EFFECTS OF CERTAIN VITAMINS AND MINERALS ON CALCIUM AND PHOSPHORUS HOMEOSTASIS

September 1982

Prepared for

BUREAU OF FOODS
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
WASHINGTON, D.C. 20204

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edited by

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FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in work in specific areas of biology and medicine.

This technical report was developed for the Food and Drug Administration (FDA) in accordance with the provisions of Contract No. FDA 223-79-2275. It was prepared and edited by Sue Ann Anderson, Ph.D., R.D., Staff Scientist, LSRO, FASEB.

The LSRO acknowledges the contributions of the investigators and consultants who assisted with this study. The report reflects the opinions expressed by an ad hoc study group that met at the Federation on January 25-26, 1982. The study participants reviewed a draft of the report and their various viewpoints were incorporated into the final report. The study participants and LSRO accept responsibility for the accuracy of the report; however, the listing of these individuals in Section XII does not imply that they specifically endorse each study conclusion.

The report was reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent Society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures, the report was approved and transmitted to FDA by the Executive Director, FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of each individual member of the FASEB constituent Societies.

September 30, 1982

Kenneth D. Fisher, Ph.D.
Director
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ABSTRACT

This report considers roles of vitamins D, A, and K, and magnesium, zinc, and fluoride in calcium and phosphorus metabolism as discussed by an ad hoc review group. Low plasma concentrations of 25-hydroxyvitamin D suggest that persons at risk of low vitamin D status include the elderly, the grossly obese, and some vegetarians. Effects of deficiency on bone diseases other than osteomalacia are ill-defined. High intakes of vitamin D are associated with ectopic calcifications and arteriosclerosis in animals. Evidence of a role of vitamin D in arteriosclerosis in humans is not available. Inadequate and excessive intakes of vitamin A are associated with abnormal bones and teeth. Limited data indicate that caries susceptibility may be increased by low vitamin A intake. Fractures may occur in patients given massive amounts of vitamin A and in persons ingesting large dietary supplements of vitamin A. Vitamin K is implicated in bone metabolism by osteocalcin, a vitamin K-dependent protein that binds calcium. The relationship of vitamin K status to osteocalcin function and bone metabolism has not been determined. Magnesium deficiency is associated with degenerative changes in the cardiovascular system and ectopic calcifications. Epidemiologic data from several countries suggest an association between mortality rates from cardiovascular disease and consumption of soft water. Zinc is required for normal bone growth in children. Short-term balance studies in humans indicate that large doses of zinc inhibit the absorption of calcium when calcium intakes are low. Epidemiologic data suggest an inverse relationship between incidence of osteoporosis and level of fluoride in drinking water. Recent clinical trials indicate that fluoride administration together with various combinations of calcium, estrogen, and vitamin D therapy increased bone mass and reduced rates of vertebral fracture in postmenopausal women with osteoporosis. Specific topics requiring additional research are identified.
SYNOPSIS

INTRODUCTION

This report considers roles of selected nutrients (vitamins D, A, and K, and magnesium, zinc, and fluoride) on maintenance of calcium and phosphorus homeostasis. Regulation of calcium and phosphorus metabolism requires a complex interplay of body reserves with physical activity, hormonal agents, and dietary factors. Dietary intakes must supply sufficient amounts of each mineral to replace losses; further, intakes of dietary factors associated with calcium and phosphorus metabolism must also meet nutritional needs. However, simply supplying adequate amounts of nutrients cannot assure positive calcium and phosphorus balance.

VITAMIN D

Quantitative determination of the amount of vitamin D contributed by foods is difficult. Few foods contain the vitamin naturally; vitamin D-fortified foods, including milk and dry cereals, supply most of the dietary intake. Estimates of vitamin D status are complicated by large variations in exposure to sunlight.

Together with parathyroid hormone and calcitonin, vitamin D acts on intestine, bone, and kidney to influence calcium and phosphorus metabolism. 1,25-Dihydroxyvitamin D is considered the most important vitamin D metabolite affecting absorption and disposition of these minerals. Serum concentrations of 1,25-dihydroxyvitamin D vary greatly with different physiologic states and fluctuate too widely to be useful in assessing vitamin D status. This metabolite may influence intestinal adaptation to changes in dietary calcium intake or metabolic demand. Serum 1,25-dihydroxyvitamin D concentrations are correlated negatively with calcium intakes in adults and are doubled or tripled in women in the late stages of pregnancy. The relationship between plasma concentrations of 1,25-dihydroxyvitamin D and calcium absorption in postmenopausal women with osteoporosis has yet to be determined.

25-Hydroxyvitamin D is the form of the vitamin present in largest concentrations in human plasma. It shows little metabolic activity at physiological concentrations. Plasma concentrations vary during the year, but this measure is considered the best biochemical measure of vitamin D status. Other hydroxylated derivatives have been identified in human plasma.

Low plasma 25-hydroxyvitamin D concentrations in a limited fraction of several subgroups in the United States population suggest that persons at risk of subnormal vitamin D status include the elderly, the grossly obese, and some vegetarians. Serum
25-hydroxyvitamin D concentrations do not differ significantly between normal women and age-matched postmenopausal patients with osteoporosis. Effects of vitamin D status on bone diseases other than osteomalacia are poorly understood.

Excessive bone demineralization may occur with therapeutic use of large doses of vitamin D or its metabolites or by self-administration of large doses of the vitamin. The resultant excess of serum calcium may be deposited in kidney, heart, and aorta.

Feeding diets containing levels of vitamin D only a few times greater than dietary requirement is associated with development of arteriosclerosis in squirrel monkeys. Some evidence indicates that oxidation products of sterols may be involved in arteriosclerosis. It is tempting to speculate that oxidation products of vitamin D might be included. However, experimental evidence neither supports nor disproves such speculation.

Assessment of a role of dietary vitamin D in the development of arteriosclerosis is difficult because the disease is multifactorial in etiology. Concerns about this issue require additional data: intake of vitamin D including that contributed by meats and fish; serum and tissue concentrations in humans; and effects of chronic, mild overdoses of vitamin D in animals, giving due consideration to comparability of serum lipid levels with those of humans.

**VITAMIN A**

In early studies of vitamin A deficiency in domestic animals, bony overgrowths resulted in blindness by constriction of the optic nerve or lack of coordination because of effects on bones of the inner ear. More recent experiments show effects on glycosaminoglycan biosynthesis in the organic matrix and mineral deposition in bones and teeth of experimental animals. Caries susceptibility in humans may be increased by low vitamin A intake, although these data are limited. Decreased urinary calcium excretion and occurrence of calcified lesions in the urinary tract of animals suggest that vitamin A deficiency may affect calcium metabolism, leading to changes in extra-osseous tissues.

Excessive intakes of vitamin A result in bone changes that are essentially the reverse of abnormalities observed with vitamin A deficiency. Bone fractures may occur in patients given massive therapeutic doses of the vitamin and in persons ingesting large dietary supplements. A direct effect of vitamin A on calcium metabolism has yet to be shown.
VITAMIN K

Vitamin K is implicated in bone metabolism by the presence in bone of osteocalcin, a protein that binds calcium and contains γ-carboxyglutamic acid. This amino acid residue in osteocalcin is carboxylated in a reaction requiring vitamin K. The protein comprises 1 to 2% of total bone protein. Proteins other than osteocalcin containing γ-carboxyglutamic acid are found in ectopic calcifications including renal calculi. The function of the protein remains elusive. Postulated functions include a role in maturation of bone mineral and direct or indirect regulation of calcium metabolism in bone. Its synthesis by osteosarcoma cells and concentration in plasma are increased by 1,25-dihydroxyvitamin D, suggesting a possible association between vitamin D and vitamin K functions in bone metabolism.

Osteocalcin concentrations in plasma are elevated in certain disorders of bone and calcium metabolism. Changes in urinary excretion of γ-carboxyglutamic acid may indicate alterations in metabolism of proteins containing the amino acid. Further study and correlation with biochemical tests, nuclear medical procedures, and histology will be required to determine the value of osteocalcin and γ-carboxyglutamic acid assays as a means of detection and evaluation of bone diseases. The relationship of osteocalcin content of bone and plasma and vitamin K status remains to be determined.

MAGNESIUM

After calcium, magnesium is the principal cation in bone. Magnesium is not an integral part of bone mineral; it is found only in the hydration shell and crystal surface. It may play a role in normal metabolism and various disorders of mineralized tissues. Few data are available to evaluate effects of magnesium intake on phosphorus metabolism; however, magnesium deficiency in animals results in defective bones and teeth. Effects of magnesium deficiency on calcium metabolism differ among rats and most other species, including man. Magnesium deficiency in humans usually occurs only as a part of pathologic conditions. Hypocalcemia in magnesium-deficient patients is not prevented by adequate intakes of calcium or reversed by oral administration of calcium, nor is it caused by increased renal losses of calcium. Some evidence suggests impaired function of the parathyroid gland or dulling of renal sensitivity to parathyroid hormone action.

Degenerative changes in the cardiovascular system, abnormalities in electrocardiograms, and deposition of calcium in soft tissues are observed during magnesium deficiency. Epidemiologic studies in several countries show an association between higher mortality rates from cardiovascular disease and consumption of soft water. However, widespread use of water softeners complicates studies of effects of water hardness in the United States.
Analysis of epidemiologic data suggests that the mean calcium to magnesium ratio in diets in seven countries, including the United States, is highly correlated with the mortality rate from coronary heart disease. Considerations of the ratios of mineral intakes (and possibly mineral interactions), as well as knowledge of absolute amounts consumed, appear necessary to develop an understanding of the roles of minerals on development of cardiovascular disease.

**ZINC**

Zinc is required for normal bone growth in children. Short-term balance studies in humans indicate that large doses of zinc inhibit the absorption of calcium when calcium intakes are low. The effects of large doses of zinc on phosphorus nutrition are not known.

**FLUORIDE**

Limited epidemiologic evidence suggests an inverse relationship between the incidence of osteoporosis in a geographic area and the concentration of fluoride in the drinking water. Determination of total fluoride intake in epidemiological studies in the United States is complicated by consumption of foods processed in areas where the fluoride content of water is different from that of the area where the food was consumed.

Recent clinical trials indicate that administration of fluoride together with various combinations of calcium, estrogen, and vitamin D therapy increases bone mass and reduces vertebral fracture rates in postmenopausal women with osteoporosis. However, except for studies of mineral balance, knowledge of effects of fluoride on calcium and phosphorus metabolism is limited.
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I. INTRODUCTION

The Food and Drug Administration (FDA) is responsible for ensuring the safety and adequacy of the American food supply and for providing nutrition information to the public. Concerns over inadequate intake of essential nutrients by certain subgroups of the American population, and conversely, extensive use of nutrient supplements by many individuals, have prompted the FDA to reexamine current food fortification practices. As a part of this effort, the Office of the Associate Director for Nutrition and Food Sciences, Bureau of Foods, FDA, requested that the Life Sciences Research Office (LSRO) review the influence of selected nutrients on calcium and phosphorus homeostasis and evaluate their role in osseous disorders and ectopic calcifications. An earlier report, focused on aspects of skeletal integrity, discussed the complex interactions of calcium and phosphorus and the influence of protein and vitamin D on the absorption and metabolism of these minerals (Chinn, 1981). The present report, based on the discussion of an ad hoc review group (see Section XII), addresses the broader subject of calcium and phosphorus homeostasis and examines the roles and interactions of vitamins D, A, and K and of magnesium, zinc, and fluoride in the physiological disposition of calcium and phosphorus.

It is recognized that nutrient interactions represent only one facet of the complex homeostatic controls of calcium and phosphorus in the body. Physical, and especially hormonal, factors are crucially involved in these controls. Parathyroid hormone (PTH), calcitonin (CT), estrogens, and other hormones are essential factors in calcium homeostasis. However, detailed consideration of their involvement is beyond the scope of this report.
II. BACKGROUND

A. REGULATION OF CALCIUM AND PHOSPHORUS HOMEOSTASIS

The concentration of calcium in serum is normally maintained between 8.5 and 10.5 mg/dl (Scully, 1978) by a highly integrated and sensitive system involving hormonal controls, nutrient influences, and body reservoirs of the mineral. This homeostatic mechanism responds rapidly to slight changes of calcium concentration to restore normal levels in extracellular fluids. Maintenance of normal serum calcium concentrations depends largely upon the actions of two polypeptide hormones: PTH and CT. The former responds to reduced, and the latter to elevated, levels of serum calcium (Guyton, 1981).

PTH is synthesized by the parathyroid glands and secreted when serum calcium falls below the normal range. It acts to restore serum calcium levels, in part by stimulating the hydroxylation in the kidney of 25-hydroxyvitamin D [25-OH-D] to its more potent metabolite, 1,25-dihydroxyvitamin D [1,25-(OH)₂D]. This compound stimulates calcium absorption by the gut and together with PTH stimulates renal reabsorption of calcium and mobilization of bone calcium, all actions tending to elevate serum calcium. Conversely, elevated levels of 1,25-(OH)₂D and serum calcium depress PTH secretion, thus effecting efficient feedback control (Lee et al., 1981a; Parfitt and Kleerekoper, 1980a). Elevation of serum calcium also stimulates the synthesis and secretion by the thyroid gland of CT which lowers circulating levels of calcium and phosphorus by inhibiting bone calcium mobilization.

Increased bone loss has often been reported in postmenopausal women; however, a recent study reported that vertebral bone mass was decreased 28% in premenopausal amenorrheic women athletes in comparison to normal bone mass values of age-matched menstruating controls (Cann et al., 1982). The steroid hormone estrogen generally inhibits bone loss, as evidenced by its use in treatment of postmenopausal osteoporosis (Riggs et al., 1982). Other hormones, including insulin, prolactin, and growth hormone, modify calcium metabolism, but their mechanism of action and significance are not fully elucidated (Pahuja and DeLuca, 1981; Raisz, 1977). A protein that stimulates bone growth has been isolated from human bones (Farley and Baylink, 1982). Physical activity and certain disease entities are also known to affect skeletal integrity and calcium balance.

Hormones exert less rigid control over phosphorus concentrations in serum than over calcium levels. Dietary intake of phosphorus appears to determine renal handling of phosphate which is the primary means of adjusting serum phosphate. Thus, urinary phosphate excretion directly reflects dietary intake even in vitamin D-deficient or thyroid-parathyroidectomized animals (Brautbar and Kleeman, 1982), even though PTH appears to play a major role.
in stimulating renal excretion of phosphate. Under normal physiologic conditions, serum inorganic phosphorus, including both \( \text{HPO}_4^{2-} \) and \( \text{H}_2\text{PO}_4^{-1} \), varies between 3.0 and 4.5 mg/dl in adults and may be as high as 6.0 mg/dl in infants less than 1 year of age (Scully, 1978).

B. EFFECTS OF IMBALANCES OF CALCIUM AND PHOSPHORUS

Total plasma calcium consists of protein-bound calcium (about 50% of total calcium), diffusible calcium complexes (about 5%), and ionized calcium (the remaining 45%) (Guyton, 1981). Ionized calcium is the most labile of these fractions. Most clinically important disturbances of plasma calcium affect this fraction (Massry et al., 1982).

When the calcium concentration of lymph and blood falls below normal, the restorative processes described above are activated. Unless adequate dietary calcium is provided, repeated demands upon the skeletal reservoir may result in significant depletion of bone mineral. When such adaptations fail to restore the calcium concentration to the normal range, the central and peripheral nervous systems become more excitable. At levels of approximately 7.0 to 8.5 mg/dl, latent tetany can occur; when serum calcium falls to about 7.0 mg/dl, nerve cells fire spontaneously and elicit tetanic muscle contractions (Guyton, 1981). McCarron et al. (1982) suggested that a relative state of calcium depletion may be associated with hypertension. Their statement was based on results of a pilot survey in which calcium intakes of 46 hypertensive adults were lower (668 mg/d) than those of 44 normotensive subjects (886 mg/d).

Hypercalcemia, greater than about 12 mg/dl, causes central nervous system depression, sluggish and weak muscle reflexes, decreased QT interval of the electrocardiogram, constipation, and lack of appetite (Guyton, 1981). At extremely high concentrations, greater than 17 mg/dl, calcium phosphate may precipitate throughout the body.

Unlike calcium, there is no evidence that plasma phosphate is protected from significant declines by a sensitive hormonal regulatory system. Severe hypophosphatemia can elicit skeletal abnormalities, rhabdomyolysis, cardiac dysfunction, central nervous system dysfunction, disturbances of renal function and electrolyte metabolism, metabolic acidosis, and abnormalities in hematopoiesis and function of leukocytes and platelets (Brautbar and Kleeman, 1982; Knochel, 1981; Lee et al., 1981b). Abnormalities in energy production and utilization (Brautbar et al., 1982a), carbohydrate uptake (Brautbar et al., 1980), and lipid metabolism (Brautbar et al., 1982b) were also recently described and may explain some of the clinical abnormalities in phosphate depletion. Hypophosphatemia is likely to result from excessive alcohol ingestion,
liver cirrhosis, untreated diabetes, or extreme losses from the gastrointestinal tract due to excessive use of antacids or prolonged diarrhea and vomiting.

Development of osteoporosis involves abnormally large losses of bone mass. Ectopic calcifications indicate abnormal patterns of mineral deposition and have been associated with the presence of osteoporosis (Boukhris and Becker, 1972; Smith and Rizek, 1966). However, specific changes in mineral metabolism have not been identified as causal factors for either of these conditions.
III. VITAMIN D

Vitamin D has long been known to be a major factor in calcium and phosphorus metabolism. Cholecalciferol (vitamin D₃) is synthesized from 7-dehydrocholesterol in the skin and is found in animal tissues (Esvelt et al., 1978; Holick and Clark, 1978). Ergocalciferol (vitamin D₂) is synthesized from ergosterol found in plant tissues and has a slightly different side chain from cholecalciferol (Askew et al., 1931). Both are used as food ingredients (Select Committee on GRAS Substances, 1978). It is now known that the hydroxylated derivatives of vitamin D rather than the vitamin itself are the active principles. Metabolic steps for activation are almost identical for vitamins D₂ and D₃ (Jones et al., 1976). While both are utilized equally well by humans and rats (Hess et al., 1925), cholecalciferol is preferentially utilized by chicks and New World monkeys (Chen and Bosmann, 1964; Hunt et al., 1967).

A. METABOLITES

1. 25-Hydroxyvitamin D

Vitamin D is first hydroxylated to 25-OH-D by vitamin D-25 hydroxylase in liver mitochondria (Bhattacharyya and DeLuca, 1974; Madhok and DeLuca, 1979) and then to 1,25-(OH)₂D in the kidney (Fraser and Kodicek, 1970; Lawson et al., 1971; Mawer et al., 1971). The second hydroxylation is the rate-limiting step. It is stimulated in vivo by PTH (Garabedian et al., 1972) and suppressed by the buildup of 1,25-(OH)₂D₃ (Larkins et al., 1974; Omdahl and Hunsaker, 1978; Tanaka et al., 1975).

25-OH-D shows little metabolic activity at physiological concentrations, although it may have effects at very high concentrations. Some investigators have attributed the toxic effects of hypercalcemia D to high concentrations of this metabolite (Counts et al., 1975; Hughes et al., 1976; Shepard and DeLuca, 1980). Circulating levels vary during the year depending upon exposure to sunlight (Haddad and Stamp, 1974; Neer et al., 1977; Roginsky et al., 1974; Stryd et al., 1979). Serum concentrations average about 30 ng/ml in the United States and 17 ng/ml in Europe, and may provide a reliable basis for assessing vitamin D status. Concentrations in excess of 500 ng/ml have been reported in cases of hypercalcemia D (Counts et al., 1975; Hughes et al., 1976; Shepard and DeLuca, 1980).

2. 1,25-Dihydroxyvitamin D

1,25-(OH)₂D is considered the most important vitamin D metabolite for maintaining calcium and phosphorus homeostasis. Serum concentrations normally approximate 30 pg/ml, although they
vary with different physiologic states; concentrations of 200 pg/ml may be reached during periods of rapid bone calcification. Its half-life in blood is only 2 to 4 hours (Coburn et al., 1981; Gray et al., 1978).

1,25-(OH)₂D acts upon three target tissues — intestine, bone, and kidney tubules (Figure 1) to maintain plasma concentrations of calcium and phosphorus within quite narrow limits (DeLuca, 1980a). As indicated in the introduction, two hormones, PTH and CT, are essential for maintenance of calcium and phosphorus homeostasis. 1,25-(OH)₂D may act independently of these hormones, for example, in stimulating intestinal absorption of calcium (Garabedian et al., 1974). PTH, in addition to its direct effects on bone cells (Parfitt and Kleerekoper, 1980a), interacts with 1,25-(OH)₂D to mobilize bone calcium (Garabedian et al., 1974). It is also necessary for stimulation of renal 25-OH-D-1α-hydroxylase to produce 1,25-(OH)₂D (Garabedian et al., 1972).

In the intestine, the primary effect of 1,25-(OH)₂D is stimulation of calcium and phosphorus absorption. Its effect on absorption of other minerals is less clear, but some reports indicate that absorption of magnesium, zinc, and lead may also be affected (Becker and Hoekstra, 1971; Chan et al., 1981; Hodgkinson et al., 1979; Mykkänen and Wasserman, 1982; Smith et al., 1981). The compound can be detected within intestinal mucosa 2 to 4 hours following administration (Norman, 1980; Stumpf et al., 1979) and calcium absorption is stimulated, by apparently differing processes, 6 and 24 hours later (Halloran and DeLuca, 1981). This time course is comparable with that of other steroid hormones which stimulate the synthesis of RNA and of specific proteins. Myrtle and Norman (1971) and Norman (1980) have shown that after administration of 1,25-(OH)₂D₃ to rachitic chicks, [³H]uridine is incorporated into RNA and [¹⁴C]leucine is incorporated into proteins, calcium-binding protein appears, and calcium transport increases. Accumulating evidence suggests that a biphasic response of intestinal calcium transport requires entry of 1,25-(OH)₂D₃ into the nucleus of the mucosal cell and the transcription and translation of genetic information into functional proteins before calcium transport begins (DeLuca, 1980b; DeLuca et al., 1982) (Figure 2). However, synthesis of a calcium-binding protein may not be necessary for all vitamin D-mediated calcium transport as Spencer et al. (1976a) have reported stimulation of calcium transport before messenger RNA for calcium-binding protein could be detected.

Although most calcium absorption occurs in the duodenum, it also occurs throughout the remainder of the small intestine (Harrison and Harrison, 1960). In fact, some calcium can be absorbed against an electrochemical gradient as distally as the colon in vitamin D-replete, but not in vitamin D-deficient, rats (Harrison and Harrison, 1969). Inorganic phosphate accompanies the calcium absorbed by active transport. Active transport of phosphate itself is a distinct process (DeLuca and Schnoes, 1976)
Figure 1. Mechanism for Calcium Homeostasis (from DeLuca, 1980a, with permission).

A fall in extracellular fluid (ECF) calcium concentration stimulates the parathyroid glands (PTG) to secrete PTH. PTH stimulates renal production of $1,25-(OH)_2D$ which stimulates intestinal absorption of calcium. PTH and $1,25-(OH)_2D$ act together to mobilize bone calcium and increase reabsorption of calcium by the kidney. PTH secretion is inhibited by the resulting rise in ECF calcium level. If ECF calcium rises above the normal level, the "C" cells of the thyroid gland secrete CT which lowers ECF calcium levels by inhibiting release of calcium from bone.
Figure 2. $1,25-(OH)_2D_3$ Initiated Intestinal Calcium Transport (from DeLuca, 1980b, with permission).

$1,25-(OH)_2D_3$ interacts with a 3.7S receptor in the cytosol of the intestinal epithelial cell and is transferred into the nucleus where it stimulates synthesis of RNA for calcium-binding proteins. The mucosal surface becomes more permeable to calcium and the ion enters the epithelial cell. Calcium is then transported, probably by mitochondria or vesicles, to the serosal membrane where it is exchanged for sodium and expelled into the extracellular fluid.
requiring the presence of calcium and sodium and is enhanced by vitamin D (Harrison and Harrison, 1961; 1980). Phosphorus absorption is decreased in vitamin D-deficient individuals (Albright and Sulkowitch, 1938), but Harrison (1976) states that intestinal absorption of phosphorus would be adequate to maintain phosphate balance, even during vitamin D deficiency, if urinary excretion of phosphate is minimal.

In calcium-deprived animals, net calcium absorption is related to concentrations of 1,25-(OH)$_2$D$_3$ in the intestinal mucosa (Bar et al., 1977). Administration of graded amounts of 1,25-(OH)$_2$D$_3$ to vitamin D-deficient animals increases proportionately active transport of calcium in the intestine (McNutt and Haussler, 1973; Tanaka et al., 1973). In some cases, therapeutic doses of 1,25-(OH)$_2$D$_3$ or 1α-OH-D$_3$ to humans have resulted in hypercalcemia (Davies et al., 1977; Haussler and Cordy, 1982). In patients with hyperparathyroidism, and perhaps in others, increased intestinal absorption of calcium may contribute to development of hypercalcemia.

There is less experimental evidence to determine the effect of vitamin D on calcium metabolism in bone than its effect on intestinal absorption of calcium. The vitamin facilitates transfer of calcium from bone to plasma when plasma calcium concentrations fall (Carlsson, 1952). PTH is the agent primarily responsible for mobilizing bone calcium and 1,25-(OH)$_2$D$_3$ is also required for this process under normal physiologic conditions (Garabedian et al., 1974; Reynolds et al., 1974). In osteoporosis, accelerated bone resorption sometimes accompanied by decreased bone formation appears responsible for the excessive bone loss (Stanbury, 1980), but the roles of vitamin D and PTH in this condition have not been determined. Stern (1980) and Baylink et al. (1980) have reviewed effects of vitamin D on many aspects of bone metabolism.

In the renal tubule, 1,25-(OH)$_2$D$_3$ has been reported to stimulate the distal reabsorption of calcium (Steele et al., 1975; Sutton and Dirks, 1978), but the physiologic significance of this action is unknown (DeLuca, 1979; Haussler and McCain, 1977). The vitamin under some circumstances may improve tubular reabsorption of phosphate in intact and parathyroidectomized animals (Puschett et al., 1972), but it is considered unlikely that vitamin D plays a role in reabsorption of phosphate under normal physiologic conditions (DeLuca and Schnoes, 1976).

Other tissues in which vitamin D may act are the parathyroid gland, muscle, pituitary, and brain. Negative feedback by 1,25-(OH)$_2$D$_3$ on PTH secretion in vitro has been reported (Chertow et al., 1975), but in vivo experiments have shown varied results (Care et al., 1976; Coburn et al., 1975; Oldham et al., 1979). Muscle weakness has been reported in vitamin D-deficient patients (Isenberg et al., 1982). According to Haussler and McCain (1977), low serum phosphate concentrations have been thought responsible,
but reports of improvement in muscle weakness following treatment with vitamin D metabolites may indicate a specific function of vitamin D in muscle (Birge and Haddad, 1975; Sørensen et al., 1979). Specific binding of 1,25-(OH)$_2$D$_3$ to nuclei has been identified in cells of rat brain and pituitary (Stumpf et al., 1979; 1982). The authors suggested that 1,25-(OH)$_2$D may act to maintain normal brain functions, not only by calcium and phosphorus homeostasis, but perhaps by selective effects on neurons as well. Both deficiency and excess of the vitamin have been reported in cases of retarded mental growth (Seelig, 1969).

3. Other metabolites

In addition to 1,25-(OH)$_2$D$_3$, whose hormonal character has been clearly demonstrated, other dihydroxy derivatives have been identified in plasma and tissues. The roles of these compounds in man, if any, have not been determined.

Hydroxylation of 25-OH-D may result in 1,25- or 24,25-dihydroxy compounds; synthesis of one is usually accompanied by a suppression of synthesis of the other (DeLuca, 1979). Synthesis of 24,25-(OH)$_2$D$_3$, however, does not depend on the presence of PTH (Mawer, 1980). 24,25-(OH)$_2$D$_3$ has been found in human plasma (Gray et al., 1974; Horst et al., 1979). Results of studies in humans of its effects on intestinal calcium absorption (Kanis et al., 1977) and mineralization of bone (Rasmussen and Bordier, 1978) have not been supported by continued investigation and recent experiments (Ameenuddin et al., 1982; Halloran et al., 1981; Tanaka et al., 1979) do not support reports of its role in embryonic development and bone mineralization in chicks (Henry and Norman, 1978; Ornoy et al., 1978). A third dihydroxylated compound, 25,26-(OH)$_2$D$_3$, has also been identified (Suda et al., 1970), but no physiologic role has been determined for it (DeLuca, 1982).

In addition, a trihydroxy metabolite of vitamin D, 1,24,25-(OH)$_3$D$_3$ and a lactone, 25-OH-D$_3$-26,23-lactone, have been identified, but their functions remain to be identified. DeLuca (1979) suggested that the lactone may be involved in the vitamin D intoxication syndrome, but no direct evidence for this role has been shown. A synthetic analog, 1α-OH-D$_3$, is converted to 1,25-(OH)$_2$D$_3$ by humans (Holick et al., 1973; 1977) and shows high biologic activity. It is used for clinical treatment of a number of disorders (Haussler and Cordy, 1982).

B. EFFECTS OF VITAMIN D STATUS ON CALCIUM METABOLISM

Increased intestinal absorption of calcium has been associated with increased concentrations of 1,25-(OH)$_2$D in serum. Wilz et al. (1979) reported a positive correlation between plasma levels of 1,25-(OH)$_2$D and net calcium absorption in vitamin D-replete patients eating similar diets. Circulating levels of
1,25-(OH)₂D₃ in the young were two to three times those in mature rats, chickens, or humans (Chesney et al., 1980; Pike et al., 1977). Women in late stages of pregnancy had serum concentrations of 1,25-(OH)₂D₃ two to three times greater than those of nonstressed adults (Kumar et al., 1979; Pike et al., 1977). In advanced pregnancy, women doubled their absorption of calcium while maintaining the same intake of dietary calcium (700-900 mg calcium per day) (Kumar et al., 1979). Similarly, pregnant women in India receiving adequate amounts of vitamin D and consuming 400 to 450 mg calcium per day increased calcium absorption from 30% in the first trimester to 53% in the second and third trimesters (Shenolikar, 1970).

Lactation does not seem to impair vitamin D, calcium, or phosphorus status in women receiving adequate amounts of these nutrients. Mean concentrations of 25-OH-D in serum of lactating women remained within normal ranges between 3 and 26 weeks postpartum although a statistically significant decrease was reported at 26 weeks (Greer et al., 1982). Mean serum concentrations of 1,25-(OH)₂D were similar to those of normal nonpregnant, nonlactating women during the first 12 weeks of lactation, but an increase in serum 1,25-(OH)₂D was noted after 26 weeks. Over this time period the calculated mean intake of vitamin D was greater than 500 IU/d while the calculated mean intake of calcium dropped from 1199 mg/d at 3 weeks to 890 mg/d at 26 weeks. Based on these findings, Greer et al. (1982) suggested that "lactating mothers receiving at least 1000 mg/day of calcium, 1300 mg/day of phosphorus, and 500 IU vitamin D/day are able to compensate for these losses in human milk during the early months of lactation." Work of Chan et al. (1982) also indicated no change in serum concentrations of 25-OH-D of lactating women receiving daily supplements of 400 IU of vitamin D and 250 mg calcium over a 6 month lactation period. None of these women showed evidence of decreased bone mineral content as determined by photon absorptiometry of the distal left radius.

Serum concentrations of 1,25-(OH)₂D₃ show an inverse relationship to calcium intake of adults having different dietary intakes of calcium (Adams et al., 1979; Gallagher et al., 1979; Greer et al., 1982), suggesting that this metabolite may mediate intestinal adaptation to changes in dietary calcium intake. Serum levels of 1,25-(OH)₂D rose within 48 hours following consumption of diets containing only 40 to 120 mg calcium per day or fell within 18 to 24 hours following loading with calcium carbonate (1500-5200 mg calcium per day). These effects were apparently mediated by PTH (Adams et al., 1979). Plasma levels of 1,25-(OH)₂D also rose in response to dietary phosphorus in healthy women (Dominguez et al., 1976), but, in contrast to the response to calcium, the effects seemed to be independent of PTH. Decreased intakes of calcium by lactating women were also accompanied by increased concentrations of 1,25-(OH)₂D in serum (Greer et al., 1982).

The mean serum concentration of 1,25-(OH)₂D was 37 pg/ml in 12 normal children and adolescents ages 6 to 17 years (Scriven et al., 1978). Normal adults less than 65 years of age had circulating levels similar to those of children (34 pg/ml) while subjects
over 65 years of age had levels significantly lower (20 pg/ml) (Gallagher et al., 1979). The percent of calcium absorption decreased significantly with age (Gallagher et al., 1979), confirming results of earlier studies (Avioli et al., 1965; Bullamore et al., 1970; Ireland and Fordtran, 1973). Serum concentrations of 25-OH-D were 17 ng/ml for nonelderly adults and 18 ng/ml for elderly subjects. Fractional calcium absorption was positively correlated to serum 1,25-(OH)₂D concentrations, but not to 25-OH-D levels (Gallagher et al., 1979).

In 20 postmenopausal osteoporotic patients and 27 age-matched normal postmenopausal control subjects, mean serum concentrations of 25-OH-D were similar: 19.5 and 15.9 ng/ml, respectively (Gallagher et al., 1979). Mawer et al. (1975) reported that formation of radiolabeled 1,25-(OH)₂D₃ also was not decreased in vitamin D-deficient elderly or in osteoporotic patients, but this method does not provide the necessary sensitivity to detect small changes. Although another study of vitamin D metabolites in postmenopausal osteoporotic patients and age-matched controls showed no difference in plasma 1,25-(OH)₂D concentrations (Crilly et al., 1981), at least two other studies reported lower circulating levels of 1,25-(OH)₂D in osteoporotic women versus age- and sex-matched controls (Gallagher et al., 1979; Slovik et al., 1981). It is of interest to note that vitamin D (50,000 IU given once or twice weekly) did not reduce vertebral fracture rate when administered with various combinations of calcium, estrogen, and fluoride therapies (see page 44) (Riggs et al., 1982). However, another recent report demonstrated that 1,25-(OH)₂D₃ administration to women with postmenopausal osteoporosis increased trabecular bone volume and reduced fracture rate (Gallagher et al., 1982).

In vitamin D-deficient patients with osteomalacia, serum concentrations of 1,25-(OH)₂D rose above normal levels to concentrations as high as 200 pg/ml within 72 hours after treatment with vitamin D (Papapoulos et al., 1980; Stanbury et al., 1981). These increased levels of 1,25-(OH)₂D and improvement in osteomalacia occurred before levels of circulating 25-OH-D reached normal. The concentration of 1,25-(OH)₂D remained elevated for 1 to 2 months. Serum 24,25-(OH)₂D concentrations increased even more slowly and only after serum 25-OH-D had increased to normal levels. Similar responses in serum levels of 1,25-(OH)₂D and 25-OH-D were reported following a single exposure to ultraviolet irradiation (Adams et al., 1982).

Studies by Bordier et al. (1978) in adults with vitamin D-deficiency osteomalacia indicated that administration of both 1,25-(OH)₂D and 24,25-(OH)₂D were required to restore normal mineralization processes to the same extent as administration of 25-OH-D. However, administration of 1,25-(OH)₂D may have been too infrequent and the therapeutic period too short to maintain a constant supply of the metabolite in the circulation and to effect mineralization (Tanaka and DeLuca, 1974; Tanaka et al., 1972).
Most clinical evidence points to 1,25-(OH)\(_2\)D as the vitamin D metabolite directly governing calcium absorption in humans. Increased serum concentrations are directly related to increased calcium absorption in pregnant and lactating women. Although decreased serum levels of 1,25-(OH)\(_2\)D have been associated with decreased calcium absorption in healthy adults and normal elderly subjects, a consistent relationship between serum 1,25-(OH)\(_2\)D and calcium absorption in patients with osteoporosis remains to be described.

1. **Evidence of compromised status**

Quantitative determination of the amount of vitamin D contributed by foods is difficult. Few foods contain the vitamin naturally; vitamin D-fortified products, including milk and dry cereals, supply most of the dietary intake. Estimates of vitamin D status are further complicated by large variations in exposure to sunlight. For the adult, adequate exposure to sunlight can usually supply sufficient vitamin D (National Research Council, 1980). However, this source may not be adequate under certain conditions, i.e., in adverse climatic conditions or chronic air pollution, during pregnancy and lactation, or during chronic illness. In such circumstances a dietary supply of the vitamin is recommended. For children, a dietary source is recommended to assure adequate vitamin D and calcium for skeletal growth (National Research Council, 1980).

Assessment of vitamin D status by biochemical means gives a more accurate picture of vitamin D nutrure than estimates derived from food intakes. This is especially true for vitamin D because skin synthesis following exposure to sunlight contributes varying amounts to total body stores. Circulating levels of metabolites of vitamin D may be measured as an index of vitamin D status. Of the major metabolites, 25-OH-D seems to be the best indicator of vitamin D status (Preece et al., 1975). Although serum concentrations of 1,25-(OH)\(_2\)D are directly related to intestinal absorption of calcium (Wilz et al., 1979), concentrations of this metabolite fluctuate too widely in response to physiologic conditions to provide an accurate assessment of vitamin D status (DeLuca, 1982).

Plasma 25-OH-D varies according to season of the year and differs by 10 to 20 ng/ml between the highest and lowest monthly means (Neer et al., 1977; Roginsky et al., 1974; Stryd et al., 1979). Plasma 25-OH-D levels peak about 2 months after maximal exposure to sunlight (Beadle et al., 1980; Devgun et al., 1981) and fall more slowly after exposure to sunlight is stopped than after oral vitamin D therapy is terminated (Stanbury et al., 1980).

Some investigators report that vitamin D status, as measured by 25-OH-D concentrations in plasma, decreases with age (Baker et al., 1980; Lawson et al., 1979; Lester et al., 1977;
Lund and Sørensen, 1979; Weisman et al., 1981), although other studies indicate that older persons have levels of 25-OH-D similar to those of younger persons (Corless et al., 1979; Toss et al., 1980). The capacity of the skin to synthesize vitamin D from 7-dehydrocholesterol does not appear to decline with age (Davie and Lawson, 1980). The lower plasma levels of 25-OH-D observed in elderly persons have been attributed primarily to decreased exposure to sunlight (Parfitt et al., 1982). In Great Britain, intake of vitamin D-containing foods by the elderly is decreased (Lawson et al., 1979) and, together with decreased exposure to sunlight and an apparent decrease in efficiency of intestinal absorption of the vitamin by the elderly (Montgomery et al., 1978), it may contribute to lower serum concentrations of 25-OH-D.

Most studies of vitamin D status completed to date have been conducted in European countries and in Israel. In the United States, a study by Parfitt et al. (1982) of the vitamin D status of elderly patients in nursing homes indicated that the mean plasma 25-OH-D concentration of these patients was 19 ng/ml, only slightly lower than the level in healthy adults, 23 ng/ml. However, 13 of 46 nursing home patients had 25-OH-D levels below 10 ng/ml, a much higher proportion than normal, and the plasma concentration in one individual was below 5 ng/ml. Other groups that may have low vitamin D status are the grossly obese (Compston et al., 1981) and some groups of vegetarians (Isenberg et al., 1982). Compromised vitamin D status can progressively deplete calcium and probably phosphorus stores, resulting in osteomalacia. In countries where vitamin D deficiency and osteomalacia are widespread, hip fractures are commonly observed (Chalmers et al., 1967; Vaishnava and Rizvi, 1974). Effects of vitamin D deficiency on other bone diseases such as osteoporosis are less certain.

2. Effects of excess vitamin D

Excessive bone demineralization and the attendant problems of hypercalcemia may occur with therapeutic use of large doses of vitamin D or its metabolites, particularly 25-OH-D, or by self-administration of large doses of the vitamin (50,000-100,000 IU/d) (Fieser and Turner, 1947; Gwinup, 1961; Losito et al., 1967). The resultant excess of serum calcium may be deposited in tissues such as kidney, heart, and aorta. In subacute toxicity tests in the rat, administration of 1α-OH-D₃ was associated with degeneration and necrosis of the intima of the arterioles of the heart, voluntary muscle, and smooth muscle of the digestive tract (Makita et al., 1976); the authors reported hypercalcemia as the cause of death.

Children having idiopathic hypercalcemia develop renal and cardiovascular lesions. The reported incidence of idiopathic hypercalcemia increased in Great Britain during a period when infants received as much as 4000 IU/d of the vitamin (British
Paediatric Association, 1956). Fortification practices were subsequently altered so that infants received no more than 1500 IU/d. The role of high vitamin D intakes in the etiology of this disease is not certain, as the incidence of idiopathic hypercalcemia remained high for several years thereafter and increased serum vitamin D concentrations were not consistently reported in children with idiopathic hypercalcemia (British Paediatric Association, 1964). Fomon et al. (1966) found no adverse effects on growth of children consuming 1380 to 2370 IU/d of the vitamin, intake levels common in many children in the United States. However, DeLuca (1980c) cautioned that amounts of vitamin D greater than 1000 IU/d should be taken only if prescribed by a physician and being certain that serum calcium concentration or fasting 24-hour urinary calcium levels are monitored on a monthly basis.

Occurrence of excessive concentrations of vitamin D in milk without addition of supplemental vitamin is unlikely. A 13-fold increase in vitamin D in the ration of dairy cows produced only a doubling of the concentration of the vitamin in milk (40 IU/l of vitamin D activity in milk when rations contained 15,000 IU/d and 80 IU/l when rations contained 215,000 IU/d) (Reeve et al., 1982).

Some evidence suggests an association of vitamin D with development of arteriosclerosis (Peng and Taylor, 1980). Liu et al. (1979) induced atherosclerosis in rhesus monkeys by administering high levels of vitamin D together with cholesterol and nicotine. Each of these agents alone failed to induce similar lesions. However, in a colony of 558 Macaca mulatta monkeys inadvertently fed a diet containing 162,000 USP units of vitamin D in a daily ration for 3 months, extensive calcium deposits and inflammation were observed in the heart and kidney (Kent et al., 1958). The lesions regressed and became minimal by 1 year after the high vitamin D diet was terminated. Taura et al. (1979) also induced lesions in aortas, coronary arteries, and hearts of swine given massive doses of vitamin D (250,000 IU/kg diet) for 4 months. Some, but not all, of these lesions were reversible when animals were subsequently fed the basal ration containing a normal level (387 IU/kg) of vitamin D for 3 months. Immune system involvement for vitamin D-induced arteriosclerosis has been suggested by the finding that active complement is necessary for the development of this lesion in Zymosan-decomplemented rats (Geertinger and Sørensen, 1970; Geertinger et al., 1970).

Peng et al. (1978) also reported that significant arteriosclerosis was induced in squirrel monkeys by the administration of 500 IU of vitamin D plus 0.5% cholesterol or 1000 IU of vitamin D alone, doses only a few times greater than the amount considered to be the dietary requirement for that species. There are also in vitro experiments suggesting that vitamin D may have a direct effect on arteries (Eisenstein et al., 1969). These latter experiments, however, used massive amounts of the vitamin and were done before vitamin D metabolites were available.
Assessment of a causal role in the development of arteriosclerosis for vitamin D at levels of intake usual in the American diet is difficult because the disease is multifactorial in etiology. There is evidence, however, that moderate excess of vitamin D can cause arterial calcification in experimental animals, particularly over long periods of time, and that agents such as diphosphonates can prevent the development of an accelerated form of arteriosclerosis in rabbits (Rosenblum et al., 1975) and Macaca fascicularis monkeys (Kramsch et al., 1981). Remaining concerns about this issue require additional data: intake of vitamin D including that contributed by meats and fish; serum and tissue concentrations in humans; and effects of chronic, mild overdoses of vitamin D in animals, giving due consideration to comparability of serum lipid levels with those of humans.

Oxidation of sterols, possibly including vitamin D, to sterol hydroperoxides may be necessary for their role in arteriosclerosis. Imai et al. (1976) have shown that 25-hydroxycholesterol can accelerate smooth muscle degeneration in the aorta of rabbits. Cholesterol autoxidation products have been found in human aortal tissues and plaques (Smith, 1981). It is tempting to speculate that oxidation products of sterols might be responsible for the observed occurrence of arteriosclerosis in experimental animals fed diets containing high levels of vitamin D. However, at present, experimental evidence is not available to support or disprove such speculation.
IV. VITAMIN A

Vitamin A collectively designates several biologically active compounds: retinol, retinal, and retinoic acid, each of which is required specifically by different target organs (Lui and Roels, 1980). The function of the vitamin in the visual cycle is well established (Wald, 1953), but its biochemical role in development and maintenance of skeletal and epithelial tissues and in reproduction is poorly understood. Bone abnormalities are evident at both extremes of vitamin A nutriture, that is, during severe depletion and after massive intakes (Wolbach, 1947). However, an interaction of vitamin A with calcium in bone metabolism remains to be investigated.

A. EFFECTS OF DEFICIENT INTAKE ON BONE

Vitamin A deficiency characteristically induces overgrowth of bone, which interferes with key physiological processes. Early studies of vitamin A-deficient calves and dogs described such bony overgrowths that resulted in blindness by constriction of the optic nerve channel (Moore, 1939) or incooordination by their effects on the bones of the inner ear (Mellanby, 1938). Overgrowths of mandibular, presphenoid, and femoral bone were also reported. Diets fed in these studies were undefined and the bone changes described have not been duplicated with the laboratory animals and defined diets used today. Additional work under well-controlled conditions is needed to determine the effects of vitamin A deficiency on bone. The overgrowth obviously reflects a distortion of normal bone remodeling, in which bone accretion markedly exceeds bone resorption. This was variously ascribed to alterations in endochondral growth (Wolbach, 1947), increased osteoblastic activity (Irving, 1949), reduced osteoclastic activity (Brown and Hayes, 1975), or combined abnormal activities of osteoblasts and osteoclasts (Hayes et al., 1968; Mellanby, 1938).

Both matrix formation and mineral deposition are affected by vitamin A deficiency, with the former process predominating. Several investigators (Frandsen and Becks, 1962; Howell and Thompson, 1967; Irving, 1949) reported that total ash content of bone was not substantially changed by vitamin A deficiency. Zile et al. (1972; 1973) noted an increase in plasma hydroxyproline (a measure of matrix formation) in vitamin A-deficient rats, with no change of serum calcium or of calcium mobilization from bone. Harris and Navia (1977), utilizing a model system in which only newly formed bone is studied, detected increased matrix component and degree of sulfation (glycosaminoglycans) and less calcium deposition in vitamin A-deficient than in control rats.

On the other hand, Firschein (1970) reported decreased amounts of both calcium and hydroxyproline content in femora of vitamin A-deficient rats, with no change in the mineral:collagen...
content of the bone. Measurements of collagen metabolism with radiolabeled sulfate or phosphate yielded conflicting results. Dziewiatkowski (1954) found decreased uptake of sulfate and phosphate by bone of vitamin A-deficient rats. However, Frane et al. (1959) reported increased uptake of sulfate in the costochondral junction in deficient guinea pigs and Havivi and Wolf (1967) also observed increased uptake in vitro of the radiolabeled sulfate by the epiphyseal matrix of tibia slices of vitamin A-deficient chicks. Increasing concentrations of retinoic acid induced changes in morphology of neonatal rat chondrocytes and in the amount and type of glycosaminoglycans produced by these cells (Shapiro and Mott, 1981).

Studies of other parameters of bone composition, including levels of calcium and uronic acid and activities of alkaline phosphatase and t-RNA methylase, have not resolved questions regarding changes in bone metabolism in vitamin A-deficient animals (Cousins et al., 1969; Havivi and Tal, 1974a,b). As pointed out by Navia and Harris (1980), interpretation of the experimental results is complicated by the use of different animal models and differing experimental conditions. Furthermore, these studies utilized experimental procedures which assumed that the analyzed bones were formed in part prior to the onset of vitamin A deficiency, which could mask changes caused by the deficiency itself.

Various investigators have reported that vitamin A-deficient rats have increased susceptibility to dental caries (Harris and Navia, 1980; Marshall, 1927; McCollum et al., 1922). Recent studies by Harris and Navia (1980) indicated increased caries susceptibility, but no differences in solubility, of tooth enamel from rats made vitamin A-deficient for a limited time during development and just prior to tooth eruption, thereby inferring that the defect was in the internal dentinal tissues. These results led Harris and Navia (1980) to suggest that "Caries initiated at the enamel surface would meet a less effective barrier at the enamel-dentine junction leading to development of severe, deeply penetrating lesions of the type found in molars of vitamin A-deficient rats."

Navia (1979) has summarized studies of effects of vitamin A deficiency on caries susceptibility in man. Most were retrospective studies that attempted to correlate enamel hypoplasia with vitamin A intake. A positive correlation was reported in some instances (Barden, 1978; Boyle, 1933; Mellanby and Pattison, 1926; Mellanby et al., 1924), but results are inconclusive.

Mean serum levels of vitamin A were reported to be low (<20 µg/dl) in almost 10% of American black children ages 1 to 5 years (Abraham et al., 1979) and in low-income Spanish American children and adolescents ages 2 to 16 years (U.S. Department of Health, Education, and Welfare, 1972). Effects of vitamin A status on dental health of these groups have not been explored.
Vitamin A may also play a role in renal stone formation because urinary calcium excretion of rats is reduced in early stages of vitamin A deficiency (Zile et al., 1972). Degenerative lesions with calcifications also occurred in the kidney and urinary tract in about 30% of vitamin A-deficient germ-free rats (Beaver, 1961).

B. EFFECTS OF EXCESSIVE INTAKE ON BONE

The pathological changes of bone which occur with excessive intakes of vitamin A are essentially the reverse of those observed with vitamin A deficiency. Rather than bone overgrowth, typical of the deficient state, thinning and marked resorption of bone occur in hypervitaminosis A (Navia and Harris, 1980; Stewart, 1975; Wolbach, 1947). Many osteoclasts are evident on the periosseal surface of cortical bone and resorption, rather than accretion, dominates the remodeling process. On the endosteal surface, the matrix is poorly calcified which contributes further to a generalized weakening of the bone (Navia and Harris, 1980). Fractures are common sequelae of hypervitaminosis A. In animals fed excessive amounts of vitamin A, spontaneous fractures near the ends of long bones and rarefaction and thinning of long bones have been reported (Navia and Harris, 1980; Stewart, 1975). Even when fractures do not result, other signs of bone weakness are evident, such as capillary engorgement and small hemorrhages. However, calcium deficiency superimposed on hypervitaminosis A in rats does not further increase the occurrence of fractures (Moore and Sharman, 1979).

The bone changes of hypervitaminosis A have been documented in man as well as experimental animals. Gerber et al. (1954) described osteoporotic changes in a 24-year-old woman consuming 500,000 IU/d of vitamin A for 8½ years; Jowsey and Riggs (1968) found increased resorptive surfaces and bone turnover, enlarged osteocyte lacunae, and hypermineralization of certain areas of bone of an 18-year-old woman consuming 200,000 to 300,000 IU of vitamin A daily for about 1½ years. Frame et al. (1974) reported skeletal pain and hypercalcemia in three male patients, 7, 16, and 46 years old. In the latter study, the adult had consumed 25,000 IU of vitamin A and 1250 IU vitamin D per day for 6 to 7 years. Other indications of bone involvement in hypervitaminosis A, especially among young children and infants, are bone pain, periosteal calcification, increased intracranial pressure, craniotabes of the skull, tender swelling of the limbs, and elevated serum calcium (Arena et al., 1951; Farris and Erdman, 1982; Frame et al., 1974; Lippe et al., 1981; Marie and Sée, 1954; Navia and Harris, 1980; Persson et al., 1965; Rothman and Leon, 1948; Wason and Lovejoy, 1982). Despite the evident bony involvement in hypervitaminosis A, it is often difficult to identify early signs of such intoxication. Shaywitz et al. (1977) were unable to detect adverse structural changes in bone by conventional x-ray
examination in a 4-year-old child who had consumed large amounts of vitamin A for some time. A bone scan, however, demonstrated bone abnormality and the investigators suggested that this might be a useful technique for early detection of vitamin A intoxication.

Evidence for an interaction of vitamin A with calcium in skeletal tissue and its mode of action in bone cells is lacking. It has been suggested that the vitamin stimulates osteoclast activity directly, causing release of lysosomal enzymes which destabilize lysosomal membranes (Wang et al., 1976), or that it acts indirectly through stimulation of the parathyroid glands (Chertow et al., 1977). No persuasive evidence has been advanced to explain any of these hypotheses, but some roles for vitamin A in bone metabolism are evident from data and observations from animal and human studies.
V. VITAMIN K

Vitamin K is required for the post-translational carboxylation of certain glutamic acid residues to \( \gamma \)-carboxyglutamic acid (Gla) (Esmon et al., 1975). This amino acid was first recognized as a component of prothrombin (factor II) and clotting factors VII, IX, and X (Stenflo et al., 1974). Subsequent work has shown that Gla-containing proteins are found in bone, tooth dentin (but not enamel), ectopic calcifications, kidney, spleen, lung, and placenta (Bell, 1980; Buchthal and Bell, 1980; Friedman et al., 1979; Hauschka, 1977; Hauschka et al., 1975; Hauschka et al., 1976; Levy et al., 1979; Lian et al., 1976; Price, 1980; Price et al., 1976a,b). For a review, see Gallop et al. (1980).

A. VITAMIN K-DEPENDENT PROTEINS IN BONE

The major vitamin K-dependent, Gla-containing protein of bone is termed osteocalcin (Hauschka and Gallop, 1977) or bone Gla protein (BGP) (Price, 1980). It is a small protein of 49 amino acid residues (6000-6800 daltons) (Poser et al., 1980; Price et al., 1976b) comprising 10 to 20% of the noncollagenous protein and 1 to 2% of the total protein of bone matrix (Hauschka and Gallop, 1977; Hauschka et al., 1975). The amino acid sequences from human, monkey, cow, chicken, rat, and swordfish osteocalcin show many identical structural features (Carr et al., 1981; Hauschka et al., 1982a; Linde et al., 1980; Poser et al., 1980; Price et al., 1977). Gla residues are located at positions 17, 21, and 24 and a disulfide bond joins Cys\(_{23}\) and Cys\(_{29}\) in all species. Analysis of the 15 amino-terminal residues of the protein established that it is not a fragment of the Gla-containing blood-clotting factors (Price et al., 1976a).

Osteocalcin isolated from chicken bones has two types of calcium binding sites and binds divalent cations in the following rank order: \( \text{Ca}^{2+} > \text{Mg}^{2+} > \text{Sr}^{2+} > \text{Ba}^{2+} \) (Hauschka and Gallop, 1977). Osteocalcin bound to hydroxyapatite is protected from thermal decarboxylation, a reaction which eliminates the ability of the free protein to bind calcium, its affinity for hydroxyapatite, and its ability to slow the precipitation of calcium phosphate salts from supersaturated solutions at physiological pH (Poser and Price, 1979). Binding of osteocalcin to both hydroxyapatite and fluorapatite is stimulated by \( \text{Ca}^{2+} \), but strongly inhibited by \( \text{Mg}^{2+} \) (Wians et al., 1982). Both cations are capable of inducing a major \( \alpha \)-helical transition in osteocalcin. All Gla side chains are located on the same face of one \( \alpha \)-helix and are spaced at intervals of 5.4 Å, closely paralleling the interatomic separation of \( \text{Ca}^{2+} \) in the hydroxyapatite lattice (5.45 Å) (Hauschka and Carr, 1982). These data suggest that Gla may be involved in the formation of a high affinity protein-mineral complex.
Osteocalcin is synthesized de novo in bone-derived cells (Lian et al., 1978; Nishimoto and Price, 1980). The presence of a residue of 4-hydroxyproline in calf osteocalcin indicates that prolyl hydroxylase has modified the protein which suggests that osteoblasts rather than osteoclasts synthesize the protein (Luben et al., 1976; Price et al., 1976a). Several lines of evidence suggest that the 49 residue osteocalcin sequence may originate from a larger Gla-containing molecule (pro-osteocalcin). High molecular weight species (10,000, 35,000, 60,000, and 80,000 daltons) which contain Gla and share antigenic determinants with osteocalcin have been identified (Hauschka et al., 1982b). Furthermore, a 70,000 dalton vitamin K-dependent protein is synthesized by chick bone in organ culture and bone microsomai preparations (Lian and Héroux, 1980).

The timing of osteocalcin appearance in developing bone differs among species. In bone of human fetuses, the concentration of osteocalcin (g BGP/mol bone PO₄) reaches the adult level at week 16 of gestation. It appears in bone shortly after phosphate is detectable in the bone mineral, i.e., after about 8 to 10 weeks of gestation (Price et al., 1981). Hauschka and Reid (1978) found that Gla appearance coincided with the onset of calcium accumulation in the skeleton of the chick embryo. In contrast, osteocalcin was present only in small amounts during the initial mineralization of bone in the rat fetus although fetal rat bone contained about 30% of the adult level of protein-bound Gla (Allen et al., 1981; Hauschka et al., 1982b). In newborn rats the level of osteocalcin increased 100-fold during the first 16 days postpartum (Price et al., 1981). This increase suggests that osteocalcin may be necessary for the maturation of bone mineral to hydroxyapatite or for epitactic alignment of hydroxyapatite crystals along collagen fibers (Price et al., 1979; 1980a; 1981).

Biosynthesis of osteocalcin by osteosarcoma cells is increased by addition of 1,25-(OH)₂D₃ in the culture medium (Price and Baukol, 1980). Alkaline phosphatase biosynthesis is also increased in this system (Manolagas et al., 1982). Intravenous administration of 1,25-(OH)₂D₃ to rats resulted in elevated serum levels of osteocalcin (Price and Baukol, 1981). The time course of this response was similar to that in the osteosarcoma cells. In addition, plasma osteocalcin levels are increased during vitamin D therapy in patients with vitamin D-dependent rickets and x-linked hypophosphatemia (Gundberg et al., 1982a).

The content of osteocalcin and Gla in normal bone increases linearly with increasing bone density. However, density fractionation of undermineralized rachitic bone reveals an elevated content of Gla compared to normal bone of the same density, but there is no accompanying increase in osteocalcin measured by radioimmunoassay (Lian et al., 1982). This is similar to the situation found in embryonic bone. In view of these findings, it is possible that
vitamin D regulates the biosynthetic rate of osteocalcin either directly or indirectly by affecting the processing of the high molecular weight pro-osteocalcin.

In addition to its presence in bone, osteocalcin is found in calf and human plasma (Price and Nishimoto, 1980). In normal human plasma, the mean level is 6 ng/ml. The source of the plasma protein appears to be newly synthesized osteocalcin released directly into plasma (Price et al., 1980a). Measurement of osteocalcin in plasma may have clinical application in evaluation of certain bone diseases. Price et al. (1980b) measured the level of this protein in 109 normal subjects and 112 patients with bone diseases. In normal subjects the levels of osteocalcin were higher for males (7.9 ng/ml) than for females (4.9 ng/ml). In normal females, but not in males, osteocalcin decreased as age increased. Delmas et al. (1982), however, showed an elevation in serum osteocalcin levels that increased linearly with age in normal post-menopausal women. Circulating levels of osteocalcin in children declined from 30 ng/ml at 1 year of age to the adult level at the age of puberty (Gundberg et al., 1982a). The high levels in children may be due to the rapid bone remodeling during normal growth. Patients with Paget's disease, bone metastases, hyperparathyroidism (primary and secondary), and osteopenia all had levels of plasma osteocalcin significantly higher than those of normal subjects (Price et al., 1980b). Osteocalcin levels were also elevated in patients with recent hip fractures and in patients with osteolytic bone metastases. On the other hand, glucocorticoid therapy was associated with significantly lower concentrations of osteocalcin (Price et al., 1980b; Slovik et al., 1982).

Urinary excretion of Gla may also indicate alterations in the metabolism of Gla-containing proteins. In humans, Gla does not undergo metabolic degradation, but is excreted quantitatively (Fernlund, 1976) as free Gla and Gla-containing peptides. Concentrations of Gla in 24-hour urine samples of normal children and normal adults decreased from a high of 100 μM/g creatinine at age 2 years to 44 μM/g creatinine at puberty at which time the daily excretion stabilized (Gundberg et al., 1982a). The pattern of Gla excretion as a function of age closely resembled the pattern of circulating osteocalcin. Adult patients undergoing stable warfarin therapy excreted less than one-third of normal levels of free Gla in the urine (Levy and Lian, 1979). This excretion probably represents largely Gla proteins of extrahepatic origin, but contributions from turnover of Gla proteins of extrahepatic tissues remain to be determined. Nonetheless, Gla excretion is increased in adults with various metabolic bone diseases and connective tissue disorders, including osteoporosis (Gundberg et al., 1982b), immobilization bone loss (Kuttan et al., 1981), scleroderma, and dermatomyositis (Lian et al., 1982). Further study and correlation with biochemical tests, nuclear medical procedures, and histology will be required to determine the value of osteocalcin and Gla measurements as means of detection and evaluation of bone disease.
Although osteocalcin has been studied extensively, its biologic role remains elusive. Lian et al. (1980) postulated an active role for osteocalcin in mineralization of bone and soft tissue (aorta). However, Price et al. (1981) reported that appearance of the protein follows, rather than precedes, initial accumulation of phosphate in bone during fetal development and postulated that the protein may be necessary for the maturation of the initially deposited mineral to hydroxyapatite. Additional experiments have shown the presence of osteocalcin in fetal rat bone (Allen et al., 1981; Hauschka et al., 1982b), supporting the theory that osteocalcin may be involved in calcification.

A second hypothesis for the function of the protein has been suggested by Price et al. (1981). Osteocalcin may act as a hormone to maintain mineral homeostasis. This theory is based on: a) the size of the protein (49 amino acid residues) which is compatible with a hormone function; b) the stimulation of its synthesis by 1,25-(OH)₂D₃; and c) the elevated levels of serum osteocalcin in patients with bone diseases characterized by high rates of bone turnover. However, the abundance of this protein in the skeleton argues against a hormonal role as the sole function of the protein.

Another possible function might be the regulation of calcium homeostasis with osteocalcin mediating the movement of calcium into and out of bone. Only 10⁻⁴ to 10⁻⁵ of the total body osteocalcin is freely circulating. Hauschka and Carr (1982) have shown that free [Ca²⁺] can strongly affect both the conformation of osteocalcin and its affinity for hydroxyapatite. Physiological fluctuation or pathological perturbation of [Ca²⁺] could change adsorption or desorption of the protein to bone surfaces thereby increasing or decreasing serum osteocalcin levels while allowing calcium to be resorbed from bone.

B. VITAMIN K-DEPENDENT PROTEINS IN ECTOPIC CALCIFICATIONS

Proteins containing Gla are found in calcium-containing renal calculi including oxalate, hydroxyapatite, and mixed stones of apatite and struvite (Lian et al., 1977). Proteins containing this amino acid were not associated with uric acid, cystine, and pure struvite stones. The Gla content of renal calculi related more closely to the total calcium content of the stones than to the amount of matrix protein, or to the presence of Gla in the urine (Lian et al., 1977).

In addition to proteins of renal calculi, Gla was found in proteins associated with several types of ectopic calcifications, including calcified tissues of patients with dermatomyositis and scleroderma (Lian et al., 1976; 1982) and autopsy samples from patients with atherosclerotic lesions (Levy et al., 1979). These proteins were not, however, the bone protein osteocalcin. Levels of Gla increased proportionately as calcium content increased in the atherosclerotic lesions leading the authors
to suggest that Gla-containing proteins might play a role in the calcification of these tissues (Levy et al., 1979). Excretion of Gla was elevated in patients with ectopic calcification disorders (Lian et al., 1979).

C. RELATIONSHIP OF VITAMIN K STATUS TO VITAMIN K-DEPENDENT PROTEINS

Except in infancy, primary vitamin K deficiency rarely occurs in humans. The vitamin is widely distributed in foods, as phylloquinone in plant sources and as menaquinones in animal tissues. In addition, intestinal microorganisms synthesize the menaquinones in quantities sufficient to meet part of the requirement for the vitamin (Olson, 1980). Rietz et al. (1970) reported that about half of the vitamin K stored in human liver was phylloquinone with the rest a mixture of the menaquinones. Malabsorption syndromes (Clark et al., 1939), liver diseases, or disorders involving bile production or distribution (Olson, 1980) reduce absorption of vitamin K. Administration of vitamin K-antagonists or antibiotics may depress prothrombin values which are then responsive to vitamin K administration (Frick et al., 1967; Udall, 1965). Some evidence suggests that such conditions contributed to vitamin K-responsive lowered prothrombin values in elderly hospitalized patients (Hazell and Baloch, 1970).

Use of certain drugs may also have the potential to affect abnormalities in metabolism of vitamin K-dependent Gla-containing proteins of bone as well as prothrombin and clotting factors. Exposure of the human fetus to warfarin during the first trimester of pregnancy causes serious bone defects (fetal warfarin syndrome) (Hall et al., 1980). It is possible that development of fetal warfarin syndrome may involve defective osteocalcin synthesis. However, histology, mineral content, and composition of bones of vitamin K-deficient chicks were not significantly affected by long-term treatment with anticoagulants, despite failure of these chicks to carboxylate osteocalcin (Hauschka and Reid, 1978). Similarly, bone of warfarin-treated rabbits contained less than 5% as much osteocalcin as control animals, but did not differ in morphology, strength, or mineral and protein content (Price et al., 1979). Bone disease has also been associated with use of anticonvulsant medication. Alterations in vitamin D metabolism (Dent et al., 1970) were thought responsible, but anticonvulsants, phenytoin in particular, have several other side effects. These include the occurrence of a vitamin K-dependent hemorrhagic disease in offspring of mothers taking the medication (Keith and Gallop, 1979) and prolonged prothrombin time in some adults on long-term phenytoin therapy (Andreassen et al., 1973). In some patients taking phenytoin, urinary Gla excretion was decreased to the range determined for patients taking warfarin (Keith et al., 1980). Serum osteocalcin levels were also elevated in children and adults taking phenytoin alone and phenytoin plus phenobarbital (Keith et al., 1982).
The information presented in this chapter is not intended to imply a direct relationship between vitamin K-dependent proteins in bone and skeletal health or calcium and phosphorus metabolism. It is possible that these proteins are involved in such functions, but such roles remain to be established. Certain instances of vitamin K deficiency induced by disease or by therapy suggest that vitamin K status might contribute to development of bone abnormalities. However, at the present time there is no direct evidence that skeletal health is impaired in patients having low steady-state prothrombin levels resulting from anticoagulant therapy. Continued investigation of patients undergoing long-term therapy with anticoagulants or anticonvulsants may help to establish the possibility of a role of vitamin K-dependent proteins in bone metabolism.
VI. MAGNESIUM

Apart from calcium, magnesium is the principal cation of bone, but its role in normal metabolism and various disorders of this tissue remains largely speculative. Total body magnesium content is 20 to 28 g. About half is present in bone (Shils, 1980). Magnesium is not an integral constituent of bone mineral, but is restricted to the hydration shell and crystal surface (Parfitt and Kleerekoper, 1980b). About 1% is exchanged daily. In addition to the short-term gains or losses resulting from heteroionic exchange, the magnesium content of newly synthesized bone varies with the plasma magnesium concentration, resulting in long-term changes due to normal bone turnover (Parfitt and Kleerekoper, 1980b).

The magnesium ion reduces the rate of calcium phosphate growth on hydroxyapatite seed crystals (Tomazic et al., 1975) and inhibits the formation of calcium-phospholipid-phosphate complexes, which have served as in vitro models for bone mineralization (Boskey and Posner, 1980). Boskey and Posner (1980) speculated that initiation of calcification may depend upon the Mg:Ca ratio in the calcifying tissue.

A. ABSORPTION AND EXCRETION

Seelig (1964) estimated that the average intake of magnesium for healthy individuals in the United States and Europe ranged from 15 to 40 mEq (360-960 mg) per day. The recommended dietary allowance for magnesium for adults is 300 mg/d (National Research Council, 1980). Magnesium is widely distributed in foods and dietary deficiency of the mineral seems to be rare. Although the main part of dietary magnesium is supplied by foods, drinking water may contribute significantly to the intake of this mineral; for example, about 13% of the total magnesium intake in a community having drinking water containing 3.7 mg magnesium per dl (Hankin et al., 1970).

Under normal conditions, about 30 to 40% of dietary magnesium is absorbed (Seelig, 1964). Although absorption is influenced by a number of factors, the most important appears to be the amount of magnesium and phosphate in the diet. The efficiency of magnesium absorption, as that of calcium, varies inversely with its intake. Thus, 24% was absorbed from an intake of 47 mEq/d; 44% of 20 mEq/d; and, 76% of 1.9 mEq/d intake (Graham et al., 1960). Conflicting reports have appeared on the effect of calcium on magnesium absorption. Some investigators have reported that large calcium intakes induce negative magnesium balances (Clarkson et al., 1967; MacIntyre et al., 1961), while others have found little or no effect (Leverton et al., 1961). Spencer et al. (1980) attributed much of the conflicting data concerning the magnesium/calcium relationship to the short duration of most human balance studies. These investigators determined metabolic balances of magnesium and calcium in adult males under controlled dietary
conditions for several weeks and found no alteration of the magnesium balance even with a range of calcium intakes, from 200 to 2000 mg/d (Spencer et al., 1978). This group also reported that increase of magnesium intake from 230 mg/d to 820 mg/d did not change the percent of calcium absorbed at calcium intakes of 200, 800, or 1400 mg/d (Spencer et al., 1978). Similar magnesium levels did not affect calcium absorption or retention in adolescent boys fed two levels of dietary protein (Schwartz et al., 1973).

In rats and guinea pigs, increased dietary phosphate reduced magnesium absorption and appeared to accentuate symptoms of magnesium deficiency (O'Dell, 1960). In man, high phosphate intakes reduced magnesium absorption slightly when magnesium intake was also high, but had no significant effect with diets low in magnesium (Spencer et al., 1979).

Like phosphorus, magnesium balance is regulated primarily by the kidney and urinary output reflects dietary intake quite closely over a wide range of intakes (Brautbar and Kleeman, 1982; Quamme and Dirks, 1980). Studies of magnesium reabsorption in the nephron indicate that its transport has very distinctive features. In contrast to other cations, including calcium, magnesium is reabsorbed slowly from the proximal tubule, but is reabsorbed rapidly and efficiently by the ascending limb of the loop of Henle. The renal tubule is less permeable to magnesium than to other cations (Quamme and Dirks, 1980) and hypermagnesemia has been reported only in patients with renal insufficiency following treatment with magnesium-containing drugs (Shils, 1980).

The interaction of calcium and magnesium transport in the nephron is only partially characterized, but hypercalcemia is known to inhibit magnesium reabsorption in the kidney tubule and hypocalcemia to stimulate it (Dirks and Quamme, 1980). Both PTH and CT appear to influence magnesium reabsorption, but their significance and relationship to calcium in influencing renal magnesium reabsorption are not yet clear.

Physiological doses of vitamin D sterols significantly improved the absorption of magnesium in vitamin D-depleted rats (Levine et al., 1980), but experiments with human subjects have given disparate results. Administration of 1,25-(OH)₂D₃ to children with chronic renal failure increased intestinal absorption of magnesium (Chan et al., 1981). Similarly, vitamin D administration increased magnesium absorption in patients with various disorders of calcium or bone metabolism, although the increase was only about one-tenth that experienced by calcium with the same vitamin dosage (Hodgkinson et al., 1979). The authors suggested that the effect on magnesium absorption might be incidental to the increased calcium absorption and that the calcium-binding protein produced in the intestine under vitamin D stimulation appeared to have less affinity for other alkaline earth metals (such as magnesium) than for calcium. In contrast, Wilz et al. (1979) reported
that increased concentrations of serum 1,25-(OH)₂D did not change the percentage of magnesium absorbed by 16 healthy subjects consuming normal diets; they concluded that factors other than 1,25-(OH)₂D must regulate magnesium absorption under normal conditions.

High intakes of vitamin D, however, were reported to increase magnesium requirements and exacerbate the effects of magnesium deficiency (Richardson and Welt, 1965; Wallach et al., 1966). Administration of pharmacologic doses of vitamin D failed to elicit a calcemic response in hypomagnesemic hypocalcemic patients (Medalle et al., 1976).

B. RELATIONSHIP WITH CALCIUM

A comprehensive discussion of the interrelations of magnesium and calcium (and of phosphate) in the various body tissues and organ systems was beyond the scope of the ad hoc meeting. It was recognized that derangements of the normal plasma and tissue levels of magnesium might manifest themselves as disturbances of nerve, skin, muscle, or other tissues (Shils, 1980). Major consideration was given to those interactions which modify bony structures, cause ectopic calcifications, or are associated with coronary heart disease.

1. Skeletal effects

Defective bones and teeth are classic signs of magnesium deficiency and have been produced experimentally in various animal species (O'Dell et al., 1960). Reduced bone strength was reported in rats chronically fed a diet low in magnesium (Héroux et al., 1975). Normal bone formation was also impaired in magnesium-deficient rats with or without parathyroidectomy (Hunt and Bélanger, 1972). Further studies with intramuscular bone matrix implants in rats showed osteoporosis and accumulation of fibrocartilaginous tissue (Bélanger et al., 1975).

Magnesium deficiency is usually observed only in pathological conditions. Parfitt and Kleerekoper (1980b) have compiled a listing of causes of magnesium deficiency and hypomagnesemia. In addition, specific conditions such as alcoholism, congestive heart failure, thyroid disease, and malabsorptive disorders may cause disturbances in magnesium metabolism (Parfitt and Kleerekoper, 1980b).

Use of laboratory rats as the experimental model in early studies caused confusion, for the rat reacts to magnesium deficiency with hypercalcemia or normocalcemia, whereas humans and most other species respond with hypocalcemia (Shils, 1980). Hypocalcemia in magnesium-deficient individuals is not prevented by adequate dietary calcium or reversed by oral administration of
calcium, nor is it caused by increased renal losses of the mineral (Shils, 1980). It has been attributed to impaired functioning of the parathyroid glands with a consequent reduced mobilization of bone calcium. Anast et al. (1976) reported low concentrations of immunoreactive parathyroid hormone (iPTH) in one patient having a defect in the intestinal transport of magnesium. Intravenous administration of a magnesium salt induced dramatic increases of iPTh: a doubling within 1 minute and an eight-fold increase within 5 minutes. Rude et al. (1978) reported similar results among 17 hypocalcemic patients. This rapidity of action suggests a defect in the secretion or release of PTH, rather than in its synthesis.

Another possible effect of magnesium deficiency is the dulling of end-organ sensitivity to PTH stimulation. Forbes and Parker (1980) reported that kidneys of magnesium-deficient rats were less sensitive to PTH stimulation than those of control animals. Calcium balance was not changed in hypomagnesemic hypocalcemic patients after administration of magnesium (Medalle et al., 1976). However, the concentration of serum calcium increased and this response was attributed to facilitated mobilization of calcium from bone. Magnesium deficiency affected bone cell differentiation and delayed the onset of mineralization and bone remodeling in rats (Jones et al., 1980), suggesting that abnormalities at the cellular level may further contribute to the hypocalcemia of magnesium depletion. In addition, Freitag et al. (1979) found that bone responsiveness to PTH was reduced in magnesium-deficient animals. PTH uptake during in vitro perfusion with the hormone was markedly reduced in bones of dogs maintained on a magnesium-deficient diet for 4 to 6 months. Production of cAMP, which normally increases markedly upon PTH administration, was also markedly reduced.

Although data are still too sparse to define precisely the role of magnesium in the metabolism of bony structures, the available evidence strongly suggests that it is essential for the formation and maintenance of normal mineralized tissues.

2. Ectopic calcifications

Magnesium deficiency causes a decrease in serum calcium and allows deposition of calcium in soft tissues, including heart, aorta, and kidney (Bunce et al., 1962; Heggtveit et al., 1964; Moore et al., 1938; Tufts and Greenberg, 1936; Vitale et al., 1961). The occurrence of these lesions varies with species: ectopic calcifications have been found in species such as rats, dogs, and calves, but not in monkeys (Vitale et al., 1965). Response to high intake of magnesium also varies among species. Oral administration of magnesium salts reduced nephrocalcinosis in the rat (Shah et al., 1980), but did not affect the rate of crystallization of calcium salts in urine of human subjects (Fetner et al., 1978).
The cardiac lesions of magnesium deficiency in susceptible species appear quite similar to those of potassium deficiency and include focal necrosis, calcification, and fibrosis (Bajusz, 1961). However, electron microscopic examination reveals that ultrastructure changes of cardiac muscle in magnesium deficiency differ from those of potassium deficiency (Heggtevit et al., 1964). In vitro biochemical studies of calcium distribution in rat aorta indicate that a low concentration of magnesium ions increased total exchangeable and intracellular calcium levels, while high concentrations of magnesium decreased total exchangeable and membrane-bound calcium levels but did not affect the intracellular calcium concentration of the aorta (Turlapaty and Altura, 1978). Such shifts in calcium distribution may play a role in initiation of ectopic calcifications.

3. Cardiovascular disease

Magnesium deficiency has been associated with degenerative changes in the cardiovascular system. Increased fat deposition was observed in aortas of rats consuming atherogenic diets containing low levels of magnesium (Vitale et al., 1959) and electrocardiographic changes were reported in dogs fed magnesium-deficient diets (Vitale et al., 1961). Magnesium is lost from cardiac tissue during development of ischemia (Karppanen et al., 1978). The absence of magnesium permits increased uptake and deposition of calcium in heart mitochondria (Silver and Sordahl, 1973) and small changes in the plasma ratio of calcium to magnesium affect coronary resistance (Haddy et al., 1963; Scott et al., 1961), nerve transmission, and myocardial contraction (Karppanen et al., 1978). Electrocardiographic changes in magnesium-deficient individuals are similar to those of hypocalcemia and hypokalemia. It is not clear whether these changes are a direct result of hypomagnesemia or of complex electrolyte shifts occurring during magnesium deficiency. Nonetheless, administration of magnesium to severely malnourished children (Caddell, 1969), to magnesium-deficient patients (Bajpai et al., 1972), and to alcoholics (Flink et al., 1954; 1957) has reversed electrocardiographic abnormalities.

Epidemiologic data from Finland have shown an association of increased incidence of ischemic heart disease with consumption of soft water (low magnesium and calcium content) and with low content of magnesium in soils (Karppanen and Neuvonen, 1973; Karppanen et al., 1978). Similar findings were reported in Canada (Anderson et al., 1973; 1975; Neri et al., 1975). The higher mortality rates among residents of soft water areas were attributed entirely to the high number of sudden deaths caused by fatal arrhythmias (Anderson et al., 1973; 1975). The concentration of magnesium was decreased in myocardial tissues, but not in diaphragm or pectoralis muscle of young adult male victims of accidents living in areas of Canada with soft water (Anderson et al., 1973; 1975). Concentrations of other minerals (calcium, zinc, copper, chromium, lead, and cadmium) in these tissues were not affected by water hardness (Anderson et al., 1975).
Use of water softeners in hard water areas is a confounding factor for studies of effects of water hardness in the United States. While 10% of households in the United States use water softeners, in some communities as many as 70% of households use them (Comstock, 1979). In the United States, water hardness is highly correlated with both magnesium and calcium concentrations and concentrations of the two ions are highly correlated with each other (Sauer et al., 1970). In epidemiologic studies in the United States, concentrations of calcium and of magnesium in water have strong correlations with death rates from coronary disease (Sharrett, 1979).

Administration of calcium exacerbated manifestations of magnesium deficiency in rats (Vitale et al., 1959). Varo (1974) reported a relatively high calcium intake in Finland. An analysis of epidemiologic data of Keys (1970) and Varo (1974) suggests that the mean calcium to magnesium ratio in the diets in seven countries, including Finland and the United States, is highly correlated ($r=0.90$) to the death rate from coronary heart disease in these countries (Karppanen et al., 1978).

Epidemiologic data from several countries indicate that a "water factor" may be directly or indirectly related to mortality from cardiovascular disease. Magnesium is the mineral most strongly implicated by these data, but consideration of the ratios of mineral intakes (and possibly mineral interactions), as well as knowledge of absolute amounts consumed, appears necessary to develop an understanding of the roles of these minerals on development of cardiovascular disease.

C. RELATIONSHIP WITH PHOSPHORUS

In magnesium deficiency, serum phosphate remains normal or only slightly low and urinary phosphate excretion is unchanged (Shils, 1980). Coburn and Massry (1970) reported that urinary excretion of magnesium increased early during experimental phosphate deprivation and Kreussler et al. (1978) found phosphate deprivation caused hypomagnesemia in rats. Interestingly, magnesium balance was maintained by increasing the dietary concentration of magnesium (Brautbar et al., 1979). In humans, phosphorus balance was maintained during intakes of magnesium of about 200 or 800 mg/d and intakes of calcium at these same levels (Spencer et al., 1979). The pattern of phosphorus excretion was changed, however, with a decrease in urinary excretion and an increase in fecal phosphorus excretion. Spencer and coworkers (1979) suggested that formation of an insoluble magnesium ammonium phosphate complex in the intestine might be partially responsible for this shift in routes of excretion.
High concentrations of urinary magnesium inhibit tubular reabsorption of calcium, causing excessive renal excretion of this element (Samiy et al., 1960). These observations raised the possibility that the high levels of urinary magnesium might contribute to the elevated excretion of calcium which is a feature of phosphate deprivation. Brautbar et al. (1979) studied this possibility in phosphate-deprived rats fed magnesium-deficient diets. They found that hypercalciuria of phosphate-deprived rats occurred both among controls and those receiving magnesium-poor diets. The investigators concluded that the increased loss of calcium during phosphate deprivation was not the result of increased magnesium excretion.
VII. ZINC

The studies by Prasad and coworkers (1963) and Halsted and coworkers (1972) have clearly established the essentiality of zinc in human nutrition. A consistent finding in these studies has been retarded growth of the deficient subjects. Sandstead et al. (1967) reported that the apparent bone age of zinc-deficient children was lower than their chronological age. Malnourished boys receiving zinc supplements responded with increases in height and bone maturity (Ronaghy et al., 1974). These findings, coupled with early studies indicating a relatively high concentration of zinc in bone (Hove et al., 1938) and the localization of bone zinc in sites of calcification (Haumont, 1961), suggest that zinc has a significant role in bone formation.

A. ABSORPTION AND EXCRETION

Sandstead (1981) has summarized ranges of zinc contents of diets of subpopulations of the United States. For adults, zinc contents compiled from a number of studies ranged from about 4 mg/d to about 20 mg/d. For elderly subgroups, the maximal dietary zinc content was somewhat lower (about 14 mg/d) than for younger adults. The recommended dietary allowance of zinc for adults is 15 mg/d (National Research Council, 1980). Determination of the zinc requirement of humans has been hindered because of complications induced by differing techniques, experimental designs, dietary regimens, physiological status, and other factors. The principal technique employed in these studies has been the balance method. However, as Halsted et al. (1974) point out, "... the apparent enteroenteric circulation of zinc makes precise estimates of absorptions and excretions impossible by a simple measurement of intake and output." In experiments, when mixed diets were fed, zinc balances in men were generally in equilibrium on zinc intakes of 12.5 mg/d (Spencer et al., 1976b). Regression analysis of balance data showed that 13.3 mg dietary zinc per day was required to maintain zinc balance (including dermal losses) in men consuming a diet in which protein supplied 16% of dietary energy (Sandstead et al., 1979). When dietary protein intake was lower (8% of dietary energy), the apparent zinc requirement was 7.8 mg/d. Although protein intake influenced zinc requirement, Sandstead (1981) reported that multiple regression analysis indicated phosphorus intake as the major predictor of zinc requirement, accounting for 62% of the variance.

Study of the interactions of zinc, as well as other nutrients, with phosphorus is complicated by the variety of phosphorus compounds consumed in the diet. For example, some forms of polyphosphates are not completely hydrolyzed by digestive enzymes and biological effects may differ among phosphate compounds (Mahoney
and Hendricks, 1978; Zemel and Linkswiler, 1981). Phytate, a hexaphosphate inositol, reduced zinc absorption in humans (Reinhold et al., 1973) and animals (O'Dell and Savage, 1960). A more recent study has shown that zinc bioavailability was not decreased by feeding of preformed complexes of calcium-zinc-phytate or zinc-phytate to rats; however, zinc content of bone was less after feeding diets containing these complexes than after feeding diets containing zinc sulfate (Ellis et al., 1982). Phytate consumption may be a factor among populations with a strong dependence on legumes and cereal grains, but is unlikely to be a major factor where animal protein is the principal dietary source (Sandstead et al., 1978). A number of animal studies have demonstrated an apparent calcium--zinc antagonism when diets containing plant protein were fed (Lewis et al., 1957; Tucker and Salmon, 1955). This effect was not demonstrable when swine were fed animal protein diets (Whiting and Bezeau, 1958).

Spencer et al. (1965) observed no significant effect on zinc absorption in human subjects fed animal protein when calcium was varied ten-fold in the diet. Also, intakes of phosphorus up to 2000 mg/d had no effect on zinc absorption. Greger and Snedeker (1980) reported that increasing daily phosphorus intake from 1010 mg to 2525 mg increased fecal zinc losses, but not sufficiently to affect apparent retention or serum levels of zinc. Calcium intake for this study was 500 mg/d. Administration of large doses of zinc given over a period of several weeks coupled with a low-calcium diet (200 mg/d) resulted in reduced absorption of $^{47}$Ca and lowered plasma $^{47}$Ca concentrations and increased fecal excretion of calcium (Spencer et al., 1980; 1982). With normal calcium intakes (800 mg/d), high dietary zinc had no effect on calcium absorption. Spencer et al. (1976b) also maintained subjects for up to 70 days on a zinc-deficient diet, but could detect no change in serum calcium or phosphorus. Nielsen suggested that there may be a slight negative effect of zinc on calcium balance and provided the following empirical formula:*

$$\text{Ca balance} = -0.16 + 0.51(\text{mg dietary Ca}) - 0.017(\text{mg dietary Zn})$$

Contradictory reports have appeared on the effect of vitamin D on zinc absorption. Wasserman (1962) and Koo et al. (1980) detected no significant changes in $^{65}$Zn absorption when

* Derived from multiple regression analysis of 130 balance studies on healthy men studied at the U.S. Department of Agriculture, Agriculture Research Service, Human Nutrition Research Center at Grand Forks, North Dakota, and presented by Dr. Forrest Nielsen at ad hoc conference, January 25-26, 1982, with permission of the Agricultural Research Service.
rachitic chicks were fed vitamin D₃ or 1,25-(OH)₂D₃. However, other investigators reported that zinc absorption and its uptake by bone were enhanced when vitamin D₃ was fed (Becker and Hoekstra, 1971; Chang et al., 1969; Worker and Migicovsky, 1961). Administration of 1,25-(OH)₂D₃ to children with chronic renal failure improved zinc absorption from 17 to 33% and increased balance from 607 to 1157 μg/m²/d (Chan et al., 1981). Serum zinc concentrations did not rise, suggesting accumulation in other tissues.

Zinc is excreted almost entirely via the feces (McCance and Widdowson, 1942; Spencer et al., 1976b). In normal subjects, less than 1 mg daily is excreted in the urine, although increased excretion has been reported to accompany various pathological conditions (Halsted et al., 1974). In persons undergoing catabolism related to surgical stress, urinary total zinc correlated closely with urinary nitrogen, suggesting that it may reflect muscle catabolism (Fell et al., 1973). In 198 normal individuals, urinary zinc excretion was positively correlated (r=0.35) with dietary zinc intake (Sandstead, 1981).

B. ROLE IN BONE

Zinc has been identified as an essential component or cofactor of many enzyme systems affecting a number of metabolic processes throughout the body. Investigation of the role of zinc in osteogenesis and bone metabolism has focused primarily on its presence in alkaline phosphatase. This enzyme is required for normal calcification processes. Its activity was decreased in bones of zinc-deficient rats (Prasad et al., 1967). Serum alkaline phosphatase in zinc-deficient humans increased when they were given zinc supplements.

Calhoun and coworkers (1974; 1975) have demonstrated significant differences in ectopic bone deposition between zinc-deficient and zinc-replete rats. All rats receiving sufficient dietary zinc demonstrated bone or cartilage formation in the model compared with only 45% of the zinc-deficient animals. Zinc and calcium concentrations in the ectopic bone formed at these sites were significantly lower in zinc-deficient than in zinc-sufficient animals. Zinc deficiency delayed, and zinc repletion accelerated, the formation of ectopic bone. Bélanger et al. (1977) reported that intramuscular bone implants in zinc-deficient rats filled with mesenchyme which slowly differentiated into defective cartilage. Poor development of blood vessels at the implantation site appeared to be an important factor in cell proliferation and abnormal differentiation in the zinc-deficient animals.

Skeletal development of chick embryos was grossly impaired by withholding zinc from the maternal diet (Kienholz et al., 1961). Zinc deficiency in growing chicks was associated with altered sulfate metabolism in the epiphyseal plate and primary spongiosas of
the tibia, suggesting some change in mucopolysaccharide metabolism in the elongation region of bone (Nielsen et al., 1970). Further study in rats showed that zinc deficiency adversely affected shearing strength, histological appearance, and protein utilization in the epiphyseal plate of the tibia (Suwarnasarn et al., 1982).

Utilizing a different model for studying bone formation, Hsieh and Navia (1980) were unable to detect changes in the calcium content of newly formed bone. They extracted an incisor tooth from guinea pigs and implanted a nylon mesh tube in the resulting cavity. The implants were later removed and analyzed for bone formation. Zinc-deficient guinea pigs had significantly less zinc in the alveolar transplants, but the calcium and phosphorus contents were not significantly reduced. The investigators concluded that under these conditions, zinc deficiency did not affect calcium and phosphorus deposition in bone, but they pointed out that the calcified tissue in their nylon implant may represent a different response to zinc deficiency than that of long bone because it does not contain cartilage as long bone does.

Brown et al. (1978) reported that the calcium and phosphorus contents of rat femora were significantly lower in zinc-deficient rats than in pair-fed zinc-sufficient controls. However, femur calcium and phosphorus accretion continued during the 23-day experimental period and the Ca:P ratio remained relatively constant. The femur zinc content of the deficient rats decreased markedly during the experimental period, a finding also reported by Calhoun et al. (1978). Excessive zinc (100 ppm in drinking water) administered to skeletally mature beagles for 9 months increased the bone zinc concentration slightly, but did not affect the circulating iPTH levels and had little or no effect on haversian bone remodeling (Anderson and Danylchuk, 1979).

Evidence is accumulating that zinc is necessary for normal bone formation, but its exact role is not yet discernible from the conflicting results of the various experimental techniques and models employed.
VIII. FLUORIDE

Interest in the possible utility of fluoride in treating osteoporosis or other skeletal rarefactions arose from observations of individuals exposed to unusually high levels of this element. Chronic ingestion of large amounts of fluoride has been known for over 50 years to produce fluorosis (Møller and Gudjonsson, 1932), a condition in which the mineral content of the skeleton is increased. Similarly, miners of cryolite (an aluminum fluoride mineral) may develop a condition of increased bone density (osteosclerosis) which in many ways is the reverse of osteoporosis (Roholm, 1937). This condition has also been reported among persons from areas with high concentrations of fluoride in drinking water (Jolly, 1970) and in patients undergoing hemodialysis with fluoridated dialysis fluid (Lough et al., 1975).

Fluoride is the only halogen element incorporated into bone mineral in any quantity. Maximal storage of fluoride by the skeleton has not been established, but factors influencing its deposition in bone include age, dosage, history of exposure, and presence of serious renal disease (Hodge and Smith, 1981). In addition to its deposition in bone and tooth enamel, fluoride is distributed both intra- and extracellularly in soft tissues (Armstrong and Singer, 1980; Whitford et al., 1979). Plasma concentrations of fluoride in humans tend to increase with age, with the highest mean concentrations reported in persons over 60 years of age (Singer and Ophaug, 1979). Factors responsible for the higher plasma fluoride levels in older persons were not identified, but increased concentrations of plasma fluoride may reflect decreased skeletal uptake of fluoride from plasma or greater release of the mineral from bone (Hodge and Smith, 1981; Singer and Ophaug, 1979). Plasma fluoride levels are also directly related to concentrations in water supplies (Guy et al., 1976) and do not differ between males and females (Fuchs et al., 1975; Singer and Ophaug, 1979).

A. INTAKE AND EXCRETION

Intakes of fluoride for people living in the United States vary considerably depending on geographic areas. Kramer et al. (1974) analyzed hospital diets from 16 cities. Dietary fluoride intake ranged from 0.78 to 1.03 mg/d in four cities not having fluoridated water supplies and from 1.73 to 3.44 mg/d in 12 cities where the water received fluoride treatment. Dietary fluoride did not correlate with the fluoride content of the drinking water in the latter cities, a finding that Kramer et al. (1974) attributed in part to the widespread use of processed foods prepared in areas where the fluoride content of the water differed from that of the locale where the diet was served. Assuming a daily intake of 2 liters (approximately eight glasses) of drinking water, an
additional 0.2 to 0.9 mg/d of fluoride in areas not having fluoridated water and 1.0 to 2.5 mg/d in areas with fluoridated water could be consumed. This produces a rough estimate of total daily fluoride intakes of about 1 to about 7 mg.

Fluoride intakes of 5.4 and 10.4 mg/d maintained positive fluoride balance in ambulatory adult males while intakes of 0.4 mg/d by control subjects were associated with slightly negative balance (Maheshwari et al., 1981). The negative balance in the controls was related to relatively high urinary excretion of fluoride and was attributed to the sudden lowering of dietary fluoride intakes for the metabolic study. A steady state of fluoride balance was reported in persons consuming water containing 0.5 ppm fluoride for several years (Largent, 1961). In contrast to the findings of positive fluoride balance in ambulatory subjects, intakes of 10 mg fluoride per day during bed rest produced negative fluoride balance and did not protect against calcium loss during immobilization (Maheshwari et al., 1982). A total intake of fluoride of 1.5 to 4.0 mg/d is tentatively recommended as safe and adequate for adults; a maximal level of 2.5 mg/d is suggested for younger age groups in order to avoid mottling of teeth (National Research Council, 1980).

Renal losses account for about 90% of fluoride excretion or about 60 to 75% of fluoride ingested at intakes of about 5 to 10 mg/d (Maheshwari et al., 1981; Spencer et al., 1970a). Higher fluoride intakes were associated with increasingly positive balances (Maheshwari et al., 1981; Spencer et al., 1970a; 1975) and with higher plasma concentrations of fluoride (Ekstrand, 1978; Guy and Taves, 1973; Maheshwari et al., 1981). Based on the repeated associations of serum fluoride concentration with intake, Maheshwari et al. (1981) suggested that serum fluoride levels might be useful as an indicator of bone fluoride concentration of persons with extended exposure to constant levels of fluoride.

High intakes of either calcium or fluoride may decrease the absorption of the other element. High calcium intakes decreased fluoride absorption significantly in dogs (Largent, 1954) and rats (Wagner and Muhler, 1960), but high calcium intakes by humans did not decrease their intestinal absorption of fluoride (Spencer et al., 1975). Conversely, moderate amounts of fluoride (10 mg fluoride as sodium fluoride) decreased absorption of calcium while subsequent studies using larger doses (40-45 mg fluoride as sodium fluoride) did not affect the intestinal absorption of calcium (Spencer et al., 1969; 1970b). However, with normal intakes of these elements, little influence on each other's absorption has been noted.
B. EPIDEMIOLOGIC EVIDENCE OF EFFECTS ON BONE

Limited epidemiologic studies suggest an inverse relationship between the incidence of osteoporosis in a locality and the fluoride concentration of its drinking water. However, criteria for diagnosing osteoporosis and techniques for evaluating bone response have varied among studies and comparison of results is difficult.

Leone et al. (1955) studied long-term skeletal changes of 237 persons aged 15 to 68 years who had lived in two Texas communities for a minimum of 15 years. The subjects were approximately equally divided between regions with 8.0 ppm and 0.4 ppm fluoride in their respective water supplies. After 10 years, only one new case of osteoporosis was detected among subjects utilizing the high fluoride water supply while eight new cases appeared among the low-fluoride group. However, increased vertebral density, as determined roentgenologically, occurred with equal frequency among members of both groups. In no case did the increased vertebral density approach the degree and extent described by Roholm (1937) in his examples of fluoride intoxication.

Bernstein et al. (1966) compared the incidence of osteoporosis among two groups of subjects of German and Scandinavian origin living in rural North Dakota. Subjects were essentially similar in regard to geographic, dietary, racial, socioeconomic, and climatic factors, but differed in the fluoride content of their drinking water. The "high-fluoride area" provided drinking water, mainly from artesian wells, containing 4.0 to 4.8 ppm. The "low-fluoride area" contained 0.15 to 0.3 ppm. The incidence of collapsed vertebrae and of reduced bone density of the lumbar spine was significantly higher among women (55 years and older) in the latter area. In contrast to these reports, Korns (1969) detected no reduced incidence of osteoporosis in areas whose drinking water was rich in fluoride in comparison to areas having water with low concentrations of fluoride.

C. THERAPEUTIC TRIALS

Rich and coworkers (1964), noting the skeletal effect of fluoride intake and the relatively benign nature of even large doses, speculated that fluoride therapy might be helpful in conditions of reduced skeletal mass or of accelerated bone resorption. Consequently, they administered small doses of sodium fluoride for short periods to osteoporotic patients. Because the medication was well tolerated at this level, the investigators increased the dosage to 60 mg fluoride per day and extended the duration of treatment to 14 weeks. After several weeks the patients demonstrated improved calcium balances with decreased rates of calcium excretion. During the two decades since this study, numerous clinical and experimental reports have appeared on the effects of fluoride.
Subsequent studies reported either no (Rose, 1965) or slight (Bernstein and Cohen, 1967) sustained calcium retention after treatment with fluoride alone. The predominant effect of fluoride appears to be the stimulation of osteoid tissue (Jowsey et al., 1968). When fluoride is given alone, the newly formed osteoid tissue is poorly mineralized. However, the simultaneous administration of high levels of calcium during fluoride therapy allows mineralization of the new bone tissue. Jowsey et al. (1972) administered a combined therapeutic regimen of sodium fluoride, calcium supplements, and pharmacologic doses of vitamin D to 11 osteoporotic patients for 1 to 1.5 years. A three-fold increase of bone-forming surfaces was observed coupled with an appreciable decrease in bone-resorbing surfaces. On the basis of these studies, the investigators recommended that osteoporotic patients be treated with 50 mg sodium fluoride and 900 mg or more of calcium per day together with 50,000 IU of vitamin D twice weekly.

Riggs et al. (1980) initiated a long-term clinical trial treating 36 osteoporotic patients with 40 to 65 mg fluoride and 1.0 to 1.5 g calcium carbonate daily for up to 6 years. Twenty-four of the patients also received 50,000 IU of vitamin D twice weekly. No reduction in the frequency of fractures was reported in 1980 and a high incidence of side effects (42%) was noted. Inclusion of large doses of vitamin D induced hypercalcemia and hypercalciuria in some patients. However, it is significant that the vertebral bone mass increased in one-third of the treated patients and that among this select group, the fracture rate was only one-sixth that of the other subjects.

Riggs and coworkers (1982) extended these studies with some modifications of the therapeutic design and have recently reported positive findings. Patients with postmenopausal osteoporosis were given supplemental calcium, fluoride, estrogen, and vitamin D in various combinations. The rates of vertebral fracture were used as indices of effectiveness. The fracture rate (per thousand person years) was 834 among untreated patients, 419 among those receiving supplemental calcium (1500-2500 mg/d), 304 among those given fluoride (50-60 mg/d) and calcium (800-1500 mg/d), 181 among the estrogen and calcium group, and 53 among those receiving fluoride, calcium, and estrogen. The addition of vitamin D (50,000 IU once or twice weekly) to therapeutic regimens had little or no effect on the fracture rate. It is evident that treatment with calcium reduced the fracture rate to half that experienced by the untreated group. However, the addition of fluoride or of estrogen significantly reduced the rate still further, and the combination of estrogen, calcium, and fluoride was the most effective therapy. The vertebral bone mass increased in 60% of the fluoride-treated patients and the fracture rate in this group was only one-seventh that of patients showing no increase in mass. The investigators hypothesized that an intrinsic abnormality of osteoblast function is present in the
latter group, preventing significant stimulation of bone formation by fluoride. They speculated further that sodium fluoride therapy may serve as a probe to identify this subgroup with impaired osteoblastic activity.

High doses of fluoride appear to stimulate osteoid forma-
tion. However, whether increased bone mass can be equated with
to bone strength is still unsettled. As Riggs (1979) cautioned,
"... such bone ... has increased crystallinity and decreased
elasticity, and thus does not have normal strength." Riggins
et al. (1976) reported that strength of bones from rats, quail,
and roosters was reduced after fluoride supplementation. However,
these bones were obtained from calcium-deprived animals. Inkovaara
et al. (1975) in a double-blind study, reported more fractures
among a fluoride-treated group of patients (25 mg fluoride per day
for 8 months) than among an untreated group. Kruse et al. (1978)
treated 23 osteoporotic patients with an average of 31.1 mg fluoride
per day for 25 months. No change in their condition was noted in
thirteen patients; six patients improved, four became worse.

Despite encouraging results with fluoride therapy, little
is known of the long-term effects and many investigators caution
against its unrestricted use. This sentiment was emphasized by
Marx (1978) who voiced the concerns of an ad hoc committee which
included many investigators active in bone research. "... outside
of an investigational setting ... fluoride should not be prescribed
for generalized or localized osteopenia until investigators have
documented the efficacy of high doses without unacceptable toxicity."
IX. CONCLUSIONS

The role of vitamin D in calcium and phosphorus homeostasis has been studied more extensively and is relatively well understood compared to the roles of other nutrients discussed by the ad hoc group. Thus, extant knowledge on vitamin D may overshadow the importance of other nutrients because comparable research has not been done. Further, lack of data on interactions of nutrients impedes our understanding of the effects of such interactions on calcium and phosphorus homeostasis.

VITAMIN D

- The plasma concentration of 25-hydroxyvitamin D is currently the most widely accepted indicator of vitamin D status. Assessments of vitamin D status (excess or deficiency) have been attempted in few population subgroups. However, measurement of plasma 25-hydroxyvitamin D concentrations in a limited fraction of several population subgroups in the United States suggests that those persons who are at risk of having poor vitamin D status include the elderly, the grossly obese, and some vegetarians. Factors responsible for reduced plasma 25-hydroxyvitamin D measurements in these groups have not been completely identified.

- Intestinal absorption of calcium is directly related to plasma levels of 1,25-dihydroxyvitamin D, a hydroxylatation product of 25-hydroxyvitamin D. This metabolite may influence observed changes in intestinal calcium absorption in response to fluctuations in dietary calcium intake. Concentrations of this metabolite fluctuate too widely in response to physiologic conditions to allow its measurement to be an accurate assessment of vitamin D status.

- The percentage of dietary calcium absorbed by adults decreases with age. This depressed calcium absorption may be related to suboptimal vitamin D status associated with decreased exposure to sunlight, decreased consumption of foods containing vitamin D, and less efficient absorption of the vitamin. However, evidence suggests that the capacities to synthesize vitamin D and 1,25-dihydroxyvitamin D are not impaired in the elderly.

- Metabolites of vitamin D that have been identified but whose role in mineral and vitamin D metabolism remains to be determined include 24,25-dihydroxyvitamin D; 25,26-dihydroxyvitamin D; and 1,24,25-trihydroxyvitamin D.
High intakes of vitamin D are associated with ectopic calcifications and arteriosclerosis in experimental animals. Epidemiologic or clinical evidence for a role of vitamin D in development of arteriosclerosis in humans is not available.

Compromised vitamin D status can progressively deplete calcium and probably phosphorus stores, resulting in clinically evident osteomalacia. Effects of vitamin D deficiency on other bone diseases such as osteoporosis are less certain.

**VITAMIN A**

Vitamin A deficiency in experimental animals affects bone structure by altering glycosaminoglycan biosynthesis in the organic matrix and mineral deposition in bones and teeth. In experimental animals, faulty remodeling results in bony overgrowths in the inner ear that may produce lack of coordination. The exact mechanism by which vitamin A deficiency results in defective bone formation remains unknown.

Retrospective studies offer tentative evidence that low vitamin A intake by humans increases susceptibility to dental caries. It is not known whether poor vitamin A status as measured by low serum levels of vitamin A, such as those reported in a significant proportion of American black children and low-income Spanish American children and adolescents, affects caries susceptibility.

Calcifications in the urinary tract and decreased calcium excretion in experimental animals indicate that vitamin A deficiency may influence calcium metabolism in tissues other than bone.

Excessive intake of vitamin A results in an imbalance in bone formation and resorption, with resorption predominating. Thin and poorly calcified, easily fractured bones have been observed in experimental animals as well as patients receiving massive doses of vitamin A for therapeutic purposes and in persons ingesting large supplements in addition to normal dietary levels. Whether these abnormalities represent a continuum of the defective mechanism operative in vitamin A deficiency or whether a separate mechanism is involved remains to be determined.
VITAMIN K

- Vitamin K-dependent proteins seem to be ubiquitous in several species, yet the functions of most of these proteins have not been fully elucidated. Postulated functions for the major vitamin K-dependent bone protein (osteocalcin or bone Gla protein) include a role in maturation of bone mineral, direct regulation of calcium metabolism in bone, or indirect hormonal mediation of mineral metabolism in bone.

- Elevated levels of osteocalcin have been observed in plasma in certain disorders of bone and calcium metabolism. These findings suggest a possible association between vitamin K and vitamin D functions in bone metabolism.

- Vitamin K deficiency in humans affects the function of prothrombin, a protein containing γ-carboxyglutamic acid. It is possible that functions of other proteins containing this amino acid are affected by vitamin K deficiency.

MAGNESIUM

- Magnesium is important in calcium and phosphorus metabolism; however, the effects of magnesium deficiency are poorly understood and appear to vary with the species under study. Hypomagnesemia in patients with certain diseases interferes with calcium metabolism, resulting in hypocalcemia. Much additional information from studies of experimental animals and humans is required before a coherent picture can emerge of the intricate relationships among these three minerals.

- Severe magnesium deficiency of dietary origin, comparable to that induced experimentally in animals, is unlikely to occur spontaneously in man because of magnesium's wide distribution in foods and water supplies. However, chronic marginal intakes are possible and little is known of the effects of marginal intakes over long periods of time.

- Epidemiologic data from several countries indicate that higher magnesium concentrations in water may be directly or indirectly related to lower mortality from cardiovascular disease. However, consideration of the ratio of calcium to magnesium intake, as well as knowledge of absolute amounts consumed, is necessary to determine the role of these minerals in cardiovascular disease.
ZINC

- Zinc is required for normal bone growth, as evidenced by retardation of bone growth in zinc-deficient children. Decreased activity of alkaline phosphatase, a zinc-dependent enzyme, is thought to be partially responsible, but it is not known whether additional factors are involved in the bone growth inhibition.

- Short-term balance studies in humans indicate that large doses of zinc inhibit the absorption of calcium when intakes of calcium are low. The effects of large doses of zinc on phosphorus absorption and utilization are not known.

FLUORIDE

- Limited epidemiologic evidence suggests that fluoride in drinking water is associated with a lower incidence of osteoporosis. Interpretation of total fluoride intake in epidemiological studies in the United States is complicated by consumption of foods processed in areas where the fluoride content of water is different from that of the area where the food is consumed.

- Fluoride administration, together with combinations of estrogen, calcium, and vitamin D therapy, is used by some clinicians in the treatment of osteoporosis. There is evidence of increased bone mass and reduced fracture rate from some clinical trials with fluoride; however, whether increased bone mass can be equated with increased bone strength is still unsettled. Except for studies of mineral balance, knowledge of effects of fluoride on calcium and phosphorus metabolism is limited.
X. SUGGESTIONS FOR FUTURE RESEARCH

VITAMIN D

- Research defining the function of vitamin D metabolites should continue as a basis for study of therapeutic applications of vitamin D metabolites to clinical problems related to calcium and phosphorus homeostasis.
- Automated methodology is needed for determination of vitamin D and its metabolites in biological samples. Concentrations of vitamin D and its metabolites in the food supply should be accurately determined by collaborative studies.
- Total vitamin D exposure from food, supplements, and sunlight should be ascertained for such population subgroups as pre- and postmenopausal women, the elderly, some vegetarians, grossly obese persons, and patients who have occlusive vascular disease.
- Information is needed on the vitamin D status of elderly persons in the United States, including persons in independent living circumstances, institutional situations, and those with disorders of calcium metabolism.
- Amounts of vitamin D stored in human tissues should be quantified and turnover kinetics of the stored vitamin characterized.
- Effects of additional selected minerals such as silicon, aluminum, and lead on vitamin D and calcium status should be investigated.
- In order to evaluate the role of vitamin D in development of arteriosclerosis, additional data are needed on intake of vitamin D, including intake contributed by meats and fish; serum and tissue concentrations of the vitamin in humans; and effects of chronic, mild overdoses of vitamin D in animals, giving due consideration to comparability of serum lipid levels with those of humans. Suggestions of a possible role of vitamin D in development of ectopic calcifications and arteriosclerosis provide sufficient justification for further research in this area.
VITAMIN A

- Few recent studies have utilized advanced methodology to study molecular effects of vitamin A deficiency and excess on bone. Such roles of vitamin A might include the recruitment and differentiation of osteoblast progenitor cells and conversion of macrophages to osteoclasts. Results from such studies should pave the way for clinical study of skeletal abnormalities in vitamin A-deficient children.

- Effects of vitamin A deficiency and excess may be mediated by different biochemical mechanisms and should be explored as separate problems.

- New approaches to measurement of vitamin A status should include procedures which can be automated and adapted for routine clinical use. The relationship of bone metabolism, and possibly calcium status, to vitamin A status should be considered in development of new methods.

VITAMIN K

- In light of the discovery of vitamin K-dependent protein components of bone and other tissues, relationships between the blood coagulation system and other processes involving vitamin K-dependent proteins should be investigated.

- The possible utility of urinary excretion of γ-carboxyglutamic acid as an early indicator of excessive bone loss in osteoporosis or other osteopenic conditions should be examined.

- Additional studies are needed to define the functional or structural role of osteocalcin in bone metabolism. Effects of chronic use of vitamin K-antagonist anticoagulants and other drugs on bone composition should be evaluated.

- Preliminary evidence indicates that vitamin K and vitamin D may interact. Further research should be considered to confirm this interaction and to explore its role in bone metabolism and bone disease. Research should include long-term studies of vitamin K status in various subpopulations.
MAGNESIUM

- Little is known of the effects of modest deficiencies of magnesium intake. Long-term clinical studies of subjects receiving reduced intakes of magnesium are necessary before its role in calcium and phosphorus homeostasis can be evaluated realistically. The effect of graded levels of magnesium intake should be studied, not only on calcium and phosphorus balance, but also on parathyroid function, and perhaps on other physiological parameters.

- Assessment of changes in the cardiovascular system should be included in any long-term animal and clinical studies of magnesium deficiency.

- Preliminary evidence suggests an interaction of magnesium with vitamin D. Possible effects of such an interaction on calcium and phosphorus metabolism need further elaboration.

- More accurate data are required on the actual magnesium intakes of various population subsets. Low magnesium intakes are common among alcoholic patients and alcohol enhances renal excretion of the mineral. The contribution, if any, of magnesium deficiency to symptoms and signs that characterize chronic alcoholism should be investigated.

- The interaction between calcium and magnesium has broad implications for such clinical conditions of ectopic calcifications, renal calculi, and coronary heart disease, as well as for bone calcification. Basic laboratory studies at the cellular level must supplement clinical investigations to uncover the fundamental mechanism of action.

- Laboratory tests are needed that will provide accurate estimates of intracellular magnesium concentration and total body magnesium status. Additional studies of the relationship between tissue and plasma levels of magnesium should be a part of this development.

ZINC

- Zinc deficiency has been demonstrated among populations relying heavily upon cereal diets. Zinc deficiency in the United States is probably uncommon, but certain population subsets on unconventional diets might be vulnerable. Additional epidemiological evidence of possible zinc deficiency would be useful.
Comparison of measurements of zinc status from different laboratories is difficult because there is no adequate measure of overall zinc nutriment. Development of improved measures of zinc status would facilitate establishment of zinc requirements in man. Little is known of the effect of age, disease, pregnancy, and activity upon human zinc requirements. Such studies are recommended.

Estimates of zinc intake are difficult because measurement of zinc in foods is complicated. Better methods for determination of the availability of zinc in foods are needed.

The effects of ingestion of quantities of zinc in excess of average dietary intakes are poorly defined. Whether high zinc intakes compromise calcium and phosphorus metabolism needs further study.

**FLUORIDE**

- In view of the increasing use of large doses of fluoride for osteoporosis, the most obvious and critical need is the determination of the long-term effects of this measure with particular emphasis on bone strength and elasticity and hematologic, endocrinologic, pharmacologic, and possible irreversible toxicologic actions of the mineral in animals.

- In addition to the need for information on the long-term pharmacologic effects, there is a need to evaluate the nutritional aspects of moderated intakes of fluoride. Total fluoride intake of population groups exposed to different environments needs to be quantified. Bioavailability of fluoride consumed in the diet and the interaction between fluoride and protein malnutrition should also be determined.

- Long-term prospective studies are recommended in which fluoride intake, especially in the range of 5 to 10 mg/d, is related to bone density, fractures, bone morphology, and other relevant parameters. Two readily available groups were suggested as useful experimental populations for such chronic studies: those residing in areas with high levels of fluoride in drinking water and workers in aluminum factories whose fluoride intakes may have exceeded 5 mg/d for many years. However, the interpretation of fluoride studies of workers exposed to aluminum will be difficult and will differ from results obtained in persons residing in high fluoride areas.
Fluoride apparently increases bone mass, but the mechanism remains obscure. Additional basic studies are essential to elucidate this process and to learn the role of fluoride as a nutrient rather than as a pharmacologic agent.

More studies are necessary to define the amount of fluoride required to maintain optimal bone structure during growth, maturity, and old age.

Improved methodology is needed for determination of fluoride concentrations in biological samples.
XI. LITERATURE CITED


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