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Title: THE INFLUENCE OF VEGETABLE FIBERS ON MINERAL BALANCE IN THE RAT

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Dietary fiber has been suggested as interfering with mineral utilization. The influence of broccoli florets and wastes, cauliflower heads and wastes, wheat bran and Metamucil on mineral uptake by the rat was determined using balance studies. All results were compared to control animals fed diets containing 10% hydrolyzed cellulose. Utilization of Ca, Mg, Cu, Zn and Fe were determined to be 62, 33, 23, 42, and 52% respectively for control animals. The uptake of Mg, Zn and Fe were significantly reduced to 12, 16 and 18% respectively from broccoli florets. Broccoli wastes decreased absorption of Zn to 14% and created a negative iron balance. Cauliflower heads improved Zn assimilation to 61%. Cauliflower wastes depressed Ca absorption to 26% and caused Mg balance to be negative. Wheat bran improved utilization of Ca and Zn to 88 and 54% respectively. Metamucil increased uptake of Ca to 88% and Mg to 53%. Copper balance was unaffected by any test diet. To better

understand results of balance trials, the bioavailability of Fe from some sources was measured. The relative biological values (RBV) of endogenous Fe in broccoli, cauliflower, Brussel sprouts and wheat bran were determined to be 93, 88, 83, 98% respectively. These values were obtained using rats fed control diets containing 5% hydrolyzed cellulose and ferrous sulfate (RBV = 100). The efficiency - true bioavailability of utilizing dietary Fe is approximately one-half of the RBV. These values agree well with apparent uptake of iron by animals in the balance trials for all diets except broccoli. This exception may be due to differences in the amount and source of broccoli fed. High dietary levels of broccoli florets and wastes appear to interfere with utilization of Fe and Zn. Cauliflower wastes depress absorption of Ca and Mg. With the exceptions noted, dietary fiber from plant sources does not appear to decrease the utilization of minerals by the rat. The nature of mineral complexes in plants may be more important in defining the degree of its absorption.

The Influence of Vegetable Fibers on Mineral Balance in the Rat

by

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INTRODUCTION

Recent epidemiological evidence has indicated an increased incidence in the number of diseases associated with Western civilization. Changes in food consumption habits, especially a reduced intake of cereals and other sources of dietary fiber, have been suggested as contributing factors. Diseases of the gastrointestinal tract and heart are highly correlated with the lower fiber intakes observed in North American and European populations. These conditions are generally absent in South Africa and other cultures where fiber intake is high. The possible beneficial nature of fiber in the diet has resulted in physicians, nutritionists and other health professionals advocating its increased consumption.

At present, there is no concise definition for fiber. It consists of a complex group of substances that differ in chemical and morphological structure and physiological effect. The heterogeneity of plant fibers makes the design and comparison of experiments difficult. Experimental results may vary with the fiber composition of a food, which is dependent on the age, species and source of the plant. The use of different animal models give observations that

are often difficult to correlate with man. An initial attempt to standardize a fiber source has been made. Wheat bran of uniform composition and in adequate supply for comparative analytical and nutritional studies is now available through the American Association of Cereal Chemists (AACC).

Most evidence strongly indicates the value of fiber in the diet. The exceptions are studies indicating that diets high in fiber may present problems with mineral availability and balance. Phytate has long been believed to be the agent responsible for impaired mineral utilization from plants. The possible negative effect of fiber on mineral utilization must be resolved. Most of the research on this problem has focused on cereal fibers and relatively few studies have dealt with vegetable fibers. This is somewhat surprising, considering the magnitude and importance of vegetables in man's diet.

The Pacific Northwest produces an abundance of vegetables. The waste portion of some of these plants are high in fiber and may represent a valuable and readily available source for food use. Before using these materials, a number of economic and feasibility considerations must be investigated. One criteria would be to determine their nutritional contribution and influence on mineral utilization.

Broccoli, cauliflower and Brussel sprouts are examples of plants having a high amount of waste with substantial fiber content. The level of fiber and minerals in these

plant foods and their effect on mineral balance and bio-availability in the rat was the object of this study.

LITERATURE REVIEW

Fiber Nomenclature

Although the role of fiber in nutrition and health has received much attention through the years, there is no agreement on a definition. Originally, fiber was defined to be the indigestible portion of foods. A fiber analysis was intended to identify the non-nutritive value of a food stuff (Cummings, 1976) and thereby assess the nutritive quality of animal feeds by difference. This avoided the need for a more costly proximate analysis. As our knowledge of the physiological and nutritional role of fiber increased, it became apparent that a better definition was necessary. VanSoest (1973) has reviewed the criticisms of using the term "crude fiber".

Citing the need for more nutritionally correct nomenclature, Trowell (1972) proposed the term "dietary fiber". This was defined as the portions of plant cell walls resistant to degradation by endogenous secretions of the human gastrointestinal tract. The partial digestion of some major fiber components leaves a degree of compromise in this definition. Godding (1976) suggested the term "edible fiber" replace "dietary fiber". This term would include all components defined by dietary fiber as well as algal polysaccharides, chitins from crustaceans and fungi, partially

synthetic, undigested, fiber-like polysaccharides (e.g., methylcellulose), and undigested, fiber-like materials of animal origin (e.g., aminopolysaccharides present in connective tissues and internal organs). At present, dietary fiber is the most widely used term and can be inferred to imply a compilation of the above listed terms. This definition of dietary fiber is implied during the course of this study.

Fiber Components

The polysaccharides of dietary fiber are the celluloses, hemicelluloses, pectins, gums and mucilages. The non-polysaccharide components include lignin, cuticular substances and products of non-enzymatic browning, as well as proteins, fats and carbohydrates whose digestibility is impaired due to their association with the plant cell walls. It is this diversity of components that continually leads to the lack of an exact definition for dietary fiber.

The interests of nutritional and health scientists have focused largely on the roles of cellulose, hemicellulose, lignin and pectin in our diet.

Cellulose is perhaps the best known and most widely distributed fiber component. Its primary structure is that of an unbranched polymer of anhydro-D-glucopyranose units joined by (1-4) glucosidic bonds in a β-configuration. X-ray

diffraction studies have shown that the molecule folds into a flat, ribbon-like structure (Rees, 1967) with an helical conformation. There may be from 100 to 1,000,000 individual glucose residues per molecule (Lang and Briggs, 1976). The structure is stabilized by extensive hydrogen bonding, both inter- and intramolecular, which is the basis for the crystalline structure.

Because of the linear, unbranched nature of the polymers, they can associate into bundles of parallel chains to form fibrils. These fibrils are cemented together by a matrix of hemicelluloses, pectins and extensin. Extensin is a protein which is covalently attached to the cellulose fibrils. Like its animal-tissue counterpart, collagen, extensin is rich in hydroxyproline residues (Lehninger, 1975). Although cellulose is insoluble in water, it is capable of binding great amounts of water. Bacterial cellulases in the large intestine account for its apparent digestibility in man. The bacterial breakdown of cellulose in the colon is estimated to be about 40% (Cummings, 1976).

Hemicelluloses are not structurally related to cellulose. At least 250 known polymers exist which contain a mixture of pentose and hexose sugars. Many of these are highly branched. The number of possible configurations appear to be unlimited. Hemicellulose molecules are generally much smaller than those of cellulose, with between 150 and 200 sugar residues per molecule. They are more

amorphous than cellulose. Their water holding capacity is assumed to be high, although experimental evidence to verify this is lacking. Hemicelluloses are difficult to isolate from the cell wall without serious structural modification. A research grade of pure hemicellulose is not currently available.

The hemicelluloses are subdivided into groups depending on their biochemical and biophysical characteristics. Polyuronide hemicelluloses are so named because of their high uronic acid content. Others include A and B hemicelluloses. Their designation depends on their precipitation by either dilute acid (A) or by dilute acid and subsequent addition of alcohol (B) (Cummings, 1976).

Digestibility of hemicelluloses by man has been estimated to be about 55% (Cummings, 1976). This degradation appears to be a result of both endogenous and bacterial enzymatic activity.

Lignin is a part of the plant cell wall which provides structural rigidity. It is defined as a polyphenolic polymer containing phenylpropane structural units. Most studies on the chemical structure and properties have been conducted on lignin derived from wood (Hartley, 1978). Molecular weights in the thousands have been reported (Freudenberg, 1965). The lignin structure in annual plants is also based on phenylpropane units, but molecular weights are probably lower than that of wood lignin (Hartley, 1978).

Lignin is determined as that portion of foodstuffs undissolved in 72% (v/v) H_2SO_4. This would include cuticular substances, products of the Maillard reaction and tannin-protein complexes. Lignin and its breakdown products are highly indigestible (Gordon, 1978).

Although present in smaller amounts than other cell wall components, the pectins are common to all plant cell walls and are present in the intercellular layers as well. Their structures are not well defined. Molecular weights range from 60,000 to 90,000 (Worth, 1967). Most are heteropolysaccharides containing mainly D-galacturonic acid, D-galactose, L-arabinose, D-xylose, L-rhamnose, and L-fucose (Cummings, 1976). In general, the molecules are highly water soluble and capable of forming viscous gels. This property has long been known and utilized advantageously by the food industry.

The fate of pectins in the gastrointestinal tract has not been thoroughly investigated. Only about 5% are recovered in the feces (Wersch and Ivy, 1941). It is assumed that digestion takes place primarily in the colon by bacterial degradation.

Gums and mucilages are not strictly cell wall components. They appear to have similar structural, physical and chemical properties. Their structure makes them resistant to enzymatic hydrolysis of digestion (Lang and Briggs, 1976). For these reasons, they are classified under the general

heading of dietary fiber.

Plant gums are sticky exudates formed at the site of injury to plants. They may be obtained by deliberate incision of plants or trees (Cummings, 1976). Commercially, they are used as emulsifiers, thickeners and stabilizers in the food industry.

Biochemically, gums are complex, highly branched, uronic acid containing polymers. These are mainly composed of glucuronic and galacturonic acids.

Mucilages are usually mixed with strong polysaccharides of plant seeds. They function as water binders to protect seeds against dessication (Aspinall, 1970). Structurally, they resemble gums but are not classified with them because of their occurrence in a distinct part of the plant. Many mucilages are neutral polysaccharides although some acidic ones exist. They are used as thickeners by the food industry and are utilized by the pharmaceutical industry in laxatives and toothpaste.

Cuticular substances may be divided into two groups: waxes and cutins. Waxes include a mixture of paraffins, aliphatic acids, and alcohols. These form the waterproof coating on fruits, seeds, and leaves. Cutines are polymers of mono, di-, tri-, and polyhydroxy fatty acids. These complex molecules are formed by ester linkages between hydroxyl and carboxyl groups (Cummings, 1976). Cuticular substances are extremely resistant to digestion and are believed to

impair the digestibility of other cell wall components (VanSoest and McQueen, 1973).

Methods of Fiber Analysis

Fiber nomenclature is often defined by its methods of analysis. Historically, the Weende system of crude fiber analysis has been used to estimate the indigestible matter in foods. The inadequacies of this procedure have been well documented (Paloheimo, 1953; VanSoest, 1975).

Basically, this system of analysis consists of a nitrogen determination, an ether extraction, a total ash and crude fiber analysis. Crude fiber is the material remaining after treating a foodstuff with sequential extractions of dilute acid, dilute alkali and alcohol. The difference between 100 and the sum of nitrogen x 6.25, the ether extraction, ash and crude fiber is called the nitrogen free extract. This resulting quantity is assumed to contain the soluble carbohydrates.

The sequence of steps involved in a crude fiber analysis results in the recovery of 50 -90% of the cellulose and about 20% of the insoluble hemicelluloses. Lignin is only measured at 10 -40% of its actual level (Cummings, 1976). The term "crude fiber" was an appropriate description. It can easily be seen why this value is totally inaccurate.

Other procedures exist for determining the fiber content of foods. Most are not without some fault or

shortcoming. Southgate et al. (1976) recommends measuring the individual sugar components of fiber. In this procedure, pigments, lipids and soluble sugars are extracted from a freeze dried sample by refluxing with hot aqueous alcohol. Pectic substances are removed with 0.5% w/v aqueous ammonium oxalate solution. Hemicelluloses are dissolved by sequential extractions with 5% and 24% w/v KOH. These yield the A and B hemicelluloses respectively. The residue remaining after these steps contains the cellulose and lignin. Hydrolysis with 72% w/v H_2SO_4 leaves lignin. Cellulose is determined by difference. Fractions containing the soluble sugars, pectic substances or hemicelluloses can be analyzed for hexoses, pentoses and uronic acids. This gives an extremely accurate and valuable description of dietary fiber in foods. The time necessary for analysis limits its usefulness as a routine analytical procedure.

Because fiber is assumed to represent substances resistant to animal digestive enzymes, it was only natural that an enzymatic method for fiber measurement would be developed. Thomas (1972) designed in vitro procedures that measured the insoluble residues after peptic and amylitic digestion. These methods are time consuming and difficult to reproduce between laboratories (VanSoest, 1978). Another drawback is the lack of information it yields regarding the composition of the undigested residue. For these reasons, enzymatic determinations of fiber are not widely used.

The methods of Goering and VanSoest (1970) were used in this study. This system provides for the determination of two fractions: neutral detergent fiber (NDF) which includes cellulose, hemicellulose, lignin; acid detergent fiber (ADF) which yields cellulose and lignin. Lignin is determined as that part of the ADF fraction insoluble in 72% (v/v) H_2SO_4.

The NDF analysis is based on the ability of sodium dodecyl sulfate (SDS) to dissolve polysaccharides, proteins and nucleic acids and solubilize lipids in a solution at neutral pH. Cetyl trimethyl ammoniom bromide serves the same purpose in the ADF method. The acid pH of this detergent solution also dissolves those hemicelluloses that are insoluble at neutral pH. Neutral detergent fiber is the most complete estimate of total dietary fiber.

The measurement of ADF is the method of choice when cellulose and lignin values are of interest (Southgate, 1976). The NDF procedure is not well suited for foods that contain high levels of starch or lipids. Neutral detergent fiber does not provide any information regarding the types of noncellulosic polysaccharides present.

Food Sources of Dietary Fiber

Dietary fiber can be obtained from a wide variety of foods. Fruits and vegetables, because of their high water content appear to contain little fiber, but it represents a substantial proportion of their dry weight. Starchy

vegetables and potatoes can supply large amounts of fiber (3.5 g/100 g potato) when eaten in quantity. Vegetables contain very little lignin. Fruits generally contain less than 1% lignin, but significant quantities are found in those containing lignified seeds (e.g. strawberry), or lignified flesh (e.g., pears). Fruits are usually rich in pectin.

Substitution of wholemeal flour for white flour and inclusion of wheat bran in the diet can greatly increase total intakes of cellulose, hemicellulose and lignin. Cereal fibers are easily obtained through the many high fiber breakfast cereals currently available.

The research arm of the food industry has responded to consumer interest by developing fiber-rich breads. Powdered, food-grade cellulose is the fiber component most often added to these products. Cellulose and methylcellulose are recognized food additives, often used as thickeners in foods.

Incidental sources of fiber include starch analogs and gums used as food texture modifiers. Pectins are added to jams and jellies. Mucilages are medically recommended for their laxative properties.

Many potential sources of dietary fiber for food supplementation have not been investigated. High levels of fiber are often concentrated in plant materials that are considered wastes and relegated as animal feeds (Andres, 1977). For example, broccoli and cauliflower plants yield

about 25% edible and 75% waste materials. These plants may provide a fiber with qualities (e.g., laxative, altered transit time, cholesterol lowering capacity) superior to fibers currently in use.

Fiber in the Gastrointestinal Tract

It has been suggested that fiber behaves as a sponge with both fibrous and amorphous characteristics (Eastwood and Kay, 1979). The physiological action of this sponge, as it passes along the gastrointestinal tract are dictated by the physiochemical properties of its components (e.g., gel formation, water holding, cation exchange, antioxidant and digestibility).

Depending on the capacities of the fiber for gel formation and water absorption, transit time may increase or decrease in different parts of the gastrointestinal system. In the small intestine, the physical and chemical properties of the sponge matrix may be modified by pH, osmotic conditions and electrolyte concentration. Fiber is largely resistant to digestion by the enzymes of the small intestine and consequently, structural changes are relatively minor. Most fibers swell in the aqueous medium of the small intestine, but certain fibers (e.g., pectins and gums) form viscous gels characterized by cross-linkages (Rees, 1967). Soluble materials may become trapped within the gel

structure. Fiber may thus reduce the rate of diffusion towards the absorptive site.

Fiber also has a cation exchange capacity due to carboxyl groups on the sugar residues. Essential minerals may be bound to fiber and escape absorption in the upper intestine (Reinhold et al., 1976).

In the colon, bacteria are capable of degrading fiber. The resulting changes brought about by the breakdown of fiber in the colon might alter microbial populations (Finegold and Sutter, 1978). These changes have been suggested as possible factors in the etiology of colon cancer (Walker, 1978).

Lignin may act as a free radical scavenger by virtue of its reducing phenolic groups. This is potentially important because free radical or other oxidative processes may have a role in carcinogenisis (Eastwood and Kay, 1979). Lignin has a complex, cross-linked, three dimensional structure (Southgate, 1976) that is more resistant to enzymatic degradation in the colon than the linear cellulose and hemicellulose molecules.

Nutritional and Health Implications of Dietary Fiber

The laxative effects of wheat bran have been known since the time of Hipprocrates. In the 1940's it was believed brown breads were slimming and increased satiety, compared to white breads, which were fattening (McCance and

Widdowson, 1942). Kellogg (1923) advocated the inclusion of bran in American diets and stimulated fiber-related research. Most of these early investigations centered on the laxative action of wheat bran (Cowgill and Sullivan, 1933). Concurrent research in Britain reported the successful use of wheat bran in the treatment of constipation, hemmorrhoids, colitis and irritable colon (Dimock, 1936; 1937).

In India, McCarrison (1936) reported better health among those people who ate whole wheat bread, legumes and fruit rather than diets based on polished rice. These observations came before vitamins had been identified.

McCance and Walsham (1948) demonstrated that high fiber cereals decreased total energy absorption while low fiber cereals had the opposite effect. At the time, this information was used to promote the benefits of white bread. In retrospect, it might appear to constitute a prescription for obesity.

In South Africa, changes in food consumption habits, especially reduced intakes of fiber, were found to be highly correlated with the increased incidence of gastrointestinal disorders (e.g., bowel motility, appendicitis, gallstones), diabetes, atherosclerosis and coronary heart disease (Walker, 1966; 1974). In Britain, Cleave et al. (1966), Burkitt (1973), Burkitt et al. (1972, 1974), Painter (1969), and Trowell (1973) expanded this hypothesis to include the emergence and increasing incidence of many other diseases:

obesity, diverticular disease, colonic cancer, varicose veins, hemorrhoids and hiatus hernia.

The implication of fiber depletion as a cause of heart disease interested many workers. Kirtchevsky (1964) called attention to the lack of agreement of results conerning the atherogenicity in rabbits of cholesterol-free diets containing saturated fats. A review of available data revealed that feeding saturated fats with a semipurified diet resulted in significant atherosclerosis, whereas the same fats fed with commercial rabbit diets resulted in little or no atherosclerosis. He surmised that some other dietary component (e.g., carbohydrate, roughage or mineral) present in the commercial diet was exerting a protective effect. Similar evidence was reported for rats (Kritchevsky et al., 1973). Available evidence suggests that fiber was the beneficial material.

The effects of dietary fiber on cholesterol metabolism seem to be linked, at least in part, to fiber's effect on bile acid metabolism. Portman (1960) has reviewed several experiments on the effects of diet on bile acid metabolism. When compared to rats fed a commercial laboratory ration, rats fed semipurified diets containing either no fiber or cellulose excreted much less cholic acid and β-hydroxy sterols. Another fiber associated material, pectin, has been shown to be responsible for a hypocholesteremic response linked to increased bile acid excretion and reduced

cholesterol absorption (Leveille and Sauberlich, 1966). Alfalfa and cellulose have been shown to increase bile acid excretion (Kritchevsky et al., 1974; 1975).

Eastwood and Boyd (1967) found that significant quantities of bile acids were bound to nonabsorbable materials in the small intestine. This was also observed with in vitro experiments (Eastwood and Hamilton, 1968). Lignin was shown to actively bind bile salts from phosphate buffer solutions. Similar observations were reported by Kritchevsky and Story (1974). This information suggested fiber inhibited cholesterol absorption by binding bile salts. Binding would result in a failure to form micelles essential for cholesterol absorption. This, in turn, would increase bile acid excretion, effecting an increase in bile acid synthesis from endogenous cholesterol to replace the lost bile salts. Both events would reduce blood cholesterol.

Eastwood (1974) has theorized that fiber in the large intestine results in a physiological chromatography column whose gel filtration and ion exchange properties could influence bile acid absorption and hence metabolism. With the magnitude of information indicating the binding and chelating properties of fibers on lipid components, similar effects on minerals would be expected.

Various nutrient deficiencies, including minerals, have long existed among and within world populations. The striking observation only realized in the early 1970's, is the

apparent correlation with mineral deficiencies and diets high in plant phytates and/or fiber. To explain or refute a cause-effect relationship between these dietary components and poor mineral nutriture is the subject of intense investigation.

The Phytate Hypothesis

Breads made with high extractions flours were initially suspected as the cause of hypogonadal dwarfism, a symptom of Zn deficiency, observed in adolescent Iranians (Reinhold, 1975). High extraction flours are those which retain many of the original grain components. Although the diets were sufficient in all minerals to fulfill daily requirements (Malèki, 1972; Haghshenass et al., 1972), Zn deficiencies were apparent. Subsequent observations indicates these populations to be marginally lacking in Fe (Haghshenass et al., 1972) and Ca (Reinhold, 1976). Interference with absorption of bivalent metals was generally attributed to phytates remaining in high extraction flours.

Phytic acid was first described by Pfeffer in 1872. Its exact structure (Figure 1) was only recently established (Johnson and Tate, 1969). It occurs primarily in plant tissues such as seeds and whole grains (deBoland et al., 1975). The large number of phosphoric radicals in its structure allows phytic acid to form simple salts with one metal, or mixed salts with several metals in the same molecule.

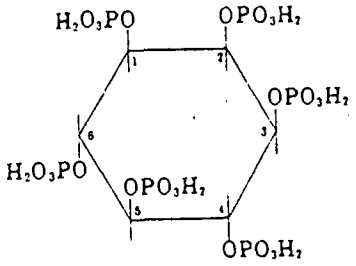

Figure 1. Structure and configuration of phytic acid.

Salts of Ca, Fe, Mg, Zn, and Cu are practically insoluble.

Phytic acid and phytates can be decomposed by prolonged exposure to heat treatment, acid hydrolysis, or enzymatic action or phytase. Pillegi (1959) discovered that rats possess an intestinal phytase. Wheat bran also contains a phytase (Ranhotra and Loewe, 1975). The net contribution of these two phytases on mineral absorption is unknown.

Reinhold et al. (1975) investigated the in vitro binding of Ca and Zn by wheat bran and wholemeal breads. A portion of these minerals was released by the action of phytase. However, totally dephytinized wheat bran and wholemeal breads showed enhanced ability to bind minerals. Southgate and Durnin (1970) and Ismail-Beigi et al. (1977) also demonstrated increased mineral binding ability in dephytinized breads. It was becoming apparent that the phytate hypothesis did not totally explain the observed disturbances in mineral metabolism.

The Mineral Binding Properties of Fiber

A number of experiments have been designed to test the *in vitro* cation binding capacity of dietary fibers. Reinhold *et al.* (1975) observed a downward drift in pH when Zn was added to suspensions of bread, bran or cellulose. This suggested that adsorption involved an ion exchange process. Such an exchange had been described by Belford *et al.* (1959) for Cu and was attributed to the presence of free carboxyl groups. Reed (1973) demonstrated the prevention of Ca binding to polyglucose matrices as a result of acetylation.

McConnell *et al.* (1974) reported cation exchange capacities ranging from 0.6 mequiv./g acetone dried powder for pears to 3.1 mequiv./g for lettuce. Carrots, spring cabbage and lettuce were shown to have ion-exchange potentials equalling commercially available, weak cation exchange resins.

Branch *et al.* (1975) found that dietary fiber from plants low in phytate bound Ca *in vitro* in proportion to its uronic acid content. This suggests that binding by non-cellulosic polysaccharides could reduce the availability of Ca for small intestinal absorption. Microbial digestion in the colon is believed to liberate this Ca. Absorption of Ca in the colon has been recognized (Harrison and Harrison, 1974).

Although *in vitro* evidence demonstrates cation binding by dietary fiber, it doesn't necessarily follow that *in vivo* mineral availability will be altered. Fiber-mineral complexes may dissociate prior to reaching absorptive sites, thereby showing no apparent interference with mineral uptake. It is also possible that such complexes may actually enhance the availability of various cations (Stiles, 1976; Chao-Lo, 1979).

In Vivo Studies of the Influence of Fiber on Mineral Bioavailability

Numerous balance studies and feeding trials have attempted to define the effects of various fibers on mineral availability. Kelsay (1978) has reviewed a great deal of the literature available and many of the results are contradictory. The earliest investigations involved breads of high extraction flours in the diet. Widdowson and McCance (1942) reported that subjects absorbed more Fe from white bread than from brown bread, in spite of the fact that intakes of Fe were 50% higher in the brown bread diets. Björn-Rasmussen (1974) observed reduced absorption of radioactive Fe (^{59}Fe) from breads and rolls baked with added bran. Serum iron levels have been shown to decrease as a result of increased consumption of dietary fiber; 36 g/day (Jenkins *et al.*, 1975) and bran (Perrson *et al.*, 1975).

No significant changes in hemoglobin levels were noted during a 19 week balance trial involving 35 men fed 181 g of high extraction, wholemeal breads each day (Heaton et al., 1976). Similarly, Sandstead (1978) could find no significant changes in Fe balances of five men fed 26 g of wheat bran/day for a period of 30 days.

Wheat bran and high extraction flours have been shown to decrease absorption of Ca and Mg (McCance and Widdowson, 1942; McCance and Walsham, 1948) or decrease serum levels of Ca (Heaton and Pomare, 1974). Connell et al. (1975) could not demonstrate any significant differences in serum Ca levels between groups fed white or wholemeal breads. Heaton et al. (1976) was unable to reduce serum Ca levels with diets high in wheat bran as he had earlier with wholemeal bread diets.

It has been demonstrated that cellulose (Ismail-Bergi et al., 1977) and wholemeal breads (Reinhold et al., 1976) increase fecal excretion and create negative balances of Zn in man. Sandstead et al. (1978) noticed no change in Zn balance of men fed 26 g wheat bran per day. In the same study, Cu balance was observed to improve during the trial period.

Considering most of the available data, the possibility exists that fiber might interfere with the absorption of all minerals. A review of the literature appears to indicate Ca, Mg, Fe, Cu, and Zn to be the primary minerals affected.

These five were selected for evaluation in this study.

Other Factors that Might Influence Mineral Availability

In addition to the effects of fiber, and phytate previously discussed, different chemical forms of endogenous minerals might affect their availability.

Oxalic acid occurs as natural water soluble salts with sodium and potassium. The calcium salt is practically insoluble at neutral or alkaline pH but becomes soluble in acid (Gontzea and Sutzescu, 1968). Oxalates are widely distributed in the plant kingdom. Oxalate content of plants depends on species, climate, and soil conditions. The absorption of oxalates by man is poor, ranging from 1-6% under non-fasting conditions (Fassett, 1973).

The effects of dietary oxalates on the absorption of essential minerals has received little attention. The insolubility of most simple oxalates and the coprecipitation of other minerals with Ca oxalates (Krishnamurty and Harriss, 1961) suggest that minerals in plants containing high amounts of oxalates (e.g., spinach) may not be very readily available.

Jones (1978) has suggested that surface sianol groups of amorphous silica associated with the plant cell wall might adsorb metal cations and impair their availability. However, little is known about the exact matrix in which

minerals are held in plants. This information would be beneficial in understanding and optimizing the absorption of all minerals.

Bioavailability Studies

Bioavailability has been defined as the ratio of a nutrient consumed to the amount determined by biological assay to be incorporated by the body. Hemoglobin repletion is perhaps the most widely used method for measuring the bioavailability of Fe.

The standard bioassay for determining inorganic and non-heme Fe bioavailability is based on hemoglobin test advanced by Fritz (Pla and Fritz, 1970; Fritz and Pla, 1972). This procedure is continually being reevaluated and improved. Hemoglobin regeneration by the anemic rat fed the test Fe source is the basis of this test. Results are reported in terms of relative biological value (RBV). Reagent grade ferrous Fe is the reference standard. This value, RBV, is defined as the ratio of equivalent ppm Fe ($FeSO_4$)/ppm Fe of test source x 100 that produces the same hematological response. An Fe source which is less available than ferrous sulfate will have an RBV of less than 100. A sample which is a better source of available Fe will have an RBV in excess of 100. The true bioavailability of Fe from ferrous sulfate in rats is approximately 50%. Relative biological value is only a comparative figure. This method of rating

Fe sources does not take into account differences in amount of Fe consumed, or differences in weight gained by the animal. Hemoglobin concentration does not reflect the total amount of hemoglobin regeneration. This is important when changes in body mass result between animal groups on different dietary regimes.

Determining the RBV of Fe from non-heme Fe sources offers a second way of measuring the real utilization of a mineral by the body. The other way, as employed in this project, is by balance study (Bieri, 1979).

MATERIALS AND METHODS

Experimental Design

The design of the experiment is summarized as follows:

1. Purpose
   a) Measure the fiber and mineral composition of the edible and waste portion of broccoli, cauliflower and Brussel sprouts.
   b) Evaluate the effect of selected plant, or fiber, sources (a above) on mineral utilization.
      1) Determine the utilization of Ca, Mg, Fe, Cu, and Zn from the edible and waste portions of broccoli and cauliflower by balance studies with the rat as an animal model.
      b) Determine the relative biological value of Fe from broccoli, cauliflower and Brussel sprouts in the anemic rat.
2. Experimental animal
   a) Male rats (3 animals per group)
   b) Male rats (6 animals per group)
3. Experimental groups
   a) Three experimental periods, each involving one cellulose control and two test diets: broccoli florets and wastes for period one, cauliflower heads and wastes for period two, and wheat bran

and Metamucil for the final period. Each trial period was of 13 days duration.

b) Initial Fe depletion period of 28 days followed by 14 days on respective test diets.

4. Parameters measured

a) 1) Food consumption
2) Body weight
3) Daily fecal weight
4) Daily fecal mineral excretion
5) Daily urinary mineral excretion
6) Fecal neutral and acid detergent fiber
7) Liver lipid and cholesterol
8) Serum cholesterol

b) 1) Food consumption
2) Body weight
3) Hemoglobin concentration

Balance Trials

Source and Description of Fiber Sources

Gem broccoli grown in Brooks, Oregon was obtained through the Stayton Canning Company Cooperative of Stayton, Oregon. It was harvested on July 3, 1978. Snowball White cauliflower, grown in Salinas, California, was provided after harvest in July, 1978 by Interharvest of Salinas, California. Both vegetables were obtained as whole plants,

having been cut at ground level.

The plants were throughly washed and divided into two portions corresponding to the commercially utilized and waste materials. Yields for both vegetables were approximately 25% edible and 75% waste. The samples were freeze dried by Oregon Freeze Dry Foods Inc., Albany, Oregon and ground in a Thomas hammer mill to pass through a 1.0 mm mesh screen.

Protein, lipid, fiber and mineral content of fiber sources are presented in Table 1.

Soft white wheat bran is a uniform product made available through the Association of American Cereal Chemists (AACC). Hydrolyzed cellulose (cellufil) was obtained from Nutrition Biochemical Corporation (Cleveland, Ohio). Metamucil is a product of Searle Laboratories (Division of G.D. Searle and Co., Chicago, Illinois), and is composed of equal weights of psyllium seeds and dextrose. Psyllium seeds contain high levels of mucilages and are promoted for their laxative and fecal bulking properties.

Diets

Diet formulations are outlined in Table 2. All diets were formulated and standardized to contain 10% neutral detergent fiber (NDF). Protein, lipid, vitamin and mineral levels were in accordance with the recommendations of the

Table 1. Protein, lipid, fiber, and mineral composition of fiber sources and casein - balance studies.[1]

	Moisture	Protein[2]	Lipid	NDF	ADF	Hemicellulose[3]	Cellulose[4]	Lignin	Ca	Mg	Cu	Fe	Zn
	%								mg/g		µg/g		
Broccoli florets	6.93[5]	27.34	3.64	16.89	13.97	2.92	12.40	1.57	3.06	1.19	3.97	47.9	32.9
Broccoli wastes	4.87[5]	23.00	6.26	17.28	14.21	3.07	11.57	2.64	10.90	1.53	2.82	83.0	31.1
Cauliflower heads	5.31[5]	23.20	3.44	14.48	8.65	5.83	7.27	1.38	1.87	1.46	4.17	58.7	29.3
Cauliflower wastes	6.43[5]	21.80	4.50	21.80	17.12	4.68	14.65	2.47	1.14	2.54	4.34	81.3	29.1
Wheat bran (AACC)	11.04[5]	13.91	4.21	38.64	12.31	26.33	9.98	2.33	.51	4.83	11.34	116.6	55.8
Reported values	10.4[5]	14.3	5.22	40.2	11.9	28.3	8.7	3.2	1.20	4.30	15.6	122.0	54.5
Metamucil	--	--	--	--	--	--	--	--	.01	.001	0.12	5.5	1.16
Casein	--	92.20	--	--	--	--	--	--	.06	.01	1.1	9.1	41.3

[1]Mean values of triplicate determinations.

[2]Kjeldahl N x 6.25

[3]Hemicellulose = NDF - ADF

[4]Cellulose = ADF - Lignin

[5]Freeze dried original moisture level in %; broccoli florets, 90.17; Broccoli wastes, 91.28; cauliflower heads, 93.72; cauliflower wastes, 90.83.

[6]Reported values (AACC, letter dated Dec. 7, 1976).

Table 2. Diet formulations - balance studies.

Ingredient	Cellufil control	Broccoli florets	Broccoli waste	Cauliflower heads	Cauliflower waste	Wheat bran	Metamucil
				Diet			
	No. 1,4,7	No. 2	No. 3	No. 5	No. 6	No. 8	No. 9
	----------	----------	----------	g/100 g	----------	----------	----------
Glucose monohydrate	60.13	19.86	25.43	11.81	26.14	49.70	65.13
Casein (vitamin free)	20.00	10.40	12.26	12.17	13.31	15.78	20.00
Corn oil	5.00	2.70	1.40	2.62	2.52	3.91	5.00
D L Methionine	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Choline Chloride	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Vitamin mix[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mix[2]	3.50	-	-	-	-	-	3.50
Mineral mix II[3]	-	1.64	1.64	1.64	1.64	1.64	-
Calcium hydrogen phosphate-dibasic	-	1.11	-	1.33	-	1.72	-
Cupric carbonate	-	0.64×10^{-3}	0.80×10^{-3}	0.58×10^{-3}	0.67×10^{-3}	0.44×10^{-3}	-
Ferrous sulfate-heptahydrate	-	1.94×10^{-3}	-	-	-	1.90×10^{-3}	-
Zinc carbonate	-	0.96×10^{-3}	1.30×10^{-3}	1.81×10^{-3}	2.68×10^{-3}	2.98×10^{-3}	-
Cellufil[4]	10.00	-	-	-	-	-	-
Broccoli florets[5]	-	62.90	-	-	-	-	-
Broccoli waste[5]	-	-	57.90	-	-	-	-
Cauliflower heads[5]	-	-	-	69.06	-	-	-
Cauliflower waste[5]	-	-	-	-	55.01	-	-
Wheat bran (AACC)	-	-	-	-	-	25.88	-
Metamucil[6]	-	-	-	-	-	-	5.00

[1]AIN vitamin mixture, U.S. Biochemical Corp. (in g/kg of vitamin mix), thiamin HCl, 0.6); riboflavin, 0.6; pyridoxine HCl, 0.7; nicotinic acid, 3.0; D-calcium pantothenate, 1.6; folic acid, 0.2; D-biotin, 0.02; cyanocobalamine, 0.001; retinyl palmitate, 0.8; DL-α-tocopherolacetate 20.0; cholecalciferol, 0.0025; menaquinone, 0.005.

[2,3]See Table 3; [4]Cellufil - hydrolyzed, U.S. Biochemical Corp.; [5]Freeze dried; [6]Searle Laboratories.

American Institute of Nutrition (AIN) for small animals (Bieri et al., 1977). Plant materials were added to a basal diet formula with appropriate reductions in casein, corn oil, mineral salts and glucose monohydrate to maintain comparable levels in all diets. Calcium, Mg, Cu, Zn and Fe were the minerals of principal concern. To balance mineral levels between diets, two salt mixtures were used (Table 3). For control (No. 1,4,7) and the Metamucil diets (No. 9), a complete mineral mixture (I) was used. To vegetable diets containing endogenous Ca, Mg, Cu, Zn, and Fe, a mineral mix (II) lacking these minerals was added. Appropriate additions of these five minerals were made (Table 2) to obtain uniform levels. The degree to which identical fiber and mineral levels were actually achieved between diets is indicated in Table 4. In substituting plant protein for casein, vegetable protein was considered to have a protein efficiency ratio (PER) of one half that of casein (Schupman, 1970). Metamucil and cellufil contained negligible amounts of protein, lipid and minerals and therefore only replaced equal weights of glucose monohydrate in the diets.

There were three experimental periods involving a total of seven diets. Each period had a cellufil control diet and two test diets: broccoli florets and wastes in period one, cauliflower heads and wastes in period two, wheat bran and Metamucil in period three.

Table 3. Composition of mineral mixes I and II.

Component	Mineral Mix I[1]	Mineral Mix II[2]
	g/100 g	
Sodium chloride	7.4	7.4
Potassium citrate	22.0	22.0
Potassium sulfate	5.2	5.2
Manganous carbonate	0.35	0.35
Potassium iodate	0.001	0.001
Sodium selenite	0.001	0.001
Chromium potassium sulfate	0.055	0.055
Calcium phosphate	50.0	--
Magnesium oxide	2.4	--
Ferrous sulfate-heptahydrate	0.497	--
Zinc carbonate	0.16	--
Cupric carbonate	0.03	--
Glucose monohydrate	11.906	11.906

[1] 3.50% of diet
[2] 1.64% of diet

Table 4. Protein, lipid, fiber and mineral levels in test diets - balance studies.[1]

No.	Diet	Protein[2]	Lipid	NDF	ADF	Hemi-cellulose[3]	Cellu-lose[4]	Lignin	Ca	Mg	Cu	Fe	Zn
		%							mg/g		μg/g		
#1	Cellufil	18.52	5.07	9.58	9.37	0.21	9.02	0.35	3.05	0.38	5.75	49.0	40.0
#2	Broccoli florets	26.07	4.78	10.40	8.57	1.83	7.40	1.17	3.70	0.76	7.25	44.2	34.5
#3	Broccoli waste	25.52	4.69	9.39	7.61	1.78	6.01	1.60	5.30	0.875	3.50	70.0	35.5
#4	Cellufil	18.52	5.07	9.58	9.37	0.21	9.02	0.35	3.05	0.38	5.75	4-.0	40.0
#5	Cauliflower heads	24.40	4.84	10.23	6.16	4.07	5.12	1.04	4.15	0.95	5.00	40.7	50.0
#6	Cauliflower waste	24.22	4.85	10.33	8.18	2.15	6.76	1.42	6.85	0.95	5.00	67.0	46.5
#7	Cellufil	18.32	5.10	9.81	9.62	0.19	9.19	0.43	3.53	0.39	5.85	44.3	45.8
#8	Wheat bran	18.31	4.82	10.39	2.97	7.42	2.25	0.72	3.99	0.89	6.49	42.1	42.7
#9	Metamucil	18.51	5.27	-	-	-	-	-	4.22	0.46	6.15	41.2	38.9
Theoretical[5]		18.50	5.00	-	-	-	-	-	5.20	0.50	6.00	35.0	30.0

[1]Mean value of triplicate determinations.

[2]Kjeldahl N x 6.25.

[3]Hemicellulose = NDF - ADF

[4]Cellulose = ADF - Lignin

[5]AIN recommendations (Bieri, et al., 1977).

Animals and Housing

Weanling, male, Long-Evans rats ranging from 45 to 60 g in weight were obtained from Simonsen Laboratories of Gilroy, California. Animals were housed in small rodent metabolism units (Maryland Plastics Inc., model E100) and fed diets and deionized water ad libitum for 13 days. Daily weight gain and feed consumption were recorded.

Fecal and urine collections were made at 24 hour intervals. After drying, each fecal sample was divided into two portions of ca. equal weight. One portion was used to form composite samples for each animal corresponding to days 1-5, 6-9, and 10-13. These composite samples were used in the determination of fecal NDF and ADF. Daily urine collections and the remaining daily fecal samples were assayed for minerals.

Animals were sacrificed at the end of the 13 day balance period. Livers were removed and serum collected.

Statistical Analysis

Statistical analysis of data was carried out using the paired *t* test for populations of unequal variance (Snedecor and Cochran, 1976).

Analytical Procedures

Protein

Protein was measured according to the Association of Official Analytical Chemists (AOAC) Kjeldahl organic nitrogen method (AOAC, 1975a) using a micro-Kjeldahl apparatus. Protein levels are reported as Kjeldahl nitrogen X 6.25.

Lipid

The method of Folch et al. (1957) was adapted for lipid determination. Lipid was measured gravimetrically from an aliquot of the chloroform:methanol (2:1, v/v) sample extracts.

Moisture

Water content of dietary fiber sources and rat fecal material was determined after drying to a constant weight in an oven at 105°C. This is not the AOAC recognized method for moisture determination in dried foods (AOAC, 1975b). The high moisture levels obtained may be explained, in part by the use of this high heat drying process.

Minerals

Wet ashing and atomic absorption spectrophotometry (AAS) were used for sample digestion and mineral determination

respectively. All samples were digested in HNO_3:$HClO_4$ (4:1, v/v) and evaporated to dryness on a hotplate at a moderate temperature. Sample size and acid volume varied with the nature of the sample. Diet samples were of approximately one gram and acid volume was 25 ml. Fiber source samples were two grams in 35 ml. Fecal samples were ca. 0.5 g in 20 ml. Daily urine collections were digested in 20 ml of the acid solution.

When the samples reached dryness, they were dissolved in 20 ml of 0.1 N HCl. For the determination of calcium and magnesium, samples were diluted with 0.1 N HCl containing 0.2% lanthanum oxide.

A Perkin-Elmer Model 403 atomic absorption spectrophotometer, using a single element hollow cathode lamp was employed. Absorption of samples was measured at 421.6 nm, 258.2 nm, 213.9 nm, 249.4 nm, and 210.8 nm for Ca, Mg, Zn, Fe, and Cu respectively (Perkin-Elmer, 1979). Minerals levels were determined from calibration curves prepared from standards (1000 µg/ml, Harlco, Philadelphia, Pa.).

Dietary Fiber

For dietary fiber analyses the methods of Goering and VanSoest (1970) were employed. These procedures for the following determinations: neutral detergent fiber (NDF) which includes cellulose, hemicellulose and lignin; and acid detergent fiber (ADF) which is the sum of cellulose and

lignin. Hemicellulose is determined by subtracting ADF from NDF. Cellulose is determined by subtracting lignin from ADF. Lignin was measured as the portion of ADF insoluble in 72% (v/v) H_2SO_4.

Determination of NDF: Transfer an analytically weighed 0.5-1.0 g sample(S) into a 200 ml refluxing flask in the following order: 100 ml cool (room temperature) neutral detergent solution[1], 2 ml technical grade decahydronapthalene and 0.5 g reagent grade sodium sulfite. The flask was heated to boiling, then refluxed 60 min from onset of boiling. The contents of the flask were transferred quantitatively to a 50 ml tared Gooch crucible and filtered under low vacuum. The mat was broken up, washed twice with hot water and twice with acetone. Final residue was dried at 100°C overnight and weighed (W). Percent NDF = W/S x 100.

Determination of ADF: Approximately 1 g of sample (S) analytically weighed and transferred to a 200 ml refluxing flask containing 100 ml acid detergent solution[2] at room temperature. The solution was heated to boiling and refluxed for 60 min from the onset of boiling. The contents

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[1]To 1 liter distilled water add: 30 g sodium dodecyl sulfate, USP; 18.61 g disodium dihydrogen ethylenediaminetetraacetic dihydrate, reagent grade; 6.81 g sodium borate decahydrate, reagent grade; 4.56 g disodium hydrogen phosphate, anhydrous, reagent grade; and 10 ml 2-ethoxyethanol, purified grade. Agitate to dissolve. Check pH and adjust to 6.9-7.1.

[2]Dissolve 20 g technical grade cetyltrimethyl ammonium bromide in 1.00 N H_2SO_4 (1 liter), previously standardized.

of the flask were transferred quantitatively to a tared 50 ml, coarse porosity, pyrex Gooch crucible (W_1). Filtration was completed using minimum suction. The filtered mat was broken up and soaked for 30 sec with hot (90-100°C) water and dried under vacuum. This operation was repeated once with water and twice with acetone. The remaining mat was dried overnight at 100°C and weighed (W_2). Percent ADF was calculated by the formula:

$$100(W_2 - W_1)/S$$

Determination of Lignin: One g acid washed asbestos (medium fiber, J.T. Baker) was added to the crucible containing ADF. The contents of the crucible were covered with cool 72% (v/v) H_2SO_4, and stirred with a glass stirring rod to a smooth paste, breaking all lumps. The crucible was refilled with 72% (v/v) H_2SO_4 and stirred hourly as the acid drained. After three hours, the acid was removed under vacuum and the residue was washed with hot water until acid-free to pH paper. Crucibles were dried at 100°C overnight and weighed (W_3). All organic materials were removed by ashing at 500°C and the crucibles weighed (W_4). A 1.0 g asbestos blank was carried through the entire procedure to record any loss in weight through ashing (W_5). Percent acid insoluble lignin was calculated by the formula:

$$\frac{(W_3 - W_4 - W_5)}{5} \times 100 \ .$$

Determination of Serum and Liver Cholesterol

Serum cholesterol was determined by an enzymatic procedure employing reagents and standards obtained from Pierce Chemical Co., Rockford, Illinois (HDL/Cholesterol Rapid Stat Kit - 44110). This assay is based on the work of Trinder (1969). Enzyme reagent is dissolved in a 0.2% phenol reagent solution. This enzyme reaction mixture contains: 1) a cholesterol esterase to liberate free cholesterol, 2) cholesterol oxidase converting free cholesterol to cholest-4-en-3-one and hydrogen peroxide, 3) a peroxidase which degrades the hydrogen peroxide yielding oxygen that complexes with, 4) 4-aminoantipyrine. The serum samples and cholesterol standards are added to the enzyme reagent and incubated for 15 min at 37°C. The tubes are removed and allowed to cool for 2 min. The resulting pink quionemine dye complex formed is quantitated colorimetrically at 510 nm. Serum cholesterol is calculated from the standards run at the same time.

Cholesterol in livers was measured by a modified method of Abell *et al.* (1952). Liver lipid was extracted by the method of Folch *et al.* (1957). A three ml aliquot of the chloroform:methanol (2:1) solution was evaporated to dryness. After the addition of one ml of 1 N NaOH the samples

were saponified in a hot water bath (70°C) for 30 min. One ml of water and two ml of hexane were added. The samples were vigorously mixed and then centrifuged. A one ml aliquot of hexane was removed, evaporated, and then the residue dissolved in one ml glacial acetic acid. After adding five ml Liebermann Burchard reagent (Abell et al., 1952), samples were placed in a covered 25°C water bath for 30 min. Absorbance was read after 30 min at 620 nm. Cholesterol content was determined from a standard calibration curve.

Relative Biological Value

Diets and Dietary Components

Insufficient material used in balance studies required obtaining a second set of broccoli, cauliflower and Brussel sprouts. Initial plans did call for balance studies to be conducted with Brussel sprouts. These vegetables were grown in Oregon during the 1978 season and obtained freeze dried from Oregon Freeze Dry Foods, Inc., Albany, Oregon. Proximate, fiber and Fe analysis methods were as described previously. Values are presented in Table 5. Additional sources of fiber and/or Fe were: cellufil (pure cellulose, no detectable Fe) and soft white wheat bran (AACC). The fiber and Fe content of this bran was reported in Table 2.

Table 5. Protein, lipid, fiber and iron levels in freeze dried broccoli, cauliflower and Brussel sprouts - RBV studies.[1]

	Broccoli	Cauliflower	Brussel sprouts
Protein, %	28.55	24.13	23.95
Lipid, %	5.39	5.06	3.57
NDF, %	18.8	15.8	20.2
ADF, %	14.8	11.1	11.4
Hemicellulose, %	4.0	4.7	8.8
Cellulose, %	11.6	9.4	6.9
Lignin, %	2.4	1.7	1.9
Iron, µg/g	70.1	65.1	53.3

[1]Mean value of triplicate determinations.

Control diets contained three levels of Fe ($Fe_2SO_4 \cdot 7H_2O$): 6.25 ppm (No. 11); 12.5 ppm (No. 12); and 25 ppm (No. 13). Diets formulated to contain corresponding Fe levels from wheat bran resulted in levels of 5% (No. 14), 10% (No. 15) and 15% (No. 16) of this fiber source. The three remaining test diets each contained 25% broccoli (No. 17), cauliflower (No. 18), and Brussel sprouts (No. 19). Animals were initially maintained on a control diet containing no added iron (No. 10). Composition of diets to determine RBV of plant FE are given in Table 6.

Animals

Weanling male Long Evans rats were obtained from Charles River Breeding Laboratories (North Wilmington, MA.). These animals were 23 days upon receipt. They were housed in stainless steel cages with wire mesh floors. Uniform lighting for 12 hours (7:00 am to 7:00 pm) and darkness was controlled automatically. Distilled water (in rubber-stoppered glass bottles) and feed (in aluminum cups) was supplied ad libitum. All animals were maintained on the Fe free control diet (No. 10) with feed consumption and weights measured at ca. four day intervals to remove animals of marginal growth performance.

Table 6. Composition of diets for determining relative biological value of plant iron.

Ingredient	Control[1]	Wheat bran			Broccoli	Cauliflower	B. sprouts
	Diet No.						
	10-13	14	15	16	17	18	19
	g/100 g						
Glucose monohydrate	64.7	64.7	59.7	49.7	49.94	49.21	48.84
Casein	20.0	20.0	20.0	20.0	16.11	16.76	16.76
Corn oil	5.0	5.0	5.0	5.0	3.65	3.73	4.10
Cellufil	5.0	-	-	-	-	-	-
Vitamin mix[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mix[3,4]	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Choline chloride	0.07	0.07	0.07	0.07	0.07	0.07	0.07
DL methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Wheat bran	-	5.0	10.0	20.0	-	-	
Broccoli	-	-	-	-	25.0	-	-
Cauliflower	-	-	-	-	-	25.0	-
Brussel sprouts	-	-	-	-	-	-	25.0

[1]One control-basal diet, iron-free and 3 control diets supplying 6.25, 12.5 and 25.0 g/Fe/g diet. Iron supplied as $FeSO_4$ $7H_2O$.

[2]AIN vitamin mixture 76, United States Biochemical Corp. (in g/Kg vitamin mix), thiamin CHl, 0.6; riboflavin, 0.6; pyridoxine HCl, 0.7; nicotinic acid, 3.0; D-calcium pantothenate, 1.6; folic acid, 0.2; D-biotin, 0.02; cyanocobalamine, 0.001; retinyl palnitate, 0.8; and DL-α-tocophenol, 20.0; cholecalciferol, 0.0025; menaquinone, 0.005.

[3]Control diet No. 10, wheat bran diets 14, 15 and 16, broccoli diet 17, cauliflower diet 18, and Brussel sprouts diet 19 contained mineral mix I minus ferrous sulfate-hystohydrate; see Table 3.

[4]Control diet No. 11, 12, 13 contained mineral mix I with 0.089, 0.178, and 0.356 g ferrous sulfate heptohydrate per 100 g mineral mix; see Table 3.

Iron Bioavailability

The Fe free diet was fed for 28 days. At this time, the hemoglobin concentration was measured. Depleted animals were assigned to 10 groups of 6 animals each in such a manner that the mean and standard deviation of hemoglobin concentration were similar (6.7 ± 0.1 g/100 ml). One group of rats was maintained on the Fe free diet (No. 10) and the remaining nine groups were placed on the test diets (No. 11-19). After a 14 day repletion period, hemoglobin concentration was again measured. Weight of animals at the start and end of the repletion period was also recorded. This protocol is similar to the procedure outlined by Fritz and Pla (1972).

Blood Drawing and Hemoglobin Determination

Animals were subjected to blood drawing at the end of depletion and regeneration periods. The rats were immobilized in a stainless steel animal container. Blood taken from the tail was analyzed for hemoglobin by the cyanmethemoglobin method (Crosby *et al.*, 1954) using a Bausch and Lomb Spectronic 20 colorimeter. Drabkin reagent and hemoglobin standard were obtained from Sigma Chemical Co. (St. Louis, MO).

The cyanmethemoglobin standard solution yields a transmittance equivalent to 18 g Hb/100 ml whole blood. Zero,

2.0, 4.0, and 6.0 ml of cyanmethemoglobin standard were pipeted into 6.0, 4.0, 2.0, and 0.0 ml of Drabkin's solution, respectively, to construct a calibration curve. These mixtures have transmittance levels corresponding of blood hemoglobin 0.0, 6.0, 12.0, and 18.0 g/100 ml. Samples were assayed by adding 20 µl whole blood to tubes containing 5 ml Drabkin's solution. The pipets were rinsed several times. Samples and standards were mixed and allowed to stand 15 min at room temperature protected from light. Drabkin's solution was used as the blank. Transmittance was measured at 545 nm. Sample hemoglobin levels were obtained directly from the calibration curve of transmittance (%T) vs. hemoglobin concentration (g/100 ml).

RESULTS AND DISCUSSION

Dietary Components

The proximate, fiber and mineral composition of dietary plant components used for balance studies are presented in Table 1. The well defined composition of AACC wheat bran (Mullen, 1978) made it useful as a standard reference material. All values determined for the wheat bran, except for Ca, agreed with the reported composition.

A totally reliable and consistent Ca analysis could not be achieved. A great deal of variation was observed in multiple determinations of any sample. While a level of 1.2 mg Ca/g wheat bran was reported, values observed were generally 50% lower. Recovery of known amounts of Ca added to purified diets was also low. Reasons for these inconsistencies could not be identified. Although analytical problems were encountered, dietary levels of Ca were considered to be adequate. Errors in Ca determination were reflected in inconsistent values from control groups during balance studies. For these reasons, all data concerning Ca levels and Ca balance studies cannot be considered absolute and are presented with reservations. However, it is hoped the error is uniformly low throughout and comparisons are presented with the possibility of providing some useful information.

Fiber Composition of Plants

Neutral detergent fiber, ADF and lignin levels in broccoli and cauliflower were similar in all samples although obtained at different times (Tables 1 and 5). These levels are also consistent with values reported by other investigators (Robertson, 1978; Shipley, 1978) for freeze dried samples. Acid detergent fiber content of all vegetables including Brussel sprouts was similar to wheat bran. Vegetables are rather low in hemicellulose. For this reason, their NDF values were only half that of wheat bran. Lignin content of vegetables wastes and wheat bran were comparable. Levels in edible portions of vegetables were lower. Original moisture content of broccoli and cauliflower used in balance studies (Table 1) allows conversion of compositional data to a wet weight basis.

Proximate and Mineral Composition of Plants

Protein (Kjeldahl N x 6.25) and lipid composition of the edible portions of vegetables agreed with values reported in United States Department of Agriculture Handbook No. 8 (Watt and Merrill, 1963) when compared on a wet weight basis. Vegetables have about twice as much protein as does wheat bran. Lipid levels were similar for all plants, but tended to be slightly higher in the leafy waste portions of vegetables.

Wheat bran is much richer in minerals than any of the vegetables. Calcium content of broccoli florets and cauliflower heads were also found to be higher than reported (on a wet weight basis, Watt and Merrill, 1963). The extent of error in Ca analysis is unknown.

Actual Dietary Composition - Balance Studies

Diets formulated (Table 2) for balance studies were analyzed for actual fiber and nutrient composition (Table 4). Calcium recovery in formulated diets was found to be low. Although diets were intended to provide equivalent levels of minerals as recommended for the rat (Bieri, 1977) some problems were encountered. The high Mg content of all plant materials tested resulted in these diets exceeding the AIN recommendation of 0.5 g/100 g diet. Similarly, Fe was high in all diets, exceeding 35 µg/g. Copper in the diets appeared to be lower and Zn higher. Endogenous plant mineral contributions, exogenous mineral salt additions to diets and analytical analysis could have all contributed to levels varying from theoretical.

Feed Consumption, Weight Gain and Feed Conversion

Feed consumption, weight gain and feed conversion for animals maintained during balance trials are presented in Table 7. The small number of animals involved in these

Table 7. Pooled feed consumption and weight gain.[1,2]

No.	Diet	Feed consumption, g	Final body weight, g	Weight gain, g	Weight gain (g)/ food intake (g)
1	Cellufil	158.8±35.9	121.5±12.5	68.5±13.1	0.43
2	Broccoli florets	131.2±14.1	112.6± 2.4	61.4± 5.0	0.47
3	Broccoli wastes	112.6±14.8	90.7±11.5	36.6±12.1	0.33
4	Cellufil	165.0±24.8	127.7±10.5	76.7±12.4	0.46
5	Cauliflower heads	114.5± 7.9	90.7±17.5	39.7±14.2	0.35
6	Cauliflower wastes	144.4±13.8	106.7± 2.9	55.7± 0.5	0.39
7	Cellufil	148.8±27.3	135.6±24.4	78.3±24.5	0.53
8	Wheat bran	177.4±11.5	142.4±17.3	89.1±19.2	0.50
9	Metamucil	140.0±17.2	122.7± 6.6	67.1± 6.5	0.48

[1]Over 13 days

[2]Mean ± S.D. 3 animals per diet.

trials and the resulting wide variations within each dietary group prevented statistical evidence of significant differences. Trends were apparent.

Decreased feed consumptions and weight gain were observed in animals fed cauliflower heads and broccoli wastes. Feed conversion was also impaired in these same diets. These differences were not seen in animals fed broccoli florets. A number of explanations for these differences can be suggested. 1) Palatibility may have been a factor, especially in diets no. 3 and no. 5 where very low consumption was noted. 2) The protein content of the diets may have been inadequate. Although plant protein was assumed to have a PER of one-half that of casein, adequate levels of certain amino acids might not have been present. Broccoli and cauliflower are noticeably deficient in methionine and tryptophan (Altman and Dittmer, 1968). In this regard a 2 to 1 substitution of plant protein for casein may not have been satisfactory. 3) A factor, or factors, may be present in these vegetables that interfere with nutrient utilization and subsequent growth. Metamucil and wheat bran diets were similar to the cellufil control.

Fecal Weights and Moisture Content

No significant differences were noted between average daily dry fecal weights of test and control animals (Table 8). Although results ranged widely, Metamucil-fed rats

Table 8. Daily fecal weight and moisture content.[1]

No.	Diet	Fecal matter: Net weight g	Fecal matter: Dry weight g	% H_2O	Significance[2]
1	Cellufil	1.88± .55	1.23± .50	34.6±1.0	
2	Broccoli florets	7.20±1.73	1.98± .77	72.5±2.3	$p < .002$
3	Broccoli wastes	4.72± .48	1.63± .55	65.5±3.2	$p < .002$
4	Cellufil	1.71± .30	1.30± .42	24.0±2.6	
5	Cauliflower heads	4.23±1.50	1.24± .45	70.7±8.1	$p < .002$
6	Cauliflower wastes	8.59±2.02	2.35± .60	72.6±4.3	$p < .001$
7	Cellufil	1.73± .63	1.20± .37	30.6±2.7	
8	Wheat bran	2.61± .71	1.35± .42	48.3±3.2	$p < .02$
9	Metamucil	1.28± .28	0.59± .20	53.9±2.8	$p < .01$

[1]Mean ± S.D. of 13 days, 3 rats per diet.

[2]Significance of difference between fecal moisture content of test and control animals.

were low with 0.59 g/day versus 2.35 g/day for those fed cauliflower wastes. Values for all other diets were grouped near the middle of this range. Differences became apparent when moisture content of the feces was considered. The water holding capacity of feces from animals on test diets ranged from 48.3% to 72.6% for wheat bran and cauliflower waste fed animals respectively. In general, values for test diets were about twice that of control animals (30%). This could not be explained by the amount of any one fiber component in the diet. Vegetable fiber would appear unique in its water binding capacity.

Eastwood and Mitchell (1976) reported widely varying water holding capacities for a number of acetone dried vegetable powders of similar fiber composition. McConnell et al. (1974) suggested that these values might be used as a basis for predicting increases in stool weights. In this experiment, the hypothesis proved true for all plant materials.

The fecal moisture content of Metamucil-fed rats agreed with values given by Forsythe et al. (1978). Data presented by Forsythe et al. for wheat bran and cellulose-fed animals were lower than observed herein; 12% vs. 30% for cellulose, 30% vs. 48% for wheat bran. Differences are explained, in part, by the higher levels of fiber fed in this experiment; 10% vs. 6% fed by Forsythe.

Wheat bran has long been promoted for its fecal bulking, water holding and consequent laxative properties. The development of Metamucil and similar products is a relatively recent occurrence. Although these products have been proven effective, vegetable fibers were found to be superior for increasing fecal water holding capacity. This information suggests that rather than advocating increased consumption of wheat bran, or ingestion of expensive mucilage products, perhaps more emphasis should be placed on the value of vegetables.

Daily Fecal Fiber and Mineral Excretion and Urinary Mineral Excretion

Daily fecal fiber and mineral excretion, as well as daily urinary mineral excretion are presented in Table 9.

Excretion of minerals in the urine was less variable between groups than was fecal mineral elimination. Although the data are not highly correlated, increased mineral loss through the urine are reflected in increased fecal excretion (decreased absorption) of these elements.

This observation is most apparent when cauliflower waste-fed rats are compared to the respective control animals. The meaning of this is not fully understood.

Table 9. Daily fecal fiber and mineral excretion, and daily urinary mineral excretion.[1,2]

	NDF	ADF	Ca		Mg		Fe		Zn		Cu	
	F[3]	F	F	U[4]	F	U	F	U	F	U	F	U
	g/day		mg/g				µg/g					
1 Cellufil	0.890±.363[a]	0.858±.367[a]	19.90±10.15[a]	0.30± .32[a]	2.44±1.17[a]	0.48± .27[a]	282.6±120.2[a]	12.4±4.9[a]	307.8±134.9[a]	9.5± 5.4[a]	48.7±20.5[a]	1.8± .9[a]
2 Broccoli florets	0.898±.357[a]	0.734±.314[a]	24.57±13.50[a]	0.47± .53[a]	5.83±2.55[a]	0.99± .49[a]	355.1±186.5[a]	12.9±3.8[a]	273.3±108.9[a]	17.1± 9.8[a]	54.4±18.7[a]	2.1±1.3[a]
3 Broccoli wastes	0.682±.235[a]	0.607±.224[a]	30.93±14.24[a]	2.37±2.14[a]	4.94±2.06[a]	1.38± .85[a]	628.4±319.6[a]	15.7±5.9[a]	247.6± 15.7[a]	13.6± 5.9[a]	36.6±10.2[a]	1.1± .5[a]
4 Cellufil	0.954±.316[a]	0.912±.305[a]	14.95± 5.34[a]	0.25± .12[a]	2.64± .94[a]	1.03± .32[a]	282.0± 78.4[a]	8.9±3.4[a]	330.9±102.1[a]	9.2± 4.9[a]	39.0±18.7[a]	2.4±1.1[a]
5 Cauliflower heads	0.454±.152[a]	0.370±.135[a]	10.24± 3.32[a]	0.59± .46[b]	5.99±2.38[b]	1.25± .98[a]	172.6± 55.7[a]	10.2±5.6[a]	190.8± 75.2[a]	13.4± 8.0[a]	35.6±12.7[a]	2.0±1.0[a]
6 Cauliflower wastes	0.808±.192[a]	0.775±.189[a]	53.67±17.19[a]	3.70± .17[a]	19.84±5.82[b]	2.52±1.10[a]	387.0±144.7[a]	13.0±4.0[a]	262.7± 71.7[a]	50.7±20.6[a]	42.6±11.2[a]	3.4±1.5[a]
7 Cellufil	0.881±.286[a]	0.835±.273[a]	8.60± 3.66[a]	0.20± .33[a]	2.23±7.11[a]	0.62± .31[a]	225.8± 85.4[a]	8.3±3.0[a]	311.1± 90.1[a]	11.3± 4.9[a]	48.8±16.0[a]	1.9±1.0[a]
8 Wheat bran	0.753±.232[a]	0.347±.253[a]	6.56± 2.23[a]	0.22± .15[a]	7.36±2.73[a]	1.90± .71[a]	251.8± 63.1[a]	8.9±3.6[a]	255.9± 93.0[a]	14.2±12.1[a]	56.0±20.5[a]	2.6±1.4[a]
9 Metamucil	N.A.	N.A.	4.61± 1.90[a]	0.14± .14	1.97± .70[a]	0.33± .18[a]	262.7±153.1[a]	7.1±3.1[a]	244.9± 92.3[a]	10.6± 6.4[a]	43.0±13.0[a]	1.9± .9[a]

[1]Values followed by different letters are significant different ($p < 0.05$).

[2]Dry weight.

[3]Fecal.

[4]Urinary.

Apparent Digestibility of Fiber Components

The apparent digestibility of hemicellulose from all plant sources except broccoli florets exceeded 50% (Table 10). This low digestibility may be attributed to the resulting different intestinal flora promoted or maintained by the broccoli florets. Hemicellulose from plant wastes appeared to be more degraded than from edible portions. The 59.6% breakdown of wheat bran hemicellulose agreed with values reported by Williams and Olmstead (1936). Southgate and Durnin (1970) observed 87.2% digestion of the fiber fraction.

Because the lignin content of all fiber sources was low, the apparent digestibility of the ADF fraction mainly reflects cellulose breakdown. Only ca. 75% of ingested cellufil was recovered in the feces of control animals. The same was true for the ADF fraction for wheat bran-fed rats. Cellulose from cauliflower heads was most resistant to intestinal degradation. The breakdown of the ADF fraction of edible portions of vegetables was greater than vegetable wastes. The reverse was observed regarding digestibility of hemicelluloses.

Cellulose was long thought to be indigestible. The original aim of the crude fiber assay was the determination of cellulose. This was thought to provide, by difference, the amount of digestible matter in the foodstuff. It is

Table 10. Apparent digestibility of fiber components.[1,2]

Diet	NDF	ADF	Hemicellulose[3]
1 Cellufil	24.0±11.1[a]	25.3±12.3[a]	-
2 Broccoli florets	14.9± 8.5[a]	15.7± 8.1[a]	11.6±11.5[a]
3 Broccoli wastes	16.0± 6.9[a]	7.9± 9.4[a]	50.2± 9.4[a]
4 Cellufil	21.1± 2.8[a]	23.1± 1.6[a]	-
5 Cauliflower heads	49.1± 8.6[b]	31.3± 9.1[a]	7.5± 9.4[a]
6 Cauliflower wastes	31.1± 3.1[ab]	16.6± 3.3[a]	86.5± 3.3[b]
7 Cellufil	24.9± 0.5[a]	27.3± 0.9[a]	-
8 Wheat bran	47.0± 2.3[b]	26.1±10.2[a]	59.6± 8.2
9 Metamucil	..	-	-

[1]Values followed by different letters are significantly different ($p < 0.05$).

[2]Apparent digestibility = $\frac{\text{total intake - total excretion}}{\text{total intake}} \times 100.$

[3]Hemicellulose = NDF - ADF.

apparent now that significant degradation of ingested cellulose does occur. Cummings (1976) reviewed the available data and concluded that cellulose utilization by man was about 40%.

Reported values for digestibility of fiber fractions are often open to question. The inclusion of microbial polysaccharides of bacterial cell walls in fecal samples can complicate studies of fiber degradation (VanSoest, 1978). Obvious differences in results are often apparent when comparing fibers from different sources. Purified sources of fiber available for nutritional studies have generally been structurally modified during extraction. Extrapolation of these data to predict the digestibility of native forms of fiber may prove inaccurate.

Comments on Mineral Balance Trials Using Rats

Isaksson and Sjögren (1967) have reviewed the problems inherent in mineral balance studies. A number of factors make interpretation of results difficult. Data from balance trials are seldom absolute. Normal experimental error and differences in experimental design often limit the comparisons that can be made between results of different researchers. One such consideration is the choice of animal to predict responses of human subjects. The limitation of directly comparing results obtained from experiments dealing with man and rats is acknowledged.

Calcium Balance

From the work of Reinhold et al. (1976), decreased Ca absorption would be expected as a result of fiber consumption. Data presented in Figure 2 indicate that with the exception of cauliflower wastes, fiber had no significant detrimental effects on Ca uptake. Calcium utilization by control animals was 62% (average of 9 animals over 3 test periods). Improved Ca absorption was observed in rats fed wheat bran and Metamucil ($p < 0.001$). The cauliflower waste diet provided the greatest amount of Ca on a per gram basis, but its absorption was reduced to 26% ($p < 0.025$).

Wheat bran and high extraction flours have long been shown to interfere with uptake and decrease body levels of Ca in man (McCance and Widdowson, 1942; McCance and Walsham, 1948; Reinhold et al., 1976). These effects were not apparent in rats in this investigation.

As with other animals, the chemical form of Ca in plants may affect its availability. Calcium pectates are assumed to be fairly well utilized (VanSoest, 1978). The types and availability of other forms are not well described.

Magnesium Balance

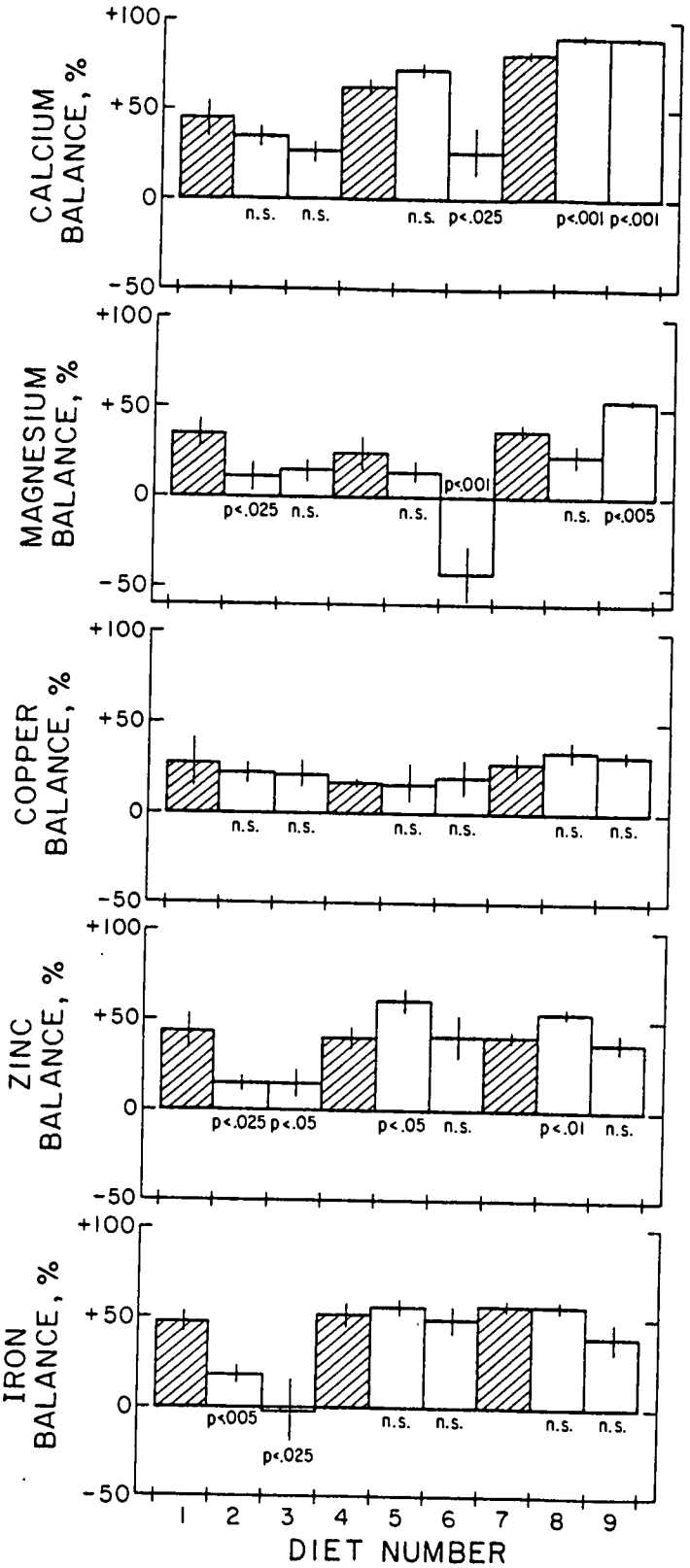
Control animals (9 observations) utilized 33.8% of the Mg in their diet (Figure 2). Metamucil improved the

Figure 2. Mineral balances in rats fed fibers from different sources.

(a) Balance is defined as:

$$\frac{\text{Total intake - Total excretion}}{\text{Total intake}} \times 100$$

(b) Diet 1, cellufil control; diet 2, edible broccoli; diet 3, broccoli wastes; diet 4, cellufil control; diet 5, cauliflower heads; diet 6, cauliflower wastes; diet 7, cellufil control; diet 8, wheat bran (AACC); diet 9, Metamucil.


CALCIUM BALANCE, %
+100
+50
0
-50
n.s. n.s. n.s. p<.025 p<.001 p<.001
MAGNESIUM BALANCE, %
p<.025 n.s. n.s. p<.001 n.s. p<.005
COPPER BALANCE, %
n.s. n.s. n.s. n.s. n.s. n.s.
ZINC BALANCE, %
p<.025 p<.05 p<.05 n.s. p<.01 n.s.
IRON BALANCE, %
p<005 p<.025 n.s. n.s. n.s. n.s.
1 2 3 4 5 6 7 8 9
DIET NUMBER

absorption of Mg to 53.3%. Broccoli waste, cauliflower heads and wheat bran had no significant effect. Decreased uptake of Mg was noted in rats fed broccoli florets ($p < 0.025$). Animals fed cauliflower wastes displayed a very negative balance for this mineral ($p < 0.001$). This observation appears to warrant additional study.

It has been suggested that Mg and Ca availability should be similarly affected by fiber consumption (Cummings, 1978). This phenomenon was demonstrated by all fiber sources in this experiment, except wheat bran.

High extraction flours have generally been used as the fiber source in Mg balance studies. McCance and Widdowson (1942) and McCance and Walsham (1948) demonstrated that Mg from this source was not utilized by man to the degree found in white flours. Reinhold et al. (1976) observed negative balance for this mineral in men fed great amounts of bread made with flours high in bran and fiber.

Although statistically significant evidence was not always observed, all plant sources of Mg were less well utilized than the magnesium oxide used in control diets.

Copper Balance

No significant differences were noted in the ability of rats on any test diet to utilize Cu (Figure 2). The average uptake of this mineral by rats was 23.4%.

Information on the effects of fiber on Cu balance is limited. In studies with human subjects, Sandstead et al. (1978) demonstrated improved Cu balance when diets were supplemented with 26 g wheat bran/day. Stiles (1976) reported decreased availability of Cu in rats when peanut hulls or corn pericarp were added at any level compared to a basal, fiber-free diet.

Based on the results of Stiles (1976), Cu might be more available to rats fed diets low in fiber although uniform utilization was basically observed for all animals in this investigation. Apparently, fiber has a minimal effect on the utilization of Cu.

Zinc Balance

A 41.6% uptake of Zn was seen in control animals (Figure 2). Utilization by animals on broccoli floret and waste diets was only 16.4% ($p < 0.025$) and 14.4% ($p < 0.05$) respectively. Cauliflower wastes and Metamucil displayed the same levels of utilization as found in control animals. Uptake of Zn from cauliflower heads and wheat bran were significantly higher at 61% ($p < 0.05$) and 53.7% ($p < 0.01$) respectively.

Ismail-Beigi et al. (1977) reported increased fecal excretion of Zn in man due to inclusion of 10 g cellulose/day in the diet. In a subsequent study, Reinhold et al. (1976) also observed a negative balance for this mineral

in adults fed high levels of wheat bran. This observation is not supported by the present study. This might be due to differences between rats and man in their ability to absorb available Zn. Sandstead (1972) has published results that conflict with those of Reinhold et al. Zinc balance was reported to be unchanged when diets of men were supplemented with either white bread, hard red spring wheat bran, corn bran, or soy bean hulls.

Iron Balance

Data in Figure 2 show that uptake of Fe from diets containing cellufil was 51.6%. Iron from both broccoli florets and wastes was not as well utilized ($p < 0.025$). Broccoli waste fed animals showed slight negative balances. What is unique about broccoli is not readily apparent. Metamucil appears to depress, but not significantly so, the utilization of Fe. Since the fiber of Metamucil can be classified as a mucilage, it might be interesting to further investigate the role of mucilages in Fe utilization. Observed balances of animals fed wheat bran and cauliflower heads and wastes compare favorably with those of control animals. This suggests that Fe from these sources is readily available and their fiber effects minimal.

Conclusions drawn from reported utilization of endogenous Fe from wheat bran are not consistent. The influence of wheat bran on Fe absorption was the subject of some of

the earliest fiber-related research. Some studies indicated the Fe from wheat bran was not well utilized (Widdowson and McCance, 1942) and might also interfere with the uptake of Fe supplied by other dietary components. Sandstead et al. (1978) did not observe any detrimental effects on iron balance of men fed 26 g/wheat bran/day. Chao-Lo (1979) found 70% of the Fe in wheat bran to be utilized versus 52% from control diets containing 5% cellufil and Fe supplied as ferrous sulfate.

Very little information exists regarding the chemical and physical nature of iron present in plants. The availability of endogenous iron of plant origin has not been fully investigated. Research in this area would greatly enhance the ability to assess the availability of iron from these important components of our diet.

Additional Special Comment - Balance Studies

Balance trials are not ideally suited for determining the effects of fiber on mineral utilization. Unless the same mineral source is used for all diets, decreased absorption may reflect the availability of a mineral salt rather than a specific fiber effect. Methods exist that are used to determine the bioavailability of minerals from various sources. The bioavailability of iron can be measured by the hematological response of anemic rats to an iron source (Fritz et al., 1970). This procedure was employed to better

define the role of fiber in iron utilization by rats. Results of this study are discussed in the following sections.

Serum Liver Cholesterol and Total Liver Lipid

Serum cholesterol levels were elevated in rats fed wheat bran and broccoli florets, but liver cholesterol and total liver lipid were unaffected (Table 11). All other fibers displayed no significant influence on serum and liver cholesterol of total liver lipid. Serum samples for animals on diets No. 1 and 3 were lost.

The *in vitro* binding of bile salts by fiber has been demonstrated (Eastwood and Hamilton, 1968; Story and Kritchevsky, 1976). The hypothesis has been advanced that this binding would reduce serum cholesterol levels and, perhaps, decrease the incidence of atherosclerosis (Story and Kritchevsky, 1978). Results of *in vivo* studies have not been conclusive.

Tsai *et al.* (1976) and Story *et al.* (1977) observed no changes in liver or serum cholesterol when either wheat bran of cellulose were incorporated into diets fed to rats. Forsythe *et al.* (1978) noticed increased serum cholesterol in rats fed coarse (16 and 30 mesh) wheat bran. This effect was not observed in animals fed 80 mesh wheat bran, psyllium or cellulose.

Interpretation of the effects of fiber on cholesterol metabolism are often difficult. Results are complicated by

Table 11. Liver weight, liver lipid, and liver and serum cholesterol.[1]

No.	Diet	Liver weight (g)	Liver lipid (%)	Liver cholesterol (mg/g)	Serum cholesterol (mg/100 ml)
1	Cellufil	4.67± .24	4.43±.30	2.48±.08	-
2	Broccoli florets	5.06± .17	4.17±.13	2.17±.07	111.5± 8.4
3	Broccoli waste	3.67± .67	4.15±.22	2.46±.19	-
4	Cellufil	5.62±1.66	3.73±.76	2.21±.60	94.8±18.1[a]
5	Cauliflower heads	4.44±1.06	3.96±.28	2.00±.06	72.0±13.5[a]
6	Cauliflower waste	4.80± .21	3.87±.33	1.96±.17	65.3±10.4[a]
7	Cellufil	5.88±1.65	4.25±.03	2.47±.06	69.4± 5.5[a]
8	Wheat bran	6.12±1.23	4.62±.19	2.42±.16	100.5± 9.6[b]
9	Metamucil	4.70± .20	4.22±.25	2.25±.22	75.3±13.2[a]

[1]Values reported as mean ± SD for 3 animals, values not followed by the same superscript letter are significantly different at $p < 0.05$.

the lack of standardization of the types and levels of dietary components fed. It appears that fiber has the potential to affect cholesterol metabolism. More investigations to determine the mode of action and potential applications of fiber in this area are necessary to include vegetable fiber.

Relative Biological Value - Iron

Growth

Feed consumption, actual dietary Fe levels and initial and final weight and hemoglobin levels are presented in Table 12. Rats fed wheat bran diets showed slightly higher, but not significant, weight gains compared to controls which provided more Fe. This might be due to the fact that the wheat bran diets contained a full complement of casein (20 g/100 g diet) in addition to the endogenous protein of the bran itself. The possibility of palability or other anti-nutritional factors in feeding such large amounts of the vegetables investigated remains unknown. The largest increase in weight was observed in animals fed the broccoli based diet. Growth of cauliflower-fed animals was comparable to animals fed control diets with moderate (12.5 ppm) Fe content. Depressed weight gains were apparent for broccoli floret and cauliflower head-fed rats in balance studies (Table 7), but not as pronounced when compared to

Table 12. Feed consumption, dietary iron levels, hemoglobin and body weight during RBV repletion trial.[1]

No.	Diet	Food consumption[a] ---- g ----	Dietary iron[3] g/g	Hemoglobin Initial ------- g/100 ml -------	Hemoglobin Final ------- g/100 ml -------	Body weight Initial ---------- g ----------	Body weight Final ---------- g ----------
10	Control-basal	147.2±43.6[a]	0	5.9±1.7[a]	4.9±1.6[a]	136.4±27.0[a]	151.4±34.2[a]
11	Control	173.4±24.5[a]	6.25	6.7±1.3[a]	6.2±0.8[a]	141.8±36.2[a]	165.0±21.7[a]
12	Control	212.4±12.4[a]	12.5	6.7±1.3[a]	8.7±0.8[ab]	143.3±37.5[a]	186.0±26.2[a]
13	Control	234.0±21.8[a]	25.0	6.7±1.3[a]	11.8±0.9[c]	146.7±29.9[a]	195.8±33.0[a]
14	Wheat bran	183.4±13.9[a]	5.25	6.7±1.3[a]	6.9±0.4[abd]	139.3±18.7[a]	169.5±18.7[a]
15	Wheat bran	208.4±13.1[a]	10.5	6.7±1.3[a]	7.9±0.1[ab]	136.5±29.0[a]	189.2±32.6[a]
16	Wheat bran	234.0±18.1[a]	21.0	6.7±1.3[a]	11.0±1.8[bcd]	140.0±22.2[a]	192.3± 8.2[a]
17	Broccoli	222.6±29.1[a]	17.5	6.7±1.3[a]	8.8±1.1[abcd]	127.7±15.4[a]	201.4±31.7[a]
18	Cauliflower	184.6±27.9[a]	16.3	6.7±1.3[a]	8.4±1.0[abcd]	146.7±27.6[a]	190.3±28.5[a]
19	Brussel sprouts	198.6±19.5[a]	13.3	6.7±1.3[a]	8.5±0.9[abcd]	161.0±27.8[a]	173.7±19.4[a]

[1]Values followed by different letters are significantly different ($p < 0.05$).

[2]Over 14 day repletion period.

[3]Theoretical, based on added ingredients.

RBV studies. The smallest weight increases were noted in the Brussel sprouts fed animals. These rats grew less than those on the basal-Fe free diet (No. 10). McCance and Walsham (1948) reported decreased energy utilization due to high fiber diets. This might explain, in part, these observations.

Hemoglobin Levels

The normal hemoglobin level of a healthy rat is approximately 14 g/100 ml blood. None of the animals in this experiment attained that level (Table 12). The greatest regeneration of hemoglobin occurred in rats fed the control diet supplying the highest level of ferrous sulfate. Wheat bran fed rats showed degrees of repletion comparable to those on corresponding control diets. Feed consumption between control and wheat bran fed animals was comparable.

Final hemoglobin levels of rats on vegetable diets were similar. The small differences noted are believed due to slight inequalities of dietary Fe levels (broccoli < cauliflower < Brussel sprouts). Feed consumption observed for these animals was not significantly different than control or wheat bran fed animals.

Relative Biological Value - Iron

Two methods were employed to determine the bioavailability of plant Fe. Relative biological value, as described

by Fritz and Pla (1970), evaluates test Fe by comparing its ability to replenish hemoglobin against that of Fe from ferrous sulfate. The ratio of test Fe to that of ferrous sulfate that gives the same hematological response is determined. This method was modified to take into consideration changes in body weight (Chao-Lo, 1979). A standard dose-response curve, obtained by plotting graded Fe levels ($FeSO_4$) against the product of final hemoglobin and body weight was used for comparative purposes. A linear regression equation was obtained using the basal (No. 10) and three cellufil control diets (Nos. 11-13):

$$Fe = -10.714 + 0.015 \times (\text{final Hb} \times \text{final body weight}),$$
$$(r = 0.9927).$$

From this equation the test source Hb x body weight is used to obtain an iron value. This method does not take into account differences in feed consumption between test groups.

A second method employed evaluated an Fe source according to the efficiency by which its Fe is converted to hemoglobin Fe. Available Fe, or percent true Fe efficiency, is defined as

$$\frac{\text{final mg Hb Fe - initial mg Hb Fe}}{\text{total dietary Fe intake}} \times 100$$

Determination of blood levels of hemoglobin Fe is based on the assumptions that 6.5% of the body weight of the rat is blood and hemoglobin contains 3.4 mg Fe/g.

Results of both methods for determining Fe with utilization are reported in Table 13.

Iron Bioavailability of Wheat Bran

The RBV of wheat bran Fe was greater than or equivalent to that of ferrous sulfate when NDF levels of these and control diets were similar (Table 13). When wheat bran provided 8 g NDF/100 g diet (Diet No. 18), the calculated RBV dropped to 86. This suggests fiber does affect Fe uptake. As fiber (wheat bran) level increases, the effect becomes more pronounced. However, it is apparent that wheat bran offers a readily utilizable form of Fe. Morris and Elliss (1976) have found monoferric phytate to be the major Fe salt present in wheat bran. They demonstrated that assimilation of monoferric phytate was high and suggested that wheat bran should be a source of readily available iron. This study supports that conclusion. Chao-Lo (1979) reported an RBV of 140 for Fe from wheat bran.

Other studies have not been as positive about the effects of wheat bran in the diet. Stiles (1976) reported decreased uptake of iron by rats due to inclusion of cellulose, peanut hulls or corn pericarp in their ratios, Björn-Rasmussen (1974) observed decreased incorporation by man of radioactive iron (^{59}Fe) from breads and rolls containing more than 3.3% wheat bran. Widdowson and McCance (1942) and Reinhold et al. (1976) feeding wheat bran or high fiber

Table 13. Relative biological value and true availability values of iron from wheat bran, broccoli, cauliflower and Brussel sprouts.[3]

No.	Product	Relative Biological value, %[1]	Percent efficiency of utilization
11	Control (5% cellufil)[2]	-	13[a]
12	Control	-	54[b]
13	Control	-	49[b]
14	Wheat bran	113[a]	53[b]
15	Wheat bran	96[a]	58[b]
16	Wheat bran	86[a]	52[b]
17	Broccoli	93[a]	51[b]
18	Cauliflower	83[a]	45[bc]
19	Brussel sprouts	88[a]	33[c]

[1]Based on regression equation from control diets containing 5% cellufil and Fe supplied as $FeSO_4 \cdot 7H_2O$.

$$-10.714 + 0.015 \text{ (final weight x final Hb)}; \quad (r = 0.9927)$$

[2]Control diets 11, 12, 13 provide 6,25, 12.5 and 25.0 ppm Fe respectively.

[3]Values followed by different letters are significantly different ($p < 0.05$).

flours as 40-50% of total calories, found subjects invariably displayed negative Fe balances. More moderate intakes of wheat bran (26 g/day) had no effect on Fe balance (Sandstead et al., 1978).

Iron from wheat bran appears to be readily available. Consumed in moderation, fiber effects on Fe, utilization are negligible. When high levels of this fiber are fed, the uptake of Fe from this source may be impaired by the binding action of fiber.

Bioavailability of Vegetable Iron

Endogenous iron of the vegetables evaluated in this study proved to be very available (Table 13). Relative biological values for all plants exceeded 80. Iron from broccoli (RBV = 93) was the best source. To the author's knowledge, this is the first study actually determining the availability of endogenous Fe in these plants using the rat.

Utilization of Fe from these sources exceeded reported values for endogenous iron of spinach and equalled those for soybean isolate. Chao-Lo (1979) observed an RBV of 71 for spinach Fe. The RBV of Fe from soybean isolate has been reported to range from 66.5 (Steinke and Hopkins, 1978) to a high of 125% (Fritz et al., 1970). The variability of these latter results may be attributed to differences in experimental conditions and the type of product evaluated. The RBV's of these vegetables were not significantly different

than values found for wheat bran or the theoretical values (RBV = 100) established for controls.

True Iron Bioavailability

Iron bioavailability studies based on RBV's are only relative measurements of Fe utilization. A more realistic value is obtained when differences in Fe consumed due to unequal food consumption are considered. True Fe bioavailability compares the amount of hemoglobin Fe gained to the amount of Fe consumed. It is reported as percent efficiency of utilization. In calculating these values, intake was computed as the product of dietary Fe level (μg/g) times total food consumption in g (Table 12). Hemoglobin Fe was determined as the difference between initial and final hemoglobin Fe content. Both initial and final hemoglobin Fe levels were calculated as the product of body weight (Table 12) times 0.065 g blood/g body weight times hemoglobin concentration (Table 12) times 3.35 mg Fe/g hemoglobin.

In this experiment, 54 and 49% of the Fe in diets containing 12.5 and 25.0 ppm Fe ($FeSO_4$), respectively, was utilized for hemoglobin regeneration. The conversion efficiency is in agreement with Mahoney et al. (1974) and Fritz et al. (1970). The poor conversion in the diet containing low Fe (6.25 ppm as $FeSO_4$) cannot be explained, but has been previously observed (Chao-Lo, 1979).

Comparing the utilization of the mean from the two control diets (Nos. 12 and 13), which was 52%, versus the three wheat bran diets (Nos. 14, 15, and 16) with a mean of 57%, wheat bran Fe utilization was not significantly higher. There was no significant difference between the means of controls against broccoli and cauliflower. The utilization of Fe from Brussel sprouts was significantly lower ($p < 0.05$).

True Fe bioavailability is generally one-half of reported RBV's. This relationship was observed for all diets except that containing Brussel sprouts. The RBV of Fe from Brussel sprouts was higher than would be predicted by the results of true Fe bioavailability.

Comparison of True Iron Bioavailability and Balance Studies

When results of true Fe bioavailability studies (Table 13) are compared to balance trials (Figure 2) a more clear picture of fiber effects can be demonstrated. Iron utilization for control animals in balance studies was ca. 50%. Similar results were observed for rats fed 12.5 and 25.0 ppm Fe ($FeSO_4$) in the RBV trials. Likewise, wheat bran and cauliflower-fed rats displayed nearly equal uptake of Fe in both investigations. The only discrepancy was observed in the broccoli-fed animals. The true availability of the endogenous Fe of broccoli was found to be 51% (Table 13).

Apparent uptake of this Fe from balance trials was only 18% (Figure 2). These results might be explained by the different levels and sources of broccoli fed in the two experiments.

The NDF content of diets used in balance studies was twice that of diets used in RBV trials. The close agreement observed in Fe utilization by all except the broccoli-fed groups suggests that fiber does not seriously interfere with Fe availability.

SUMMARY AND CONCLUSIONS

The influence of broccoli florets and wastes, cauliflower heads and wastes, wheat bran and Metamucil on mineral balance was determined. Minerals of concern were Ca, Mg, Cu, Zn and Fe. Balance studies were employed using the rat as an experimental model. The relative biological value (RBV) of endogenous Fe in broccoli, cauliflower, Brussel sprouts and wheat bran was measured by the hemoglobin repletion technique.

Animals were fed diets containing 10% neutral detergent fiber (NDF) from the various plant sources. Minerals were contributed from the plants and from inorganic salts such that levels were comparable in all diets. Balance was measured as the percent of ingested minerals not recovered in the feces and urine. The apparent utilization of Ca, Mg, Cu, Zn and Fe by control animals fed 10% cellulose was determined to be 62, 33, 23, 42, and 52% respectively. Broccoli florets decreased absorption of Mg, Zn, and Fe to 12, 16 and 18% respectively. Broccoli wastes depressed uptake of Zn to 14% and caused Fe excretion to exceed uptake (-1%). Cauliflower wastes decreased utilization of Ca to 26% and created a negative balance of Mg (-40%).

Not all results were negative. Calcium absorption was improved by both wheat bran and Metamucil from 62% (control)

to 88%. Metamucil also improved utilization of Mg from 33% (control) to 53%. Zinc uptake was increased in rats fed cauliflower heads and wheat bran from 33% (control) to 61 and 54% respectively. Copper balance was not significantly affected by any plant source.

Results of these balance trials were complicated by the fact that the source of minerals fed was not consistent in all diets. The chemical form of minerals is known to vary between plants. Furthermore, minerals are capable of complexing with various plant components. The effects of these factors in mineral availability are not clearly defined. The relative biological value (RBV) of endogenous iron from some plants used in the balance trials was measured to aid in understanding the true influence of fiber on iron balance.

Animals made anemic over a 28 day period were fed plant sources of iron for 14 days. The product of hemoglobin regeneration times body weight in the test animals was compared to controls fed ferrous sulfate (RBV = 100%). Values determined for broccoli, cauliflower and Brussel sprouts were 93, 84 and 88% respectively. Wheat bran was fed at 5, 10 and 20% levels and RBV's were measured as 113, 96 and 89% respectively. The true bioavailability, or percent efficiency, of an iron source was measured as the ratio of total iron gained as hemoglobin to the amount of iron consumed. These values were found to be one-half of the RBV's. Good

agreement was observed between results of iron bioavailability studies and the apparent utilization of iron by all animals in balance trials except those fed broccoli. Broccoli may offer some unique answers to divalent cation utilization, especially Fe.

The NDF level of diets in the balance study was 10%. Diets in the RBV trial had NDF contents of from 2% for a wheat bran diet to 5% for the Brussel sprouts diet. The lack of disparity in results for all except the broccoli diet suggests that fiber does not significantly effect the utilization of iron in the rat. The inverse relationship between RBV of wheat bran iron and the level of wheat bran in the diet suggests that increased fiber consumption may impair uptake of endogenous iron of wheat bran, but this effect was slight.

During the course of the balance study it was noted that all test fibers increased fecal moisture content to twice that observed for control animals. Cellulose and wheat bran are often promoted for their fecal bulking properties. All vegetable fiber sources used in this study were superior in this regard.

As a freeze dried product, vegetables and vegetable wastes can provide 14-20% NDF and more than 20% protein (Kjeldahl nitrogen x 6.25). Although not as rich in minerals as wheat bran, these plants contain highly available iron. Before being considered for food uses, the

functional properties (i.e. texture, taste, etc.) of these products should be evaluated.

These experiments have indicated that with few exceptions, vegetable fibers did not detrimentally influence mineral balance in rats. Extrapolation of these results to human nutrition is inappropriate in view of documented specie differences. Additional research should be directed towards determining the exact nature of minerals in foods and the bioavailability of these native forms.

BIBLIOGRAPHY

Abell, L. L., B. B. Levy, B. B. Brodie, and F. E. Kendall. 1952. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J. Biol. Chem. 195:357.

Altman, P. L. and S. Dittmer (eds.). 1968. Metabolism. Fed. of American Soc. Exp. Biol., Bethesda, Md., p. 53.

Andres, C. 1977. Fiber forms, functionality, applications, availability. Food Processing 38:37.

Association of Official Analytical Chemists. 1975. "Official Methods of Analysis," 12th ed., Washington, D. C., p. 15, 2.049.

Association of Official Analytical Chemists. 1975. "Official Methods of Analysis," 12th ed., Washington, D. C., p. 601, 32.036.

Aspinall, G. O. 1970. Pectins, plant gums, and other plant polysaccharides. In: The Carbohydrates. Edited by W. Pigman and D. Horton. Academic Press, New York, Vol. IIb, Chap. 39, p. 515.

Belford, D. S., A. Meyers, and R. C. Preston. 1959. A study of the ordered adsorption of metal ions on the cellulose microfibril. Biochem. Biophys. Acta. 34:47.

Bieri, J. G., G. S. Stowesand, G. M. Briggs, R. W. Phillips, J. C. Woodward, and J. J. Knappa. 1977. Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. J. Nutr. 107:1340.

Bjorn-Rasmussen, E. 1974. Iron adsorption from wheat bread. Influence of various amounts of bran. Nutr. Metabol. 16:101.

Blisel, W. R. 1979. Metabolic balance studies - their continuing usefulness in nutritional research. Am. J. Clin. Nutr. 32:271.

Branch, W. J., D. A. T. Southgate, and W. P. T. James. 1975. Binding of calcium by dietary fibre: Its relationship to unsubstituted uronic acids. Proc. Nutr. Soc. 34:120A.

Burkitt, D. P. 1973. Some disease characteristics of Western Civilization. Br. Med. J. 1:274.

Burkitt, D. P., A. R. P. Walker and N. S. Painter. 1972. Effects of dietary fiber on stools and transit times, and its role in the causation of disease. Lancet 2: 1408.

Burkitt, D. P., A. R. P. Walker and N. S. Painter. 1974. Dietary fiber and disease. J. Am. Med. Assoc. 229: 1068.

Chao-Lo, S. L. 1979. Influence of fish on the bioavailability of plant iron in the anemic rat. Master's thesis, Oregon State University.

Cleave, T. L., G. D. Campbell, and N. S. Painter. 1966. Diabetes, coronary thrombosis and the saccharine disease. Bristol, Wright.

Connell, A. M., C. L. Smith and M. Somsel. 1975. Absence of effect of bran on blood lipids. Lancet 1:496.

Cowgill, G. R. and A. J. Sullivan. 1933. Further studies on the use of wheat bran as a laxative. J. Am. Med. Assoc. 100:795.

Crosby, W. H., J. I. Munn and F. W. Furth. 1954. Standardizing a method for clinical hemoglobinometry. U.S. Armed Forces Med. J. 5:693.

Cummings, J. H. 1976. What is fiber? In: Fiber in Human Nutrition. Edited by G. A. Spiller and R. J. Amen, Plenum Press, New York.

Cummings, J. H. 1978. Nutritional implications of dietary fiber. Am. J. Clin. Nutr. 31:S21.

deBoland, A. R., G. B. Garner and B. L. O'Dell. 1975. Identification and properties of "phytate" in cereal grains and oilseed products. J. Agr. Food Chem. 23: 1186.

Dimock, E. M. 1936. Their treatment of habitual constipation by the bran method. M.D. Thesis, Cambridge.

Dimock, E. M. 1937. The prevention of constipation. Br. Med. J. 1:906.

Eastwood, M. A. 1974. Dietary fibre and nutrition. Proc. Nutr. Soc., No. 5, Edinburgh Univ.

Eastwood, M. A. and G. S. Boyd. 1967. The distribution of bile salts along the small intestine of rats. Biochem. Biophys. Acta. 137:393.

Eastwood, M. A. and . . Hamilton. 1968. Studies on the absorption of bile salts on nonabsorbed components of diet. Biochem. Biophys. Acta. 152:165.

Eastwood, M. A. and R. M. Kay. 1979. An hypothesis for the action of dietary fiber along the gastrointestinal tract. Am. J. Clin. Nutr. 32:364.

Eastwood, M. A. and W. D. Mitchell. 1976. Physical properties of fiber: A biological evaluation. In: Fiber in Human Nutrition. Edited by G. A. Spiller and R. J. Amen. Plenum Press, New York, p. 109.

Fassett, D. W. 1973. Oxalate. In: Toxicants Occurring Naturally in Foods. 2nd ed., pp. 346. National Academy of Sciences, Washington, D. C.

Finegold, S. M. and V. L. Sutter. 1978. Fecal flora in different populations, with special reference to diet. Am. J. Clin. Nutr. 31:S116.

Folch, J., M. Lees and G. H. Stanley-Sloane. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497.

Forsythe, W. A., W. L. Chenoweth and M. R. Bennink. 1978. Laxation and serum cholesterol in rats fed plant fibers. J. Food Sci. 43:1470.

Freudenberg, K. 1965. Lignin: Its constitution and formation from p-hydroxycinnamyl alcohols. Science 148:595.

Fritz, J. C. and G. W. Pla. 1972. Application of the animal hemoglobin repletion test to measurement of iron bioavailability in foods. J.A.O.A.C. 55:1129.

Fritz, J. C., G. W. Pla, T. Roberts, J. W. Boehne and E. L. Hove. 1970. Biological availability in animals of iron from common dietary sources. J. Agr. Food Chem. 18:647.

Godding, E. W. 1976. Dietary fiber redefined. Lancet 1:1129.

Goering, H. K. and P. J. VanSoest. 1970. Forage fiber analysis. Agriculture handbook No. 379. A.R.S., U.S.D.A., Washington, D.C., U.S. Gov't Printing Office.

Gontzea, I. and P. Sutzescu. 1968. Substances depressing utilization of minerals. I. Phytic acid. In: Natural Antinutritive Substances in Foodstuffs and Forages. pp. 55-84. S. Karger-Basel (Switzerland), New York.

Gordon, A. J. 1978. The chemical structure of lignin and quantitative and qualitative methods of analysis in foodstuffs. In: Topics in Dietary Fiber Research, edited by G. A. Spiller and R. J. Amen, Plenum Press, New York, pp. 59-104.

Haghshenass, M., M. Mahloucji, J. G. Reinhold and N. Mohammadi. 1972. Iron-deficiency anemia in an Iranian population associated with high intake of iron. Am. J. Clin. Nutr. 25:1143.

Harrison, H. E. and H. C. Harrison. 1974. Intestinal absorption, edited by D. H. Smith, London, p. 793.

Hartley, R. D. 1978. The lignin fraction of plant cell walls. Am. J. Clin. Nutr. 31:S90.

Heaton, K. W., A. P. Manning, and M. Hartog. 1976. Lack of effect on blood lipids and calcium concentrations of young men on changing from white to wholemeal bread. Brit. J. Nutr. 35:55.

Heaton, K. W., and E. W. Pomare. 1974. Effect of bran on blood lipids and calcium. Lancet 1:49.

Isaksson, B. and B. Sjogren. 1967. A critical evaluation of the mineral and nitrogen balance in man. Proc. Nutr. Soc. 26:106.

Ismail-Beigi, F., B. Faradji and J. G. Reinhold. 1977. Binding of zinc and iron to wheat bread, wheat bran and their components. Am. J. Clin. Nutr. 30:1721.

Jenkins, D. J. A., M. A. Hill, and J. H. Cummings. 1975. Effect of wheat fiber on blood lipids, fecal steroid excretion and serum iron. Am. J. Clin. Nutr. 28:1408.

Johnson, L. F. and M. E. Tate. 1969. Structure of "Phytic acids". Can. J. Chem. 47:63.

Jones, L. H. P. 1978. Mineral components of plant cell walls. Am. J. Clin. Nutr. 31:S94.

Kellogg, J. H. 1923. The new dietetics: A guide to the scientific feeding in Health and Disease. Battle Creek.

Kelsay, J. L. 1978. A review of research on effects of fiber intake on man. Am. J. Clin. Nutr. 31:142.

Krishnamurty, K. U. and G. M. Harres. 1961. The chemistry of the metal oxalate complexes. Chemical Rev. 61:213.

Kritchevsky, D. 1964. Experimental atherosclerosis in rabbits fed cholesterol-free diets. J. Atheroscler. Res. 4:103.

Kritchevsky, D., R. P. Casey and S. A. Tepper. 1973. Isocaloric, isogravic diets in rats. II. Effect on cholesterol absorption and excretion. Nutr. Rep. Int. 7:61.

Kritchevsky, D. and J. A. Story. 1974. Binding of bile salts in vitro by non-nutritive fiber. J. Nutr. 104: 458.

Kritchevsky, D., S. A. Tepper and J. A. Story. 1974. Isocaloric, isogravic diets in rats. III. Effects on non-nutritive fiber (alfalfa or cellulose) on cholesterol metabolism. Nutr. Rep. Int. 9:301.

Kritchevsky, D., S. A. Tepper, H. K. Kim, D. E. Moss and J. A. Story. 1975. Experimental atherosclerosis in rabbits fed semi-purified diets. IV. Investigation into the source of cholesteremia. Exptl. Mole. Pathology 22:11.

Lang, J. A. and G. M. Briggs. 1976. The use and function of fiber in diets of monogastric animals. In: Fiber in Human Nutrition edited by G. A. Spiller and R. J. Amen, Plenum Press, New York, p. 151-170.

Lehninger, A. L. 1975. Biochemistry. Worth Publishers, Inc., New York, 1104 p.

Leveille, G. A. and H. E. Sauberlich. 1966. Mechanism of the cholesterol-depressing effect of pectin in the cholesterol-fed rat. J. Nutr. 88:209.

Mahoney, A. W., C. C. VanOrden and D. G. Hendricks. 1974. Efficiency of converting food iron into hemoglobin by the anemic rat. Nutr. Metabol. 17:223.

Maleki, M. 1972. Food consumption and nutritional status of 13 year old village and city school boys in Fars province of Iran. Ecol. Food. Nutr. 1:39.

McCance, R. A. and E. M. Widdowson. 1942. Mineral metabolism of healthy adults on white and brown bread diets. J. Physiol. 101:44.

McCance, R. A. and C. M. Walsham. 1948. The digestibility and absorption of the calories, proteins, purines, fat and calcium in wholemeal wheaten bread. Brit. J. Nutr. 2:26.

McCarrison, R. 1936. Nutrition and Health. Cantor lectures before Royal Society of Arts, London, Faber and Faber (reprinted 1961), pp. 29-31, 103 and 104.

McConnell, A. A., M. A. Eastwood and W. D. Mitchell. 1974. Physical characteristics of vegetable foodstuffs that could influence bowel function. J. Sci. Food Agric. 25:1457.

Morris, E. R. and R. Ellis. 1976. Isolation of monoferric phytate from wheat bran and its biological value as an iron source to the rat. J. Nutr. 106:753.

Mullen, J. O. 1978. Dietary fiber sources for human studies. Am. J. Clin. Nutr. 31:S103.

Painter, N. S. 1969. Diverticular disease of the colon, A disease of this century. Lancet 2:586.

Paloheimo, L. 1953. Some persistent misconceptions concerning crude fiber and the nitrogen free extract. J. Sci. Agric. Soc. Finland 25:16.

Perkin-Elmer Corp. 1973. Analytical Methods for Atomic Absorption Spectrophotometry. Perkin-Elmer Corp., Norwalk, CT.

Persson, I., K. Raley, P. Fonns-Bech and E. Jensen. 1975. Bran and blood lipids. Lancet 2:922.

Pileggi, V. J. 1959. Distribution of phytase in the rat. Arch. Biochem. Biophys. 80:1.

Pla, G. W. and J. C. Fritz. 1970. Availability of iron. J.A.O.A.C. 53:791.

Portman, O. W. 1960. Nutritional influences on metabolism of bile acids. Am. J. Clin. Nutr. 8:462.

Ranhotra, G. S. and R. J. Loewe. 1975. Effect of wheat phytase on dietary phytic acid. J. Food Sci. 40:940.

Reed, K. C. 1973. Binding of calcium by cellulose membrane and sephadex. Biochem. Biophys. Res. Commun. 50:1136.

Rees, D. A. 1967. The Shapes of Molecules. Oliver and Boyd, Edinburg.

Reinhold, J. G., F. Ismail-Beigi and B. Faradji. 1975. Fibre vs. phytate as determinant of the availability of calcium, zinc and iron of bread stuffs. Nutr. Rep. Int. 12:75.

Reinhold, J. G., B. Faradji, P. Abadi and F. Ismail-Beigi. 1976. Decreased absorption of calcium, magnesium, zinc and phosphorous by humans due to increased fiber and phosphorous consumption as wheat bread. J. Nutr. 106:493.

Robertson, J. B. 1978. The detergent system of fiber analysis. In: Topics in Dietary Fiber Research, edited by G. A. Spiller and R. J. Amen, Plenum Press, New York, p. 203.

Sandstead, H. 1972. Human zinc requirements: Effects of dietary fiber on zinc metabolism. Fed. Proc. 31:254.

Sandstead, H., Munoz, R. A. Jacob, L. M. Klevay, S. J. Reck, G. M. Logan, F. R. Dintzis, G. E. Inglett and W. C. Shevy. 1978. Influence of dietary fiber on trace element balance. Am. J. Clin. Nutr. 31:S180.

Schupman, W. 1970. Control of plant protein. The influence of genetics and ecology on food plants. In: Proteins as Human Food, edited by R. A. Lawrie, AVI Publishing Co., Inc., Westport, Conn. pp. 245-265.

Shipley, E. A. 1978. Dietary fiber content of foods. In: Topics in Dietary Fiber Research, edited by G. A. Spiller and R. J. Amen, Plenum Press, New York, p. 203.

Snedecor, G. W. and W. G. Cochran. 1976. Statistical Methods. 6th ed. The Iowa State Univ. Press, Ames, Iowa. 593 p.

Southgate, D. A. T. 1976. The chemistry of dietary fiber, In" Fiber in Human Nutrition, edited by G. A. Spiller and R. J. Amen, Plenum Press, New York, p. 31-72.

Southgate, D. A. T., B. Bailey, E. Collinson and A. Wasker. 1976. A guide to calculating intakes of dietary fiber. J. Human Nutr. 30:303.

Southgate, D. A. T. and J. V. G. A. Durnin. 1970. Calorie conversion factors. An experimental re-assessment of the factors used in the calculation of the energy value of human diets. Brit. J. Nutr. 24:517.

Steinke, F. H. and D. T. Hopkins. 1978. Biological availability to the rat of intrinsic and extrinsic iron with soybean protein isolates. J. Nutr. 108:481.

Stiles, L. W. 1976. Effect of fiber on the availability of minerals. In: The role of fiber in the diet, edited by D. L. Downing, N. Y. State Ag. Exp. Station. Special bulletin No. 21.

Story, J. A. and D. Kritchevsky. 1976. Comparison of the binding of various bile acids and bile salts in vitro by several types of fiber. J. Nutr. 106:1292.

Story, J. A., S.K. Czarnecki, A. Baldine, and D. Kritchevsky. 1977. Effect of components of fiber on dietary cholesterol in the rat. Fed. Proc. 36:1134.

Story, J. A. and D. Kritchevsky. 1978. Bile acid metabolism and fiber. Am. J. Clin. Nutr. 31:S199.

Thomas, B. 1972. Enzymatic procedures for the determination of structural cell wall constituents for their interpretation. Intern. Assoc. for Cereal Chemistry Congr., Vienna.

Trinder, P. 1969. Simple turbidimetric method for the determination of serum cholesterol. Ann. Clin. Biochem. 6:165.

Trowell, H. 1972. Ishemic heart disease and dietary fiber. Am. J. Clin. Nutr. 25:926.

Trowell, H. 1973. Dietary fiber, ishemic heart disease and diabetes mellitus. Proc. Nutr. Soc. 32:151.

Tsai, A. C., J. Elias, J. J. Kelley, R. C. Lin and J. R. K. Robseon. 1976. Influence of certain dietary fibers on serum and tissue cholesterol in rats. J. Nutr. 106:118.

VanSoest, P. J. 1973. Collaborative study of acid detergent fiber and lignin. J.A.O.A.C. 56:781.

VanSoest, P. J. 1975. Physiochemical aspects of fibre digestion. In: Procedures of the IV International Symposium on Ruminant Physiology, edited by I. W. McDonald and A. C. I. Warner, The University of New England Publishing Unit, p. 351.

VanSoest, P. J. 1978. Component analysis of fiber in food, summary and recommendations. Am. J. Clin. Nutr. 31: S75.

VanSoest, P. J. and R. W. McQueen. 1973. The chemistry and estimation of fiber. Proc. Nutr. Soc. 32:123.

Walker, A. R. P. 1966. Nutritional, biochemical and other studies on South African populations. S. Afr. Med. J. 40:814.

Walker, A. R. P. 1974. Dietary fiber and pattern of diseases. Editorial, Ann. Intern. Med. 80:663.

Walker, A. R. P. 1978. The relationship between bowel cancer and fiber content in the diet. Am. J. Clin. Nutr. 31:S248.

Watt, B. K. and A. C. Merrill. 1963. Composition of Foods. Agricultural Handbook No. 8, U.S.D.A. Gov't Printing Office, Washington, D. C.

Wersch, S. C. and A. C. Ivy. 1941. On the fate of ingested pectin. Am. J. Dig. Dis. 8:101.

Widdowson, E. M. and R. A. McCance. 1942. Iron exchanges of adults on white and brown bread diets. Lancet 1:588.

Williams, R. D. and W. H. Omstead. 1936. The effect of cellulose, hemicellulose and lignin on the weight of stool. A contribution to the study of laxation in man. J. Nutr. 11:433.

Worth, H. G. J. 1967. The chemistry and biochemistry of pectic substances. Chem. Rev. 67:465.