B COMPLEX VITAMINS IN WHOLE WHEAT, ENRICHED FLOUR AND THREE MILL STREAMS

by

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TABLE OF CONTENTS

INTRODUCTION

Scarcity of food is an increasing problem in the world. One practical solution is to explore additional food sources, especially in underdeveloped countries where food production has not kept pace with population growth. The Third World Food Survey by the Food and Agriculture Organization of the United Nations (1963) indicated that no less than half of the world's population suffers from varying degrees of undernutrition and malnutrition. Undernutrition is defined as a lack in quantity of food. Malnutrition implies that people eat enough food to keep them alive, but the food lacks those nutritive elements which are essential for growth, vitality and resistance to disease. Dietary deficiency diseases such as kwashiorkor, pellagra and beriberi are prevalent in various parts of the world.

Recognition of the importance of vitamins of the B complex in nutrition has created widespread interest in their distribution in cereal grains. The presence of vitamins of the B complex in cereals that have a comparatively high carbohydrate content is particularly important, since most of these vitamins are concerned with the metabolism of carbohydrate.

Wheat is one of the world's major cereals. It is generally considered the staple food cereal of Europe, America and parts of Asia and Africa. Pyler (1952) stated that wheat may contribute approximately 25% of the total energy in the United States diet and up to 50% in most European diets. A nutritional survey in Great Britain, as cited by Kent-Jones and Amos (1957),

indicated that wheat not only provides 25 to 30% of the total calories, but also contributes important proportions of the total intake of protein, vitamins of the B complex and iron.

Most of the wheat produced in the United States is milled into flour and during the milling a by-product, millfeed, is produced. Millfeed is composed of several mill streams, including red dog, bran, shorts and germ. The germ and bran contain a large proportion of vitamins of the B complex of the wheat kernel (Dawbarn, 1949; Hegsted et al., 1954). Millfeed generally is used as a livestock feed. However, Bradley (1963) suggested that millfeed could be made into a nutritive food for young human beings.

The development of reliable chemical and microbiological assay methods for vitamins of the B complex has made it possible to study further their distribution in wheat and its milled products. The purpose of this study was to determine the content of vitamins of the B complex in whole wheat, enriched flour and 3 mill streams.

REVIEW OF LITERATURE

Background Information about Wheat

Pyler (1958) stated that the epic of wheat closely parallels the epic of man's civilization. In the earliest of prehistoric times, man relied on grain and cultivated it. Wheat formed the basic foodstuff of human existence nurturing not only his body but also the beginnings of culture and civilization. The

cultivation of wheat began somewhere in the region extending from the Nile Valley of Egypt to the Persian Gulf. Archaeological discoveries indicated that implements used to mill wheat were known as early as 6000 B.C. Wheat is mentioned as one of the important crops of China as long as 5000 years ago.

Production. Wheat represents the second largest field crop in the United States and from the standpoint of human nutrition, it far exceeds in importance all other cereals. Geddes (1951) explained that wheat grown in the United States belongs to three botanical species. By far the most important of these is Tricitum vulgare or common wheat, which comprises nearly 95% of the total production. Pyler (1958) stated that there are more than 300 distinct varieties of common wheat. They are grouped into four major categories: hard red spring, hard red winter, soft red winter and winter and spring white wheats. Hard red winter wheat is planted in the fall in moderate temperature regions and is harvested in early summer. Kansas is one of the principal states producing hard red winter wheat. Spring wheat is sown in the spring and harvested in late summer. According to Agricultural Statistics (U.S. Dept. of Agriculture, 1964), the total United States wheat production for 1962-1963 was 1,138 million bushels which included: hard red winter, 544 million; soft red winter, 212 million; hard red spring, 162 million; durum, 50 million; and white, 170 million.

Hard wheat varieties find their principal use in the

production of bread flours. Soft wheats are particularly adapted to the production of cakes, pastries, cookies and other chemically leavened products. Durum wheat yields flour for macaroni and noodles. The usefulness of a wheat for a particular purpose is determined primarily by its protein content. Hard wheats generally possess a higher protein content than soft wheats (Pyler, 1952).

Milling. Pyler (1952, 1958) stated that there are three distinct structural parts in a wheat kernel, namely, bran, germ and endosperm. The bran coat consists of several distinct layers. The outer layers comprise epidermis, epicarp, cross layer and endocarp. The inner layers consist of testa, episperm, and aleurone. The aleurone layer is next to the endosperm. However, it adheres to the perisperm or inner bran layers and is removed with the bran during the milling. The embryo or germ lies at the base of the grain on its round side. The scutellum is a layer of epithelial cells around the germ and functions to secrete enzymes during germination and to absorb and conduct food material from endosperm to the growing embryo. The endosperm is made up chiefly of starch embedded in a matrix of protein.

The aim of milling wheat is to separate as completely as possible the bran and germ portions of the kernel from the endosperm and to reduce the endosperm to fine flour. Although the wheat kernel is approximately 80 to 85% endosperm, with 13 to 17% bran and 2 to 3% germ, generally flour contains only

72 to 75% of the kernel. Because of variation in quality, different varieties of wheat are blended to yield a flour with uniform baking characteristics. After the wheat has been blended, it is cleaned and tempered or conditioned to improve its milling performance before actual grinding. The milling process involves passing the wheat and the resultant milled products through a long series of both corrugated and smooth paired iron rolls, revolving toward each other at different speeds. Each passage through the rolls is followed by shifting and separation of the ground material into several streams for further reduction and separation. The further series of breaks and reduction produce many flour streams that are derived from different portions of the endosperm and differ in degree of refinement. Beginning with the first middlings separation which is the most highly refined, the flour contains increasing proportions of bran and germ at each successive reduction. The flour obtained from the final reduction in the milling process is called red dog. Fine bran usually is obtained from the last of the five breaks during the first phase of milling. Scalp (germ stock) contains wheat germ and some endosperm and bran.

Vitamin content. The principal vitamins present in wheat and its milled products are vitamins of the B complex and vitamin E (Pyler, 1952; Bailey, 1944; Kent-Jones and Amos, 1957). The other recognized vitamins are either completely absent or occur in insignificant amounts.

The thiamine content of wheat and wheat products has been

extensively investigated. Downs and Cathcart (1941) indicated that the thiamine content of wheat is influenced by both variety and environment. On the whole, spring wheat and hard winter wheat have somewhat higher thiamine values than soft wheats. Thiamine is not uniformly distributed in the wheat kernel. Somers and Coolidge (1945) and Somers et al. (1945) found that thiamine was concentrated principally in the scutelum, aleurone layer and outermost endosperm and was very low in the inner endosperm. As a result, mill products comprising different parts of the kernel show marked variation in their thiamine content.

Andrews et al. (1942a) stated that the riboflavin content of wheat does not seem to be markedly affected by environmental factors, but does vary with variety. They also indicated that riboflavin is not uniformly distributed in the wheat kernel and tends to be concentrated in the red dog, germ and bran portions. Somers et al. (1945) found that the embryo and aleurone layer contain the greatest concentration of riboflavin. Hinton et al. (1953) estimated the amounts of vitamins of the B complex in different parts of the wheat kernel and commented upon the high concentration of riboflavin, nicotinic acid and pantothenic acid in the aleurone layer.

Barton-Wright (1944) specified that niacin is concentrated mainly in the bran. In contrast, Moran (1945) found a higher concentration of niacin in the endosperm, immediately adjacent to the aleurone layer, than in the bran itself. Whereas the germ is a relatively rich source of thiamine and riboflavin, only a small amount of niacin is found in the germ.

The other vitamins of the B complex differ somewhat in their distribution in the wheat grain. Calhoun et al. (1960) found that the greatest proportion (50 to 90%) of pantothenic acid, folic acid, biotin and p-aminobenzoic acid was in the bran and shorts.

The effect of milling upon the vitamin content of wheat mill products is largely explained by their distribution in the kernel and the parts removed by various degrees of milling. The concentration of vitamins of the B complex in flour progressively declines as the degree of extraction falls below 100% (Dawbarn, 1949). Calhoun et al. (1958) stated that thiamine, riboflavin, niacin, folic acid, biotin and p-aminobenzoic acid underwent a reduction of approximately 80% in the milling of wheat into white patent flour, whereas pantothenic acid suffered about a 50% milling loss.

Enrichment with vitamins of the B complex. Cereals are the most important source of dietary energy for mankind. The actual contribution of wheat to nutrition falls short of its potential value because of the losses of vitamins during the milling process. Enriched or high extraction flours have been suggested to improve the nutritive value of flour. England decided to use an 80 to 85% extraction flour which included substantial portions of germ and bran and retained greater parts of the vitamins of the B complex (Kent-Jones and Amos, 1957). In the United States, the enrichment of flour with thiamine, riboflavin and niacin was inaugurated by the National Nutrition Conference for Defense in 1941, for the purpose of

the best possible utilization of the nation's food resources (Committee on Cereals of Food and Nutrition Board, 1958). The enrichment program became compulsory throughout the nation at that time. After World War II, the enrichment program lost its mandatory character except in 26 states, including Kansas, that adopted state regulations requiring bread and flour enrichment. However, the enrichment program for flour has met with a favorable and widespread volunteer acceptance and is evidently constructive and beneficial to public health.

The original purpose of flour enrichment was to raise the level of certain nutrients in white flour to the presumed levels at which these nutrients occurred naturally in whole wheat (Pyler, 1952). The enrichment standards which have remained unchanged since their inception, require that enriched flour contain thiamine, riboflavin and niacin at the following levels: thiamine, 2.0 to 2.5 mg/lb; riboflavin, 1.2 to 1.5 mg/lb; and niacin, 16.0 to 20.0 mg/lb. The thiamine and niacin contents of flour enriched to the minimum level approach the corresponding figures for whole wheat, whereas the riboflavin content of enriched flour exceeds that of whole wheat (Griswold, 1962). The riboflavin assays of whole wheat at the time enrichment started were high owing to the analytical method in use. Although better analytical methods have been introduced, the high enrichment standard for riboflavin is still used because of the prevalence of low riboflavin intakes in the United States.

Importance of Vitamins of the B Complex

The vitamins of the B complex are organic compounds which are: a) constituents of natural food, b) water soluble, c) essential for normal health and growth of animals, d) coenzymes or activators of enzymatic processes, e) present in normal food in extremely small concentration and physiologically effective in minute amounts and f) causes of deficiency diseases when they are absent from the diet or not properly absorbed from the diet (Kleiner and Orten, 1962; Wagner and Folkers, 1964). These vitamins include thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folic acid, p-aminobenzoic acid and vitamin B_{12}. Pyridoxine will not be reviewed since appropriate methods of analysis in cereals are not available (Calhoun et al., 1958). Vitamin B_{12} also will be omitted because it generally is not present in vegetable foods. Inositol and choline frequently are discussed with vitamins of the B complex, but both normally occur in animal tissues in amounts greater than are generally associated with vitamin values. Neither appears to be a coenzyme nor a part of one.

Thiamine. Thiamine in acid solution is fairly stable to heat and oxidation, but is readily broken down in neutral or alkaline solution into its constituent pyrimidine and thiazole moieties (Horwitt, 1964). It is stable in the dry state. The thiamine content of most food is essentially unaffected by freezing, but considerable losses are sustained during cooking.

Thiamine is absorbed from the small intestine and is

phosphorylated in the intestinal mucosa. It is excreted in the urine, the amount closely paralleling the dietary supply or administration of the vitamin (Goldsmith, 1964). Thiamine is found in all tissues, largely in the form of monophosphate or pyrophosphate. Body stores are not great.

Biological role. Thiamine is an essential nutrient for all mammalian species and functions in its coenzyme form, thiamine pyrophosphate (cocarboxylase). Thiamine pyrophosphate participates in important reactions of intermediary mammalian metabolism which have been summarized by Handler (1958) and Wuest and Furness (1962). It is the prosthetic group of pyruvic dehydrogenase and α-ketoglutaric dehydrogenase. These enzymes are involved in two enzymatic oxidative decarboxylations occurring in the Kreb cycle in mammalian metabolism. Pyruvic acid, an end product of carbohydrate metabolism in the anaerobic process, is introduced into the Kreb cycle system after oxidative decarboxylation to "active acetyl" through the participation of thiamine pyrophosphate, lipoic acid and coenzyme A. Oxidative decarboxylation of α-ketoglutaric acid is essential for the formation of succinyl CoA, an important intermediate in mammalian metabolism.

Brin *et al.* (1958, 1961) and Brin (1962, 1963) indicated that thiamine pyrophosphate is also an essential cofactor for transketolase activity. The transketolase reaction is important for the enzymic cleavage of ribulose-5-phosphate in the "direct oxidative pathway" of glucose metabolism. This pathway is essential for synthesis of ribose for nucleotide formation. It

also results in formation of $NADPH_2$ which is necessary for fatty acid biosynthesis.

Thiamine appears to be a cofactor for α-glycerophosphate dehydrogenase (Van Eys, 1960 and 1961). Thiamine deficiency results in a significant lowering of the soluble glycerophosphate dehydrogenase.

Requirement. Thiamine requirement is related to rate of metabolism and is expressed best in terms of caloric intake. Requirement also depends on the composition of the diet, particularly on the quantity of carbohydrate ingested. Fat and protein exert a sparing action and there is some evidence that alcohol may have a similar effect (Westerfeld and Doisy, 1945).

Many studies have been conducted on adult subjects to determine minimum thiamine requirement (Keys et al., 1943; Najjar and Holt, 1943). Findings indicated a need of 0.2 to 0.3 mg per 1000 Cal for prevention of clinical thiamine deficiency. Holt et al. (1949) reported that deficiency in infants can be prevented by an intake of 0.14 to 0.2 mg daily or with approximately 30 µg per kg of body weight. Horwitt (1953) stated that the minimum requirement for the infant in relation to calories was similar to that of the adult and he assumed that the same values would prevail for various ages of childhood, adolescence and old age. However, Oldham (1962) indicated that the minimum requirement for thiamine was greater for older than for younger women. As judged by urinary thiamine excretion, the older women reacted more rapidly to thiamine depletion and responded more slowly to partial repletion than did the younger women.

The present recommended dietary allowance of the Food and Nutrition Board (1964) for thiamine is 0.4 mg per 1000 Cal for all age groups. An additional 0.2 mg per day is recommended during the second and third trimester of pregnancy and during lactation, since thiamine requirement appears to be increased during these periods.

Effect of deficiency. The classical pathological syndrome of thiamine deficiency in the human is called beriberi. Beriberi is the most important nutritional disease in South China, and is prevalent throughout Asia (Platt, 1958). This disease is caused by eating polished rice as the staple food in the diet. Clinical beriberi, although usually associated with long-standing deficiency of thiamine is properly regarded as a multiple deficiency syndrome which does not occur if an adequate supply of thiamine is utilized.

The clinical symptoms in thiamine deficiency were described in great detail by Sebrell (1962). Fatigue, weight loss and anorexia are among the early symptoms of this disease. As the deficiency progresses, gastrointestinal complaints and neurological and cardiovascular manifestations appear. Far advanced neuropathesis appears in the peripheral nerves, particularly, in the lower extremities. Circulatory and cardiac involvement usually result in an enlarged dilated heart and generally lead to edema and irregular cardiac rhythm.

Infantile beriberi, resulting in infantile mortality, is associated frequently with children of mothers with beriberi (Burgess, 1958). Secondary or conditioned thiamine deficiencies

are in evidence throughout the world. Among the more common forms are alcoholic polyneuritis caused by a combination of high caloric intake and defective thiamine absorption and polyneuritis of pregnancy caused by increased metabolic demands of the fetus.

Riboflavin. Riboflavin is heat stable, but highly sensitive to light and is readily decomposed by both visible and ultraviolet light (Kleiner and Orten, 1962).

Riboflavin is absorbed readily from the small intestine in its free form which occurs in food (Horwitt, 1954). The bacterial flora of the intestinal tract synthesize riboflavin that is partially available to man. When the diet is rich in this vitamin, it is easy to saturate the tissues. It is excreted in the urine and feces, the amount being dependent on dietary intake, degree of saturation of tissues, physical activity, heat, stress and thiamine deficiency.

Biological role. When riboflavin is combined with phosphoric acid or adenylic and phosphoric acid, it acts as the prosthetic or active group of important flavoproteins present in every living cell of higher forms of life. These enzymes are involved in cell respiration as components of the electron transport system. Wagner and Folkers (1964) stated that riboflavin-5-phosphate and flavin adenine dinucleotide (FAD) are the coenzyme forms of riboflavin. The enzymes which contain riboflavin-5-phosphate are Warburg's yellow enzyme, cytochrome C reductase of yeast and L-amino acid dehydrogenase (Horwitt, 1954; Bro-Rasmussen, 1958a). The enzymes which contain FAD are crossed

yellow enzyme, cytochrome C reductase of liver, Haas enzyme, diaphorase, xanthine dehydrogenase (oxidase), liver aldehyde dehydrogenase, diamine dehydrogenase, glucose oxidase, D-amino acid dehydrogenase, glycine dehydrogenase, fumaric acid dehydrogenase and pyruvic acid oxidase. These enzymes are all dehydrogenases and can be alternately oxidized and reduced by accepting and donating hydrogen atoms one at a time through the cytochrome system or by direct combination with oxygen.

Physiological, nutritional and clinical observations have disclosed a striking similarity between the metabolism of riboflavin and nicotinic acid. Both riboflavin and nicotinic acid combine with labile proteins to form oxidative enzymes. Henderson et al. (1955) stated that riboflavin-containing enzymes are important for the conversion of tryptophan into nicotinic acid.

Riboflavin has been shown to be present in the retinal pigment of the human eye, where it appears to play a role in light adaptation (Robinson, 1951). It has the property of transforming rays of short wave length into yellowish-green fluorescent light for which the sensitivity of the eye is maximal. It also protects the cones of the retina against excessive light. The ocular manifestations of ariboflavinosis are due to the inability of the body to make good the losses of riboflavin that occur on exposing the eye to light.

Requirement. Riboflavin is involved both in protein and energy metabolism. Bro-Rasmussen (1958a, b) thoroughly reviewed the riboflavin requirements of animals and man and concluded that the requirements were more related to energy expenditure than to protein intake. In addition, the amount of riboflavin required is influenced by variations in heredity, growth, environment, age, activity and health of the body (Horwitt, 1954).

Bro-Rasmussen (1958b) estimated that the amount of riboflavin required to essentially saturate the tissues of children was 0.7 to 0.8 mg per 1000 Cal. The minimum need seems to decrease with advance in age, perhaps in relation to rate of growth. For the adult, the usual signs of riboflavin deficiency must be used as criterion of requirement instead of growth. The minimum requirement of riboflavin to prevent clinical deficiency appears to be 0.3 mg/1000 Cal. Intake of less than 0.25 mg per 1000 Cal produced experimental deficiency in adult human subjects (Horwitt et al., 1949; Sebrell et al., 1941). The riboflavin allowance recommended by the Food and Nutrition Board (1964) is 0.6 mg/1000 Cal for all age groups. An additional amount is recommended during pregnancy and lactation.

Effect of deficiency. Riboflavin deficiency in man is due to an inadequate dietary intake or to some conditional factor which impairs absorption or utilization or increases the requirement for the vitamin (Goldsmith, 1964). Deficiency is more common during periods of physiologic stress, that is, during

rapid growth periods, pregnancy, lactation or illness.

The primary effect of riboflavin restriction in young animals is the cessation of growth. Sure and Romans (1948) observed that riboflavin deficiency reduced in parallel the utilization of food with protein utilization, as expressed in weight gain per unit of protein intake. Mayfield and Hedrick (1949) found that the biological value of dietary protein as measured by nitrogen balance in rats was reduced in riboflavin deficiency. Kaunitz et al. (1954) and Coons and Moyer (1960) stated that negative nitrogen balance was closely associated with reduced utilization and retention of riboflavin. The interdependence of utilization of riboflavin and protein arises from the fact that flavoproteins in general are the most labile proteins in the body. When protein supply is not adequate, riboflavin deficiency may appear. Kwashiorkor, primarily a protein deficiency, is frequently associated with signs of riboflavin deficiency (FAO, 1955).

Bro-Rasmussen (1958a, b) indicated that loss of labile oxidative enzymes, flavoproteins in particular, obviously could lead to the inhibition of carbohydrate breakdown and accumulation of fat. This loss would reduce the rate of the biological oxidation process so that less oxygen would be consumed.

Early symptoms of riboflavin deficiency are soreness and burning of the lips, mouth and tongue, visual complaints such as photophobia, lacrimation, burning and itching of the eye and visual fatigue (Goldsmith, 1964). Later manifestations include

cheilitis, angular stomatitis, seborrheic dermatitis, glossitis and superficial vascularization of the cornea. The symptoms often are complicated with those of pellagra.

Various other effects of riboflavin deficiency have been observed. Miller et al. (1962) suggested that a critical low level of FAD might be the reason for embryo malformations. Riboflavin deficiencies in rats (Axelrod and Pruzansky, 1955) and in swine (Harmon et al., 1963) were shown to cause moderate suppression of antibody production.

Niacin (Nicotinic acid). Mammalian cells require a supply of nicotinic acid, nicotinamide or their precursor, L-tryptophan, in order to synthesize the coenzymes of nicotinamide-adenine dinucleotide (NAD) and nicotinamide-adenine dinucleotide phosphate (NADP) (Wagner and Folkers, 1964). The amino acid, tryptophan, was found to be a precursor of niacin in many animal species (Krehl et al., 1945; Rosen et al., 1946) and in man (Sarett and Goldsmith, 1947 and 1949). Administration of tryptophan was followed by an increase in urinary excretion of niacin metabolites. Pyridoxal phosphate plays a coenzyme role in the conversion of tryptophan to niacin. In pyridoxine deficiency, conversion of tryptophan to niacin was found to be impaired (Krehl, 1949).

Horwitt et al. (1956) and Goldsmith (1958) indicated that studies in normal adults showed that an average of 3.3% of administered tryptophan was converted to niacin compounds. On a molar basis, approximately 60 mg of dietary tryptophan appears to be equivalent to 1 mg of niacin in man.

Biological role. Many enzymes which require NAD or NADP as coenzymes are important links in the series of reactions usually associated with carbohydrate, lipid and protein metabolism (Mundley, 1954). The NAD- and NADP-containing enzymes play an important role in the biological oxidation and reduction systems by virtue of their capacity to serve as hydrogen-transfer agents. The nicotinic acid moiety of the coenzymes operates in these systems by reversibly alternating between an oxidized and a reduced state. All of the dehydrogenases, except succinic dehydrogenase, of the Kreb cycle require either NAD or NADP to accept hydrogen atoms and to initiate electron transfer along the electron transport chain of respiratory carrier to oxygen. Similarly, NAD is required for activities of dehydrogenases of glycolysis, and for the oxidative degradation of fatty acids. NADP is essential for glucose-6-phosphate dehydrogenase activity of the phospho-gluconate oxidative shunt, and its reduced form, $NADPH_2$ is required for the synthesis of fatty acid. Either NAD or NADP is demanded for the reversible oxidative deamination of L-glutamic acid. It provides a link for the biological synthesis of an amino acid utilizing a compound which can be derived from carbohydrate. Conversely, it provides a means whereby the body can derive energy from protein by converting the protein to glycogenic fragments.

Requirement. Niacin requirements have been studied intensively by Horwitt et al. (1956) and Goldsmith (1958). They found that the niacin-tryptophan requirement appeared to be

related to body weight and caloric intake. The amount and quality of protein in the diet influenced the niacin requirement. This was shown in experimental animals in which amino acid imbalance had been produced. Horwitt (1958) and Goldsmith (1958) reported that the minimum niacin requirement, including niacin formed from tryptophan, for the prevention of pellagra is about 9 to 12 mg daily for adults, and 6 mg for infants. For pregnancy and lactation, an additional amount is needed.

Recommended dietary allowances for niacin of the Food and Nutrition Board (1964) are expressed as niacin equivalents. Allowances for adult males are 15 to 19 mg niacin equivalents daily; for females, 13 to 14 mg; and for infants, 6 mg. Allowances for pregnancy and lactation are based on the recommended increase in caloric intake during these periods.

Effects of deficiency. Pellagra, a characteristic niacin deficiency disease, was long prevalent in southern Europe, southern United States and Central America. Most cases occurred in the low income groups, where diets were restricted to a few cheap foodstuffs, such as corn, that contained low amounts of niacin and tryptophan (Sydenstricker, 1958). Niacin deficiency may occur as a complication of pathologic states in which food intake is limited or in which there is interference with absorption or utilization of nutrients (Goldsmith, 1964).

The clinical findings of niacin deficiency, or pellagra, are characterized by dermatitis, inflammation of mucosal membranes, alimentary disorders such as achlorhydria, pigmentation and thickening of the skin (Goldsmith, 1964). Nervous

disorders and mental disturbances often occur. The pathologic lesions in pellagra are presumably related to defects in coenzyme function.

Pantothenic acid. Pantothenic acid is stable to oxidizing and reducing agents. It is destroyed by dry heat and by heating in an alkaline or an acid medium. The intake of pantothenic acid is reflected in the urinary excretion (Ralli, 1954; Pudelkewicz and Roderuck, 1963). The constancy of fecal excretion is indicative of good absorption of the vitamin. The coenzyme form of pantothenic acid in animal tissue is coenzyme A (CoA). Of the tissues, liver is highest in CoA and adrenal gland is next.

Biological role. Pantothenic acid as a component of CoA is essential for life. CoA plays a central role in two carbon unit metabolism by facilitating acyl and acetyl transfer (Lipmann, 1954). It is essential to aerobic metabolism of carbohydrate for the oxidative decarboxylation of pyruvate to acetyle CoA, and it is required for the continuance of Kreb cycle activity in the oxidative decarboxylation of α-ketoglutrate to form succinyl CoA. Furthermore, it is involved in the synthesis of various amino acids, as glutamic acid and proline, from carbohydrate sources (Gallagher, 1964). Through the succinyl transfer by succinyl CoA, CoA participates in erythrocyte metabolism and synthesis of the pyrrole ring in the heme molecule (Kikuchi et al., 1958). CoA is required for activation of fatty acids during lipogenesis to longer chain derivatives

or oxidative breakdown to units of acetyl CoA and propionyl CoA. Wakil (1960) reported that fatty acid synthesis in cytoplasm requires conversion of acetyl CoA to malonyl CoA prior to condensation in the presence of biotin-containing enzymes. Rudney (1955) stated that CoA played a role in sterol and terpene synthesis.

Requirement. Daily requirement of pantothenic acid for adults was estimated by Ralli (1954) to be 3 to 5 mg. The approximate daily dietary intake of pantothenic acid in human adult subjects in the United States varies from 10 to 20 mg (Mangay Chung et al., 1961). Judging from the amounts excreted daily, the amount of pantothenic acid in the diet is more than adequate. Ralli (1954) suggested that the infant and growing child need relatively more pantothenic acid than the adult. In addition, in conditions of stress and in situations where the metabolic rate is significantly increased, the requirement of pantothenic acid is increased.

Effect of deficiency. Pantothenic acid is widely distributed in foodstuffs, so there seems to be no pathological evidence of deficiency in man. The experimental syndromes of pantothenic acid depletion in man have been characterized by a burning sensation, muscle weakness, abdominal disorder, frequent infection and depression (Lubin et al., 1956; Anonymous, 1959). In addition, erratic changes in acetylation of p-aminobenzoic acid, glucose tolerance and adrenal dysfunction have been observed. Antibody production was reduced

in swine during pantothenic acid deficiency (Harmon et al., 1963). The disease syndrome associated with pantothenic acid deficiency is principally due to CoA depletion, with its disruptive effects on oxidative carbohydrate metabolism, Kreb cycle and fatty acid metabolism.

Biotin. Biotin is heat stable. It is widely distributed in both free and combined states in animal and vegetable tissues. The combined form is easily liberated by the action of proteolytic enzymes since the linkage is peptide in nature (Kleiner and Orten, 1962).

Biological role. Biotin plays a coenzyme role in the carbon dioxide fixation reaction. The biotin-containing enzymes include aspartic acid deaminase, serine and threonine deaminase, oxaloacetate decarboxylase and succinate decarboxylase (György, 1954). Recently, biotin was indicated to be the prosthetic group of other enzymes, such as acetyl CoA carboxylase, β-methylcrotonyl CoA carboxylase, propionyl CoA carboxylase, methylmalonyl oxaloacetic transcarboxylase and pyruvate carboxylase (Mistry and Dakshinamurti, 1964).

These biotin-containing enzymes participate in a variety of important biological reactions, as summarized by Terroine (1960) and Wagner and Folkers (1964), that include: a) deamination of aspartic acid, threonine and serine, b) carboxylation of pyruvate, adenine and guanine, c) decarboxylation of oxaloacetate and succinate, d) oxidation of pyruvate, lactate and propionic acid, e) biosynthesis of citrulline, some purines and certain saturated and unsaturated long chain fatty acids and

f) degradation of leucine and tryptophan. Wakil and Gibson (1960) and Oxman and Ball (1961) found that biotin-containing enzymes participated in fatty acid biosynthesis by catalyzing the carboxylation of acetyl CoA to malonyl CoA in the presence of ATP. Mistry and Dakshinamurti (1964) also indicated that biotin might be involved in some initial steps in the utilization of glucose; these effects were the indirect result of biotin controlled CO_2 fixation reactions.

Intensive studies with numbers of biotin enzymes by Kaziro and Ochoa (1961) and Waite and Wakil (1963) indicated the formation of a CO_2 -biotin enzyme as the intermediate of a biotin-catalyzed carboxylation reaction. The mechanism of action of biotin enzymes has been thoroughly reviewed (Anonymous, 1963). A two-step mechanism is involved in the binding of CO_2 to the biotin moiety of the enzyme with simultaneous hydrolysis of ATP and subsequent transfer of the "high energy" CO_2 to an acceptor.

Requirement. The minimum daily biotin requirement has not been established, but diets containing from 150 to 300 µg of biotin daily are considered adequate by the Food and Nutrition Board (1964).

Effect of deficiency. The biotin-containing enzymes participate directly or indirectly in the metabolism of carbohydrate, lipid, protein and nucleic acids. In biotin deficiency of rats and chicks as observed by Dakshinamurti and Mistry (1963a), the levels and activities of biotin enzymes were great-

ly depressed.

Mistry et al. (1962) found that in biotin deficiency, utilization of glucose was impaired and glycogen synthesis was reduced markedly. The effect of the reduction in glucose utilization impaired the conversion of D-glucose to L-ascorbic acid. RNA, as well as protein synthesis, was shown to be reduced markedly in biotin-deficient cells (Dakshinamurti and Mistry, 1963b). The reduction in protein synthesis was probably the result of the decreased formation of RNA or limited fixation of CO_2 into dicarboxylic acids, and therefore a limitation in energy supply for synthetic reactions. Modi et al. (1962) indicated that in biotin deficiency, the lipid content of liver mitochondria progressively decreased by about 30%, a decrease that was a direct result of reduction in the synthesis of fatty acids.

Although biotin deficiency can be produced readily by feeding avidin or large quantities of raw egg white, the disease does not seem to occur under natural conditions (Gyorgy, 1954). Nisenson (1957) indicated that infant seborrheic dermatitis and Leiner's disease were biotin deficiency diseases and biotin seemed to have a therapeutic effect.

Folic acid. Folic acid is stable to heat in neutral or alkaline solution but unstable to heat in acid media. It is inactivated by sunlight and greatly reduced by cooking (Kleiner and Orten, 1962).

Folic acid is composed of three main parts: 1) pteridine moiety, 2) p-aminobenzoic acid and 3) glutamic acid. Folic acid,

usually conjugated with two or six molecules of glutamic acid, occurs in a wide variety of foods of animal and vegetable origin. The conjugases, normally present in animal tissues, are necessary to release the free active folic acid from conjugated compounds. Folic acid is absorbed at all levels of the small intestine. An inability to absorb folic acid occurs in a variety of malabsorption syndromes such as sprue, idiopathic steatorrhea and infantile celiac disease (Luhby and Cooperman, 1964).

Biological role. The folic acid coenzymes and their metabolic functions have been reviewed by Jukes and Broquist (1961) and Luhby and Cooperman (1964). Free folic acid is converted through the dihydro to the tetrahydro form by folic acid reductase. There are 5 active coenzyme forms of tetrahydrofolic acid (THF): N^5 -formyl-THF (citrovorum factor or folinic acid), N^{10} -formyl-THF, N^5 -formimino-THF, N^5, N^{10} -methenyl THF and N^5, N^{10} -methylene-THF. Folinic acid is a naturally occurring derivative of folic acid. Santini et al. (1964) indicated that formyl derivative constituted the most abundant form of folic acid in foods. Tetrahydrofolic acid can be converted to an active coenzyme form by donation of a single carbon unit from formic acid, formaldehyde, serine or histidine. These various coenzyme forms of folic acid are interconvertible and revert to tetrahydrofolic acid when they donate their one-carbon moiety in a metabolic reaction.

Folic acid coenzymes participate in the transfer of 1-carbon units between purines, histidine, serine, thymine and

methionine (Wagner and Folkers, 1964; Stokstad, 1954). They are necessary for interconversion of glycine and serine, methylation of ethanolamine to choline, synthesis of methionine from homocysteine and synthesis of pyrimidines, thymine in particular. They are involved in synthesis and breakdown of histidine, methylation of nicotinamide to N^1-methylnicotinamide and oxidation of phenylalanine to tyrosine. Folic acid is essential for the maintenance of human normal hematopoiesis (Rundles, 1959). This may be explained by the role of folic acid coenzymes in the synthesis of purine and pyrimidine compounds that are utilized in the formation of nucleoprotein.

Folic acid metabolism is interrelated with ascorbic acid and vitamin B_{12}. Ascorbic acid, or other reducing agents, plays a prominent role in maintaining the folic acid coenzymes in the active state (Trufanow, 1959). The functions and metabolism of folic acid and vitamin B_{12} are intimately linked (Hutner and Nathan, 1959; Jukes and Broquist, 1961). Both are involved in synthesis of methionine, thymidine, RNA, DNA and nucleoprotein and a deficiency of either produces a megaloblastic anemia. Vitamin B_{12} is necessary for activities of folic acid coenzymes and conjugase systems. Clinical findings demonstrate the functional connection between folic acid and vitamin B_{12}. Folic acid permits a more efficient utilization of vitamin B_{12} in pernicious anemia, but sometimes exacerbates the vitamin B_{12} deficiency through a too sudden mobilization of vitamin B_{12}. Folic acid is probably necessary for the synthesis of vitamin B_{12}.

The protein of the diet is involved in folic acid metabolism since the conversion of folic acid into active coenzyme forms is dependent upon an enzymatic system that is protein in nature (Trufanow, 1959). In protein insufficiency, the ability of the liver to convert folic acid to folic acid coenzymes is disrupted.

Requirement. The daily requirement of folic acid for maintenance of normal nutrition is still unknown. Bacterial intestinal synthesis of folic acid or folinic acid in man may constitute an important source (Bethell, 1954).

Data are available as to the quantity of folic acid that is effective in alleviation of syndromes associated with folic acid deficiency in both animals and man (Goldsmith, 1964). These findings do not delineate requirements under normal conditions. Davidson and Jandle (1959) indicated that patients with folic acid deficiency responded well with daily doses of .4 mg or less. Mangay Chung et al. (1961) reported that the folic acid requirement of the normal adult does not exceed .4 mg of folic acid.

Effect of deficiency. A number of syndromes associated with folic acid deficiency have been observed in man. Nutritional macrocytic anemia and megaloblastic anemia in pregnancy are caused by dietary inadequacies and defective absorption of folic acid (Vilter et al., 1963). Folic acid deficiency may be encountered in patients with liver cirrhosis (Jandle and Lear, 1956), long-term administration of antiepileptic drug and hemoglobinopathy. The combined deficiencies of folic acid and ascorbic acid cause the megaloblastic anemia of infancy and scurvy (Vilter et al., 1963).

Folic acid deficiency can result in megaloblastic alteration in the bone marrow and macrocytosis in the peripheral blood. Glossitis, stomatitis, leukopenia and thrombocytopenia are common syndromes of folic acid deficiency (Goldsmith, 1964). Vilter et al. (1963) stated that the usual defect responsible for a megaloblastic anemia was an abnormality in folic acid metabolism which reduced the amount or activity of the folic acid coenzymes. The pathology of folic acid deficiency is essentially due to inhibition of mitosis in normal actively-dividing tissues such as bone marrow, skin, intestinal mucosal membrane and young growing tissues (Gallagher, 1964). Although the exact nature of folic acid participation in mitosis is not known, it is probably attributable to the requirement for tetrahydrofolic acid as a 1-carbon group transfer agent in purine, pyrimidine and nucleoprotein synthesis.

p-Aminobenzoic acid (PABA). p-Aminobenzoic acid is widely distributed in plant and animal cells in both a free and a bound form. PABA is also a moiety of folic acid and can replace it in the nutrition of some, but not all organisms (Wagner and Folkers, 1964). Thompson et al. (1943) reported that approximately 80% of the PABA content of animal tissues was in the bound form, whereas in plants the bound form represented only 44% of the total PABA.

PABA is an essential metabolite for all organisms inhibited by sulfanilamide. The sulfa drug owes its bacteriostatic activity to a competition with PABA for a position in a coenzyme necessary for bacterial reproduction (Kleiner and Orten, 1962).

PABA takes part in some enzyme reactions necessary for the life of the bacterial cell, possibly in a phenolase system.

In early studies of vitamin-like substances, Ansbacher (1941) concluded that PABA was a "vitamin" since it could correct gray hair in rats. Martin and Ansbacher (1941) and Martin (1942) in further studies produced evidence that PABA was essential for growth and prevention of achromotrichia in the rat when inositol was present in the diet. Sure (1941, 1943) stated that PABA was essential for lactation and reproduction in the rat. However, Emerson (1941) and Brown and Sturtevant (1949) could obtain no chromotrichial effect with PABA in grayed rats, although pantothenic acid was effective. Brandaleone et al. (1943, 1944) found that PABA was valueless in treatment of gray hair of human beings. A deficiency of PABA has not been demonstrated conclusively in any vertebrate, therefore it has been suggested that the compound no longer be considered as a vitamin, but rather a nutrile (Briggs and Daft, 1955; Wagner and Folkers, 1964).

Methods of Analysis

Thiochrome method for thiamine. Thiochrome procedures depend upon the oxidation of thiamine to thiochrome which fluoresces in ultra-violet light (Association of Vitamin Chemists, 1951). Under standard conditions and in the absence of other fluorescing substances, the fluorescence is proportional to the thiochrome present, and hence to the thiamine originally in the solution. The thiamine may be freed from interfering substances

by treatment with absorbents which retain thiamine but not the impurities.

Background and principle of microbiological assay. The microbiological assay method of Snell and Strong (1939) for riboflavin was the first one to show that microorganisms could be used with confidence for routine determination of a vitamin in natural materials. Subsequently, the microbiological assay of vitamins of the B complex and amino acids has been widely used to analyse for the nutritive value of food (Barton-Wright, 1961).

The basic information necessary to the development, utilization and evaluation of microbiological assay was reviewed by Snell (1948 and 1950). The complexity of the essential steps involved in microbiological assay is such that the end result may be influenced by the manner in which almost every operation of the procedure is carried out. The fundamental assumption is made that any uncontrolled variations in procedural details that occur under conditions of normal practice will affect the response of the test organism to standard and sample in a similar manner, hence they will not affect materially the accuracy of the assay result. In many cases, this assumption appears to be borne out in practice.

The principle of the microbiological method is based on the observation that certain microorganisms require specific vitamins for growth (Association of Vitamin Chemists, 1951). Using a basal medium complete in all respects except for the vitamin under test, growth responses of the organism are compared

quantitatively in standard and unknown solution. Either quantity of acid or turbidity produced by the organism is measured to determine the extent of growth, and thereby the amount of vitamin in the test solution. The majority of assays utilize lactic acid bacteria as test organisms. Yeast and neurospora mutants provide alternative organisms.

Factors influencing the growth of assay microorganisms. To obtain optimal growth of any assay organism, it is necessary to satisfy its physio-chemical requirement.

Nutritional requirements. Nutritional requirement of various lactic acid bacteria used in assay work were investigated and reported by Snell (1945) and Peterson and Peterson (1945). Cultures require one or more of the following vitamins: thiamine, riboflavin, niacin, pantothenic acid, biotin, vitamin B_6, folic acid and p-aminobenzoic acid. Lactobacillus plantarum, the commonly used assay organism, requires only biotin, niacin and pantothenic acid. Lactobacillus casei may require folic acid, riboflavin and vitamin B_6 in addition to biotin, niacin and pantothenic acid. These bacteria also need a variable assortment of amino acids, purine and pyrimidine bases, a fermentable carbohydrate and mineral salts for growth.

Neurospora has the simplest nutritional requirements (Snell, 1948). By radiating with X-rays or ultraviolet light, mutant strains of the organism can be derived which are unable to synthesize certain vitamins or amino acids and therefore cannot grow unless these substances are added to the minimal basal medium.

Temperature. As a general rule, any organism used for assay should be cultured at or near its optimal temperature. McMahon and Snell (1944) and Perlman and McCoy (1945) indicated that the exact temperature selected for incubation is not of crucial importance, so long as it lies reasonably near to the optimal temperature, in a range which permits good growth of the test organisms. It is of primary importance, however, that the temperature selected for incubation be maintained constant throughout the area occupied by the assay vessels. Temperatures used most extensively with various lactic acid bacteria range from 30° to 37°C, varying somewhat with the organism. Neurospora is most commonly incubated at 30°C.

pH. Lactic acid bacteria produce large amounts of lactic acid during growth. In order to prevent the pH of the medium from rapidly dropping to levels which would inhibit further growth of the organisms, buffers must be added to the assay medium. The pH which limits growth of different organisms varies from just over 4 to as low as 3 (Rosebury, 1932). The optimal pH for growth of lactic acid bacteria lies between 5.5 and 6.5. Since some acid is produced during autoclaving the initial pH of the medium should be somewhat higher than this, 6.8 to 7.0. It is important that all of the sample solutions for assay be adjusted to the same initial pH as the medium. The buffer content of the sample solution must not be so high that it materially changes the buffer capacity of the medium. Neurospora grows over a wide range of hydrogen ion concentrations.

Oxygen. Lactic acid bacteria are microaerophilic and

grow essentially anaerobically, although small amounts of air are not toxic (Snell, 1950). Aeration by shaking or exposure of large surfaces to air is generally deleterious. For this reason, stock cultures are carried as stabs in agar and, for assay purposes, ordinary test tubes serve well as containers since the organisms grow throughout a deep tube of medium. Neurospora grows best under aerobic conditions. In assay work, this organism is cultured in vessels where the ratio of surfaces to volume of the culture medium is high to permit ready access to air.

Microbiological assay procedures. The essential steps involved in microbiological assay are: a) preparation of media for carrying stock cultures and maintenance of these cultures; b) preparation of inoculum; c) preparation of vitamin-deficient basal medium; d) extraction of the vitamin from the sample preparatory to assay; e) setting up the assay; f) sterilization of the assay tubes and media; g) inoculation with the test organism; h) incubation; i) determination of response to the vitamin; and j) calculation of results (Snell, 1948). With the basic information about microbiological assay in mind, some of the individual steps will be considered.

Extraction of free vitamin from natural materials. One major purpose of most vitamin determinations is to assess the value of a sample as a dietary source of the vitamin for animals. The vitamins occur naturally in combined forms which animals are able to break down and utilize, but which are unavailable to test organisms. Adequate extraction for liberating free

vitamins from such combined forms to make them available for the test organism is essential (Snell, 1950). Elimination of known interfering material also is necessary. Strong and Carpenter (1942) and Neal and Strong (1943) indicated that fatty materials markedly interfere with the assay of riboflavin and pantothenic acid. Strong (1947) and Jukes (1947) suggested that either filtration of pH 4.5 and/or extraction with ether could prevent the interfering effect of fatty acids.

Basal medium. A satisfactory basal medium must be free of the vitamin to be determined (Difco Laboratories, 1964). It must be adequate in all other required nutrients. Therefore, the addition of the sample, in the amount necessary for assay should not supply substances, other than the vitamin to be assayed. It should not increase or inhibit the amount of growth obtained at the end of the recommended incubation period. The essential vitamin being assayed should be the only substance limiting growth.

Cultures and inoculum. In all cases, it is essential that pure cultures of the test organisms be maintained (Snell, 1950). If, after a period during which successful assays have been obtained, sudden transition to irregular behavior is noted, the purity of the stock culture should be checked. Aseptic technique is practiced to maintain pure cultures of the test organism. If a diluted and well washed inoculum is used, the final amount of growth obtained is dependent on the concentration of the limiting vitamin present. Fluctuations in drop size of inoculum will not materially influence results.

Determination of response to the vitamin. For assays, accurate methods for determining response of the organism to additions of the vitamin must be available (Snell, 1948). The growth of the organism is either determined directly by measurements of turbidity, weight of mycelium or by the amount of some metabolic product of the organism such as lactic acid.

Specificity, reliability and precision of microbiological assay. One of the unique advantages inherent in the microbiological assay is the sensitivity of organisms to small quantities of the vitamin and their consequent applicability to determination of the vitamin content of minute samples.

Criteria for specificity and reliability of the general microbiological procedure have been reviewed by Snell (1947). They include: a) agreement with results of other reliable methods, b) obtainment of theoretical recoveries on addition of a known amount of vitamin to a sample, c) obtainment of consistent values on repeated assay within $\pm$ 10%, d) agreement of results calculated from different assay levels within $\pm$ 10% and absence of drift and e) agreement in assay values obtained with different assay organisms having different nutritive requirements.

EXPERIMENTAL PROCEDURE

Whole Wheat, Enriched Flour and Mill Streams Used

The enriched flour and 3 mill streams (red dog, fine bran and scalp) used in the study were milled from a mixture of hard

red winter wheat varieties by the Abilene Flour Mill, Abilene, Kansas. The proximate composition of whole wheat, enriched flour and 3 mill streams as determined by the method of AOAC (1960) is presented in table 1. The flour was a 67% extraction medium patent flour. The fine bran represented approximately 10% of the wheat kernel; the red dog, 2%; and the scalp (germ stock), 1%. The samples were protected from light during handling and were stored at household refrigerator temperature until needed. The samples were finely ground previous to the assays.

Thiochrome Assay for Thiamine

Thiamine was determined by the thiochrome method recommended by AACC (1962). However, the scalp was extracted with ethyl ether for 3 hours on low heat in a Goldfish extractor before assay to prevent interference of fatty material. Since the extract was clear, purification with decalso was not needed in the preparation of sample solutions. Three samples of each product were hydrolyzed and analyzed in duplicate to obtain the mean thiamine content.

Microbiological Assays for Other 6 Vitamins

Riboflavin, niacin, pantothenic acid, biotin, folic acid and p-aminobenzoic acid were determined by microbiological methods. The detailed procedure for microbiological assay recommended by the Association of Vitamin Chemists (1951) was followed. A standard curve was constructed each time an assay

TABLE 1

Proximate composition of whole wheat, enriched flour and 3 mill streams[1]

Nutrient	Whole wheat	Enriched flour	Red dog	Fine bran	Scalp
	%	%	%	%	%
Protein[2]	10.80 (10.62)[3]	10.83 (9.54)	13.54 (10.41)	14.93 (13.80)	15.68 (14.91)
Fat[4]	1.67	1.28	2.47	3.04	3.97
Crude fiber	2.42	0.33	1.72	8.25	5.92
Moisture	13.77	12.34	10.76	12.94	13.31
Ash	1.53	0.43	1.48	4.85	3.68
Nitrogen-free extract[5]	69.81	74.79	70.03	55.99	57.44
Carbohydrates[6]	72.23	75.12	71.75	64.24	63.36

[1]Analysis by S. N. Rodgers, Chemical Services, Kansas State University.

[2]Factor used for conversion of nitrogen into protein, 5.70.

[3]Parentheses indicate protein content on a 14% moisture basis.

[4]Ether extract of fat.

[5]Nitrogen-free extract is protein, fat, crude fiber, moisture and ash subtracted from 100.

[6]Carbohydrate is nitrogen-free extract + crude fiber.

was run since conditions, such as autoclaving and temperature of incubation, that influence the standard curve reading could not be duplicated precisely from one time to the next. Seven or more levels of standard solution in duplicate tubes were used.

All the assay organisms and culture media were obtained from Difco Laboratories (Detroit, Michigan). Each test tube was filled with 5 ml of the rehydrated medium, specified amounts of the standard or test solution and sufficient distilled water to give a total of 10 ml. The tubes were capped, autoclaved, cooled and inoculated.

Three or more hydrolysates were prepared from each of the 5 products. Five different levels of each hydrolysate within the range of the standard curve were run in duplicate. The Beckman pH meter was used to adjust the pH of the hydrolysates.

Riboflavin. Lactobacillus casei ATCC 7469 and Bacto-Riboflavin Assay Medium, which was a slight modification of the medium described by Snell and Strong (1939), were used. The stock cultures of L. casei ATCC 7469 were prepared by stab inoculation into 10 ml of Bacto-Lactobacilli Agar AOAC. After 24 hours incubation at 37°C, the stock cultures were refrigerated. Transplants were made at weekly intervals, in triplicate. Inoculum for assay was prepared by subculturing from a stock culture of L. casei ATCC 7469 into 10 ml of Bacto-Lactobacilli Broth AOAC. Following incubation for 24 hours at 37°C, the culture was centrifuged under aseptic conditions and the supernatant liquid decanted. The cells were suspended in 10 ml

sterile 0.85% NaCl and centrifuged again. The cells were resuspended in 10 ml sterile 0.85% NaCl. One drop of this suspension was used to inoculate each of the assay tubes.

Each sample was hydrolyzed with 50 ml 0.1 HCl and autoclaved at 15 lb pressure for 15 minutes. After autoclaving, the sample solution was adjusted to pH 4.5 with 2.5M sodium acetate and filtered. The extract was readjusted to pH 6.8 with 0.1 NaOH and made to specified volume with distilled water. The assay was conducted under subdued light.

The standard curve for riboflavin was obtained by using riboflavin at levels of 0.0, 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 µg per assay tube. After 72 hours incubation at 37°C, the response of the test organism was determined by acid titration using 0.1 NaOH in a microburet.

Niacin. Lactobacillus plantarum ATCC 8014 and the Bacto-Niacin Assay Medium, which was prepared according to the formula described by Snell and Wright (1941) and modified by Krehl et al. (1943) and Barton-Wright (1944), were used. The preparation of the stock culture of Lactobacillus plantarum ATCC 8014 and inoculum were similar to that of the riboflavin assay.

Each sample was hydrolysed using 50 ml of 1N sulfuric acid per g of sample for 1 hour in an autoclave at 20 lb pressure. The sample was cooled and adjusted to pH 6.8 with 1N NaOH, then made to specified volume with distilled water and filtered. The standard curve was obtained by using niacin at levels of 0.0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3 and 0.4 µg per assay tube. After

72 hours incubation at 37°C, the response of the test organism was determined by acid titration using 0.1N NaOH.

Pantothenic acid. Lactobacillus plantarum ATCC 8014 and Bacto-Pantothenate AOAC Medium, prepared according to the specifications in AOAC (1960), were used. The stock culture and inoculum were prepared as described for the riboflavin assay.

Each sample was digested with 0.1 g mylase P per g of sample in sodium acetate buffer, pH 4.5 to 4.7, under toluene and incubated for 24 hours at 37°C as suggested by Ives and Strong (1946). After incubation, 75 ml of boiling water was added and the sample was allowed to stand 30 minutes with frequent shaking, cooled and filtered. The extract was adjusted to pH 6.8 with 1N NaOH and then made to specified volume with distilled water.

The standard curve was obtained by using calcium pantothenate at levels of 0.0, 0.025, 0.05, 0.075, 0.1, 0.15, 0.175 and 0.2 µg per assay tube. Acidimetric determination with 0.1N NaOH was made after 72 hours incubation at 37°C.

Biotin. Lactobacillus plantarum ATCC 8014 and the Bacto-Biotin Assay Medium were used. Preparation of the stock culture and inoculum was similar to that of the riboflavin assay. Hydrolysis of the sample was done as described for the niacin assay.

The standard curve was obtained by using biotin at levels of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mµg per assay tube. Acidimetric determination was made after 72 hours incubation at 37°C.

Folic acid. Streptococcus faecalis ATCC 8043 and the Bacto-Folic Acid Assay Medium, prepared according to the formula described by Capps et al. (1948) but modified by the use of sodium citrate instead of sodium acetate, were used. The preparation of stock culture and inoculum was similar to that of the riboflavin assay.

Each sample was digested under toluene with 20 mg of dehydrated chicken pancreas in 5 ml of 0.2M phosphate buffer (pH 7.0) per g of sample and incubated for 24 hours at 37°C. After incubation, the sample solution was heated in a boiling water bath for 5 minutes, cooled, made to specified volume with distilled water and filtered.

The standard curve was obtained by using folic acid at levels of 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 mμg per assay tube. After 18 hours incubation at 37°C, the response of the test organism was determined turbidimetrically, in a Bausch and Lomb colorimeter set at a wave length of 550 mμ. The assay was conducted under subdued light because folic acid is light labile.

p-Aminobenzoic acid (PABA). Neurospora crassa ATCC 9278 and the Bacto-Inositol-PABA Assay Medium, a modification of the medium described by Horowitz and Beadle (1943), Beadle (1944) and Agarwala and Peterson (1950), were used. The stock culture was prepared by inoculation on slants of Bacto-Neurospora Culture Agar of Difco. After 48 hours incubation at 32°C, the tubes were refrigerated. Triplicate transplants were made every week. The

inoculum was prepared by removing spores from a 48 hour stock culture and suspended in 100 ml of sterile saline. One drop of this suspension was added all assay flasks.

Each sample was autoclaved with 1N NaOH for 15 minutes at 20 lb pressure as recommended by Calhoun et al. (1958). The sample solutions were gelatin-like after autoclaving. In order to obtain a clear extract, the sample solution was centrifuged three times. Each time the supernatant was removed and the precipitate was washed with distilled water before the next centrifugation. The supernatants were combined and adjusted to pH 6.6 with 1N HCl and made to specified volume with distilled water.

The standard curve for PABA was obtained by using PABA at levels of 0.0, 0.01, 0.015, 0.02, 0.03, 0.04, 0.05, and 0.06 µg per assay flask. Ten ml of the rehydrated medium, specified amounts of the standard or test solution and sufficient distilled water to give a total of 20 ml per flask were used. Five levels of the test solution within the range of the standard curve were run. The flasks were capped, autoclaved, cooled, inoculated and incubated at 32°C for 5 days. After incubation, the flasks were steamed at 100°C for 5 minutes and cooled. The solution of each flask was filtered through weighed millipore, mean pore size of 1.2 microns (Millipore Filter Corporation, Bedford, Mass.). The mycelia collected on the millipore was dried overnight in an electric oven at 60°C. The dried mycelia pellets were weighed on an analytical balance to the nearest 0.5 mg.

Calculations

Thiochrome assay. Thiamine content was calculated according to the following formula:

$$\mu g/g = \frac{Rx-Rxb}{Rs-Rsb} \times \frac{V}{5} \times \frac{1}{S}$$

where Rx: fluorometer reading with unknown

Rxb: blank fluorometer reading with unknown

Rs: fluorometer reading with thiamine standard

Rsb: blank fluorometer reading with thiamine standard

V: volume of extraction (100 ml)

S: sample weight in g on dry weight basis

Microbiological assays. The standard curves for riboflavin, niacin, pantothenic acid and biotin were drawn by plotting ml 0.1N NaOH against the concentration of vitamin per tube in the standard series. The standard curve for folic acid was obtained by plotting % transmittance against the concentration of folic acid per tube in the standard series. The standard curve for PABA was constructed from the weights of mycelium obtained against the concentration of PABA per tube in the standard series.

The vitamin content of each sample solution was determined by interpolation of the values on the standard curves. Vitamin content was calculated for 1 ml of the test solution and converted to µg per g on dry weight basis.

$$\mu g/g \text{ on dry weight basis} = \frac{\mu g \text{ per ml of extract}}{\text{wt. of sample on dry basis}} \times \text{volume}$$

Since most cereal data is published on the basis of 14% moisture, the vitamin content was then converted to this basis.

$$\mu g/g \text{ on } 14\% \text{ moisture basis} = \mu g/g \text{ on dry basis} \times \frac{100-14}{100}$$

Milling losses. The % loss of pantothenic acid, biotin, folic acid and p-aminobenzoic acid in milling wheat to flour was calculated. The flour was a 67% extraction.

$$\%\ \text{milling loss} = \frac{\text{vitamin content of whole wheat } (\mu g/gm) - \text{content of flour } (\mu g/g \times 67)}{\text{vitamin content of whole wheat } (\mu g/gm)} \times 100$$

Statistical Analysis

Ninety percent confidence intervals on μ were calculated to estimate the true mean vitamin content of each product studied, as indicated in the following formula:

$$CI_{.90} = \bar{x} \pm t_{.10} s_{\bar{x}}$$

RESULTS AND DISCUSSION

Means and 90% confidence intervals on μ for vitamin content of whole wheat, enriched flour and 3 mill streams on 14% moisture basis are shown in table 2. All assay values on dry weight basis are shown in the appendix. The vitamins will be discussed individually.

It is difficult to make meaningful detailed comparisons between the values given in table 2 and values found in the

literature. Vitamin content of wheat may be influenced by both variety and environmental conditions. Calhoun et al. (1958) observed that the vitamin content of wheat blends increased with increasing amounts of protein. This was more marked for thiamine, riboflavin, niacin, pantothenic acid and p-aminobenzoic acid than it was for biotin and folic acid. In addition, the proportion of the whole wheat represented by each mill stream may vary from one mill to another. The differences in values obtained in various laboratories also may represent differences in analytical techniques. Taking cognizance of these facts, the results obtained in the present study will be compared to the findings of other investigators.

Complete sample descriptions and methods of determination were not available for most of the studies used for comparison, except that of Calhoun et al. (1958 and 1960). They studied the vitamin content of wheat, flour and commercial mill products. The general assay methods in the present study were similar to those in their studies, but the wheat samples were different. A blend of hard red winter wheats was used in this study; the wheat blend in their studies was 50% hard red spring and 50% hard red winter wheats. The protein content of samples (on 14% moisture basis) in this study as shown in table 1 was lower than that in the study of Calhoun et al. (1960). Their values were: wheat, 12.9%; patent flour, 11.7%; red dog, 16.4%; bran, 14.5%, and germ, 22.7%. In addition, the scalp used in the present study was a combination of germ, flour and bran instead

TABLE 2

Means and 90% confidence intervals on μ for vitamin content of whole wheat, enriched flour and 3 mill streams (on 14% moisture basis)

		Whole wheat μg/g	Enriched flour μg/g	Red dog μg/g	Fine bran μg/g	Scalp μg/g
Thiamine[1]	$\bar{x}$[2]	2.70	4.89	8.24	4.87	9.27
	$CI_{.90}$ on μ[3]	2.67 to 2.73	4.86 to 4.92	8.06 to 8.42	4.65 to 5.09	8.89 to 9.65
Ribo-flavin	$\bar{x}$	2.14	3.75	3.20	8.17	4.12
	$CI_{.90}$ on μ	2.05 to 2.23	3.63 to 3.87	2.97 to 3.42	7.94 to 8.41	4.02 to 4.22
Niacin	$\bar{x}$	45.70	38.92	33.58	181.66	94.86
	$CI_{.90}$ on μ	45.03 to 46.36	38.06 to 39.78	33.12 to 34.04	178.65 to 184.68	93.04 to 96.68
Panto-thenic acid[4]	$\bar{x}$	14.56	7.28	9.80	39.42	21.31
	$CI_{.90}$ on μ	14.33 to 14.80	7.03 to 7.53	8.18 to 10.42	37.71 to 41.13	20.21 to 22.41
Biotin	$\bar{x}$	0.084	0.061	0.063	0.371	0.242
	$CI_{.90}$ on μ	0.082 to 0.086	0.060 to 0.063	0.061 to 0.065	0.365 to 0.377	0.237 to 0.248
Folic acid	$\bar{x}$	0.431	0.129	0.579	0.901	1.197
	$CI_{.90}$ on μ	0.409 to 0.454	0.124 to 0.134	0.561 to 0.597	0.870 to 0.932	1.131 to 1.263
p-Amino-benzoic acid	$\bar{x}$	1.84	0.24	1.64	4.09	3.42
	$CI_{.90}$ on μ	1.78 to 1.89	0.23 to 0.24	1.58 to 1.70	3.93 to 4.26	3.33 to 3.51

[1] As thiamine hydrochloride.

[2] Mean.

[3] 90% confidence interval on μ.

[4] As calcium pantothenate.

of pure germ used in the study of Calhoun et al. (1960).

Thiamine

Enriched flour had a mean thiamine content of 4.89 µg/g. This value was higher than that of whole wheat, 2.70 µg/g, but similar to that of fine bran, 4.87 µg/g. Both scalp (germ stock), 9.27 µg/g, and red dog, 8.24 µg/g, had higher mean thiamine content than fine bran, whole wheat and enriched flour. Scalp had the highest value among these products.

Somers and Coolidge (1945) and Somers et al. (1945) observed that thiamine was concentrated principally in the scutellum, aleurone layer and the outermost endosperm. As a result, milled products from different parts of the kernel showed marked variation in their thiamine content. In a study of soft wheat, Shetlar and Lyman (1941) found that red dog contained 10.1 µg thiamine per g, but bran contained only 5.7 µg/g. In the studies of Sherwood et al. (1941) and Calhoun et al. (1960), red dog was unusually rich in thiamine, 28 to 29.6 µg/g, and was higher than germ, 13.5 to 22.9 µg/g, and bran, 6.29 to 8.7 µg/g. The values obtained in the present study were much lower than those of the latter studies, but closer to the values found by Shetlar and Lyman (1941).

The mean thiamine content of the enriched flour fell within the range of the thiamine enrichment standard, 4.4 to 5.5 µg/g. The mean thiamine content of the whole wheat was lower than the value, 5.2 µg/g, for hard red winter wheat found in Agriculture

Handbook No. 8 (Watt and Merrill, 1963).

Riboflavin

The mean riboflavin content of whole wheat, 2.14 µg/g, was the lowest among those of enriched flour, whole wheat and 3 mill streams. The mean riboflavin content of scalp (germ stock), 4.12 µg/g, was slightly higher than that of enriched flour, 3.75 µg/g, and red dog, 3.2 µg/g, but lower than that of fine bran, 8.17 µg/g. The fine bran had the highest mean riboflavin concentration among these products.

The mean riboflavin content of the fine bran in this study was much higher than the values, 2.8 to 3.3 µg/g, observed by Andrews et al. (1942a) and Calhoun et al. (1960). The values of red dog and scalp were in agreement with the range of values for red dog, 3.2 to 3.8 µg/g, and germ, 4.0 to 5.0 µg/g, in the findings of above-mentioned studies. Somers et al. (1945) and Hinton et al. (1953) observed that the embryo and aleurone layer contained the greatest concentration of riboflavin. The aleurone layer adheres to the inner bran layer and is removed with the bran during the milling. This might explain the high riboflavin content of fine bran in this study.

The mean riboflavin content of the enriched flour was slightly higher than the maximum value, 3.3 µg/g, of the riboflavin enrichment standard. The mean riboflavin content of the whole wheat, 2.14 µg/g, was higher than some of the values found in the literature. Bailey (1944) and Kent-Jones and Amos (1957)

indicated that wheat contained approximately 1 µg/g of riboflavin. In Agriculture Handbook No. 8 (Watt and Merrill, 1963), hard red winter wheat is reported to contain 1.2 µg/g of riboflavin. However, Barton-Wright (1942) observed the riboflavin content of wheat to range from 2.6 to 2.9 µg/g and the Council on Foods and Nutrition of American Medical Association (1941) reported that the riboflavin content of whole wheat was 2.5 µg/g.

Niacin

The mean niacin content of whole wheat, 45.70 µg/g, was higher than that of enriched flour, 38.92 µg/g and red dog, 33.58 µg/g, but much lower than that of fine bran, 181.66 µg/g, and scalp (germ stock), 94.86 µg/g. The fine bran contained the highest concentration of niacin among these products.

Barton-Wright (1944) and Moran (1945) observed that niacin was concentrated mainly in the bran and endosperm immediately adjacent to the aleurone layer. Bailey (1944), Kent-Jones and Amos (1957) and Calhoun et al. (1960) reported that bran contained the highest niacin concentration, 266 to 325 µg/g, among wheat mill products. These values are higher than the value, 181.66 µg/g, in this study. Although, they stated that germ was poor in niacin content, 45.3 µg/g, the niacin concentration of scalp in this study was 94.86 µg/g. The niacin content of red dog reported here was substantially less than values, 80 to 120 µg/g, found by Andrews et al. (1942b) and Calhoun et al. (1960).

Teply et al. (1942) and Melnick et al. (1941) observed that

whole wheat contained 45 to 70 µg niacin per g wheat. Hard red winter wheat contains 43 µg/g according to Agriculture Handbook No. 8 (Watt and Merrill, 1963). These values are similar to the value found in this study. The niacin content of enriched flour was within the range of the enrichment standard, 34 to 44 µg/g.

Pantothenic Acid

The mean pantothenic acid content of the whole wheat, 14.56 µg/g was higher than that of flour, 7.28 µg/g, and red dog, 9.80 µg/g, but lower than that of fine bran, 39.42 µg/g, and scalp (germ stock), 21.31 µg/g. The fine bran had the highest concentration of pantothenic acid among these products.

Hinton et al. (1953) found that pantothenic acid was concentrated in the aleurone layer which was removed with the bran during the milling. Calhoun et al. (1960) observed that bran contained the highest pantothenic acid content, 39.1 µg/g, among wheat mill products. This was in agreement with the value, 39.42 µg/g, in the present study. The pantothenic acid content of the scalp in this study was higher than the value, 10.4 µg/g, in the study of Calhoun et al. (1960). The pantothenic acid content of red dog in the present study was substantially less than the value, 18.2 µg/g, in their findings.

The pantothenic acid content of hard winter wheat was indicated as 14.2 µg/g by Bailey (1944). The series of all classes of bread wheat listed by Bailey (1944) contained from 9.1 to 17.5 µg pantothenic acid per g wheat. The value of whole wheat in this study was in fair agreement with these values.

The pantothenic acid of flour was slightly higher than the values, 5.5 to 6.5 μg/g, indicated by Bailey (1944) and the value, 4.83 μg/g, observed by Calhoun et al. (1960).

It was interesting to notice that the ratio between pantothenic acid content of flour and wheat was 1:2. The milling loss was 66.5%. Calhoun et al. (1958) observed the milling loss of pantothenic acid was 51%.

Biotin

The mean biotin content of whole wheat, 0.084 μg/g, was higher than that of flour, 0.061 μg/g, and red dog, 0.063 μg/g, but much lower than that of fine bran, 0.371 μg/g, and scalp (germ stock), 0.242 μg/g. The biotin content of flour and red dog were almost the same. Bran contained the highest concentration of biotin among these products.

Calhoun et al. (1960) observed that bran had the highest biotin content, 0.44 μg/g, among wheat mill products. This value was higher than the value, 0.371 μg/g, in the present study. The biotin content of scalp in this study was higher than the value, 0.174 μg/g, in the study of Calhoun et al. (1960). The biotin content of red dog reported here was substantially less than the value, 0.250 μg/g, found in their study.

The biotin content of wheat was lower than the value reported by Calhoun et al. (1960), but higher than the value observed by Lampen et al. (1942). The biotin content of flour in this study was markedly higher than the values reported by Lampen et al. (1942) and Calhoun et al. (1958, 1960). The 51.3%

loss of biotin in milling wheat to flour was much lower than the value 85.6% in the study of Calhoun et al. (1958).

Folic Acid

The mean folic acid content of whole wheat, 0.431 µg/g, was higher than that of flour, 0.129 µg/g, but lower than that of red dog, 0.579 µg/g, fine bran, 0.901 µg/g, and scalp (germ stock), 1.197 µg/g. The scalp contained the highest concentration of folic acid among these products.

Calhoun et al. (1960) reported that germ had the highest folic acid content, 2.05 µg/g, among wheat mill products. This value was higher than the value of scalp, 1.197 µg/g, in the present study. This might be explained by the fact that the scalp used in this study was a combination of germ, flour and bran, while Calhoun et al. (1960) used pure germ.

Calhoun et al. (1960) observed that folic acid content of flour, wheat and bran were 0.11, 0.50 and 0.88 µg/g, respectively. These values were in fair agreement with the findings in the present study, but the folic acid content of the red dog was markedly lower than the value, 1.2 µg/g, in their study. The 79.9 loss of folic acid in milling wheat to flour agreed quite well with the value 74.5% in the study of Calhoun et al. (1958).

p-Aminobenzoic Acid

The p-aminobenzoic acid (PABA) content of the whole wheat, 1.84 µg/g, was much higher than that of flour, 0.24 µg/g, and slightly higher than that of red dog, 1.64 µg/g, but lower than

that of fine bran, 4.09 µg/g, and scalp (germ stock), 3.42 µg/g. The fine bran contained the highest concentration of PABA among these products. This observation agreed with the findings obtained by Calhoun et al. (1960). They reported that bran had the highest concentration of PABA, 14.8 µg/g, among wheat mill products. This value was much higher than the value, 4.09 µg/g, in the present study.

The PABA content of the scalp was higher than that of red dog in this study, but Calhoun et al. (1960) observed that red dog contained a higher PABA concentration than germ. The PABA content of the scalp in this study was in fair agreement with the value, 3.70 µg/g, in their findings, but the value of red dog was substantially lower than the value, 7.81 µg/g, in their study.

The PABA content of flour and whole wheat were lower than values found in the study of Calhoun et al. (1958, 1960). The 91.6% loss of PABA in milling wheat to flour was close to the 93% in the study of Calhoun et al. (1958).

Conclusions

Concentrations of riboflavin, niacin, pantothenic acid, biotin and PABA of fine bran were higher than those of whole wheat, enriched flour, red dog and scalp (germ stock). Scalp had the highest thiamine and folic acid content among these 5 products. Although, pure germ is rather low in niacin, pantothenic acid and biotin, the scalp was a good source of these vitamins. This

was attributed to the fact that the scalp was not pure germ, but a combination of germ, flour and bran. Bran apparently brought up the niacin, pantothenic acid and biotin content of scalp as compared to samples of pure germ tested by other workers. Red dog appeared to be a good source of thiamine, but was low in the other vitamins when compared to fine bran and scalp.

Thus, fine bran and scalp appear to be much better sources of the vitamins of B complex than whole wheat, enriched flour and red dog. Therefore, utilization of these mill streams in food for human beings should contribute markedly to dietary intake of B complex vitamins.

SUMMARY

Content of vitamins of the B complex in whole wheat, enriched flour and 3 mill streams (red dog, fine bran and scalp (germ stock)) was investigated. Thiamine was determined by thiochrome assay. The other 6 vitamins were determined by microbiological assay using the following organisms: Lactobacillus casei ATCC 7469 for riboflavin; Lactobacillus plantarum ATCC 8014 for niacin, biotin and pantothenic acid; Streptococcus faecalis ATCC 8043 for folic acid; and Neurospora crassa ATCC 9278 for p-aminobenzoic acid (PABA). Ninety percent confidence intervals on μ were calculated to estimate the true mean vitamin content of each product studied.

Whole wheat had the lowest thiamine and riboflavin content

of any of the products. Niacin, pantothenic acid, biotin and PABA concentrations of whole wheat were higher than those of enriched flour and red dog, but lower than those of fine bran and scalp. Folic acid content of whole wheat was higher than that of flour, but lower than that of red dog, fine bran and scalp.

Thiamine content of enriched flour was higher than that of whole wheat and similar to that of fine bran, but lower than that of red dog and scalp. Riboflavin content of enriched flour was higher than that of whole wheat and red dog, but lower than that of fine bran and scalp. Enriched flour had a higher niacin content than red dog, but lower than whole wheat, fine bran and scalp. Among the 5 products investigated, flour had the lowest concentration of pantothenic acid, folic acid and PABA. Biotin content of flour was lower than that of whole wheat, fine bran and scalp, but similar to that of red dog.

Red dog had a lower thiamine content than scalp, but higher than whole wheat, enriched flour and fine bran. Riboflavin content of red dog was higher than that of whole wheat, but lower than that of enriched flour, fine bran and scalp. Red dog had the lowest niacin content of any of the products. Red dog had less pantothenic acid and PABA than whole wheat, fine bran and scalp, but higher than flour. Biotin content of red dog was similar to that of flour, but lower than that of the other products. Folic acid content of red dog was higher than that of whole wheat and flour, but lower than that of fine bran and scalp.

Among the 5 products investigated, fine bran had the highest concentration of vitamins of the B complex, except thiamine and folic acid. Thiamine content of fine bran was higher than that of whole wheat and similar to that of enriched flour, but lower than that of red dog and scalp. Fine bran had a lower folic acid content than scalp, but higher than whole wheat, flour and red dog.

Scalp was highest among the 5 products in thiamine and folic acid. Scalp had a lower concentration of riboflavin, niacin, pantothenic acid, biotin and PABA than fine bran, but higher than whole wheat, enriched flour and red dog.

In general, fine bran and scalp appeared to be much better sources of vitamins of the B complex than whole wheat, enriched flour and red dog. Therefore, utilization of these mill streams in food for human beings should contribute markedly to dietary intake of B complex vitamins.

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APPENDIX

TABLE 3

Thiamine[1] content of whole wheat, enriched flour and 3 mill streams (on dry weight basis)

Assay No.	Whole wheat µg/g	Enriched flour µg/g	Red dog µg/g	Fine bran µg/g	Scalp µg/g
I.	3.13	5.70	9.60	5.53	10.66
II.	3.13	5.67	9.47	5.78	10.66
III.	3.16	5.70	9.67	5.66	11.03
$\bar{x}$[2]	3.14	5.69	9.58	5.66	10.78
$CI_{.90}$ on µ[3]	3.10 to 3.18	5.65 to 5.73	9.37 to 9.79	5.40 to 5.92	10.34 to 11.22

[1]As thiamine hydrochloride.

[2]Mean.

[3]Confidence interval at 90% level on µ.

TABLE 4

Riboflavin content of whole wheat, enriched flour and 3 mill streams (on dry weight basis)

Assay No.	Whole wheat µg/g	Enriched flour µg/g	Red dog µg/g	Fine bran µg/g	Scalp µg/g
I.	2.81	3.78	3.17	8.42	4.38
	2.65	4.26	3.34	10.57	4.94
	2.68	4.37	3.72	9.45	4.76
	2.72	4.54	4.06	9.26	5.32
	2.72	4.88	4.50	9.07	
II.	2.96	4.09	3.06	10.29	4.38
	2.72	4.37	3.17	10.75	4.76
	2.92	4.48	3.72	9.82	4.66
	2.85	4.99	4.06	10.19	5.22
	2.92	4.38	4.50	9.54	
III.	2.03	4.26	3.17	8.51	4.38
	2.14	4.20	3.17	8.98	4.94
	2.44	4.20	3.61	9.16	4.94
	2.68	4.71	4.00	9.07	4.94
	2.96	4.88	4.50		
IV.	2.21	3.78			4.20
	2.48	3.76			4.85
	2.72	4.06			4.94
	3.06	4.09			4.94
		4.71			
V.	1.02				4.48
	1.93				4.66
	2.03				4.76
	2.07				4.57
	1.97				4.85
VI.					4.20
					4.94
					5.32
					4.85
					5.32
$\bar{x}$[1]	2.49	4.36	3.72	9.51	4.79
CI.90 on µ[2]	2.38 to 2.60	4.22 to 4.51	3.45 to 3.98	9.23 to 9.78	4.67 to 4.90

[1]Mean.

[2]Confidence interval at 90% level on µ.

TABLE 5

Niacin content of whole wheat, enriched flour and 3 mill streams (on dry weight basis)

Assay No.	Whole wheat µg/g	Enriched flour µg/g	Red dog µg/g	Fine bran µg/g	Scalp µg/g
I.	52.02	46.27	40.35	187.97	111.51
	50.62	44.87	40.35	206.46	111.51
	54.84	47.67	40.35	210.73	112.84
	53.43	45.43	41.36	221.50	122.66
		47.67	39.44		
II.	53.43	44.87	40.35	208.29	97.57
	53.99	46.55	38.34	215.40	105.93
	50.62	49.07	38.34	210.73	105.93
	52.30	47.67	40.35	218.45	105.93
		56.08	41.36		
III.	52.32	42.06	38.34	209.92	100.36
	50.17	44.31	38.34	205.85	94.78
	53.59	43.18	38.34	205.24	84.74
	55.23	42.06	41.36	213.37	81.40
		40.94			
IV.	54.87	43.47	37.02	216.62	143.17
	55.28	42.62	38.13	211.34	137.15
	52.32	44.14	37.02	216.42	142.17
	55.12	40.94	37.12	221.50	
V.			38.13		119.24
			41.97		120.25
			37.93		110.06
			38.34		109.04
			35.31		102.93
$\bar{x}$[1]	53.13	45.26	39.04	211.24	110.30
$CI_{.90}$ on μ[2]	52.36 to 53.91	44.25 to 46.26	38.51 to 39.58	207.73 to 214.74	108.18 to 112.42

[1]Mean.

[2]Confidence interval at 90% level on µ.

TABLE 6

Pantothenic acid[1] content of whole wheat, enriched flour and 3 mill streams (on dry weight basis)

Assay No.	Whole wheat µg/g	Enriched flour µg/g	Red dog µg/g	Fine bran µg/g	Scalp µg/g
I.	15.92	6.11	9.10	29.07	22.40
	15.41	7.43	10.67	46.17	19.43
	14.65	7.60	11.86	47.51	26.04
	15.69	7.21	12.13	46.51	28.00
		7.49	12.41	46.51	
II.	17.94	6.79	9.10	40.24	17.92
	14.65	8.10	10.67	47.74	19.43
	14.51	7.94	11.86	46.94	24.36
	16.31	7.49	12.46	45.84	28.00
	16.20	7.49	12.88	46.51	
III.	16.68	7.01	10.48	55.90	29.68
	16.31	8.22	11.86	52.21	27.61
	16.42	8.41	11.86	49.19	22.96
	16.48	8.19	11.58	45.84	29.68
				48.07	28.56
IV.	16.03	7.57	8.82	50.31	20.16
	15.94	11.22	9.93	44.72	19.60
	16.31	10.66	11.03	39.13	21.28
	15.94	11.78	11.86	45.84	25.76
		10.36	15.99	42.48	24.86
V.	18.28	7.49			22.40
	19.12	7.85			25.76
	19.26	8.52			26.88
	19.99	8.13			31.36
VI.	19.68	9.53			19.60
	19.12	10.66			22.40
	18.56	9.81			25.20
	17.99	9.53			26.88
					32.81
$\bar{x}$[2]	16.94	8.47	11.40	45.84	24.78
CI.90 on µ[3]	16.66 to 17.21	8.18 to 8.75	10.68 to 12.12	43.85 to 47.82	23.5 to 26.05

[1]As calcium pantothenate.

[2]Mean.

[3]Confidence interval at 90% level on µ.

TABLE 7

Biotin content of whole wheat, enriched flour and 3 mill streams (on dry weight basis)

Assay No.	Whole wheat mμg/g	Enriched flour mμg/g	Red dog mμg/g	Fine bran mμg/g	Scalp mμg/g
I.	104.05	72.91	67.09	447.06	285.32
	100.11	80.20	78.69	432.84	294.49
	101.80	80.76	78.69	426.74	288.38
	107.42	80.20	74.65	447.06	277.17
				424.71	
II.	86.05	64.50	67.59	398.29	280.23
	89.99	66.18	74.15	394.23	294.49
	98.99	78.52	78.69	412.52	275.13
	104.05	72.91	73.14	424.71	311.81
				445.03	
III.	92.80	75.72	67.59	432.84	298.57
	93.92	80.20	69.61	406.43	254.75
	101.24	72.91	72.64	426.74	282.26
	104.05	75.15	73.14	455.19	274.11
		78.52	85.75	459.26	274.11
IV.	90.34	71.23	60.53	432.84	295.51
	94.94	65.06	72.13	409.47	259.85
	98.00	65.06	67.59	438.94	295.51
	89.83	70.67	75.16	455.19	274.11
			79.19	451.13	255.77
V.		56.08			
		67.30			
		64.50			
		65.06			
		66.18			
$\bar{x}$[1]	97.35	71.36	73.11	431.06	281.75
$CI_{.90}$ on μ[2]	94.88 to 99.82	69.76 to 72.95	70.85 to 75.38	423.91 to 438.21	275.28 to 288.23

[1] Mean.

[2] Confidence interval at 90% level on μ.

TABLE 8

Folic acid content of whole wheat, enriched flour and 3 mill streams (on dry weight basis)

Assay No.	Whole wheat µg/g	Enriched flour µg/g	Red dog µg/g	Fine bran µg/g	Scalp µg/g
I.	0.461	0.150	0.604	1.237	1.065
	0.553	0.135	0.705	1.150	1.278
	0.571	0.175	0.705	1.140	1.420
	0.482	0.159	0.745	1.099	1.562
	0.435	0.152	0.735	1.079	1.542
II.	0.435	0.116	0.529	1.079	1.115
	0.538	0.137	0.690	1.069	1.318
	0.533	0.147	0.755	0.997	1.521
	0.435	0.146	0.735	1.099	1.582
	0.461	0.163	0.720	1.180	1.552
III.	0.320	0.127	0.654	0.946	1.572
	0.476	0.130	0.680	0.936	1.349
	0.533	0.144	0.665	1.018	1.440
	0.533	0.140	0.796	0.936	1.582
	0.551	0.145	0.780	1.120	1.095
IV.	0.448	0.123	0.705	0.967	1.572
	0.528	0.121	0.644	0.957	1.349
	0.538	0.137	0.685	0.936	1.369
	0.594	0.145	0.644	1.120	1.460
			0.654		1.095
V.	0.384	0.147	0.654	0.916	
	0.487	0.165	0.629	0.916	
	0.558	0.178	0.574	0.977	
	0.604	0.197	0.554	0.946	
	0.579	0.210	0.604	1.262	
VI.				0.977	
				1.017	
				0.957	
				0.997	
				1.374	
$\bar{x}$[1]	0.502	0.150	0.674	1.048	1.392
CI.90 on μ[2]	0.476 to 0.528	0.144 to 0.155	0.653 to 0.695	1.013 to 1.084	1.316 to 1.468

[1]Mean.

[2]Confidence interval at 90% level on μ.

TABLE 9

p-Aminobenzoic acid content of whole wheat, enriched flour and 3 mill streams (on dry weight basis)

Assay No.	Whole wheat µg/g	Enriched flour µg/g	Red dog µg/g	Fine bran µg/g	Scalp µg/g
I.	2.05	0.28	2.27	6.11	4.72
	2.18	0.29	2.09	5.09	4.21
	2.13	0.30	2.11	4.58	4.21
	2.23	0.29	2.01	4.73	4.11
	2.23	0.30		5.70	
	2.18				
	2.05				
II.	2.38	0.28	2.19	5.04	4.31
	2.31	0.30	1.91	4.48	3.70
	2.10	0.29	1.71	4.48	3.80
	2.05	0.27	1.76	5.19	3.90
		0.25	1.66	5.09	
III.	2.38	0.25	2.19	5.60	3.96
	2.20	0.27	1.91	4.94	3.70
	1.87	0.27	1.81	4.78	3.68
	1.84	0.25	1.76	4.48	3.55
	1.87	0.27	1.71	4.12	
IV.	2.38	0.30	2.01	5.60	4.26
	2.31	0.27	1.81	4.53	3.70
	2.00	0.30	1.86	4.02	3.96
	1.97	0.25	1.81	4.07	3.90
			1.74	4.12	
V.		0.27		5.34	
		0.30		4.53	
		0.29		3.97	
		0.27		4.27	
		0.27		4.17	
$\bar{x}$[1]	2.14	0.28	1.91	4.76	3.98
$CI_{.90}$ on μ[2]	2.07 to 2.20	0.27 to 0.28	1.85 to 1.98	4.57 to 4.95	3.87 to 4.09

[1]Mean.

[2]Confidence interval at 90% level on μ.

B COMPLEX VITAMINS IN WHOLE WHEAT, ENRICHED FLOUR AND THREE MILL STREAMS

by

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Content of vitamins of the B complex in whole wheat, enriched flour and 3 mill streams (red dog, fine bran and scalp (germ stock)) was investigated. Thiamine was determined by thiochrome assay. The other 6 vitamins were determined by microbiological assay using the following organisms: Lactobacillus casei ATCC 7469 for riboflavin; Lactobacillus plantarum ATCC 8014 for niacin, biotin and pantothenic acid; Streptococcus faecalis ATCC 8043 for folic acid; and Neurospora crassa ATCC 9278 for p-aminobenzoic acid (PABA). Ninety percent confidence intervals on u were calculated to estimate the true mean vitamin content of each product studied.

Whole wheat had the lowest thiamine and riboflavin content of any of the products. Niacin, pantothenic acid, biotin and PABA concentrations of whole wheat were higher than those of enriched flour and red dog, but lower than those of fine bran and scalp. Folic acid content of whole wheat was higher than that of flour, but lower than that of red dog, fine bran and scalp.

Thiamine content of enriched flour was higher than that of whole wheat and similar to that of fine bran, but lower than that of red dog and scalp. Riboflavin content of enriched flour was higher than that of whole wheat and red dog, but lower than that of fine bran and scalp. Enriched flour had a higher niacin content than red dog, but lower than whole wheat, fine bran and scalp. Among the 5 products investigated, flour had the lowest concentration of pantothenic acid, folic acid and PABA. Biotin content of flour was lower than that of whole wheat, fine bran and scalp, but similar to that of red dog.

Red dog had a lower thiamine content than scalp, but higher than whole wheat, enriched flour and fine bran. Riboflavin content of red dog was higher than that of whole wheat, but lower than that of enriched flour, fine bran and scalp. Red dog had the lowest niacin content of any of the products. Red dog had less pantothenic acid and PABA than whole wheat, fine bran and scalp, but higher than flour. Biotin content of red dog was similar to that of flour, but lower than that of the other products. Folic acid content of red dog was higher than that of whole wheat and flour, but lower than that of fine bran and scalp.

Among the 5 products investigated, fine bran had the highest concentration of vitamins of the B complex, except thiamine and folic acid. Thiamine content of fine bran was higher than that of whole wheat and similar to that of enriched flour, but lower than that of red dog and scalp. Fine bran had a lower folic acid content than scalp, but higher than whole wheat, flour and red dog.

Scalp was highest among the 5 products in thiamine and folic acid. Scalp had a lower concentration of riboflavin, niacin, pantothenic acid, biotin and PABA than fine bran, but higher than whole wheat, enriched flour and red dog.

In general, fine bran and scalp appeared to be much better sources of vitamins of the B complex than whole wheat, enriched flour and red dog. Therefore, utilization of these mill streams in food for human beings should contribute markedly to dietary intake of B complex vitamins.