UNIT 4 PROTEINS

Structure

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4.1 INTRODUCTION

The term protein (Greek: proteuo- to occupy first place) was first suggested by *Berzelius* to describe the complex organic nitrogenous substances found in animal and plant tissues. Proteins form three-fourths of the animal body on a moisture-free basis. They are essential for life processes. All the basic functions of life depend on proteins. Indeed, no form of life exists without proteins. They are found in every cell, make up the contractile elements and enzymes that catalyze the release of energy for the maintenance of life and there is no physiological function in which they do not participate. Now in this unit let us get familiarized with the chemical nature, digestion, absorption, utilization and other nutritional aspects of proteins.

Objectives

After studying this unit, you will be able to:

- discuss the classification and functions of proteins,
- identify the food sources of proteins,
- explain the processes of digestion and absorption of proteins,
- recognize the methods of estimating the protein quality, as well as, requirements at various stages of life, and
- describe the symptoms of protein deficiency.

4.2 PROTEINS – AN OVERVIEW

We just read that proteins are essential for maintaining and sustaining life. What are proteins or what constitutes proteins that make it so important for us?

Proteins contain carbon, hydrogen, nitrogen, sulphur and some also contain phosphorus. In the Nutritional Biochemistry Course, Unit 2, you may recall studying that proteins are chains of amino acids held together by peptide bonds. When a peptide chain is

extended by more and more amino acids, until a chain length of one hundred to several thousand amino acid residues is reached, it is classified as a 'protein'. The nitrogen content of proteins varies from about 14 to 20 percent. The average value of 16 percent is used commonly for converting nitrogen content of food stuffs or tissues into proteins by multiplying with the factor 6.25 (100/16) (i.e. Crude protein = Nitrogen × 6.25).

Let us now study and find out how proteins are classified.

4.2.1 Classification

Proteins vary widely in their properties. You may recall reading about the properties of proteins in the Nutritional Biochemistry Course, in Unit 2. We suggest you look up this unit once again now as the information about proteins and their properties given there will supplement your understanding of the functions and other aspects of proteins discussed here in this unit.

Let us consider the two very familiar proteins. One of them is the egg white protein, which is very sensitive. It denatures on heating, dissolves easily in water and is quite reactive, while the other one is keratin of nails and hoofs, is wholly insoluble, tough and chemically inert and resistant. Hence, it is not easy to classify proteins. We simply distinguish proteins which are insoluble and fibrous and function as structural material (scleroproteins) and globular proteins, those represented by egg white or serum proteins, which are soluble in water or salt solution and consist of spherical molecules.

Besides classifying proteins on the basis of *soluble* and *insolubl*e, proteins have been further classified based on the following attributes:

- Classification based on chemical nature
- Classification based on chemical properties
- Classification based on amino acid content

Let us get to learn about proteins based on this classification.

A) **Classification based on chemical nature**

Frequently proteins are classified based on the chemical nature of amino acids (such as solubility and prosthetic group), as *simple, conjugated* and *derived* proteins. Let us get to know each of these.

I) *Simple proteins*: Simple proteins are those which contain only amino acids or their derivatives and no prosthetic group. They yield only amino acids or their derivatives on hydrolysis. Let us see which are these and where are they found.

1) *Albumins*: Proteins such as egg albumin and serum albumin are soluble in water and coagulable by heat.

2) *Globulins*: These proteins are insoluble in pure water, but soluble in neutral salt solutions. For example, serum globulin, tuberin (potato), arachin and conarachin (peanuts).

3) *Glutelins*: These are insoluble in all neutral solvents but soluble in very dilute acids and alkalis. e.g. glutenin of wheat.

4) *Prolamins*: Proteins soluble in 70-80% alcohol. e.g. gliadin and zein.

5) *Fibrous proteins*: These proteins are characteristic of the skeletal structures of animals and also of the external protective tissues, such as the skin, hair, etc. e.g., collagen, elastin and keratin.

6) *Histones*: Soluble in water and insoluble in very dilute ammonia. On hydrolysis, they yield several amino acids, among which the basic ones predominate. The important proteins of this group are the thymus, histones and the globin of haemoglobin.

Let us now move on to the next category of proteins i.e. conjugated proteins and its types.

II) *Conjugated proteins*: Conjugated proteins contain some non-protein substances. Most proteins occur in cells in combination with prosthetic groups and hence are important for the nutritionist. These include:

1) *Glycoproteins*: Most of the naturally occurring conjugated proteins are glycoproteins. Sugar molecules are covalently bound to them, especially those secreted from the cell. They range in size from a molecular weight of 15,000 to more than one million. The carbohydrate component varies from 1 to 85%. Glycoproteins with more than 80% of their molecules as carbohydrates are called 'proteoglycans.'

2) *Lipoproteins*: These are the *multicomponent complexes of lipids and proteins that form distinct molecular aggregates*. They contain polar and neutral lipids, cholesterol or cholesterol esters in addition to protein. The proteins and lipids are held together by non covalent bonds. Lipids are primarily hydrophobic and cannot be easily transported through an aqueous environment as blood. The lipoprotein combination renders the lipid molecule hydrophilic and is transported in the blood to tissues which can use or store the lipids.

3) *Nucleoproteins*: Nucleoproteins are *combinations of nucleic acids and simple proteins, which usually consists of a large number of basic amino acids*. Nucleoproteins have very complex structures and numerous functional activities. All living cells contain nucleoproteins. Some cells, such as viruses, are composed of nucleoprotein.

4) *Other conjugated proteins*: The phospho proteins and the metallo proteins are loose (as with phosphate carrying protein) or tight (as with the phosphate in casein or the iron in ferritin) associations of proteins with phosphate groups or such ions as zinc, copper and iron.

What are derived proteins? Let us find out next.

III) *Derived proteins*: Derived proteins are *the derivatives of the protein molecule, apparently formed through hydrolytic changes in the molecule*. These are either *primary* or *secondary* protein derivatives. Let us get to know them.

1) *Primary Protein Derivatives*: These are the *derivatives of the protein molecule formed by hydrolysis involving slight alterations*. Examples include:

a) *Proteins*: These are the insoluble products which result from the incipient action of very dilute acids or enzymes. e.g. casein (curdled milk), fibrin (coagulated fibrinogen).

b) *Metaproteins*: Proteins resulting from the action of acids and alkalis whereby the molecule is sufficiently altered to form proteins soluble in weak acids and alkalis, but insoluble in neutral solvents.

c) *Coagulated Proteins*: Insoluble proteins which result from the action of heat on protein solutions or the action of alcohol on the protein. e.g. cooked egg albumin or egg albumin precipitated by alcohol.

2) *Secondary Protein Derivatives*: These are the products of further hydrolytic cleavage of the protein molecule. Examples include:

a) *Proteoses*: Soluble in water, not coagulable by heat, precipitated by saturating their solutions with ammonium.

b) *Peptones*: Soluble in water, not coagulable by heat and not precipitated by saturating their solutions with ammonium sulphate. These represent a further stage of cleavage than the proteoses.

c) *Peptides*: These are the compounds containing two or more amino acids. An anhydride of two amino acids is called a '*dipeptide*', one having three amino acids, a '*tripeptide*' and containing several amino acids, a '*polypeptide*'. Peptides result from further hydrolytic cleavage of the peptones.

Next, let us review the classification based on chemical properties.

B) **Classification based on chemical properties**

Depending on their chemical properties and optical activity, the amino acids in proteins are classified under the following heads:

1) *Mono amino mono carboxylic acids*: Examples include glycine, alanine, valine, leucine, isoleucine, serine and threonine.
2) *Mono amino dicarboxylic acids*: Examples include aspartic acid and glutamic acid.
3) *Diamino mono carboxylic acids*: Arginine and lysine.
4) *Sulphur containing amino acids*: Cysteine, cystine and methionine
5) Aromatic and heterocyclic amino acids: Phenylalanine, tyrosine, histidine, tryptophan , proline and hydroxyproline.

The next classification is based on the amino acid content. Let us get to learn about this classification.

C) **Classification based on amino acid content**

Nutritionally, amino acids are classified on the basis of the body's ability to synthesize them – as *essential* (indispensable and not synthesized in the body) and *non-essential* (dispensable and that can be synthesized in the body) amino acids. Indispensable amino acids must be a part of the diet while dispensable amino acids need not be present in food. These definitions, however, become blurred at the metabolic level. *Lysine* and *threonine* are perhaps the only metabolically indispensable amino acids because they are not transaminated to any nutritionally significant extent. This is a crucial point because lysine and threonine are the first and second limiting amino acids in cereal proteins. Lysine is the first limiting amino acid in human milk. Glutamic acid and probably serine are the only truly dispensable amino acids since these are the only amino acids which can be synthesized by the reductive amination of the appropriate keto acid. You may recall studying about these reactions in the Nutritional Biochemistry Course, in Unit 7. There is a third group of amino acids which are '*conditionally essential*', and are characterized by two features. Firstly, their synthesis uses other amino acids as carbon precursors and is confined to specific organs. This is an important metabolic distinction from the dispensable amino acids. For some conditionally essential amino acids, e.g., tyrosine, the precursor is a dispensable amino acid; while for others such as cysteine both an essential amino acid, methionine as sulphur donor and a non essential amino acid, serine are required. At the metabolic level, the organism's ability to synthesize a conditionally essential amino acid is constrained by the availability of a suitable amino acid precursor. Secondly, the maximum rate at which their synthesis proceeds may be limited and potentially restricted by developmental or pathophysiological factors. Thus, very low birth weight infants are unable to synthesize cysteine and proline and lack the ability to synthesize glycine. These factors are important because human milk proteins have low glycine content. Amino acids are classified based on the amino acid content as given in the Table 4.1.

Table 4.1: Classification of amino acids

Essential (indispensable)	Conditionally-essential	Non-essential (dispensable)
Methionine	Tyrosine	Glutamic acid
Tryptophan	Cystine	Alanine
Valine	Aspartic acid	Proline, Hydroxyproline
Isoleucine	Serine	Glycine
Leucine		
Phenylalanine		
Lysine		
Threonine		
Arginine		
Histidine (only for infants)		

We now know that proteins are composed of amino acids and that proteins differ in their amino acid make-up. Proteins lacking in one or more of the essential amino acids, cannot be utilized to meet the protein requirements of the body hence they are not good quality proteins. The nutritive value of a protein will be high if the amino acid make-up is very similar to that of the body proteins and will be low if it lacks partially or completely any one of the 10 essential amino acids (refer to Table 4.1) or if its amino acid composition is very much different from that of the body proteins. Based on their nutritive value or amino acids make-up, proteins are therefore classified as:

I) *Complete proteins* – e.g., egg proteins. These proteins promote growth and provide all the essential amino acids.

II) *Partially complete proteins* – e.g., wheat proteins. These promote moderate growth and partially lack one or more essential amino acids.

III) *Incomplete proteins* – e.g., gelatin or zein. They do not promote growth and completely lack one or more essential amino acids.

After understanding how proteins are classified, let's move on to sources of protein in our next sub-section.

4.2.2 Food Sources

The important sources of proteins in the diets of low-income groups are cereals and legumes. Meat, fish, eggs and milk are important sources of proteins of high biological value. Oilseeds, oilseed meals and soy are rich potential sources of proteins. The protein content of some important foods is given in Table 4.2.

Table 4.2: Protein content of some important foods

Foods	Protein (g / 100 g)	Foods	Protein (g / 100 g)
Cereals	3 - 13.27	Cow's Milk	3.26
Legumes	18 - 24	Buffalo Milk	3.68
Soybean	37.8	Tuna Fish	72.12
Nuts and oilseeds	2.7 - 23.6	Goat liver	20.32
Egg, country hen	72.96		

Source: Indian Food Composition Tables, National Institute of Nutrition, ICMR, 2017

We shall continue with our discussions on the metabolism and function of proteins. However, you must first attempt the check your progress exercise given below before procceeding further to recapitualate the learnt concepts.

Check Your Progress Exercise 1

1) Fill in the blanks

   a) The nitrogen content of food-stuffs are converted into proteins by multiplying the factor ..... .

   b) An example of conjugated proteins is ..... .

   c) Glycoproteins with more than eighty percent of their molecules as carbohydrates are called ..... .

   d) An example of complete proteins is ..... protein.

   e) Zein is an example of ..... protein.

2) Match the following:

A		B	
1.	Simple proteins	a)	Lipoprotein combination
2.	Truly indispensable aminoacids	b)	Soyabeans
3.	Proteins of high biologic value	c)	Zein
4.	Richest plant source of protein	d)	Albumin
5.	An anhydride of two amino acids	e)	Valine
6.	Renders the lipid molecule hydrophilic	f)	Non vegetarian foods
7.	Incomplete protein	g)	dipeptide

After having sound knowledge of what proteins are composed of, its types and food sources, let us now study about their physiological aspect, i.e. digestion, absorption and transport in the body.

4.2.3 Digestion, Absorption and Transport

Under this sub-section, we shall learn how proteins that we consume are digested, absorbed and transported to various body tissues. You may recall studying about this aspect in the Nutritional Biochemistry Course in Unit 5, sub-section 5.4.2. We suggest you look up this unit now. This will facilitate your understanding on this topic. A brief review on the process of digestion, the enzymes involved, their location and target amino acids is present next for your recapitulation.

4.2.3.1 Digestion

The daily protein intake (of about 50-100 g) and the protein of enzymes, sloughed (bled or drop off) epithelial cells and mucins, which are found in the gut is almost completely digested and absorbed. This is a very efficient process and ensures a continuous supply of amino acids to the whole body's amino acid pool. The purpose of protein digestion is to liberate the amino acids of the consumed proteins. This is accomplished through a series of enzymes which have specific target linkages as their point of action as shown in the Table 4.3.

Table 4.3 : Enzymes and their target linkages

Enzyme	Location	Target Linkages
Pepsin	Stomach	Peptide bonds involving the aromatic amino acids
Trypsin	Small intestine	Peptide bonds involving arginine and lysine
Chymotrypsin	Small intestine	Peptide bonds involving tyrosine,tryptophan, phenylalanine, methionine and leucine
Elastase peptidase A	Small intestine	Peptide bonds involving alanine, serine and glycine
Carboxy peptidase A	Small intestine	Peptide bonds involving valine, peptidase A leucine, isoleucine and alanine
Carboxy peptidase B	Small intestine	Peptide bonds involving lysine and peptidase B arginine.
Endopeptidase Aminopeptidase Dipeptidase	Cells of brush	Di and tripeptides that enter the aminopeptidase brush border of the absorptive dipeptidase cells.

The protein *hydrolases*, called as *peptidases*, fall into two categories. Those that attack internal peptide bonds and liberate large peptide fragments for subsequent attack by other enzymes are called the '*endopeptidases*' and those that attack the terminal peptide bonds and liberate single amino acids from the protein structure are called '*exopeptidases*'. The exopeptidases are further subdivided according to whether they attack at the carboxy end of the amino acid chain (carboxy peptidases) or the amino end of the chain (amino peptidases). The initial attack on the intact protein is catalyzed by the intestinal epithelial cells. The hydrolysis of proteins in the gastro- intestinal tract is completed by *proteases* secreted in the gastric juice and pancreatic juice and also by proteases in the intestinal mucosa.

In Table 4.3, you would have noticed that the digestion of protein starts only in the stomach. In contrast to carbohydrate and lipid digestion, which is initiated in the mouth with the salivary amylase and the lingual lipase, protein digestion does not begins until the protein reaches the stomach and the food is acidified with the gastric hydrochloric acid (HCl). Let us get to know about gastric digestion in greater details.

Gastric digestion

If gastric HCl production is low and not adequate to maintain the pH of the stomach contents between 2 and 3, protein digestion in the stomach may be negligible. This will happen in *achlorhydria* (a condition characterized by the failure of the intragastric pH to fall to less than 4.0), *achylia gastrica* (absence of gastric juice, partial or complete) or *pernicious anaemia*. The HCl serves several functions in gastric digestion. It acidifies the ingested food, killing potential pathogenic organisms. However, not all pathogens are killed. Some are acid-resistant or are so plentiful in the food that the amount of gastric acidification is insufficient to kill all of the pathogens. HCl also serves to denature the food proteins, thus making them more vulnerable to attack by the proteolytic enzymes present in the gastric juice namely, *pepsin* and *endopeptidase* (it hydrolyzes peptide bonds in the interior of the protein molecule).

Pepsin has a strong clotting action on milk. This factor is important in the digestion of milk proteins in infants. The optimum pH of pepsin action is about 2.0. Actually pepsin is not a single enzyme. It consists of *pepsin A* which attacks peptide bonds involving phenylalanine or tyrosine and several other enzymes which have specific

attack points. The pepsins are released into the gastric cavity as *pepsinogen*. When the food entering the stomach stimulates HCl release and the pH of the gastric contents fall below 2, the pepsinogen looses a 44-amino acid sequence. The activation of the pepsins from pepsinogen occurs by one of the two processes–*autoactivation* and *autocatalysis*. Let's understand what these are. The first one is called *autoactivation* and occurs when the pH drops below 5. At low pH, the bond between the 44th and 45th amino acid residue falls apart and the 44th-amino acid residue (from the amino terminus) is liberated. The liberated residue acts as an inhibitor of pepsin by binding to the catalytic site until pH value of 2 is achieved. The inhibition is relieved when this fragment is degraded. Degradation occurs at pH 2 or by the action of pepsin. Thus, pepsin hydrolyzes mainly peptide bonds containing phenylalanine, tyrosine or tryptophan and leucine and other acidic amino acids and since food remains in the stomach for a limited time, dietary proteins are hydrolyzed mainly into a mixture of polypeptides as highlighted herewith.

$$\text{Dietary proteins} \xrightarrow{\textit{Pepsin}} \text{Polypeptides}$$

Let us get to know about the *autocatalysis* process next. 'Autocatalysis' occurs when already active pepsin attacks the precursor pepsinogen. This is a self-repeating process and serves to ensure ongoing catalysis of the resident protein. The cleavage of the 44-amino acid residue, in addition to providing activated pepsin, also serves as a signal peptide for cholecystokinin release in the duodenum. This sets the stage for the subsequent pancreatic phase of protein digestion.

Let us get to know about protein digestion in the intestine next.

Proteolysis in the intestines: The main digestion of polypeptides produced in the stomach takes place in the intestines. *Cholecystokinin* stimulates both the exocrine pancreas and the intestinal mucosal epithelial cells to release its digestive enzymes. The proteases involved in the digestion are *trypsin, chymotrypsin* and *carboxypeptidase* (as highlighted in the Table 4.3) secreted in pancreatic juice and amino peptidases present in the intestinal mucosa. The intestinal cells release an enzyme, *enteropeptidase or enterokinase*, which serves to activate the protease trypsin, released as trypsinogen by the exocrine pancreas. This trypsin not only acts on food proteins, it also acts on other preproteases released by the exocrine pancreas, thus activating them. Thus *trypsin* acts as an *endoprotease* on chymotrypsinogen by releasing chymotrypsin, on proelastase by releasing elastase and on procarboxypeptidase by releasing carboxypeptidase. *Trypsin*, *chymotrypsin* and *elastase* are all endoproteases, each having specificity for particular peptide bonds. Trypsin and chymotrypsin act at pH 7.4 to 8.0. Trypsin hydrolyses mainly peptide linkages containing tyrosine or phenylalanine.

$$\text{Proteins and polypeptides} \xrightarrow{\textit{Trypsin and Chymotrypsin}} \text{Peptides + Amino acids}$$

Each of the three proteases i.e. trypsin, chymotrypsin and elastase have serine as a part of their catalytic site so that any compound that ties up the serine will inhibit the activity of these proteases. One such inhibitor, diisopropylphosphofluoridate reacts with serine and stops protein digestion. Through the action of pepsin, trypsin, chymotrypsin and elastase, numerous oligopeptides are produced which are then attacked by the *amino* and *carboxypeptidases* of the pancreatic juice and those on the brush border of the absorptive cells. Carboxypeptidase A hydrolyzes the end group in peptides containing aromatic or aliphatic amino acid, thus releasing free amino acids as shown herewith. Carboxypeptidase B hydrolyzes peptides containing arginine and lysine residues. The intestinal mucosa contains a group of amino peptides which complete the hydrolysis of peptides to amino acids. The intestinal mucosa also contains tripeptidase, dipeptidase, etc., which hydrolyze tri and dipeptides as highlighted in the reaction given herewith:

Peptides —(*Carboxy peptidase*, *Amino peptidases*)→ Amino acids

Dipeptides —(*Dipeptidase*)→ Amino acids

Tripeptides —(*Tripeptidase*)→ Amino acids

Thus, the ultimate products of digestion of proteins, namely, amino acids are liberated from these chains one by one and are absorbed and appear in the portal blood.

With this, we come to an end on our discussion on digestion. In the following sub-section, we will look at how amino acids formed are absorbed in our body.

4.2.3.2 Absorption

Although single amino acids are liberated in the intestinal contents, there is insufficient power in the enzymes of the pancreatic juice to render all of the amino acids singly for absorption. The brush border of the absorptive cell, therefore not only absorbs the single amino acid but also the di- and tripeptides. In the process of absorbing these small peptides, it hydrolyzes them to their constituent amino acids. There are specific transport systems for each group of functionally similar amino acids and peptides. The site of absorption is the 'small intestine'. The process of absorption requires energy. It is observed that L-isomers (natural isomers) of amino acids are more rapidly absorbed than D-amino acids and are hence biologically more important. What are L and D isomers of amino acids? We have already talked about this in the Nutritional Biochemistry Course, in Unit 2. Do look up sub-section 2.6.2 now. These isomers are transported by an active carrier system against a concentration gradient. Similarly, neutral amino acids are more rapidly absorbed than the basic amino acids and in general, amino acids compete with one another for absorption. In several instances, the carrier is a shared one, that is, the carrier transports more than one amino acid. Vitamin B_6 is essential for amino acid absorption. Let us now learn how this transport mechanism works.

4.2.3.3 Transport of Amino Acids

More than one transport or carrier system functions in the absorption of amino acids. The active carrier system for neutral amino acids shares a common membrane carrier. Neutral amino acids and those with the short or polar side chains (serine, threonine and alanine) are transported by the shared carrier system. The basic amino acids, that is, lysine, arginine and histidine share a carrier system with cystine. The mechanism of transport and uptake are depicted in Figure 4.1.

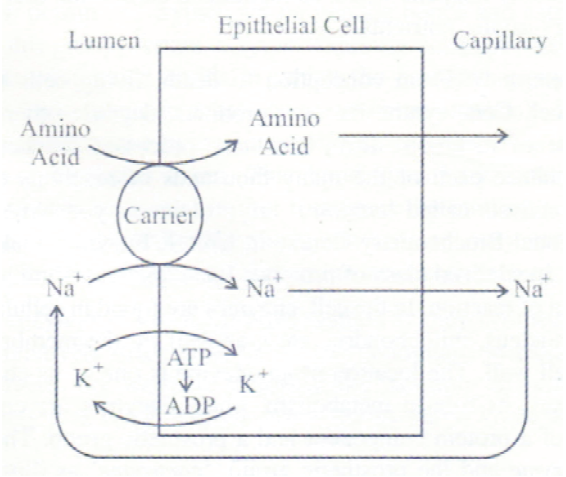


Figure 4.1: Carrier-mediated sodium-dependent amino acid transport

In Figure 4.1, you may have noticed that the amino acid transport is dependent on the Na^+ ion. The dependence of amino acid transport on Na^+ ion suggests a direct interaction between the carrier and Na^+ ion. This is similar to that observed in the absorption of glucose, as you may recall studying in the last unit. The amino acid associates with the carrier and Na^+ ion in the microvilli and the complex travels to the inner side of the membrane where it dissociates, releasing the amino acid and Na ion into the cytoplasm as illustrated in Figure 4.1. Thus the amino acid leaves the absorptive cell with sodium. The carrier, Na ion is recirculated back to the lumen for reuse. As sodium enters the cell, potassium is pumped out via a Na^+ K^+ ATPase system. As sodium leaves the cell, potassium flows back in and the electrolyte balance is maintained. The Na^+ ion is then actively transported out of the cell.

So we have looked at the digestion, absorption and transport of proteins and amino acids. What are the functions of proteins in our body? Let us study and find out next.

4.2.4 Functions

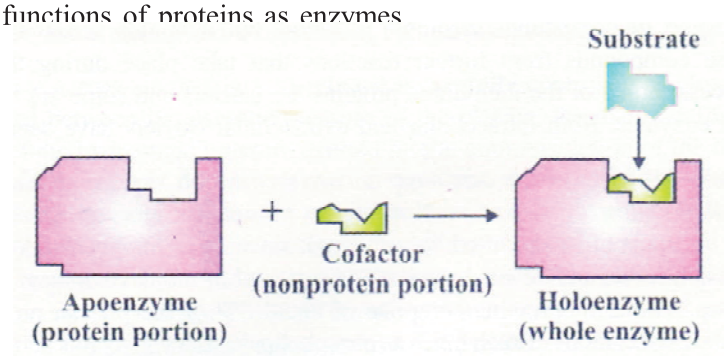
Each of the various proteins in the body serves a specific function in the maintenance of life. Any loss in body protein, in reality, means a loss in cellular function. In contrast to lipids and carbohydrates, proteins have no true body reserve. Humans when deprived of or insufficiently supplied with protein, compensate for this dietary deficiency by catabolizing some, but not all, of their tissue functionality. Cells, tissues, organs and whole systems cannot exist without proteins serving their various functions. So let us get to know the varied functions of proteins in our body. We begin our discussion on functions, first with the body-building function.

1) *Body-building functions of proteins*: The primary functions of proteins, as you might be aware, is tissue growth and maintenance. Protein contains amino acids – the building blocks - that our bodies use to build and maintain muscles, bone, skin, blood and other organs. Thus, proteins play an important role in growth and body-building. For the constant growth of human beings from birth to adulthood, a regular supply of dietary protein is required. Now this does not mean that the body does not require proteins once the growth ceases. During adulthood, worn out cells, body tissues need continuous replacement. Proteins are required for maintenance of our body. Proteins, therefore, are crucial and required through out our life span for growth, body-building and maintenance.

2) *Protein as an energy source*: Proteins contribute to the body's energy need. If diet does not furnish enough calories from carbohydrates and fats, proteins are catabolized to give energy. One gram protein yields 4 Kcal. But, what is important for us is to understand is that this is not the *major function of proteins*. This only happens when, as mentioned earlier, the diet does not supply enough energy-giving nutrients.

3) *Proteins as enzymes*: From conception to death, living cells use oxygen and metabolize fuel. Cells synthesize new products, degrade others, and generally are in a state of metabolic flux. For these processes to occur, catalysts are needed to enhance each of the many thousands of reactions occurring in the cell. These catalysts called '*enzymes*' are proteins as you have already studied in the Nutritional Biochemistry Course in Unit 4. Enzymes make up the largest and the most specialized class of proteins. Each enzyme is unique and catalyzes a specific kind of reaction. In the cell, enzymes are found in cellular compartments (cytoplasm, nucleus, mitochondria, etc.), as well as, the membranes within and around the cell wall. The location of an enzyme is one of its characteristics and dictates, in part, its role in metabolism. Many enzymes are complex proteins; they consist of a protein component and a prosthetic group. The protein part is called

apoenzyme and the prosthetic group, '*coenzyme*' as illustrated in Figure 4.2. A detailed discussion on enzymes is presented in the Nutritional Biochemistry Course, Unit 4. We suggest you read this unit now for a better understanding of the functions of proteins as enzymes.



Coenzyme + apoenzyme → (holo) enzyme

Figure 4.2: Holoenzyme

Enzymes consist of specific sequences of amino acids. The catalytic function of an enzyme is intimately related to its amino acid sequence. Enzymes must possess a shape that will complement the reactive molecular shape of the substrate in the same way as a key fits into a lock. This is commonly referred to as 'lock and key mechanism'. This shape is a function of the enzyme protein's primary, secondary, tertiary and quaternary structure. In the same way, substrates should also have specific shapes in order to be catalyzed by their respective enzymes.

$$\text{Enzyme} + \text{Substrate} \rightleftharpoons \text{Enzyme} - \text{Substrate}$$

$$E + S \rightleftharpoons E\ S$$

This is the reason why only D-sugars or L-amino acids can be metabolized by mammalian cells. These stereoisomers conform to the shape required by the enzyme which serves as its catalyst. While enzymes show absolute specificity, the specificity generally applies to the entire molecule. If however, the substrate is large and complex, the structural requirements are less stringent in that only that part of the substrate involved in the enzyme-substrate complex should have the appropriate molecular arrangement. The portion of the substrate not involved in the reaction need not be the appropriate conformation.

Some enzymes are specific for only one substrate; others may catalyze several related reactions. While some are specific for a particular substrate, others are specific for certain bonds. This is called 'group specificity'. For example, glycosidases act on glycosides, pepsin and trypsin act on peptide bonds and esterases act on ester linkages. Within this group, certain enzymes exhibit greater specificity. Chymotrypsin preferentially acts on peptide bonds in which the carboxyl group is a part of the aromatic amino acids (phenylalanine, tyrosine or tryptophan). Enzymes such as carboxypeptidase catalyze the hydrolysis of the carboxy-terminal or amino-terminal amino acid of a polypeptide chain. This bond specificity, rather than molecular specificity, is useful to the animal in that it reduces the number of enzymes needed within the organism. Incidentally, the above enzymes are very useful to the protein chemist in his / her determination of the amino acid sequence of a given protein.

Cells synthesize enzymes in much the same fashion as they synthesize other proteins; yet enzymes are relatively short lived. Cells must continually synthesize their enzymes if they are to survive. So, we have looked at the functions of proteins as enzymes. Proteins also function as carriers in the body. Let us get to know about this function next.

4) *Proteins as carriers*: A large variety of compounds are carried in the blood between tissues and organs of the body. Some of the compounds require specific protein for their transport. Not only is this specific protein necessary for the transport of compounds insoluble in blood, but it is also necessary to protect these compounds from further reactions that take place during the transport process. Some of the membrane proteins are carriers and some are both carriers and enzymes. Both intracellular and extracellular carriers have been identified.

The plasma proteins which can have a carrier function are the albumin and the α- and β-globulins. The best studied of the plasma carriers are those associated with the transport of lipid (called lipoproteins), since these *lipoproteins* (carriers plus lipids), when levels are elevated, appear to be related to the development of a variety of diseases. These lipoproteins comprise of about 3% of the plasma proteins. They are the loose associations of such lipids as phospholipids, triacylglycerols and cholesterols and represent an example of how proteins function as carriers. The lipids they carry are either from the diet or are synthesized de novo in tissues, such as the liver. The β-globulin proteins carry these lipids to such sites as muscle or adipose tissue, where they are either used or stored. The release of the lipid from the protein carrier is a complicated process. In adipose tissue, the lipoprotein is attached to a membrane receptor site-an enzyme, lipoprotein lipase cleaves the lipid from the protein. The lipid is then picked up by another protein called a lipid binding protein and is carried to the interior of the cell for storage. The β-globulin protein carrier, once free of its lipid, returns to the liver or intestinal mucosa and is recycled.

The plasma lipids, phospholipids, acylglycerols, cholesterol, cholesterol esters and free fatty acids are usually transported as loosely associated lipid-protein complexes. At least three different proteins have been identified. Albumin usually transports the free fatty acids, whereas the α and β-globulins transport the phospholipids, acylglycerols and cholesterols. The different lipoprotein complexes can be separated and identified on the basis of their antigenicity, their electrophoretic mobility and their density. The low density or β-lipoproteins contain the β-peptide, cholesterol and some phospholipids. The majority of phospholipids are carried as α-lipoproteins. With age, the lipid content of the plasma tends to rise and the rise is reflected almost entirely as an increase in β-lipoproteins. As the density of the lipoproteins decreases, the molecular weight and complexity of the lipid it carries decreases. The α-lipoproteins carry mainly (up to 60%) acylglycerols. These glyceroles are usually those synthesized in the body rather than coming from the diet. The dietary acylglycerols are usually carried as chylomicrons. These particles are the largest and least dense of the lipid-protein complexes. Read Unit 7 in the Nutritional Biochemistry Course for more information.

In addition to serving as carriers of lipids, some of the globulins in the plasma can combine with iron and copper, as well as, with other divalent cations. These combinations are called '*metalloproteins*'. The globulins serve to transport these cations from the gut into the tissues where they are used. The monovalent cations, sodium and potassium, do not need carriers but most other minerals do.

Many hormones and vitamins require transport or carrier proteins to take them from their point of origin to their active site. In addition, there are intracellular transport proteins such as the lipid binding proteins that are responsible for the transport of materials between the various cellular compartments. Lastly, there are transport proteins which carry single molecules. The classic example is haemoglobin, the red cell protein, responsible for the transport of oxygen from the lungs to every oxygen-using cell in the body.

From carrier function, we move on to the regulatory function of proteins.

5) *Proteins as regulators of water balance*: As substrates and solutes are transferred or exchanged across membranes, water has a tendency to follow to maintain equal osmotic pressure on each side of the membrane. If osmotic pressure is not

maintained, the individual cells either shrink from lack of internal water or burst from too much. You may recall studying about this phenomenon in the Food Microbiology and Safety Course, in Unit 3. The balance of water between intracellular and extracellular compartments is closely regulated.

Water balance across the capillary membrane is carefully controlled. A close balance is maintained between the osmotic pressure of the plasma, the interstitial fluids and the cells and the hydrostatic pressure exerted by the pumping action of the heart. The total osmotic pressure of the plasma and of the intra- and extracellular fluids is the result of its content of inorganic electrolytes, its organic solutes and its proteins. The concentrations of the electrolytes and organic solutes in plasma, interstitial fluid and cells are substantially the same so that the contribution to the osmotic pressure by these substances is practically equal. However, since there are more proteins in plasma than in the cells, plasma exerts an osmotic pressure on the tissue fluids. The result of this inequity of solutes is the drawing of fluids from the tissue spaces and from the cells into the blood. Opposing this force is the hydrostatic pressure, exerted by the pumping action of the heart, which moves fluids from the blood into the tissue spaces and into the cells. The hydrostatic pressure is greater on the arterial side of the capillary loop than on the venous side. There is some kind of interplay between these four kinds of pressure- *blood osmotic pressure, tissue osmotic pressure, blood hydrostatic pressure and tissue hydrostatic pressure*. This interplay results in the filtration of solutes and metabolites and the transfer of oxygen from the arterial blood into the tissues and cells it supplies and on the venous side, a resorption from the tissue space of CO_2, metabolites and solutes back into the blood supply.

Albumin plays a more significant role in maintaining the osmotic pressure than the other blood proteins because of its size and abundance. It is a small molecule and has a greater number of particles per unit volume than the other larger serum proteins. With fewer proteins in the serum, water leaks out into the interstitial space and accumulates. You may be familiar with this condition commonly referred to as 'oedema'. The oedema of protein deficiency may also be the result of the body's inability to regulate the protein hormone, particularly, Anti-Diuretic Hormone (ADH). This hormone plays a role in controlling water balance. The effect of protein is on the distribution of water amongst the various body compartments than on the total body water.

So now you have understood what important role, proteins can play in regulating the water balance. Proteins also function as biological buffers. What do we mean by this and how proteins function as biological buffers is discussed next.

6) *Proteins as biological buffers*: Proteins have the ability to accept or donate hydrogen ions and by doing so they serve as biological buffers you may recall studying under the properties of proteins in sub-section 2.6.2 in Unit 2 in the Nutritional Biochemistry Course. In blood, there are three important buffering systems – *plasma proteins, haemoglobin and carbonic acid bicarbonate*. The equilibrium reactions for each of these buffering systems is as follows:

$$H\ Protein \leftrightarrow H^+ + Protein^-$$

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

$$HHb \leftrightarrow H^+ + Hb^-$$

The first of these buffering systems, the plasma proteins, functions as a weak acid/salt buffer when the free carboxyl groups on the protein dissociate, or as a weak base/salt buffer when the free amino groups dissociate. Although the buffering ability of the plasma protein is extremely important in maintaining blood pH, it is not as important as the other two systems.

The second buffering system, carbonic acid-bicarbonate, is extremely effective because there are reactions which follow this equilibrium which will regulate either acids or

bases. The H_2CO_3 level in plasma never goes too high because it is in equilibrium with CO_2 ($H_2CO_3 \rightarrow CO_2 + H_2O$), which is expired by the lungs. In blood, this equilibrium proceeds very quickly because of the presence of carbonic anhydrase, an enzyme found in red blood cells which catalyze it. If the carbonic acid-bicarbonate reaction goes in the opposite direction, the concentration of the HCO_3^- so formed will be regulated by the kidneys.

The third important buffering system in blood results from haemoglobin. Haemoglobin has six times the buffering power of the plasma proteins. It functions well as a buffer because it is present in large amounts, it contains 38 histidine residues (Histidine residues are good buffers because they can dissociate to H^+ and the imidazole group) and because haemoglobin exists in blood in two forms, reduced haemoglobin and oxy haemoglobin. It is thus a weaker acid and a better buffer.

Next let us look at the function of proteins as structural elements.

7) *Proteins as structural elements and structural units*: The liver cell membrane analysis shows that this membrane contains 50-60% protein, 35% lipids and 5% carbohydrates. The carbohydrate present is joined primarily to the protein forming glycoproteins, compounds which constitute the receptor sites of several hormones. The protein portion of the membrane is so oriented that its hydrophilic aspects are also in close proximity to the intracellular and extracellular fluids. The protein molecules are interspersed within the lipids and lend both structural stability and fluidity to the membrane. Membrane function depends on how the proteins are placed in the membrane and on the fluidity, which results from the combination of proteins in a lipid mixture. If the lipid is more saturated, a more rigid crystalline structure will form. Many lipids being fluid and less rigid, allow the proteins to change their shape in response to ionic changes and hence these proteins function as enzymes, carriers, binding or receptor sites or entry ports for a large variety of materials binding, entering or leaving the cell. Thus proteins serve as the structural and functional units of the cell membrane.

Proteins are also important intracellular structural units. Muscle is composed of 20% protein, 75% water and 5% inorganic material, glycogen and other organic compounds. The major proteins in muscle are *myosin* – a large globular protein, and *actin* – a smaller globular protein. These two proteins, plus the filamentous tropomyosin and troponin are the molecular components of the muscles. The muscle proteins are characterized by their elasticity, which contributes to the contractile power of the tissue.

The most important structural function of protein is related to skin and connective tissue. The skin is composed of epithelial tissue which covers not only the exterior of the body but also lines the gastrointestinal tract, respiratory tract and the urinary tract. One of the major protein found in the skin is 'melanin'. Melanin is a tyrosine derivative and provides the pigmentation or characteristic colour to the skin. Persons unable to form this pigment are albinos and the disease is called '*albinism*.' *Keratin* is the protein which forms hair, nails, hooves, feathers or horns. Each of these structures is slightly different but all contain keratin. This protein is insoluble in water and is resistant to most digestive enzymes. It has a high percentage of cystine.

Connective tissue is that tissue which holds various cells and tissues together. It includes bones and teeth also, since they start with a matrix protein into which various amounts of minerals are deposited. *Collagen* and *elastin* are the two distinct types of proteins in the connective tissue. It contains proline, hydroxyproline and glycine. These proteins are not easily degradable and are inert metabolically. Even in protein deficient states, the body will synthesize collagen and elastin and these proteins will not be catabolized for needed amino acids. However, this protein can be degraded to a limited degree by boiling in acid. It is then converted to gelatin. The collagen of bone,

skin, cartilage and ligaments differ in chemical composition from that of the white fibrous tissue which holds individual cells together within muscle, liver and other organs. Elastin and chondroalbumoid are two other proteins in the connective tissue. They are present in small amounts and serve as a part of the structural protein.

Proteins as structural elements have been studied above. Interestingly proteins also surround the joints and function as lubricants. Let's look at this lubricant function of proteins next.

8) *Proteins as lubricants*: The mucous of the respiratory tract, oral cavity, vaginal tract and the rectal cavity reduces the irritation which might be caused by materials moving through these passages. This mucous is a mucoprotein, a conjugated protein which contains *hexosamine*. Proteins as lubricants also surround the joints and facilitate their movement. The absence of these lubricants or substantial decrease in their fluidity through deposition of minerals, makes skeletal movement difficult and painful.

Last but not the least, proteins also have an important role to play in the immune system. Let us find out how.

9) *Proteins in the immune system*: Proteins such as γ-globulin serve to protect the body against foreign cells. The immunoglobulins produced by lymphocytes are the large polypeptides having more than one basic monomeric unit. These proteins differ in their amino acid structure, which in turn, affect their secondary, tertiary and quaternary structures. Just as the amino acid sequence of an enzyme determines substrate specificity, the amino acid sequence of the immunoprotein assures antigen-antibody specificity. The initiation of synthesis of particular immunoglobulins by the lymphocytes requires the binding of an antigen (a foreign protein) to the cell surface at particular locations called 'antigen receptors'. The immunoglobulin synthesized binds with the foreign protein immobilizing it. Thus, the complex antigen-antibody is formed about which we have already studied in great details in the Applied Physiology Course in Unit 3. Do look up this unit now to understand this process.

With this, we come to an end of our study on the functions of proteins.

Check Your Progress Exercise 2

1) Write a note on the enzymes that are involved in protein digestion.

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2) Bring out the role of HCl in gastric digestion.

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3) List the functions of protein.

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4) Which protein plays an important role in maintaining the osmotic pressure?

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5) Give examples of proteins as carriers.

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With a clear understanding on the functions of proteins, next we shall review the methods of determination of proteins and amino acid content in foods.

4.3 METHODS OF DETERMINATION OF PROTEINS AND AMINO ACID CONTENT IN FOODS

The methods for protein quality evaluation are grouped under the following headings:

I) Analytical methods for the determination of nitrogen and amino acids in foods

II) Chemical and microbiological assays of protein quality

III) Evaluation of protein quality in experimental animals

IV) Clinical methods for evaluation of protein quality

In this section we shall review both the qualitative and quantitative evaluation of protein and amino acid content in foods. We shall begin with the quantitative/analytical method for the determination of nitrogen and amino acid in foods.

Quantitative/Analytical Method for Estimation of Nitrogen and Protein

You may recall reading earlier that proteins contain nitrogen along with carbon, hydrogen and some other substances. Nitrogen (N) in food not only comes from amino acids in protein but also exists in additional forms that may or may not be used as a part of the total nitrogen economy of humans and animals. Thus, determination of nitrogen is commonly used to determine the protein content of a sample. Total nitrogen, which is determined by the Kjeldahl procedure, is converted to crude protein by a factor of 6.25, as mentioned earlier. Thus, the conventional measure of "protein" or "crude protein" in foods is N × 6.25, and it is recommended that this one factor be used in nutritional studies in which whole diets contain more than one source of nitrogen.

The Kjeldahl's method is commonly used in estimation of protein content of foods and has been extensively used for protein estimation of various foodstuffs. Although over a period of time many other methods have emerged for determination of organic nitrogen, this method still remains an old favourite because it is reliable and has very well standardized procedures.

We shall not go into the details related to the method of this process here in this unit, since it has already been covered in the Nutritional Biochemistry Practical Course

(MFNL-002) in Practical 5. We suggest you look up this Practical now. Next, we shall review the methods for protein quality evaluation.

Qualitative Estimation of Protein/Amino Acid Content in Foods

The protein and amino acid content of foods helps to interpret nutritional differences among foods in terms of their amino acid make-up. This has been obtained only through experiments on animals or human beings. The methods available to determine protein quality are based on several parameters. They are categorized as methods based on:

I) *Growth and body weight changes*: The methods under this category include–
- Protein efficiency ratio (PER)
- Net protein ratio (NPR)
- Gross protein value
- Rat repletion method
- Nitrogen growth index
- Slope ratio method

II) *Carcass nitrogen analysis*: This method includes–
- Nitrogen retention method
- Net protein utilization (NPU)
- Rat repletion methods

III) Nitrogen balance includes–
- Nitrogen balance
- Digestibility coefficient, Biological value and net utilization of dietary proteins
- Nitrogen balance index
- Egg replacement method

IV) Regeneration of blood and liver constituents includes–
- Liver protein regeneration
- Blood protein regeneration
- Regeneration of liver enzymes

V) Availability of amino acids includes–
- Chemical methods
- Enzymic methods
- Microbiological methods
- Animal assays

VI) Miscellaneous parameters include–
- Plasma amino acid levels
- Microbiological methods

VII) Chemical scoring include–
- Chemical score
- Essential amino acid index
- Simplified chemical score

Qualitative differences in food protein sources can be determined by many methods. The traditional methods - protein efficiency ratio (PER), net protein utilization (NPU) and biological value (BV), PER, NPR and NPU - have been described in brief, followed by discussion on the more recent methods of estimation using the fecal protein and ileal amino acid (IAA) digestibility to compute the Protein Digestiblity Corrected Amino Acid Score (PDCAAS) and Digestible Indispensable Amino Score (DIAAS).

Let us get to know these methods. We shall start off with PER i.e Protein Efficiency Ratio.

Protein Efficiency Ratio (PER)

PER method was developed by *Osborne, Mendel* and *Ferry* in 1919 and is based on the growth of young rats. The diets usually contain 10% of dietary protein to be tested and are complete in all other dietary essentials. Groups of albino rats (21 days old) are fed for a period of 4 weeks on different diets. Records of the gain in body weight and protein intake of the rats are maintained. The protein efficiency ratio (PER) is calculated using the following formula:

$$\text{PER} = \frac{\text{Gain in body weight (g)}}{\text{Protein intake (g)}} = \text{gain in weight per g of protein consumed}$$

PER, therefore, *is a measurement of the efficient utilization of the proteins in the body*. You would be interested to know that the egg protein has the highest protein efficiency ratio. Hence, egg protein is the standard to which all other forms of proteins are measured. Next, let us learn about digestibility coefficient.

Digestibility Coefficient

You have earlier learnt that dietary proteins are hydrolyzed to amino acids during digestion. The digestion begins in the stomach by the action of pepsin and later completed in the intestines by trypsin, erepsin and other enzymes. Proteins differ in their digestibility. Egg and milk proteins are easily digested and converted into amino acids while pulse proteins are slowly digested to amino acids. Amino acids are absorbed into the blood stream and the unhydrolyzed portion of the protein is wasted in the faeces. Thus, digestibility coefficient of protein refers to *the percentage of the ingested protein absorbed into the blood stream after the process of digestion is complete.*

When an animal is fed on nitrogen (N)-free diet, certain amount of nitrogen is excreted in the faeces. This is derived mainly from the digestive juices. This is called '*endogenous faecal N.*' When a protein food is given, the N found in faeces consists both of endogenous N and food N lost in digestion. To find out N lost in digestion, endogenous faecal N should also be determined. For the determination of the digestibility coefficient, therefore, the data on food nitrogen intake (I_n), total faecal nitrogen excreted (F_n) and endogenous faecal nitrogen (F_e) are required. The digestibility coefficient can thus be calculated using the formula:

$$\text{Digestibility coefficient} = 100 \times \frac{\text{N intake} - (\text{N in faeces} - \text{endogenous faecal N})}{\text{N intake}}$$

$$= \frac{100 \times I_n - (F_n - F_e)}{I_n}$$

where, $F_n - F_e$ is the food nitrogen lost in digestion.

The digestibility coefficients of proteins are influenced by several factors, such as, the presence of indigestible carbohydrates like cellulose and hemi-cellulose and the presence of proteolytic enzyme inhibitors.

A term which is closely and synonymously used with protein quality is "Biological Value" of protein.

Biological Value

A method for determining the biological value of proteins was developed by *Mitchell* in 1925. It measures the *quantity of dietary proteins utilized by the animal for meeting its protein needs for maintenance and growth*. Groups of albino rats (28 days old) are fed successively on the following diets for a period of 10 days: (1) protein-free diet, and (2) diet containing 10 percent protein to be tested. Urine and faeces are collected by keeping them in metabolism cages. Records of food intake are maintained. The diet, urine and faeces are analyzed for nitrogen. Biological value is then calculated using the following formula:

$$\text{Biological value} = \frac{(\text{Nitrogen digested} - \text{Nitrogen lost in metabolism}) \times 100}{\text{Nitrogen digested}}$$

Let us determine the expression for numerator and denominator.

Nitrogen digested = N intake (I_n) – N in faeces (F_n) on the protein diet – N in faeces (F_e) on protein free diet, i.e.

Nitrogen digested = $I_n - (F_n - F_e)$

Nitrogen lost in metabolism = N in urine (U_n) on protein diet – N in urine (U_e) on an protein free diet = $U_n - U_e$

Hence, Biological value can be expressed as:

$$BV = \frac{I_n - (F_n - F_e) - (U_n - U_e)}{I_n - (F_n - F_e)} \times 100$$

Thus, *BV is a measurement of protein quality expressing the rate of efficiency with which the protein is used for growth*. On a scale with 100 representing top efficiency, Table 4.4 presents the biological values of proteins in several foods.

Table 4.4: Biological values of proteins in several foods

Food Items	BV
Whole egg	93.7
Milk	84.5
Fish	76.0
Beef	74.3
Soybeans	72.8
Rice, polished	64.0
Wheat, whole	64.0
Corn	60.0
Beans, dry	58.0

Source: Food and Agriculture Organization of the United Nations. The Amino Acid Content of Foods and Biological Data on Proteins. Nutritional Study #24. Rome (1970).

Amino Acid Score (AAS): The amino acid score determines the effectiveness with which absorbed dietary nitrogen can meet the indispensible amino acid requirement at the safe level of protein intake. By this approach we can evaluate the capacity of a given protein or mixture of proteins to meet the essential amino acid and nitrogen requirement of an individual.

Amino acid score is expressed as the ratio of mg of amino acid in 1 g of test protein to mg of amino acid in reference protein.

$$\text{Amino acid score (AAS)} = \frac{\text{mg of amino acid in 1 g of test protein}}{\text{mg of amino acid in 1 g reference protein}}$$

The 2007 FAO/WHO/UNU consultation has recommended the standard amino acid pattern presented in Table 4.5 (retained by ICMR 2020) for scoring quality of protein. Using this reference requirement pattern, the amino acid score for a test protein can be calculated.

Table 4.5: Amino acid scoring pattern for toddlers, children adolescent and adults (mg/g protein) (amended values from the 2007 WHO/FAO/UNU report)

Age (y)	His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
0.5	20	32	66	57	28	52	31	8.5	43
1-2	18	31	63	52	26	46	27	7.4	42
3-10	16	31	61	48	24	41	25	6.6	40
11-14	16	30	60	48	23	41	25	6.5	40
15-18	16	30	60	47	23	40	24	6.3	40
>18	15	30	59	45	22	38	23	6	39

Values based on the amino acid requirement and the protein requirement for each age group.

His: Histidine; Ile: Isoleucine; Leu: Leucine; Lys: Lysine; SAA: Sulphur Amino Acids; AAA: Aromatic Amino Acids; Thr: Threonine; Trp: Tryptophan; Val: Valine

Source: Dietary protein quality evaluation in human nutrition. Report of an FAO Expert consulation-FAO 2013

With our understanding of the BV and amino acid score we move to the concept of Protein Digestibility Corrected Amino Acid Score (PDCAAS).

PDCAAS:

It is a chemical score, which is derived from the ratio between the first limiting amino acid in a test protein and the corresponding amino acid in a reference amino acid pattern and corrected for true faecal N digestibility. It is expressed as:

Then, Protein digestibility corrected amino acid score (PDCAAS) =

$$\text{PDCAAS(\%)} = \text{Protein Digestibility} \times \text{Amino Acid Score}$$

$$\text{True protein digestibility(\%)} \frac{\text{* mg of amino acid in 1 g of test protein * 100}}{\text{mg of amino acid in 1 g reference protein}}$$

PDCAAS value for some protein sources is given in Table 4.6

Table 4.6: PDCAAS of Some Protein Sources

Protein Source	PDCAAS
Egg	1.0
Milk	1.0
Soy protein	1.0
Wheat	0.42
Whey protein	1.0
Casein	1.0
Peanuts	0.52

Source: Hoffman JR and Falvo MJ. *J Sports Sci Med.* 2004;3:118–130.

Schaafsma G. *J Nutr.* 2000;130(7):1865S-1867S.

Digestible Indispensable Amino Acid Score (DIAAS)

Digestible Indispensable Amino Acid Score (DIAAS) is a protein quality index which is based on true ileal (terminal end of small intestine) amino acid digestibility. It provides for a more accurate measure of the amount of amino acid absorbed by the body and the protein's contribution to human amino acid and nitrogen requirement. DIAAS has replaced the current protein ranking standard, the Protein Digestibility Corrected Amino Acid Score (PDCAAS). DIAAS is defined as follows:

DIAAS % = 100 × [(mg of digestible dietary indispensable amino acid in 1 g of the dietary protein) / (mg of the same dietary indispensable amino acid in 1 g of the reference protein)]/

Recommended amino acid scoring patterns (i.e. amino acid pattern of the reference protein) to be used for calculating protein quality for dietary assessment for different age groups is presented in Table 4.5

In calculating DIAAS the ratio should be calculated for each dietary indispensable amino acid and the lowest value designed as the DIAAS.

Net Protein Utilization (NPU)

Mitchell (1922) introduced the term 'Net Utilization of Dietary Protein' which is a *product of digestibility coefficient and biological value divided by 100,* as shown herewith.

$$NPU = \frac{\text{Digestibility coefficient} \times \text{Biological Value}}{100}$$

A direct method of estimating Net Protein Utilization (NPU) was developed by *Miller and Bender* (1955). Groups of albino rats 28 days old were used. One group was fed on a non-protein diet while the other groups were fed on the test diets containing different proteins at 10 percent level for a period of 10 days. The food intakes of the animals were measured. The animals were killed at the end of 10 days and the body nitrogen was determined by Kjeldahl method on a sample of dried and powdered carcass. The NPU is calculated according to the formula:

$$NPU = \frac{\text{Body N of the test group} - \text{Body N of the non-protein group} + \text{N consumed by non-protein group}}{\text{N consumed by test group}} \times 100$$

The main advantage of determining NPU of a food or diet is that it helps in the calculation of the net available protein of the diet. Studies have shown that there is a good correlation between the values for PER, NPU and chemical score.

Let us now understand what is a Net Protein Ratio or NPR, and how is it determined.

Net Protein Ratio (NPR)

This method was introduced by *Bender* and *Doell* (1957) and is a modification of the PER method. In this method, an allowance is made for the protein requirements for maintenance. The method consists of feeding a group of weanling rats on a diet containing 10 percent of the test protein and another comparable control group on a non-protein diet for a period of 10 days. The NPR is calculated by adding the loss in weight of the control group to the gain in weight of the test group and dividing the total weight (g) by the quantity of protein consumed by the test group according to the following formula:

$$NPR = \frac{\text{Gain in weight (g) of the test group} + \text{loss in weight (g) of the non-protein group}}{\text{Protein intake (g)}}$$

NPR values have been reported to correlate closely with NPU values.

Lastly, nutrient-to-energy ratio has attracted wide interest as indices of dietary quality. The protein-energy ratio as a measure of dietary quality is explained next.

Protein-Energy Ratio (NDP Cal%)

Platt and his colleagues (1961) are largely responsible for the introduction of the ratio of protein energy to total energy (PE ratio) as a useful measure of dietary quality in human nutrition. To take into account both the quality and concentration of the protein, they introduced the concept of the net dietary protein calories as a percentage of total calories (NDP Cal%). The NDP Cal%, is the net dietary protein value expressed as percent of total calories. The NPU or the *chemical score* could be utilized in calculating the NDP Cal% as both of them are indicative of protein value. The formula used is as under:

$$\text{NDP Cal\%} = \frac{\text{Protein calories in the diet}}{\text{Total Calories}} \times \text{NPU} \times 100$$

OR

$$\text{NDP Cal\%} = \frac{\text{Protein calories in the diet}}{\text{Total calories}} \times \text{Amino Acid Score} \times 100$$

For adults, diet with an NDP Cal% of 5% would be adequate to maintain health. In infants, children, adolescent and pregnant women, growth is supported only by a diet providing an NDP Cal% of 8% or above.

Now that we have studied and understood the various methods of determining proteins, it is important that we practically assess the protein/amino acid quality of our diet or perhaps of other population groups. To help you in this task, we have included various activities in the Practical Manual (MFNL-004) accompanying this course. So go ahead carry out these activities.

Let us now proceed further and find out the ways by which we can improve protein quality of our diets.

4.4 IMPROVEMENT OF QUALITY OF PROTEIN IN THE DIET

Since the net protein utilization (NPU) values of milk or egg proteins are higher than those of proteins of average diets consumed in different countries, a correction has to be made for this variation in the NPU of dietary proteins. This is shown herewith:

$$\text{Dietary protein requirements (g)} = \frac{\text{Safe level of intake of egg or milk proteins} \times \text{NPU of egg or milk proteins}}{\text{NPU of dietary proteins}}$$

For example, if the NPU of the dietary proteins is 45 and egg protein is 90, the correction factor will be 2. In other words, the amount of this dietary protein needed to satisfy the requirements of a given population group will be twice as high as that of egg or milk protein.

The next thing that might come to your mind is regarding the protein quality of the Indian diets. Average Indian diets as consumed in different parts of the country consists chiefly of vegetable source of protein. The amount of animal protein depends on the diet habits, with only milk providing a source of animal protein in vegetarian diets to varying amounts of meat and flesh in the non vegetarian diets. The question of whether the protein quality of our predomintantly vegetarian diets is adequate and

what are the ways in which the quality of protein in our diets can be improved has been addressed through a number of studies.

Habitually, Indian diets are cereal-based diets, limiting in lysine, an essential amino acid critical for growth and development in children. The term limiting is used to describe that *indispensable amino acid which is present in the lowest quantity in the food, in comparison with the same amino acid in a reference protein such as egg or milk*, the quantity of the amino acid expressed in terms of per g nitrogen. Further, the diet is predominantly vegetable-based, and foods of animal origin do not usually find a place because of their high cost. A large percentage of people are vegetarian and their diets include pulses, vegetables, cereals and grain products. These plant foods tend to have too little of one or more indispensable amino acids (i.e. lysine, threonine, tryptophan or methionine, particularly in legumes). In other words, individual plant foods such as cereal alone or pulses alone tend to be relatively deficient in one or more essential amino acids and thus exclusive consumption of single plant foods such as chiefly rice or wheat would result in deficiency of an essential amino acid and if this consumption pattern is continued over a long period of time, it can result in protein deficiency. However, a combination of plant foods, such as cereal-pulse-vegetable based diets are fully capable of meeting protein needs, when consumed in amounts that satisfy energy needs. Fortunately, for us, the amino acid deficient in cereals, namely lysine is present in ample quantities in pulses and green leafy vegetables. Similarly, the essential amino acid methionine which is relatively low in pulse, is present in larger quantities in cereals. Thus, the different sources of vegetable proteins complement each other in terms of the amino acids they provide. Therefore, if we ensure that diet at every meal is a combination of cereals, pulses, and vegetables with nuts and milk contributing wherever one can afford, it will take care of the protein requirements. In this context, when we consider the Indian cuisine we notice, North and West Indian meals consist of *chapatis* or *rotis* and rice as staples, eaten with a wide variety of side dishes like dals, curries, yoghurt, chutney and *achars.* South Indian dishes are mostly rice-based, sambhar, rasam and vegetables being important side dishes. The pulses/legumes, included in the diet, with their high content of lysine but low content of methionine, complement the grains (cereals), which are more than adequate in methionine and cystiene but limiting in lysine. Such cereal-pulse combination diets when consumed help the body receive all the indispensable amino acids. Hence, there is no need to worry about protein if we are eating a varied vegetarian diet! It is easy to get protein from lentils, dal, beans, curd, rice, soy milk, and cereals when eaten in combination so that their amino acid patterns become complementary. The inclusion of pulses in cereal-millet-based diets is critical not only in increasing the protein content, but also in improving the nutritional quality of the protein.

So, then, that brings us to the second question i.e. can we improve the nutritive value of protein? Yes, as discussed above, the nutritive value of a protein can be improved in two ways : (1) by *mutual supplementation,* that is, by blending two or more proteins so that the excess of essential amino acids present in one protein makes up the deficiencies of the same amino acids in another protein and (2) by *supplementation of the dietary proteins with limiting essential amino acids.* Let us understand these methods by way of few examples.

1) *Mutual supplementation*

a) *Improvement of cereal proteins*: Cereals, in general, are limiting in lysine and threonine while legumes, milk, meat and fish are good sources of the amino acids. Hence, the proteins of legumes, milk, meat and fish supplement effectively cereal proteins.

b) *Soyabean and sesame proteins*: Soyabean proteins are good sources of lysine but are deficient in methionine while sesame proteins are good sources of methionine but are deficient in lysine. Hence, the proteins of sesame supplement effectively those of soyabean.

c) *Improvement of cereal diets with legume and milk proteins*: The proteins of poor diets based on different cereals are limiting in lysine and threonine and their quality can be improved effectively by incorporation of legumes, soyabean or milk proteins in the diet.

So it is clear that mutual supplementation is the answer to improve the nutritive value of proteins. Another way to do so would be to supplement with individual amino acids as discussed next.

2) *Supplementation with individual amino acids*

a) *Improvement of cereal diets by supplementation with lysine and threonine*: Cereal diets supplemented with lysine alone or a mixture of lysine and threonine markedly increases the PER of cereal proteins or proteins of poor cereal diets.

b) *Improvement of soybean and cow's milk protein with methionine*: The proteins of soybean and cow's milk are deficient in methionine. Supplementation with methionine increases the PER of the diet from 2.0 to 2.9 for soy bean and 3.0 to 4.0 for milk proteins.

c) *Improvement of sesame and sunflower seed proteins with lysine*: Supplementation with the limiting amino acid lysine increases the PER of sesame proteins from 1.7 to 2.9 and sunflower seed proteins from 1.2 to 1.8.

The discussion so far focused on methods to determine the protein quality and also on methods one could adopt to improve the quality of protein in a particular diet. Next let us move on to protein requirements – how they are estimated and assessed?

4.5 METHODS OF ESTIMATING AND ASSESSING PROTEIN REQUIREMENTS AT DIFFERENT STAGES OF LIFE CYCLE

In this section, we are going to deal with the methods that are used to estimate protein requirements, as well as, the factors which affect it. Let's read and find out first what we mean by protein requirement and its significance.

Human protein and amino acid requirements have been studied for well over 100 years using a variety of techniques. Nutrition scientists have collected data on the quantity of protein foods consumed in health, growth and weight gain of various populations. The assumption was made that whatever healthy people ate was probably what kept them, healthy and should, therefore, be used as a standard of comparison for other diets. These standards with respect to protein were invariably high for populations having an abundance of meat, milk, poultry and fish in their diets. *Voit* and *Atwater* around the turn of the 20th century, found intakes of 118 and 125 g protein/day, respectively for an adult woman and man.

As nutrition developed as a science, more accurate methods for assessing nutrient needs were developed. Among these methods were those for assessing the intakes and excretion of nitrogen compounds. The *Kjeldahl method*, about which we learnt above, and other methods for determining the nitrogenous end products of metabolism were devised. These methods made possible the development of the concepts which today's scientists use to determine the nutrient requirements of humans, as well as, other species.

In protein nutrition, it was realized that the body consists of two pools of protein: one which has a short half life and which must be constantly renewed and one which is slowly broken down and rebuilt. If one assumes that over a short period of time, the pool having the long half life contributes almost nothing to the nitrogenous metabolic end products and then a measure of the amount of nitrogen excreted will reflect only the turnover of the short lived proteins. These proteins have to be replaced by proteins

newly synthesized from the amino acids provided by the diet. Hence, the term protein requirement means that '*amount of protein which must be consumed to provide the amino acids for the synthesis of those body proteins irreversibly categorized in the course of the body's metabolism*'. The intake of nitrogen from protein must be sufficient to balance that excreted; this basic concept is called *nitrogen balance*. This concept is useful in understanding the minimal need for protein in the diet.

Protein requirement is greatly influenced by many factors such as age, environmental temperature, energy intake, gender, micronutrient intake, infection, activity, previous diet, trauma, pregnancy and lactation. Let us take up each of these factors in detail.

1) *Age*: Protein in excess of maintenance needs is required, when a new tissue is being formed. Certain age periods, when growth is rapid, require more dietary protein than other periods. Age differences in protein turnover, as well as, protein synthesis explain some of the effects of age on protein needs. Premature infants (those born before their 10 lunar month gestation time) growing at a very rapid rate require between 2.5 to 5 g protein/kg/day if they are to survive.

Studies of full term infants have indicated that a protein intake of 2.0 to 2.5 g/kg/day resulted in a satisfactory weight gain and that further increases in protein intake did not measurably improve growth. Older infants and children, whose growth rate is not as rapid as the premature or new born infant, require considerably less protein (1.25 g/kg/day). As growth rate increases during adolescence, the protein needs increase. Again, this can be related to the demands for dietary amino acids to support the growth process. As the human completes his growth, the need for protein decreases until it arrives at a level which is called the '*maintenance level*'. It is at this level that the concept of body protein replacement by dietary protein applies. During the growth period, it is very difficult to separate the requirements for maintenance from those of growth. The impulse for growth is so strong that it will occur in many instances at the expense of the maintenance of body tissues. For example, malnourished children will continue to grow taller even though their muscles, as well as, other tissues show evidence of wastage due to dietary protein deficiency.

Growth carries with it not only a total nitrogen requirement but also a particular amino acid requirement. Maintenance, on the other hand, appears to have only a total protein requirement. The adult can make a number of short-term adjustments in his protein metabolism that can compensate for possible inequities of imbalances in amino acid intake as long as the total protein requirement is met. The young growing animal is not that flexible. The essential amino acid requirements are age dependent. Although histidine can be synthesized in sufficient quantity by the adult to meet maintenance needs, yet it is not synthesized in great enough amounts to support growth or tissue repair. Thus, *histidine is an essential amino acid for the infant, growing child and injured adult.* This is due to the nature of the growth and repair processes.

Let us move on to the next factor and find out what environment influences have to do with our protein requirement.

2) *Environmental temperature*: As environmental temperatures rise or fall above or below the range of thermic neutrality, animals begin to increase their caloric expenditure to maintain their body temperature. In environments that are too warm, vasodilation (widening of blood vessels) occurs along with sweating and increased respiration. All of these mechanisms are designed to cool the body and all require an increase in the basal energy requirement expressed as per unit of body surface area. In cool environments, vasoconstriction and shivering occurs in an effort to warm the body and prevent undue heat loss. Again, an increase in basal energy requirement is observed. *Smuts* (1934) found that nitrogen requirements were related to basal energy requirements. Through the study of a large number of species, he concluded that 2 mg nitrogen were required for

every basal kilocalorie required when the energy requirement was expressed on a surface area basis. Thus, any increase in basal energy needs due to a change in environmental temperature will be because of the relationship between protein and energy. An increase in energy needs would be accompanied by an increase in the protein requirement for maintenance. In addition, profuse sweating as occurs in very warm environments carries with it a nitrogen loss which must be accounted for in the determination of minimal protein needs.

The next factor that require attention in case of subjects selected for estimating protein needs is information regarding the previous diet i.e., the kind of diet consumed by subjects in the past.

3) *Previous diet*: The effects of previous diet in the determination of protein requirements may be rather profound. If, for example, the subjects selected for studies on protein needs have been poorly nourished prior to the initiation of the study, their retention of the protein during the study will be greater than would be observed in subjects who have been well nourished prior to the initiation of the study. In other words, malnourished subjects have a higher protein requirement than well-nourished subjects. This of course raises the issue of whether there are body protein reserves. *Voit, Wilson, Cuthbertson, Fisher* (1890) and others observed that animals fed on a protein-free diet exhibit a lag before their nitrogen excretion level is minimized. During this phase, the animal is metabolizing his protein reserve. Other investigators maintain that every protein in the body has a function and if some of these proteins are lost, there is a loss in body function. Support for this concept is seen in the reduced ability of protein depleted animals to fight infection or respond to the metabolic effects of trauma.

Whether one believes that there is such an entity as a protein reserve may depend upon whether one perceives a difference between personal opinions on how nutrient requirements should be defined. Some nutritionists believe in stating the absolute minimum requirement to sustain life and then adding on increments for each body function above mere survival. This is known as the '*particulate approach*'. Other nutritionists believe that one cannot separate and quantify the individual requirements of each function beyond survival. They advocate a protein intake sufficient for optimal function of the animal. This is known as the '*integrative approach.*' The particulate and integrative approaches each have their merits when argued intellectually. However, since humans do not merely exist, many human nutritionists tend to take the integrative approach to human nutrition requirements in their determination of protein needs.

Next, we move on to another important factor that determines our protein needs, that is, physical activity.

4) *Physical activity*: Research on protein needs for muscular work had its beginning in 1863 when *Von Leiberg* postulated that muscle protein was destroyed with each contraction of the muscle. On this basis, he recommended that heavy muscular work required a heavy protein diet. This theory has been amply disproved, yet even today many believe that a protein rich diet will contribute to athletic prowess. Today, we know that muscle contraction does not result in destruction of the muscle. It however, requires energy in the form of ATP, glucose and fatty acids and does result in the breakdown of creatine phosphate to creatine which is then converted to creatinine, a nitrogenous waste product excreted in the urine.

As the energy requirement is increased to support the increase in muscular activity, so too is the protein requirement in much the same manner as described above for the effects of temperature. In a number of studies, the athletic performance of subjects could not be directly related to the quantity of protein consumed above that determined to be the requirements, their muscular deficiencies were reduced unless a vigorous training programme was included as a part of the experiment's protocol. Since most

of the studies were of short duration and since muscle protein has a relatively long half-life, the lack of any demonstrable effect of protein intake on muscle performance is not surprising.

Other factors such as sex, pregnancy, lactation and trauma affecting the protein requirements have been studied. As can be anticipated, males due to their greater physical activity and larger body size have a larger protein requirement than females. Pregnancy, lactation and trauma increase the protein requirements.

So, now you realize that a large number of factors play a role in determination of protein requirements. Let us next find out what are the recommended allowances for proteins for different age-groups.

4.6 NUTRITIONAL REQUIREMENTS AND RECOMMENDED ALLOWANCES FOR PROTEINS AND AMINO ACIDS

Human protein requirements are determined by methods such as:

a) N balance, and

b) Obligatory N losses adjusted for efficiency of protein utilization.

Amino acid requirement:

Indicator Amino Acid Oxidation (IAAO) method is a method of measuring amino acid requirement. It is based on the concept that if one amino acid in the diet is below requirement (i.e. limiting), then all other indispensable amino acids cannot be fully utilized for protein synthesis and the excess is therefore oxidized. With increasing intake of the limiting (test) amino acid IAAO will decrease, reflecting increasing incorporation into protein. Once the requirement for the limiting amino acid is met, there will be no further change in the indicator oxidation. It is a robust, rapid, reliable and invasive method to determine amino acid requirement in a neonates, children and adults. The estimated average requirment of each essential amino acid is present in table 4.5 earlier

Factorial method for arriving RDA for proteins of Indians

Basal or obligatory N loss

The factorial method of assessing protein requirement of an individual can be in terms of obligatory loss of N through faeces, urine and skin. The obligatory loss of urinary and faecal N has been assessed by maintaining an individual on a protein-free diet and estimating the faecal and urinary N excretion.

Total obligatory N loss for Indian adult subject: 48mg/N/Kg/d

In terms of protien, this obligatory N loss corresponds to: 48mg/ x 6.25 = 0.3g protien/ kg/d

However, the efficiency of utilization of protein being relatively low at about 47% the requirement of protein would then be the amount required to replace the obligatory loss divided by the efficiency of utilization. This would be 0.64 g protein/kg/day, which is similar to the median requirement of 0.66 g protein/kg/day. In addition, assuming a CV of 12.5%, the safe intake would be 0.83 g protein/kg/d.

Factorial estimates of protein requirements for 0-18y:

The average protein requirement (APR) for years 0-18 is calculated as the sum of maintenance requirement plus protein deposited.

APR = Maintenance + (Deposition / Efficiency of utilization)

Maintenance is calculated assuming that maintenance in young children is 0.58 g/kg/d which increase at 6 months to the adult value of 0.66 g/kg/d. In the case of infants below the age of 6 months, the efficiency of utilization of protein for growth was assumed to be 66%, while beyond that age, it was assumed to be 58%. The protein requirement for growth of infants and children are given in Table 4.7 respectively.

Table 4.7: Safe level of protein intake for infants aged less than 6 months

Age (m)	Maintenance requirement[a]	Growth requirement[b]	Average requirement[c]	Safe level[d]
1	0.58	0.83	1.41	1.77
2	0.58	0.65	1.23	1.5
3	0.58	0.55	1.13	1.36
4	0.58	0.49	1.07	1.24
6	0.58	0.4	0.98	1.14

a calculated from the maintenance requirement (from N balance studies with milk/egg)

b Protein deposition rates taken from Butte et al (6.8), adjusted for 66% efficiency of utilization (from N balance studies with milk/egg)

c Sum of maintenance and protein deposition rate (latter adjusted for efficiency of utilization)

d Mean + (1.96 x root mean square of SD values for protein deposition during growth, adjusted for efficiency of utilization, and the maintenance)

Table 4.8: Safe level of protein intake for children over the age of 6 months up to 10 years

Age (y)	Maintenance [a]	Growth [b]	Total	Safe level Indian diet[c]
	g protein / kg Body weight /d			
0.5	0.66	0.46	1.12	1.31
1	0.66	0.29	0.95	1.14
1.5	0.66	0.19	0.85	1.03
2	0.66	0.13	0.79	0.97
3	0.66	0.07	0.73	0.90
4	0.66	0.03	0.69	0.86
5	0.66	0.06	0.69	0.85
6	0.66	0.04	0.72	0.89
7	0.66	0.08	0.74	0.91
8	0.66	0.09	0.75	0.92
9	0.66	0.09	0.75	0.92
10	0.66	0.09	0.75	0.91

a From N balance studies

b From efficiency of utilization of 58% from N balance studies (see text)

c Corrected for protein from Indian cereal-pulse-milk based diet having PDCAAS of 77.4% for children up to 10 years,

Protein requirement during pregnancy and lactation: Protein requirement during pregnancy has been assessed by the factorial method as the additional requirement for foetal growth and expansion of maternal tissue. Protein requirement during pregnancy is given in Table 4.9. For a Gestational weight gain (GWG) of 10 kg, the protien deposition would be 2.5 g/d and 5.3 g/day for the second and third trimesters respectively (Table 4.9). For a GWG of 12 kg, if protein deposition was assumed to be proportional to GWG, a deposition rate of 3.0 g/d and 6.4 g/d is calculated for the

second and third trimesters, respectively. Protein deposition rates of 2.0 g/d and 4.3 g/d for the second and third trimesters respectively were calculated for a GWG of 8 kg. The safe levels for additional protein intakes were thus calculated to be 7.6 g/d and 17.6 g/d respectively.

Table 4.9: Recommendation for additional protein intake during Pregnancy for 12 kg, 10 kg, gestational weight gain

Tri mester	Mid-trimester weight gain[a] (kg)	Additional protein for maintenance g/d[b]	Protein deposition g/d	Dietary protein requirement for deposition[c] g/d	Mean extra protein requirement[d] g/d	Safe in take g/d[e]
GWG 12 Kg						
2	3.0	2.0	3.0	7.2	9.1	11.4
3	9.0	5.9	6.4	15.2	21.2	26.3
GWG 10 KG						
2	2.5	1.6	2.5	6.0	7.6	9.5
3	7.5	4.9	5.3	12.7	17.6	22.0
GWG 8 Kg						
2	-	-	2.0	-	-	7.6
3	-	-	4.3	-	-	17.6

a. Mid term increase in weight x estimated average requirement for maintenance for adult 0.66 g/kg/d
b. Protein deposited, adjusted for a 42% efficacy of utilization
c. Sum of extra maintenance plus protein deposited
d. Safe Intake = Mean extra protein requirement + (1.96 x SD) assuming CV of 12.5%. This requirement refers to high quality protein that meets criteria for DIASS.

During lactation, protein requirement has been computed on the basis of secretion of 6.4 g per day of protein in milk during 0-6 months and 5.0 g during 6-24 months. Protein requirement during lactation is given in Table 4.10

Table 4.10: Additional protein requirement during lactation

Months post-partum	Milk output g/day	Milk output corrected for IWL (g/d)[a]	Protein Conc. (g/L)[b]	NPN protein equivalent (g/L)[b]	True protein secreted (g/d)	NPN protein equivalent (g/d)	Mean dietary requirement[c]	Safe intake
1-6	688	722	8.9	2.5	6.4	1.8	13.6	16.9
6-12	583	612	8.1	2.1	5.0	1.53	10.6	13.2

a Milk output corrected for insensible water loss during test weighing measurement(5%)
b Nitrogen converted to protein by using the factor 6.25
c Efficiency of milk protein synthesis taken as 47%
d Mean + 1.96 SD assuming coefficient of variation is 12.5%

Based on N Balance

Nitrogen balance is the difference between nitrogen intake and the amount excreted in urine, feces, and sweat, together with minor losses occurring by other routes. To estimate the protein requirement, levels of dietary protein below and near predicted adequate intake are fed and nitrogen balance is measured at each level. The requirement is estimated by interpolating and extrapolating the nitrogen balance data to the zero balance point (nitrogen equilibrium) for adults or to defined level of positive balance(to allow for growth) for children.

Several studies on healthy, adult Indian subjects have shown that the minimum dietary protein intake for N equilibrium ranges from 0.5 to 0.66 g protein/kg/day with a mean of 0.58 g/kg/day which after allowing for integumental loss of 11 mg N/kg/day, works out to 0.65 g protein/kg/day. Assuming a CV of 12.5%, the safe value of intake, (mean + 1.96 x SD) works out to 0.81 g protein/kg/day. The corresponding requirement based on a mixed Indian diet will be 0.98 g protein/kg/d and this figure is also close to 1g/kg/d.

Note, for people consuming predominantly cereal based diets with low quality protein, the protein requirements are 1g/kg/d. For men the protein requirement would be 65g/d and for women 55g/d. The cereal-legume-milk composition of a diet for a moderately active man should be 3.1:2.5 to meet daily protein requirement.

After having a thorough knowledge of the factorial method of estimating N requirements, surely you would be now in a position to calculate protein requirements. The nutritional requirements of protein for Indians in different age groups and physiological stages as suggested by the ICMR is given in Table 4.11.

Table 4.11: Summary of recommended protein requirements for Indians

Groups		Body weight[a] (kg)	ICMR 2020				
			EAR[b] (g/kg/d)	RDA[b] (g/kg/d)	EAR[b] (g/d)	RDA[b] (g/d)	TUL[b] (PE ratio)
Adult Men*	Sedentary Moderate Heavy Work	65	0.66	0.83	42.9	54.0	<40% <40% <40%
Adult Women#	Sedentary Moderate Heavy Work	55	0.66	0.83	36.3	45.7	<40% <40% <40%
Pregnant Women[d]	2nd Trimester 3rd Trimester				+7.6 +17.6	+9.5 +22.0	<30% <30%
Lactating Women[e]	0-6 months 6-12 months				+13.6 +10.6	+16.9 +13.2	<40% <40%
Infants[f]	0-6 months 6-12 months	5.8 8.5	1.16 1.04	1.40 1.23	6.7 8.8	8.1 10.5	<15% <15%
Children[g]	1-3y 4-6y 7-9y	11.7 18.3 25.3	0.79 0.70 0.75	0.97 0.87 0.92	9.2 12.8 19.0	11.3 15.9 23.3	<15% <15% <15%
Boys[h]	10-12y	34.9	0.75	0.91	26.2	31.8	<15%
Girls[h]	10-12y	36.4	0.73	0.90	26.6	32.8	<15%
Boys[i]	13-15y	50.5	0.72	0.89	36.4	44.9	<15%
Girls[i]	13-15y	49.6	0.70	0.87	34.7	43.2	<15%
Boys[j]	16-18y	64.4	0.70	0.86	45.1	55.4	<15%
Girls[j]	16-18y	55.7	0.67	0.83	37.3	46.2	<15%

a Body weights are taken from WHO child growth standard (for 0-5 years), WHO growth reference (for 6-17 years) and NNMB 2016 urban data (for adults)

b EAR, Estimated Average Requirement; RDA, Recommended Dietary Allowance; TUL, Tolerable Upper Limit; PE Ratio, Protein: Energy Ratio

d Additional protein requirement for pregnant women with GWG of 10 Kg, at 2nd and 3rd trimester.

e Additional protein requirement for lactating women during 0-6 and 6-12 months

f Average protein requirements of 1-6 months and 0.5-1y were used to calculate EAR and RDA of 0-6 and 6-12 month infants respectively

g Average protein requirements of 1.5-3y, 4-6y and 7-9y were used to calculate EAR and RDA of 1-3y, 4-6y and 7-9y children respectively

h Average protein requirements of 10-12y were used to calculate EAR and RDA

i Average protein requirements of 13-15y were used to calculate EAR and RDA

j Average protein requirements of 16-17y were used to calculate EAR and RDA

* For people consuming cereal based diet with low quality protein, the protein requirements are 1 g/kg per day. For men it is 65g/day

\# For women it is 55g/day

Source: Nutrient requirements for Idnians: RDA & EAR. NIN, ICMR, Hyderabad, India, 2020

Protein requirements can also be expressed as ratio of protein energy to total dietary energy requirement, expressed as Protein energy ratio (PE ratio). PE ratio, therefore describes the protein energy interrelationship. Protein utilization and deposition are dependent on intake of adequate energy. Adequate non protein energy from carbohydrate and fat is essential for dietary amino acid to be utilized for protein synthesis and for amino acid related functions in the body. If adequate dietary energy is not available, dietary protein is inefficiently utilized. PE ratio will differ for different ages and also between individuals engaged in different activities (lifestyles). Refer to Table 4.12. It presents the PE ratio for different age groups. Safe recommended intake of protein is expressed as the ratio of recommended energy intake.

Table 4.12: Protein energy ratio (%) for different age groups

Group		Protein requirement g/kg/d[a]	Energy requirement kcal/kg/d	PE Ratio of requirement
Preschool children*	1-5y	0.95	79	4.8
School children*	6-10y	0.91	68	5.4
Adolescents	11-17y (Boys)	0.88	56	6.3
	11-17y (Girls)	0.86	51	6.7
Adults				
Men (sedentary)		0.83	32	10.4
Women (Sedentary)		0.83	30	11.1
Men (Moderate active)		0.83	39	8.5
Women (Moderate Active)		0.83	37	9.0

* Assuming moderately active children and adolescents; a Safe requirement of high-quality protein

Next, let us have a look at the consequences of protein deficiency in our diets.

4.7 PROTEIN DEFICIENCY

One of the most common nutritional disorders in the world today is the deficiency of protein. Both adults and children are affected, as the populations in the less developed nations of the world exceed their food supply. Due to the ubiquitous nature of protein and its role in bodily function, protein deficiency is characterized by a number of symptoms. In many situations, not only is protein lacking in the diet but also calories. For this reason, it is difficult to segregate symptoms due solely to protein deficiency from those of energy deficit. In children, one may observe the different symptoms and visualize them all as parts of a continuum called *protein-energy* or *protein-calorie malnutrition* (PEM or PCM) rather than distinctly different nutritional disorders. '*Kwashiorkor*' was the term used to describe a disease first observed in the Gold Coast of Africa by *Dr. Cicely Williams* in 1935 and at first was regarded as a dietary state where only protein was deficient, not energy. *Marasmus*, on the other hand, was regarded as a dietary state where both protein and energy are deficient. Now it has become apparent that the symptoms of any one of the twin diseases may intermingle with the other so that a clear-cut diagnosis is impossible. You look up the unit now as you go about reading regarding PEM here in this unit. Here a very brief overview of the disorder has been presented.

A. Kwashiorkor

The term Kwashiorkor means the 'disease the first child gets when the second baby is born', that is, 'the sickness of the deposed child'. Thus, the disease could be cured by milk.

Kwashiorkor usually affects the young child after he is weaned. The child is usually between 1 and 3 years old and is weaned because the mother has given birth to another child or is pregnant and cannot support both children. If the child has no teeth, he is given the gruel. This may be a fruit, vegetable or cereal product mixed with water and hence not a good protein source. Can you think of a few reasons which could limit or decrease the protein intake? Let us see what these are. Cultural food practices or taboos may further limit the kinds and amount of protein given to the child. Apart from this, concurrent infections, parasites, seasonal food shortages and poor distribution of food amongst the family members may also contribute to the development of kwashiorkor. The deficiency develops not only because of inadequate intake but also because at this age the growth demands for protein and energy are high.

The symptoms of kwashiorkor are as follows:

- *Growth failure*: This is manifested by decreased body length and low body weight in spite of retention of water in the body (oedema) and presence of subcutaneous fat in some children. This growth retardation is primarily due to the general quantitative lack of proteins.
- *Mental changes*: Several workers have stressed on the constant finding of mental changes described as apathy and peevishness. In advanced cases, children tend to live in an inert listless condition and show no interest in the surroundings.
- *Oedema*: Oedema occurs at first in the feet and lower legs and then may involve the hands, the thighs and face. The oedema is mainly due to lowered serum albumin and probably also due to high sodium and low potassium levels in serum. There is also some evidence that the normal diuretic-antidiuretic hormonal control of urine secretion gets disturbed.
- *Muscle wasting*: Muscle wasting is a constant feature of kwashiorkor and a reduction in the circumference of the upper arm is usually evident. It is less affected by oedema than in the forearm or leg.
- *Moon-face*: The full, well-rounded face, known as moon-face, is often present in kwashiorkor.
- *Liver changes*: Liver is slightly enlarged and fatty infiltration of liver is usually present.
- *Gastrointestinal tract*: Loss of appetite and vomiting are common. Diarrhoea is present in most cases.
- *Skin and hair changes*: The characteristic skin changes of kwashiorkor are known as the 'crazy pavement' dermatosis. This is most marked on the buttocks, back of thighs and axille. These lesions consist of dark hyperpigmental brownish black areas of skin.
- *Anaemia*: Anaemia is invariably present. It is due to the deficiency of iron and folic acid. Anaemia may be aggravated by parasitic infection which prevents the absorption of nutrients.
- *Vitamin Deficiency*: Signs and symptoms of vitamin A deficiency such as xerophthalmia and keratomalacia are widely prevalent. Angular stomatitis and glossitis due to deficiency of riboflavin may be present.
- *Biochemical changes*: Several biochemical changes have been reported in children suffering from kwashiorkor.
- *Serum Albumin*: The serum albumin content is usually low ranging from 0.7 to 2.2 g/100 ml. Serum albumin level is a good index of the severity of the disease and the rise in serum albumin level during treatment is a reliable index of the rate of recovery.

- *Enzymes in Serum and Digestive Juices*: Low levels of choline esterase, alkaline phosphatase, amylase and lipase have been reported in kwashiorkor. In experimental animals fed on protein deficient diet, reduction in lipase, amylase and protease activities of pancreas have been reported.

Let us learn about marasmus next.

B. **Marasmus**

Although children of all ages and adults can suffer from deficiencies of both energy and protein, the marasmic child is usually less than one year old. In developing countries, a common cause for marasmus is the cessation of breast-feeding. Milk production by the mother may have stopped because of her poor-health, death or deliberate decision of the mother to bottle-feed her baby. This decision might have a socio-economic connotation. Can you think of it? Well, it is just that the mother may view bottle-feeding as a status symbol or she may be forced to work to earn a living and may be unable to have her baby with her, or she may not be able to lactate. While under optimal conditions of economics and sanitation, it seems that the bottle-fed child may be well fed in emerging nations but this is not always true. The mother may not be able to buy the milk formula in sufficient quantities to adequately nourish the child, she may over dilute the milk or she may use unsafe water and unsanitary conditions to prepare the formula for the child. This plus the insufficient nutrient content often precipitously leads to the development of marasmus, a form of starvation characterized by growth failure with prominent ribs, a characteristic monkey like face and 'match stick limbs' with little muscle or adipose tissue development. Tissue wastage but no oedema is present. Whereas the kwashiorkor child has a poor appetite, the marasmus child is eager to eat. The child is mentally alert but not irritable. Anaemia and diarrhoea are present for the same reasons as in kwashiorkor. The skin and hair appear to be of normal colour.

Protein hormones which regulate and coordinate the use of dietary nutrients are not found in adequate amounts. Cell hormone receptor sites are affected which further dampens the effectiveness of the hormones produced. Marasmic and kwashiorkor children have decreased blood sugar levels, decreased serum insulin and growth hormone levels and in marasmus, decreased thyroid hormone levels. The clinical features of marasmus are as follows:

- *Nutritional Marasmus*: Nutritional marasmus is principally due to the consumption of diets markedly deficient in both proteins and calories. It is seen most commonly in the weaned infants of about l year of age in contrast to kwashiorkor, which occurs more often among children of the age group 2-4 years. Nutritional marasmus usually is precipitated by diarrhoeal diseases.

***Clinical Features*:** The two constant features of nutritional marasmus are growth retardation and severe wasting of muscle and subcutaneous fat.

a) *Growth retardation*: This is usually very severe. Loss of weight is much more marked than decrease in height. The child is usually below 60 percent of the standard weight.

b) *Wasting of muscle and subcutaneous fat*: The subject is severely emaciated. The muscles are wasted. The arms are thin and the skin is loose. Subcutaneous fat is practically absent.

c) *Other changes*: The skin is dry and atrophic. The subject shows signs of dehydration. Eye lesions due to vitamin A deficiency and anaemia may be present.

d) *Biochemical changes*: There is a slight lowering of serum albumin. Vitamin A content of serum is low. The important difference in the clinical and biochemical features between marasmus and kwashiorkor are given in Table 4.13.

Table 4.13: Clinical and biochemical features of marasamus and kwashiorkor

Features	Marasmus	Kwashiorkor
Age of maximum incidence	6-18 months	12-48 months
Loss of body weight	+ + +	+ to + +
Emaciation (Loss of muscle and subcutaneous fat)	+ + + +	+ to + +
Oedema	Absent	+ to + +
Fatty infiltration of liver	0 to +	+ + +
Skin changes	+	+ + +
Serum albumin	Slightly less	Markedly less
Serum enzymes	Slightly lowered	Markedly lowered
Serum lipids:		
Triglycerides	Normal	Normal
Cholesterol	Normal	Lowered
Non-esterified fatty acids	Elevated	Elevated
Blood sugar	Normal	Slightly lowered
Response to adrenaline	Exaggerated	Lowered
Blood urea	Normal	Lowered
Increase in body weight after high protein and high calorie therapy during the first 4 weeks	Slow	Satisfactory

What is the treatment of Kwashiorkor and Marasmus?

The treatment of both kwashiorkor and marasmic children requires care and caution. As their enzymes for digestion and their protein absorption and transport systems are less active, feeding these children with large quantities of good quality protein would be harmful. Well, this might sound strange to you. But it is so. Their diets must gradually be enriched with these proteins to allow their body sufficient time to develop the metabolic pathways to handle a better diet. Giving these children solutions of either predigested proteins or solutions of amino acids may be of benefit initially, but these solutions too must be used with care. If the amino acids in excess of immediate use are deaminated and if the pathway for synthesizing urea is not fully functional, ammonia can accumulate in the child and become lethal.

We have studied about kwashiorkor and marasmus as conditions typical of protein energy malnutrition. But, interestingly, a large number of cases show signs and symptoms of both marasmus and kwashiorkor. These are the intermediate forms. Let us learn about them next.

Marasmic Kwashiorkor

In countries where the incidence of protein-calorie malnutrition (PCM) is high, a large number of cases show signs and symptoms of marasmus and kwashiorkor. These intermediate forms are called 'Marasmic-kwashiorkor'. In addition, the inter-relationship between the two major syndromes is such that the changing circumstances may result in a transition from one clinical picture to another. A child with early kwashiorkor can develop nutritional marasmus by severe infective diarrhoea and ill-advised prolonged under-feeding. Conversely, an infant with nutritional marasmus may develop kwashiorkor if fed on protein deficient carbohydrate rich foods along with adequate common salt.

With this we end our discussion on the consequences of protein deficiency, as well as, other varied aspects of protein which may influence human health. In the next unit

we shall discuss about lipids in detail. However, you must attempt the check your exercise below before reading further.

Check Your Progress Exercise 3

1) Choose the correct answers:

   i) The NPR method of evaluating protein quality is based on:

      a) Growth and body weight changes

      b) Carcass nitrogen analysis

      c) Nitrogen balance

      d) Pattern of liver regeneration

   ii) The Chemical score of gelatin is:

      a) 100 b) 99.5

      c) 50 d) 0

   iii) The most availability feature of protein malnutrition is:

      a) Weight loss b) Growth failure

      c) Oedema d) Night blindness

   iv) The response to protein calorie therapy is shown in:

      a) Marasmus b) Kwashiorkor

      c) PEM d) VAD

   v) Moon face is a symptom of:

      a) Beri beri b) Kwashiorkor

      c) Marasamus d) Scurvy

2) Define the following terms, giving their calculation formula.

   a) Digestibility coefficient

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   .....

   .....

   b) NPU

   .....

   .....

   .....

   c) Amino Acid Score

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   .....

   .....

3) How can you improve the nutritive value of a protein?

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4) List a few factors which affect protein requirements.

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5) Give clinical and biochemical features of Kwashiorkor and Marasmus.

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4.8 LET US SUM UP

This unit covered the important macronutrient proteins. Proteins, you learnt, are vital to all body cells, tissues, organs and functions of organ systems. The process of digestion, absorption and metabolism of protein are complex and involve several nutritional and non-nutritional factors. Proteins are widely distributed in nature, the richest vegetarian source being soy beans. Egg protein has the highest biologic value. Several methods can be employed to determine the protein content of foods, the most popular being those based on growth and body weight changes. Protein quality in the diet may be improved by mutual supplementation of protein rich foods and cereals. Nutritional requirements of proteins vary with age and physiological activity/stage. Deficiency of proteins leads to the twin disorders of marasmus and kwashiorkor and may be cured by dietary intervention.

4.9 GLOSSARY

Albinism : disease caused in persons unable to form melanin pigment.

Amino acids : the building blocks of proteins composed of carbon, hydrogen, oxygen and nitrogen.

Apoenzyme : protein component of an enzyme.

Autocatalysis : a self-repeating and ongoing process of catalysis.

Amino Acid Score : the ratio between the content of the most limiting amino acid in the test protein to the content of the same amino acid in reference protein expressed as a percentage.

Conjugated proteins : proteins which contain some non-protein substances.

Derived proteins : derivatives of the protein molecules formed through hydrolytic changes in the molecule.

Digestibility coefficient : the percentage of the ingested protein absorbed into the blood stream after the process of digestion is complete.

Oedema : accumulation of water in the interstitial space.

Essential amino acids : amino acids that are indispensable and are not synthesized in the body.

Kwashiorkor : disease the first child gets when second baby is born.

Limiting amino acids : the amino acids present in the least proportion in a food.

Marasmic kwashiorkor : the intermediate form of disease where signs and symptoms of marasmus and kwashiorkor are seen.

Mutual supplementation : blending two or more proteins so that the excess of essential amino acids present in one protein makes up the deficiencies of the same amino acids in another protein.

Non-essential amino acids : amino acids that are dispensable and can be synthesized in the body.

Nucleoproteins : combination of nucleic acids and simple proteins consisting of a large number of basic amino acids.

Protein requirement : amount of protein which must be consumed to provide the amino acids for the synthesis of those body proteins irreversibly catabolized in the course of the body's metabolism.

Proteoglycans : glycoproteins with more than 80% of molecules as carbohydrates.

Regulators : factors which maintain the body's internal equilibrium or balance.

Simple proteins : proteins which contain only amino acids or their derivatives and no prosthetic group.

Supplements : foods used to enhance the nutrients present in a person's diet or menu.

4.10 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1) a) 6.25, b) metallo, c) proteoglycans, d) egg, e) incomplete

2) 1. d; 5. g;
   2. e; 6. a;
   3. f; 7. c;
   4. b;

Check Your Progress Exercise 2

1) The enzymes involved in protein digestion are enumerated in sub-sub section 4.2.3.1. Read the section and write the answer on your own.

2) The HCl serves several functions in gastric digestion. It acidifies the ingested food, killing potential pathogenic organisms. However, not all pathogens are killed. Some are acid resistant or are so plentiful in the food that the amount of gastric acidification is insufficient to kill all of the pathogens. HCl also serves to

denature the food proteins, thus making them more vulnerable to attack by the proteolytic enzyme present in gastric juice namely, pepsin and endopeptidase (it hydrolyzes peptide bonds in the interior of the protein molecule).

3) Proteins act as enzymes, carriers for various compounds, act as regulators of water balance, act as buffers, act as structural elements and structural units, function as lubricants and are vital to the immune system.

4) Albumin plays an important role in maintaing the osmotic pressure.

5) Examples of proteins as carrier include α albumin, α and β globulin and haemoglobin.

Check Your Progress Exercise 3

1) i) a

ii) d

iii) b

iv) a

v) b

2) a) The percentage of ingested protein absorbed into the blood stream after the process of digestion is complete. It is calculated as:

$$\frac{100 \times I_n - (F_n - F_e)}{I_n}$$

where, I_n = N intake

F_n = N in faeces

F_e = Endogenous faecal N

b) A product of digestibility coefficient and biological value divided by 100. Biological value can be expressed as:

$$BV = \frac{I_n - (F_n - F_e) - (U_n - U_v) \times 100}{I_n - (F_n - F_e)}$$

c) The ratio between the content of the most limiting amino acid in the test protein to the content of the same amino acid in egg protein expressed as a percentage.

3) by mutual supplementation, that is, blending two or more proteins so that the excess of essential amino acids present in one protein makes up the deficiencies of the same amino acids in another protein, and by supplementation of the dietary proteins with limiting essential amino acids.

4) Factors which affect protein requirements are age, environmental temperature, energy intake, gender, micronutrient intake, infection, physical activity, previous diet, trauma pregnancy and lactation.

5) Clinical and biochemical features of marasamus and kwashiorkor are given in Table 4.10. Read the features and answer the question on your own.