as the Maillard reaction, in which the side-chain amino group of lysine reacts with the reducing aldehyde group of sugars such as glucose, maltose and lactose. A cross-link is formed that results in the loss of lysine in the food product. Because the cross-linking occurs at high temperature (>100°C) it is the crust that is affected in bread-type products whereas in biscuits or breakfast cereals, which are baked right through at a high temperature, all of the protein in the product may have a reduced biological value. In general, caramelization reactions that create desirable colour and flavour reduce the nutritional value of protein. As temperatures rise above 100°C a great variety of products are formed. Most of these complex browning product molecules are not digested and absorbed, and are lost in the faeces.

It is pertinent to note here that it is desirable to retain at least some use of biological measures of protein quality such as rat growth studies (protein efficiency ratio, net protein utilization etc.) because factors such as reduced digestibility or toxicity are detected as well as reduction in protein quality.

Smoking of food has a preservative action because aldehydes in the smoke react with side-chain amino groups in protein, cross-linking the molecules and preventing microbial growth. But smoke also contains compounds shown to be carcinogenic, thus the use of smoking as a preservative should be discouraged. Salting of food for preservation also has a long history of use, and functions by reducing water activity. It is both safe and effective but it is now recognized that the intake of salt needs to be kept reasonably low because susceptible individuals are prone to develop hypertension with continued high intake. The Food and Nutrition Board committee has recommended that intakes not exceed 6 g per day. But higher intakes may be necessary where heavy workloads under hot conditions result in high rates of sweat loss.

Crops, derived products and genetic manipulation
A large amount of research is being undertaken in crop development, including both new varieties of the major staple crops as well as lesser known plant species. Soy is one of the major crops in Western countries and has a long history of use in the East. It is an excellent source of protein. The raw seed of a typical variety has approxi-

mately one-third protein and one-third carbohydrate, and is, as well, a rich source of oil (15–20%). The nutritional value of the protein is complementary to that of cereals in that the higher lysine content makes good the relatively low lysine content of wheat. The importance of this comple-

mentary effect should not be underestimated. This is illustrated by considering as an example soy flour (full fat)
and whole meal wheat flour. Because soy flour is high in protein (37%), having a relatively high lysine content, an admixture of only 20% soy flour into wheat flour increases the protein content from 13% to 17.8% and more than doubles the lysine content from 340 mg/100 g to 750 mg/100 g. In the wealthier Western countries, lysine requirements are likely to be met by consuming high-protein animal products whereas in developing countries with greater population pressure and less land resources per head of population, it will be more efficient in terms of resources to increase protein and lysine intake by increasing the use of legumes such as soy.

Some of the legumes are coarse in texture and less attractive as food, but food technologists are putting a great deal of effort into developing novel food formulations for a wide range of products including meat alternatives and extenders, soy milks and soy milk powder, soy-based yogurt, non-dairy cheese, frozen desserts, candy bars etc. In the USA and Europe these products are being developed to capitalize on consumer sentiment, which regards soy products as ‘very healthy’,34 but a secondary factor is the reduction in the cost of raw ingredients. Developing countries can make use of these technologies to bring new foods to market that will increase variety and interest as well as increasing lysine intake. The potential down-side of these developments is that soy-based meat and dairy alternatives do not provide other key nutrients such as iron, zinc and calcium, so it is still important to increase production and use of animal products, particularly for children.

Genetically modified organisms are coming into the food supply at an increasing rate. The potential for developments in crop plants with higher productivity, disease resistance, frost and drought resistance, higher nutritional value etc., seem almost unlimited. Where a gene or genes are transferred between organisms, there are new proteins produced in the target organism. The fact that the target plant or animal is living is the first indicator that the rearranged genome and its products as food are unlikely to be dangerous to health. However, where a gene has been transferred with the express purpose of creating a component toxic to an organism that consumes the plant, it is obvious that testing must be thorough. This is exemplified in the case of the StarLink corn into which the Bacillus thuringiensis was transferred to make it toxic to the European corn borer. Basic testing rules out overt toxicity but allergy remains possible, and because the incidence may be very low it is virtually impossible to rule it out in a prospective sense. For this reason the FDA has approved Bt corn for use as animal feed but has not approved it for human use. A major incident occurred in 2000 when StarLink corn was detected in human food and the manufacturer was ordered to recall the product.

**Animal products**

Animal production is enormously important in terms of human protein supply. Animal products provide protein that is almost always of high nutritional quality, with the notable exception of gelatin (solublized collagen). Meat, including fish, eggs, milk and milk products have sufficient of the indispensable amino acids to improve the protein quality of diets in which cereals, and other staples provide the bulk of the energy but the protein quality is prejudiced by relatively low lysine, threonine, tryptophan or methionine. The production efficiency for animals is only approximately 10% of that of plants in terms of energy and land area, but key characteristics of animal products as food justify their position in the food chain. These key characteristics include (i) high energy and nutrient density; (ii) high digestibility; (iii) supply of iron in haem form (more efficiently absorbed), together with vitamin B12, calcium (milk and cheese), as well as zinc, B6 etc.; (iv) production is not constrained by seasonal availability; (v) the use by animals of vegetation and vegetable products that cannot be used by humans (grasses, shrubs); and (vi) the fact that animals can still produce under semiarid conditions where cropping is not possible.

The role of animal products is important in countering protein–energy malnutrition in developing communities, particularly in relation to children. Situations in which novel foods based on animal products are likely to make a significant impact in developing countries is in improved processing, packaging and preservation techniques. Animal products spoil rapidly in hot climates and appropriate technologies will need to be inexpensive. Suitable preservation will allow foods to be transported from production sites to areas of demand, thus allowing development of a more robust commercial food sector. New forms of packaging, refrigeration, drying, pasteurization, water activity control, fermentation and pickling may all be useful.

There are many new developments in agriculture that can improve animal product supply in developing communities. Some of these include farming of indigenous animals for meat (e.g. deer in Africa), fish farming, adaptation of waste products for animal feeds, cloning of animals to achieve a rapid increase in high productivity breeding stock, genetic modification of animals to achieve faster growth rates and better feed conversion, as well as resistance to pests and diseases. Of course the aforementioned must be achieved with due respect to the well-being of animals and conservation of the environment.

Milk products are a source of a diversity of protein products, and this range is being extended by vigorous dairy research. New formulations for milk, cheese, yoghurt and related products are being developed for both Western and Asian markets as well as more sophisticated fractionation of milk to create proteins with special properties and uses. Whey proteins have attracted attention because of their solubility, digestibility and nutritional value. Whey proteins are widely used in infant formulae and are more suitable than casein for human infants. As well whey proteins appear to have some advantageous properties such as stimulation of immune function. The feeding of whey protein was shown to reduce the incidence of tumours in rats injected subcutaneously with a procarcinogen. Liver glutathione, an antioxidant and anticarcinogen, was higher in the livers of
immunoglobulins and may be more effective than those
Eggs from immunized hens may also be a useful source of
products. Meat industries set up in developing countries will be
forced to adopt sophisticated record and tracking systems if
they are to be involved in exporting meat products. Bovine
feeding of meat meal to other animals, as well as taking care
only by slaughtering infected stock and prohibiting the
processes and the spread of the disease has been stopped
initiating a dementia that is a variant of the Creutzfeldt–Jakob
infected animals. The infective protein apparently acts by
the formation of vacuoles in the nerve tissue and leads to loss
animal and catalyzing (?) the rearrangement of the normal
brain cell (neurone) membrane but which has a
different conformation (i.e. a different 3-D arrangement of
the peptide chains). The BSE prion was transmitted through
British cattle herds by the feeding of meat meal derived from
infected animals. The infective protein apparently acts by
entering the brain cell membranes of the newly infected
animals fed whey protein, which could have been due to the
higher content of methionine and cysteine.35
Simplesse® (CP Kelco, the Philippines) is a microparticul-
ated whey protein concentrate. The microparticulated
structure of Simplesse® in foods provides a fat-like mouth
feel, allowing its substitution for fat and a reduction in
ergy density in manufactured foods such as icecream.36
Lactoferrin, and iron-binding protein in mammalian
milk, which occurs at a relatively high level in normal breast
milk, is believed to have a role in non-specific defence
against pathogens. The tight sequestration of iron strongly
inhibits microbial growth and, as well, lactoferrin appears to
have antiviral and general stimulatory effects on the
immune system. Lactoferrin appears to offer large potential
as a food component, which will assist control of infections
without side-effects.37 Bovine immunoglobulins also appear
to have beneficial uses, particularly in milk for children.
Eggs from immunized hens may also be a useful source of
immunoglobulins and may be more effective than those
produced in cows.39 It is possible to immunize cows against
pathogens such as rotavirus, and the milk then contains
antiviruses immunoglobulins, which are beneficial in over-
coming diarrhoeal disease in children.40,41 The immunoglob-
ulins at least partially survive in the human gastrointestinal
tract.42 Recent work is aimed at creating genetically modi-
ified (GM) cows that produce required proteins in their milk
as specified by the implanted gene.

One unexpected development in cattle production has
been the emergence of bovine spongiform encephalopathy
(BSE; TSE in the USA) a disease of the brain and nervous
tissue transmitted by a new infectious agent called a prion,
similar to scrapie in sheep, and kuru and Creutzfeldt–Jakob
dementia in humans. A prion is a particular protein found in
normal brain cell (neurone) membrane but which has a
different conformation (i.e. a different 3-D arrangement of
fava (broad) beans. Favism is a condition produced in individuals with a
genetic deficiency of glucose-6-phosphatase that results
in a haemolytic anaemia after consuming fava (broad) beans.
Lectins (haemagglutinins) are present in a wide range of
legumes. Red kidney beans, for example, are quite toxic if
eaten uncooked.42 There is a large potential for improving
the nutritional characteristics of the many legume species by
gene manipulation.

High-protein moist protein foods such as meat, egg and
milk products are prone to bacterial contamination and the
development of dangerous toxins if not treated correctly.
There are many incidents each year in which inadequate
hygiene, food handling procedures, cooking, refrigeration
etc. result in food poisoning. The hazard analysis critical
control point (HACCP) approach is particularly important in
preventing spoilage and food poisoning. There are numerous
bacterial toxins involved in food poisoning, some of which
are heat stable (e.g. *Staphylococcus* toxin). Food-preserving
standards are based on criteria designed to prevent survival of
the most heat-resistant bacteria and toxins. Periodically
new food poisoning outbreaks occur that force a revision of
the criteria. A number of outbreaks of food poisoning
following consumption of hamburger meat and fermented
sausage contaminated with *Escherichia coli* strain O157 in
recent years brought the haemolytic uremic syndrome into
prominence, resulting in a revision of procedures for the safe
handling of minced meats.

Fermentations have a long history of use in food preser-
vation in developing countries but also involve some degree of
risk. Wherever fermented protein foods are made it is
important that the operators know and use the correct
conditions required to produce a safe product. Poorly con-
trolled bacterial fermentation of meat can result in botulism,
which can be fatal. Fermentations with fungi can also pose
hazards; many fungi produce toxins, not all of which have

Toxins

Some foods contain low-level toxic agents that may be
protein, glycoproteins or protein fragments released by diges-
tion. The content of these molecules may be reduced by
traditional cooking practices; for example, soaking of leg-
umes. Favisin is a condition produced in individuals with a
congenital deficiency of glucose-6-phosphatase that results
in a haemolytic anaemia after consuming fava (broad) beans.
Lectins (haemagglutinins) are present in a wide range of
legumes. Red kidney beans, for example, are quite toxic if
eaten uncooked.42 There is a large potential for improving
the nutritional characteristics of the many legume species by
gene manipulation.

High-protein moist protein foods such as meat, egg and
milk products are prone to bacterial contamination and the
development of dangerous toxins if not treated correctly.
There are many incidents each year in which inadequate
hygiene, food handling procedures, cooking, refrigeration
etc. result in food poisoning. The hazard analysis critical
control point (HACCP) approach is particularly important in
preventing spoilage and food poisoning. There are numerous
bacterial toxins involved in food poisoning, some of which
are heat stable (e.g. *Staphylococcus* toxin). Food-preserving
standards are based on criteria designed to prevent survival of
the most heat-resistant bacteria and toxins. Periodically
new food poisoning outbreaks occur that force a revision of
the criteria. A number of outbreaks of food poisoning
following consumption of hamburger meat and fermented
sausage contaminated with *Escherichia coli* strain O157 in
recent years brought the haemolytic uremic syndrome into
prominence, resulting in a revision of procedures for the safe
handling of minced meats.

Fermentations have a long history of use in food preser-
vation in developing countries but also involve some degree of
risk. Wherever fermented protein foods are made it is
important that the operators know and use the correct
conditions required to produce a safe product. Poorly con-
trolled bacterial fermentation of meat can result in botulism,
which can be fatal. Fermentations with fungi can also pose
hazards; many fungi produce toxins, not all of which have

been characterized. Toxins such as aflatoxin produced by *Aspergillus flavus* in mould-infested food can cause liver damage and ultimately lead to liver cancer. The food of most concern has been peanut crops in Africa but a variety of foods can be affected. Sensitive techniques are now used to monitor peanuts for aflatoxin content. Several samples of peanuts destined for human consumption in the UK were recently found to contain aflatoxin content above the EU limit of 2 µg per kg.45

Fish toxins can be particularly potent. Ciguatera is a toxin occurring in fish in tropical waters and is apparently carried up the food chain from a dinoflagellate bloom. Consuming affected fish leads to gastrointestinal and particularly neurological symptoms, including inverse temperature perception, and in rare severe cases, convulsions, paralysis and death. Usually recovery occurs over a few days although neurological symptoms can linger on for months. The illness may be more severe if previously affected individuals again consume toxic fish. Many people are affected in tropical regions each year. Unfortunately there is as yet no simple test that will identify toxic fish. Most bulk fish industries draw their fish from relatively cold waters but as developing tropical countries increase their processed fish industries this type of fish poisoning may become more important.

Scombroid fish poisoning can occur if partially spoiled fish such as anchovies, mackerel or tuna, are consumed; the effects are caused by ingestion of pharmacologically active quantities of histamine released by microbial decarboxylation of histidine in the fish flesh. In scombroid fish poisoning, histamine produces flushing, sweating, rash, nausea, diarrhoea, and headache. Typically the onset and duration of the illness are short, and the severity of the symptoms varies according to the individual’s sensitivity to histamine. Other amines such as putrescine and cadaverine formed by decarboxylation of ornithine and lysine may act synergistically by inhibiting the histamine detoxifying enzymes, diamine oxidase and histamine N-methyl transferase.46 The reaction is not an allergy and all individuals will react to some degree to contaminated fish. One organism, *Morganella morganii*, appears to be of particular importance in generating histamine in spoiled fish. Rapid and efficient handling of fish with appropriate refrigeration is necessary to avoid scombroid poisoning. Fish are an important source of high-quality protein in developing countries and greater use of fish is to be encouraged, not least because of the contribution to iodine supply. It is important then to encourage the development of refrigeration technology for handling foods susceptible to spoilage. Under new FDA guidelines, fishery products containing histamine at more than 5 mg/g fish are subject to rejection or recall.47

**Education**

Often in developing communities, the resources are available for the production of foods that would eliminate nutrition problems such as protein–energy malnutrition in children, but there is a lack of knowledge regarding infant and toddler nutritional needs and the foods that will meet those needs. A simple example is the use of more eggs and meat and less use of bulky cereals and root crops as weaning foods. In this context it is important that education resources should be equally accessible to women as to men. There may also be lack of knowledge of relatively simple farming methods that would produce sufficient appropriate foods to overcome particular malnutrition problems, for example, growing chickens for eggs and meat. Education therefore remains one of the most important aspects of improvement in diets with regard to adequacy of protein supply. However, it is also worth bearing in mind that knowledge does not necessarily change behaviour and other strategies to change behaviour may need to be adopted.

In developed countries, literacy and education are at higher levels but it is still difficult to give prominence to basic nutrition information. One of the risks posed by a rising tide of health claims relating to novel food products is that basic information on sensible nutrition practices for the maintenance of health will become submerged in a sea of advertising promulgated by vested interests. Responsible authorities will need to continue funding for basic education on nutrition and health.

**References**


