UNIT 2

Integration of the Functional Aspects of Vitamins and Minerals

TABLE OF CONTENTS

I. OVERVIEW

At the turn of the century, scientists seeking to understand the role of diet in health maintenance began to use rats in their research on nutrient needs. When these animals were fed diets consisting of purified proteins, fats, and carbohydrates, they died. It was soon found that specific minerals and additional factors, termed accessory food factors by Hopkins, were present in an unrefined diet and were necessary to sustain life. The minerals and these "accessory factors" were needed in very small amounts. Because it was thought that the "accessory factors" all contained nitrogen, they were called amines. Casimir Funk, an early nutrition scientist, coined the term "vitamines" to indicate that these amines were vital to the survival of the animal. Later, after it was discovered that not all vitamins contained amines, the final "e" was dropped from the word.

Vitamins are a large group of potent organic compounds necessary in minute amounts in the diet. They are usually divided into two classes based on their solubility characteristics. The water-soluble vitamins are soluble in water and usually function as coenzymes in the metabolism of protein, fats, and carbohydrates. The fat-soluble vitamins are not usually soluble in water but are soluble in one or more solvents such as alcohol, ether, or chloroform.

Each of the vitamins has a specific chemical structure and many can be synthesized rather inexpensively. Thus, multivitamin supplements can be purchased in drugstores for a modest price. While specific vitamins can cure specific deficiency diseases, as indicated in Unit 1 and detailed in the sections on each of the vitamins, the use of supplements by people consuming a wide variety of raw and cooked foods may be unnecessary.

Before the vitamins were chemically isolated and described, scientists began naming the compounds. In some instances, different research groups were studying the same compound and

unwittingly gave different names to the same vitamin. This contributed confusion to the identity of vitamins. Frequently, the name chosen described the food source or the deficiency symptom. Thus, for years thiamin was known as the antiberiberi factor, vitamin K was known as the coagulation factor, and vitamin E as the wheat germ factor or the antisterility factor. As nutrition scientists began publishing their findings, it became important to establish a uniform nomenclature and one based on the alphabet was devised. Compounds having vitamin activity were alphabetized in order of their discovery. Now, however, information about the vitamins has expanded to such an extent that this nomenclature system is outmoded. Chemically descriptive terms are now being used that more correctly identify the vitamin in question. Nonetheless, alphabetical designations are still being used and the reader will encounter some of these in this text.

As scientists learned more about the vitamins they began to reclassify them according to function rather than solubility. Thus, we have vitamins that serve as membrane stabilizers, as coenzymes, or that have antioxidant properties and/or that act at the genomic level. Some vitamins fall into more than one category. For example, ascorbic acid serves as a general antioxidant, as a redox agent (as a substrate being oxidized to dehydroascorbic acid), and yet also acts at the levels of transcription and translation for the protein, procollagen. Vitamin A is another one that is multifunctional. It has a direct role in the visual cycle, is an antioxidant, stimulates the RNA transcription for the retinoic acid receptor, and when bound to this receptor serves as a transcription factor for the transcription of numerous mRNAs. As the reader progresses through the units and sections devoted to the individual vitamins, this multifunctionality will be described.

Similarly, as the roles for each of the minerals were elucidated, the minerals likewise were subdivided into two groups based not on solubility characteristics but on the magnitude of need. Thus, we have the macrominerals and the microminerals. The human need for the former is much greater per day than the need for the latter. Just as some vitamins can serve as coenzymes in intermediary metabolism, minerals serve as cofactors in many of these same reactions. Vitamins and minerals both have active roles in the formation and maintenance of the body's structure as well as its function. Minerals and vitamins are essential to the regulation of metabolism and, as well, are important components for the expression of many specific genes.

II. THE ROLE OF MICRONUTRIENTS IN GENE EXPRESSION

Among the many functions that vitamins and minerals serve in the body, one stands out in its primacy. That is, the service in gene expression. Almost every micronutrient is involved either directly as part of a cis- or trans-acting factor in RNA transcription, or as an important coenzyme in the synthesis of the purine and pyrimidine bases, or as a coenzyme in intermediary metabolism which provides substrates and energy for the support of cell replication, cell growth, DNA replication, RNA transcription, RNA translation, and protein synthesis. Figure 1 illustrates the process of gene expression and Table 1 itemizes specific effects of vitamins and minerals on this process. Some of these effects are direct, some are indirect. Many of the symptoms of vitamin deficiencies can be traced to this involvement in gene expression. Gene products and cell types with very short half-lives will be among the first to be affected by the absence of a given micronutrient. Hence, skin lesions are a frequent feature of the deficient state because epithelial cells have an average half-life of 7 days. Red blood cells have an average half-life of 60 days and many nutrient deficiencies are characterized by anemia. Similarly, vitamin- and mineral-dependent gene products (enzymes, receptors, transporters) also will be affected should that particular nutrient be in short supply. Conversely, we have instances of diversity within a population such that one individual's nutrient intake is fully adequate while another individual in the same population, consuming that same amount of that same nutrient, is in the deficient state. This contrast is due to individual genetic

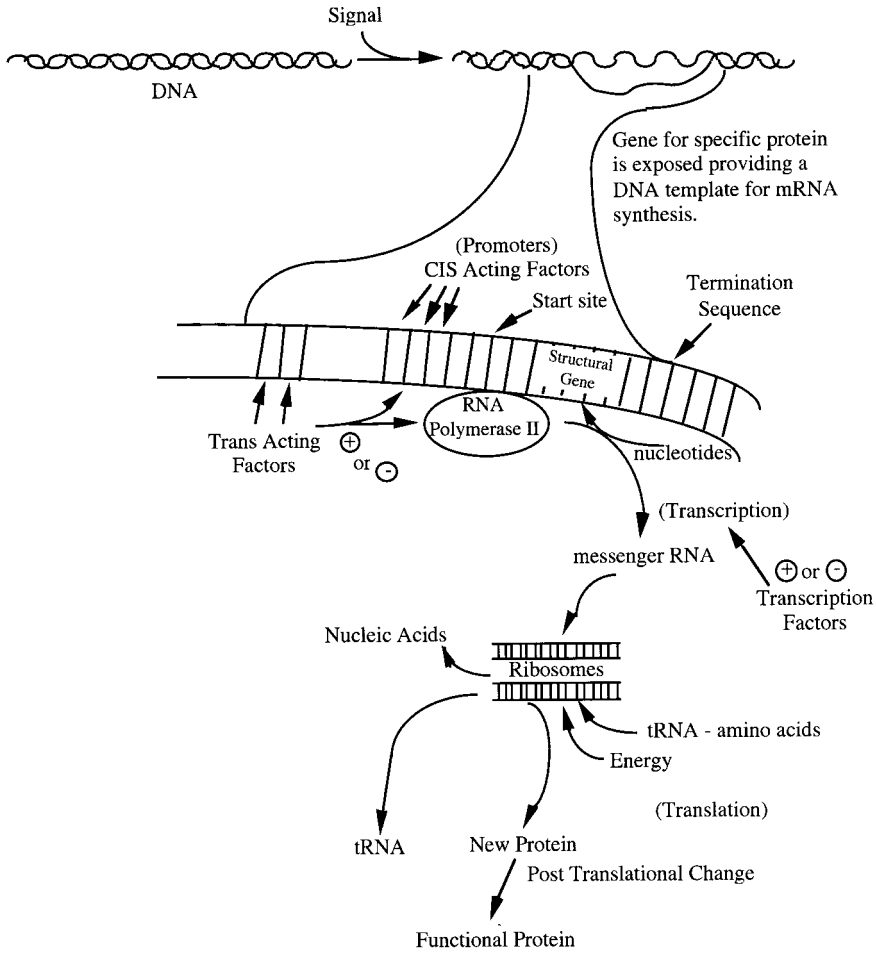

Figure 1 Overview of gene expression.

variability and can be found in every species and strain of living creatures. The explanation for this variability, not only in nutrient needs and tolerances but also in such characteristics as skin color, height, weight, or any of the myriad characteristics that distinguish one species from another and one individual from another, is in the genetic material, DNA.

The mammalian genome contains 4×10^9 base pairs (bp) and exists as a double-stranded helix with the purine and pyrimidine bases arranged in a preordained sequence and held together by phosphate and ribose groups. There is far more DNA in each cell than is used. In contrast to the DNA found in single-cell organisms (prokaryotes), eukaryotic genes contain interrupting sequences that are noncoding. That is, at intervals along a structural gene there are series of bases that do not participate in the expression of that gene. These are called introns. Exons are those base sequences that provide the coding of the genes. The introns do base pair when mRNA is transcribed, but the parts of the message transcribed by these introns are removed by splicing during nuclear RNA editing prior to export. Each mammalian cell has a complete genome in its nucleus but not all of this is transcribed. This central molecule of life consists of many discrete sequences which encode or dictate the amino acid sequence of every protein in the body, which in turn dictates the functional

Table 1 Specific Nutrient Effects on Gene Expression

Nutrient	Gene	Effect
Retinoic acid	Retinoic acid receptor and other proteins	↑ Transcription
Vitamin B_6	Steroid hormone receptor	↓ Transcription
Ascorbic acid	Procollagen	↑ Transcription ↑ Translation
Vitamin K	Prothrombin	↑ Post-translational carboxylation of glutamic acid residues
Potassium	Aldosterone synthetase	↑ Transcription
Zinc	Zinc fingers	Allows binding of cis or trans factors to specific DNA binding sites
Iron	Ferritin	When bound to ferritin mRNA allows translation to proceed
Folacin	DNA, RNA	Purine and pyrimidine synthesis
B_{12}	DNA, RNA	Purine and pyrimidine synthesis
Thiamin	All genes	As part of TPP it plays a role in bioenergetics
Riboflavin	All genes	As part of FAD it plays a role in producing ATP
Niacin	All genes	As part of NAD it plays a role in producing ATP
B_6	All genes	Purine and pyrimidine synthesis
Vitamin D	Calcium binding proteins	↑ Transcription
Vitamin E	All genes	Protects against free radical damage to DNA

attributes of each organelle, cell type, tissue, and organ. These proteins serve as structural elements, enzymes, transporters, receptors, messengers, and central integrators of the use of all the other nutrients needed by living creatures.

Gene expression is a highly controlled process. Its regulation includes transcription control, RNA processing control, RNA transport control, translational control, mRNA stability control, and post-translation control. Each of these control points have nutritionally mediated aspects. In most of the genes studied to date, more nutrients affect transcription than translation or post-translation processing. There are some exceptions, as shown in Table 1 and described later in this text. Transcription control is exerted by that portion of the DNA called the promoter region plus transcription factors that bind either to this region or to an upstream region that in turn affects the activity of either cis-acting factors or the polymerase activity. RNA polymerase II binds to the promoter region just upstream of the start codon for the gene. The promoter region is located in the 5′ flanking region upstream from the structural gene on the same strand of DNA. Cis-responsive elements are located about –40 to –200 bp from the start site. Some promoters, i.e., TATA, GC, and CCAAT boxes, are common to many genes transcribed by RNA polymerase II. These sequences interact with transcription factors that in turn form preinitiation complexes. The mechanisms of such transcriptional regulation have recently been reviewed by Semenza and by Johnson et al. Trans-acting factors are usually proteins produced by other genes which influence transcription. Trans-acting factors can be proteins or peptide hormones or steroid hormone-receptor protein complexes, or vitamin-receptor protein complexes, or minerals, or mineral-protein complexes. The mechanism for binding hormone receptors to specific regions of DNA has been reviewed and described by Freedman and Luisi (see Supplemental Readings list).

The promoter region contains the start site for RNA synthesis. RNA polymerase II binds to this specific DNA sequence and, under the influence of the various transcription factors, RNA transcription is initiated. The RNA polymerase II opens up a local region of the DNA double helix so that the gene to be transcribed is exposed. One of the two DNA strands acts as the template for complementary base pairing with incoming ribonucleotide triphosphate molecules. The nucleotides are joined until the polymerase encounters a special sequence in the DNA called the termination sequence. At this point, transcription is complete. Following this process the newly formed RNA is edited and processed. This processing removes nearly 95% of the bases. The resultant shortened

RNA then migrates out of the nucleus and becomes associated with ribosomes whereupon translation takes place.

This outline of transcription has omitted a number of important details with respect to transcription control. For example, the regulation of transcription is exerted by a group of proteins that determine which region of the DNA is to be transcribed. Cells contain a variety of sequence-specific DNA binding proteins. Nutrients can bind to these proteins and have their effect in this way. These proteins are of low abundance and they function by binding to specific regions on the DNA. The regions are variable in size but are usually between 8 and 15 nucleotides. Depending on the binding protein and the nutrient bound to it, transcription is either enhanced or inhibited and indeed cell types may differ because of these proteins. Since all cells contain the same DNA, gene expression in discrete cell types is controlled at this point simply by the binding of these very specific DNA binding proteins. Thus, genes for the synthesis of insulin, for example, could be turned on in the pancreatic β cell, but not in the myocyte, simply because the β cell has the needed specific DNA binding proteins that the myocyte lacks. At some point in differentiation, the myocyte failed to acquire sufficient amounts of these regulatory factors and thus can not synthesize and release insulin.

In many instances, specific DNA binding proteins contain zinc and as such are referred to as zinc fingers. Gene expression is regulated by the formation of these zinc fingers, yet they comprise only a part of this regulation. Most genes are regulated by a combination of regulatory factors. In some, a group of DNA binding proteins interact to control the activation or inhibition of transcription. Not all of these proteins are of equal power in all instances. There may be a "master" regulatory protein that serves to coordinate the binding of several "lesser" proteins. This is important for the coordinate expression of genes in a single pathway, as happens, for example, in the expression of the genes that encode the multienzyme complex, fatty acid synthetase.

Mutations in genes that encode any one of these transcription factors could result in disease. Mutations in genes encoding transcription factors often have pleiotropic effects because these factors regulate a number of different genes. So, too, are the effects of nutrients which are required components of these transcription factors. An example is the series of genes which encode the enzymes needed for the conversion of a fibroblast to a myocyte. The mammalian skeletal muscle cell is very large and multinucleated. It is formed by the fusion of myoblasts (myocyte precursor cells) and contains characteristic structural proteins as well as a number of other proteins that function in energy metabolism and nerve-muscle signaling. When muscle is being synthesized all of these proteins must be synthesized at the same time. In proliferating myoblasts very few of these proteins are present, yet, as these myoblasts fuse, the messenger RNAs for these proteins increase as does the synthesis of the proteins. This indicates that the expression of the genes for muscle protein synthesis is responding to a single regulatory DNA binding protein. This protein (Myo D1) has been isolated and identified and occurs only in muscle cells. Should this protein be inserted in some other cell type such as a skin cell or an adipocyte, for example, the same expression will occur. That is, the skin or fat cell will look like a muscle cell. It will take on the characteristics of a myoblast and become a myocyte.

Of interest is the fact that although all of the genes needed for synthesis in the myocyte and its master controller are present, synthesis will not occur or will occur at a very limited rate if one or more of the essential amino acids needed for this synthesis are absent or deficient in the diet. Here is an example of a gene-nutrient interaction that has control properties with respect to muscle protein synthesis and this interaction ultimately affects the overall process of growth. Turning this situation around, if the master regulator Myo D1 is aberrant, or if one or more of the genes which encode the enzymes needed for protein synthesis in the myocyte have mutated such that the enzyme in question is nonfunctional or only partly functional, muscle development will cease or be retarded. In either instance, abnormal growth will result.

As mentioned, transcription is regulated by both the nearby upstream promoter region and the distant enhancer elements. The upstream enhancer element can include a TATA box and extends

for about 100 bp. Enhancer fragments further upstream can bind multiple proteins which, in turn, can influence transcription. These factors are proteins and are labeled JUN, AP2, ATF, CREB, SP1, OTF1, CTF, NF1, SRE, and others.

One well-studied group of DNA binding proteins are those which bind steroid hormones. These are called the steroid receptors and bind to specific base sequences called steroid response elements (SREs). Steroids that enhance (or inhibit) transcription act by binding to one of these specific proteins which, in turn, binds to DNA. These complexes thus explain how cells respond to a steroid hormone stimulus. The proteins consist of about 100 amino acids and zinc. As mentioned, they recognize a specific DNA sequence. For some members of this family of proteins, the transcription-enhancing domain is localized at the amino terminus of the polypeptide chain. At the carboxy terminus is the binding site for the steroid hormone. Steroid hormones, via binding to their cognate receptors and to the hormone response element on the DNA, also enhance the transcription of mitochondrial genes. The recognition of this function of steroid hormones provides a further explanation of how these hormones function in energy balance. Enhanced mitochondrial gene expression should result in an increased mitochondrial function, i.e., enhanced activity of oxidative phosphorylation. In turn, this would result in increased ATP production which is needed for cell function and tissue growth. Although this action of specific steroid hormones has been shown to occur in mitochondria, we do not know whether vitamins A and D act in this way.

Post-transcriptional regulation of gene expression is the next stage of control. As mentioned above, newly formed mRNA is edited prior to leaving the nucleus. RNA transcription can be terminated prematurely with the result of a smaller than expected gene product. A single mRNA can be translated into several different gene products, usually peptides. These proteins or peptides may have comparable or opposing functions depending on the products in question. As described, messenger RNA is edited and processed such that only 5% of this RNA leaves the nucleus. The 95% which remains is degraded and the purine and pyrimidine bases are reused or are subject to further degradation. The RNA that leaves the nucleus does so through pores in the nuclear membrane. This is an active process, the details of which are not well understood.

Not all of the mRNA that exits the nucleus is immediately translated into protein. Translation can be blocked by specific proteins that bind at sites near the 5′ end of the molecule. This binding exerts negative translational control on gene expression. The mRNA has been made but the protein is not made. An example of this is seen in the regulation of the synthesis of ferritin by iron. Ferritin mRNA is not translated unless iron is bound to a response element that is part of the message. This allows for a rapid shift in ferritin synthesis when iron is present and an equally rapid shift away from ferritin synthesis when iron is in short supply. When iron is present, the iron response element folds away from the start site for translation making it available for use. When iron is absent, this start site is covered up by the iron response element which serves as a negative control element. Several mRNAs are subject to translational control by nutrients in this fashion.

The mRNAs have a very short half-life when compared to DNA and the other RNAs. If mRNA half-life is shortened or prolonged, gene expression is affected. Many of the very unstable mRNAs have half-lives in terms of minutes — among these are those which code for short-lived regulatory proteins such as the protooncogenes, fos and myc. This instability is probably due to an A- and U-rich 3′ untranslated region. Stability of mRNA can be affected by steroid hormones, nutritional state, and drugs.

Once the mRNA has migrated from the nucleus to the cytoplasm and attaches to ribosomes, translation is ready to begin. All of the amino acids needed for the protein being synthesized must be present and attached to a transfer RNA (tRNA). These tRNA-amino acids dock on the mRNA again, using base pairing, and the amino acids are joined to one another via the peptide bond. The newly synthesized protein is released as it is made on the ribosome and changes to its conformation and structure occur. These changes depend on the constituent amino acids and their sequence.

Post-translational modification includes a wide variety of changes. For example, nuclear-encoded proteins needed for the mitochondrial metabolism are synthesized with a leader sequence that allows them to migrate into the mitochondria. This leader is then removed as the oxidative phosphorylation system is assembled. Another example is prothrombin, which is assembled with a large number of glutamic acid residues. In the presence of vitamin K these residues are carboxylated, and this post-translational change results in a dramatic increase in the calcium binding capacity of the resultant protein. Unless prothrombin can bind calcium, it cannot function in the clotting process. This is another example of how a nutrient can affect gene expression: in this instance the expression of functional prothrombin. The site of the nutritional effect is that of post-translational protein modification.

III. SYNTHESIS OF PURINES AND PYRIMIDINES

The purines and pyrimidines are the bases that comprise DNA and RNA. They are synthesized *de novo* and this synthesis requires, both directly and indirectly, a number of vitamins and minerals. The purines are adenine and guanine while the pyrimidines are cytosine, uracil, and thymine. Uracil is used for RNA synthesis whereas thymine is used mainly for DNA synthesis. The purines form glycosidic bonds to ribose via the N(9) atoms, whereas the pyrimidines do this using their N(1) atoms. The inosine monophosphate synthesis (IMP) pathway, shown in Figure 2, is the pathway for adenine and guanine triphosphate synthesis. Also shown in Figure 2 are the minerals and vitamins needed at each step in the pathway. Lipoic acid is a cofactor but not a vitamin for the normal individual. Similarly, choline and inositol are not usually considered as vitamins yet these two compounds are also involved in intermediary metabolism. Where ATP is involved in a reaction step, all of the vitamins which serve as coenzymes in intermediary metabolism are needed. This includes niacin, thiamin, riboflavin, pantothenic acid, biotin, folacin, vitamin B_{12}, and vitamin B_6. Also needed are the minerals of importance to the redox reactions of oxidative phosphorylation (OXPHOS), i.e., iron, copper and, of course, the iodine containing hormone, thyroxine, which regulates OXPHOS, and the selenium-containing enzyme (5′-deiodinase) that converts thyroxine to its active form, triiodothyronine. Figure 3 illustrates the involvement of the vitamins and minerals in intermediary metabolism. The pyrimidine pathway (Figure 4) is simpler than the purine synthesis pathway. However, one can see where micronutrients are involved here as well. Transamination and one-carbon transfer — reactions requiring pyridoxine and folacin and of course all those minerals and vitamins needed as coenzymes for intermediary metabolism — are once again called into play so that sufficient energy is available to support the synthetic pathway. The involvement of the vitamins in the provision of energy and substrates for not only DNA and RNA synthesis but also for the synthesis of other macromolecules important to life is outlined in Figure 3.

IV. MICRONUTRIENTS AS STABILIZERS

Although vitamins and minerals serve in gene expression as just described, and as coenzymes and cofactors in the many reactions of intermediary metabolism, certain of the micronutrients have a unique role as stabilizers. They function in assuring that cells and tissues continue as intact structures and that these cells continue to reproduce themselves faithfully. This role for the micronutrients is that of protection from insult by free radicals or peroxides. Peroxides are a normal product of metabolism. They are useful agents in the defense against pathogens. However, peroxides are very reactive substances. They can damage the membranes that are the physical barriers to the cells and the organelles within the cell. They can react with DNA. The DNA, enclosed within the

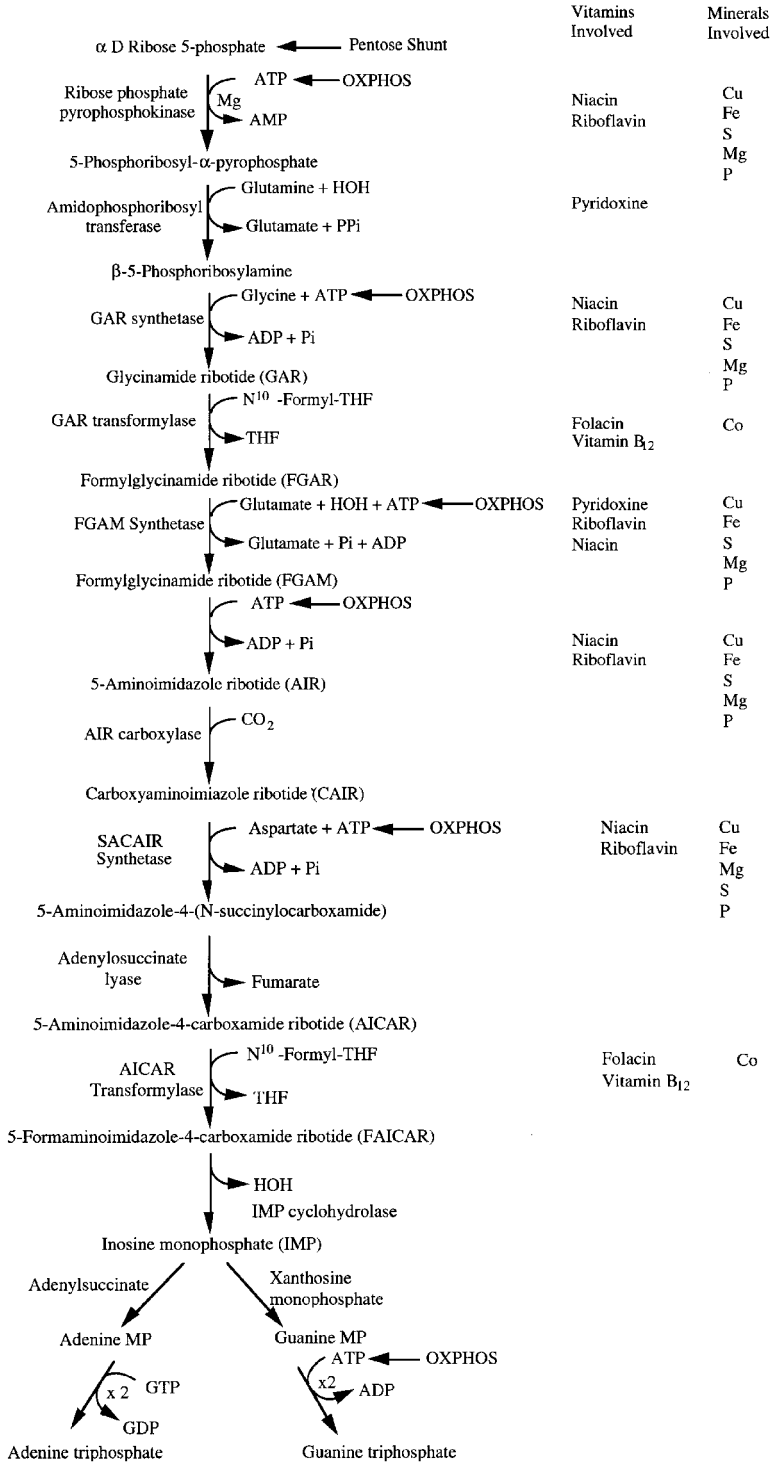

Figure 2 Purine synthesis. In this pathway the addition of ribose occurs prior to ring closure and phosphorylation.

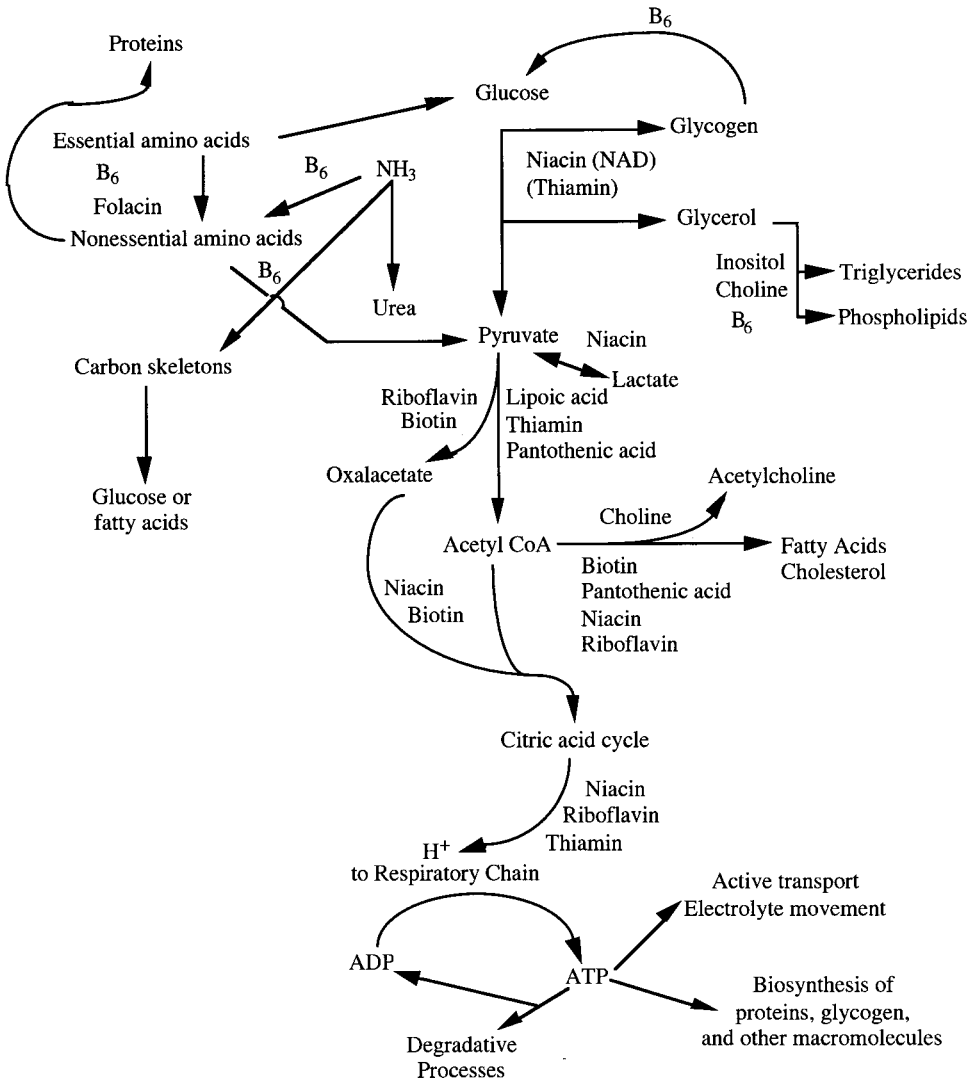

Figure 3 Involvement of the vitamins and other organic nutrients in intermediary metabolism.

nucleus, can repair itself. Occasionally, there is a missense repair and very occasionally this results in a mutation which is random. That is, the damage and subsequent missense repair can occur anywhere in the nuclear DNA and the resultant gene product could be one of more than a million products encoded by the nuclear genome. In addition, this damage might occur in only a few cells out of the many million within a given tissue or organ. Widespread damage from a single exposure is certainly possible, but probably not very frequent. Rather, slight but continued and possible cumulative damage is more likely. Whether degenerative diseases such as cardiovascular disease could be due to free radical damage to lipid-carrying proteins and/or to vascular tissue has yet to be documented. This is a very active area of nutrition research. Peroxide or free radical damage to the nuclear genome is not as serious on an individual genomic basis as damage to the mitochondrial genome. This genome encodes only 13 products but these products are important components of the mitochondrial respiratory chain and ATP synthesis. The mitochondrial DNA does not have the repair capacity of the nuclear genome. In fact, its repair capacity is quite limited. When added to the fact that the mitochondria consume about 90% of all the oxygen associated with the cell, the

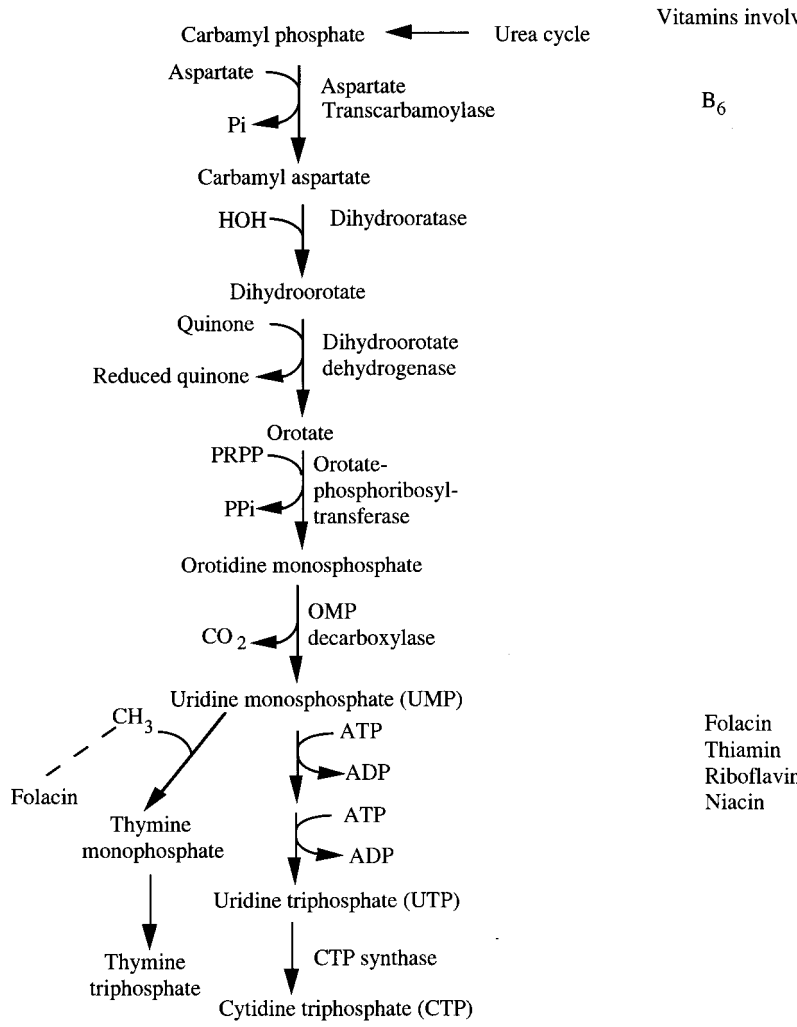

Figure 4 Pyrimidine synthesis. In this pathway the pyrimidine ring is formed before it is attached to ribose and phosphorylated.

potential for free radical damage is quite large. Fortunately, each cell has many hundreds to thousands of mitochondria so the loss of a few has little impact on the overall health and well-being of the cell or organ or whole animal. Nonetheless, should wholesale destruction of the genome occur, the results could be quite devastating. This rarely occurs.

Fortunately, there is a very active antioxidant system in place that protects against such damage. This is described in the sections devoted to vitamin E and selenium. Some of the vitamins and minerals play an important role in this system. Vitamin E quenches free radicals as they form via the conversion of tocopherol to the tocopheroxyl radical, which is then converted to its quinone. Vitamin K serves as an H^+/e^- donor/acceptor in its role to facilitate the carboxylation of the peptide glutamyl residues of certain proteins to their epoxide form. Vitamin C and vitamin A are both good H^+/e^- donor/acceptors in the suppression of free radical formation. Of course, indirectly, all those vitamins that serve as coenzymes are involved as well. Shown in Figure 5 is the free radical suppression system. Note the importance of selenium. In Unit 3, which discusses the antioxidant function of vitamin E, it is pointed out that there is a complementary role for selenium (see Unit 7).

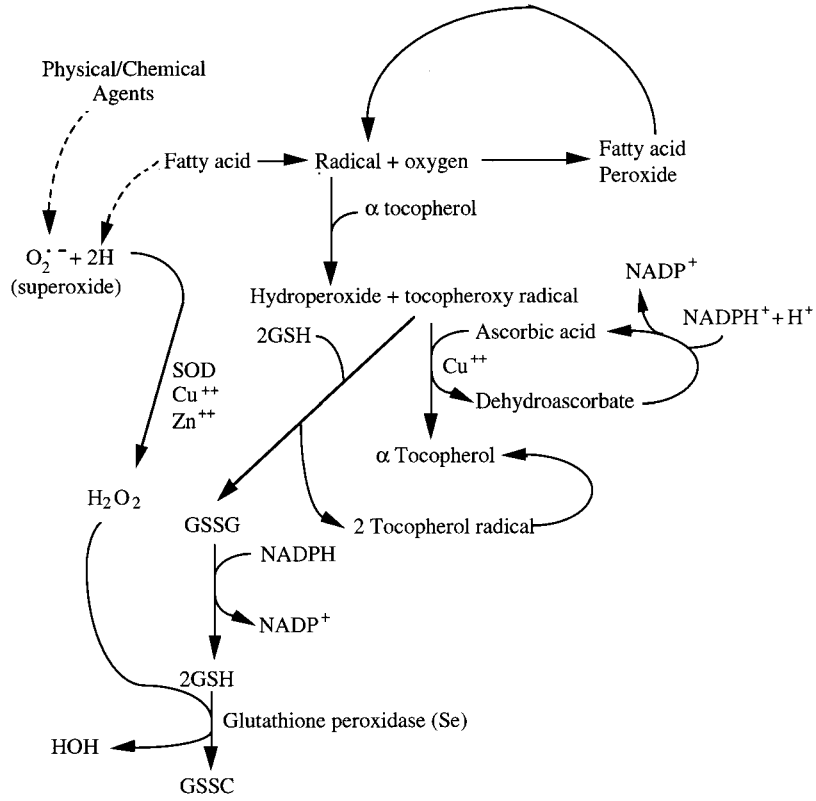

Figure 5 Roles for micronutrients in the system for the defense against free radical damage. Various agents can react with fatty acids to produce peroxides and superoxides. These very reactive materials are suppressed by the system above.

Some of the antioxidant role for vitamin E could be met if there was a sufficient intake of selenium. This mineral is important to the glutathione peroxidase enzyme which, as can be seen in Figure 5, is an important component of the free radical suppression system. Selenium plays a role in both the synthesis of this enzyme and as a required cofactor. As will be discussed in the units on minerals, several of these have roles in gene expression and these roles have overall importance to the physiological function of the body.

SUPPLEMENTAL READINGS

Atchison, M.L. 1988. Enhancers: mechanisms of action and cell specificity, *Annu. Rev. Cell Biol.,* 4:127.

Berdanier, C.D. and Hargrove, J.L., Eds. 1993. *Nutrition and Gene Expression,* CRC Press, Boca Raton, FL, 579 pgs.

Chien, K.R. 1993. Molecular advances in cardiovascular biology, *Science,* 260:916-917.

Combs, G.F. Jr. 1992. *The Vitamins,* Academic Press, New York, 528 pgs.

Demonacos, C.V., Karayanni, N., Hatzoglou, E., Tsiriyrotes, C., Spandidos, D.A., and Sekerio, C.E. 1996. Mitochondrial genes as sites of primary action of steroid hormones, *Steroids,* 61:226-232.

Derman, E. 1982. Transcriptional control in the production of liver specific mRNAs, *Cell,* 23:731-740.

Evans, R.M. 1988. The steroid and thyroid hormone superfamily, *Science,* 240:889-891.

Freedman, L.P. and Luisi, B.F. 1993. On the mechanism of DNA binding by nuclear hormone receptors: a structural and functional perspective, *J. Cell. Biochem.,* 51:140-150.

Johnson, P.F., Sterneck, E., and Williams, S.C. 1993. Activation domains of transcriptional regulatory proteins, *J. Nutr. Biochem.,* 4:386-398.

Jump, D.B., Lepar, G.J., and MacDonald, O.A. 1993. Retinoic acid regulation of gene expression in adipocytes. In Berdanier, C.D. and Hargrove, J.L., Eds., *Nutrition and Gene Expression,* CRC Press, Boca Raton, FL, pp. 431-454.

Maniatis, T., Goodbourn, S., and Fischer, J.A. 1987. Regulation of inducible and tissue specific gene expression, *Science,* 236:1237.

Semenza, G.L. 1994. Transcriptional regulation of gene expression: mechanisms and pathophysiology, *Hum. Mutat.,* 3:180-199.

Vogt, J.G. and Vogt, D. 1990. *Biochemistry,* John Wiley & Sons, New York, pg. 771-986; 1086-1178.

Yamamoto, K. 1985. Steroid receptor regulated transcription of specific genes and gene networks, *Annu. Rev. Genet.,* 19:209.