EFFECT OF HORMONES ON BONE DEVELOPMENT

LAWRENCE G. RAISZ AND PATRICIA J. BINGHAM[1]

Departments of Pharmacology and Toxicology, and Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York

Embryologically the mammalian skeleton develops by condensation of mesenchyme cells either directly to form bone (intramembranous ossification) or into cartilaginous rudiments which are then calcified, resorbed, and replaced by bone (endochondral ossification). Subsequently, linear growth depends on continuation of cartilage growth and endochrondral ossification at the epiphyses, while appositional growth and the lifelong remodeling of the skeleton depends on direct bone formation and resorption without the intervention of cartilage (1). These processes are subject to at least three kinds of regulation. (*a*) Genetic determinants are presumably responsible for the wide variation in skeletal mass and size observed in man and animals. (*b*) Variations in gravitational and mechanical stresses can determine bone shape and internal structure. (*c*) Humoral agents affecting growth can act directly or indirectly on the skeleton, controlling size, maturation, and day-to-day bone turnover. Good reviews of skeletal development and turnover (2, 3) and the hormonal control of bone resorption (4) have been published recently. The present review will emphasize the effects of hormones on bone formation and the effects of some vitamins and ions which may interact with hormones or mediate their effects on the skeleton.

HORMONAL REGULATION OF FETAL DEVELOPMENT

Although many in vitro studies of hormonal influences on bone growth employ fetal tissue, little is known concerning the roles of fetal and maternal hormones in the regulation of skeletal development in utero. Teratogenic effects of hormone excess have been demonstrated: for example, glucocorticoids can produce cleft palate in rodent fetuses (5, 6). This was ascribed to inhibition of ribonucleic acid synthesis at a highly susceptible stage in development, with resultant asymmetric growth (6). Similar mechanisms may be involved in toxic effects of large doses of estrogens (7), insulin (8), parathyroid hormone (9), and vitamin D (10), although the resulting skeletal deformities are much less specific.

[1] Holder of a Pharmaceutical Manufacturers Association Foundation Fellowship Award in Pharmacology-Morphology, 1970–72.

The parathyroid, thyroid (including thyrocalcitonin-secreting cells of ultimobranchial origin), and pituitary all have functional capacity at the time of most rapid growth and calcification of the fetal skeleton. In fetal rats, calcium accumulates slowly until the seventeenth to eighteenth day of gestation, when rapid calcification of the skeleton begins. Subsequently, fetal calcium-regulating hormones can control fetal serum calcium (11, 12). In fetal sheep feedback regulation has been demonstrated in that raising or lowering fetal serum calcium correspondingly decreases or increases the concentration of parathyroid hormone in fetal blood independent of changes in the mother (13).

There are considerable species differences in the requirements for growth hormone and thyroxine in fetal growth. Skeletal lesions due to lack of these hormones are more marked neonatally than in utero (14, 15). The importance of growth hormone may depend on the time of development of responsiveness in the target organs; for example, growth hormone-induced sulfation factor fails to stimulate embryonic rat cartilage growth but does stimulate newborn rat cartilage (16). Embryonic chick cartilage, however, does respond to sulfation factor (17).

Regulation of Growth and Remodeling

The bulk of information on hormonal influences on bone growth has been obtained by examining the effects of adding or removing a single hormone. However, normal growth is undoubtedly the result of concerted action of a number of hormones and these must affect not only bone formation but also resorption, since growth is always accompanied by remodeling. Growth hormone, thyroxine, insulin, and cortisol are all required for normal skeletal development. Sex hormones are particularly important in skeletal maturation but may also have a continuous modulating effect on bone turnover. Parathyroid hormone and thyrocalcitonin are most important for their roles in regulating bone resorption for calcium homeostasis, but effects on bone growth have also been described. In addition, vitamins A, C, and D have been implicated in the regulation of bone growth.

Growth hormone.—Growth hormone deficiency impairs, and excess increases, linear growth primarily by effects on epiphyseal cartilage. When the epiphyses close, growth hormone can still stimulate cortical bone formation (18) and increase bone and skin collagen turnover (19).

In cartilage, growth hormone increases cell activity, stimulating protein, ribonucleic acid, and mucopolysaccharide synthesis (20) and cell division (21, 22). However, these effects are indirect. Growth hormone alone does not stimulate cartilage growth in vitro (20). Administration of growth hormone to hypophysectomized animals results in the appearance of sulfation factor in the serum. This factor reproduces in vitro the in vivo effects of growth hormone on cartilage (23). It is probably produced in the liver (24) and has been partially purified (23, 25, 26) and shown to be a small protein

or polypeptide bound to a larger protein in the serum. Measurable amounts of sulfation factor first appear 6 to 12 hours after growth hormone administration and persist in the serum much longer than the hormone (20). Mediation of the long-term effects of growth hormone on the skeleton by sulfation factor is teleologically appropriate since secretion of growth hormone itself is very irregular (27). Dwarfism associated with normal or elevated growth hormone levels may be due to inability to produce sulfation factor (28, 29).

Sulfation factor can affect protein synthesis in muscle as well as in cartilage (30), but it is not known whether it has any effect on bone matrix formation or mineralization. Growth hormone itself may increase calcification in fetal long bone shafts in vitro (31). The effects of growth hormone, in vivo, on bone formation and collagen turnover could be direct, or mediated by sulfation factor or by changes in the availability of ions such as of phosphate or potassium. These ions are associated with the control of bone growth (see below) and are affected by changes in growth hormone levels.

Insulin.—Clinical evidence in diabetics does not support any specific role of insulin in growth and development. Juvenile diabetics may have advanced bone age (32), and the incidence of osteoporosis is reported to be lower in diabetics than in age-matched controls (33).

Insulin shares with sulfation factor the capacity to increase protein polysaccharide and nucleic acid synthesis in cartilage (34) but less markedly and only at high doses, 10 to 400 mU/ml. The hormone can increase collagen and, to a lesser extent, mucopolysaccharide synthesis in bone in organ culture (35, 36). Acute in vitro studies of nucleoside and amino acid uptake in isolated bone cells (37) or calvaria (38) have demonstrated insulin stimulation at concentrations of less than 10 mU/ml, but these levels are still well above the immunoreactive insulin concentration in normal human plasma, 0–75 μU/ml (39).

Thyroxine.—Thyroxine and triiodothyronine are essential for normal growth of the skeleton in childhood (40). This may be related to maintenance of growth hormone levels since hypothyroidism is associated with decreased synthesis and release of growth hormone (27). The presence of these thyroid hormones is also required for everyday bone turnover. This has been demonstrated both by calcium kinetic studies (41, 42) and by microradiography (43). Thyroid ablation reduces both bone formation and resorption and blunts responses to parathyroid hormone and thyrocalcitonin (43). Thyroxine administration returns these processes to normal. In thyrotoxicosis, excessive resorption may occur, resulting in hypercalcemia and reduced bone mass (44).

In vitro, thyroxine stimulates chondrogenesis (45). Neither thyroxine nor triiodothyronine affects bone resorption in tissue culture (46, 47) and effects on bone formation and mineralization in vitro are unknown. It is

probable that these thyroid hormones have nonspecific roles in maintaining bone cell metabolism and may increase sensitivity of the cells to other agents, such as parathyroid hormone (43).

Glucocorticoids.—The osteoporosis of Cushing's syndrome and the retardation of growth of children given glucocorticoids have been ascribed to inhibitory effects of glucocorticoids on bone. However, these impairments need not be the result of direct effects on bone (48). Glucocorticoids inhibit the release of growth hormone (48) and this could account for the reduction in the rate of cell division (49) and thinning of epiphyseal plates (50) seen in glucocorticoid-treated animals. These steroids also inhibit calcium absorption from the gut (51), thus reducing the availability of calcium. This could induce secondary hyperparathyroidism resulting in increased bone resorption and osteoporosis. Bone cell metabolism and differentiation may also be impaired (50, 52).

In organ culture low doses of cortisol (10^{-5} to 10^{-8} M) can inhibit (53, 54) or enhance (55) mucopolysaccharide synthesis in cartilage depending on the system used. Similar doses of cortisol inhibit protein and ribonucleic acid synthesis and enhance ribonucleic acid catabolism in isolated bone cells in vitro (56, 57). Glucocorticoids inhibit resorption in tissue culture (47, 58), particularly induction of resorption by brief exposures to parathyroid hormone or 25-hydroxycholecalciferol (59), a process that requires ribonucleic acid synthesis. These steroids also stabilize lysosomes, and their inhibition of release of lysosomal enzymes may contribute to their blocking of resorption in vitro (60). That the direct effects of glucocorticoids in vitro are inhibitory supports the suggestion that the increase in bone resorption observed in vivo is the indirect result of calcium deficiency and increased parathyroid activity.

Sex hormones.—The adolescent growth spurt, followed by closure of the epiphyses and cessation of linear growth, is temporally associated with an increase in sex hormone secretion. However, a causal relationship has not been proven. In man, estrogens and androgens inhibit bone resorption produced in vivo by parathyroid hormone and triiodothyronine. This is indicated by decreases in urinary hydroxyproline levels (61) and reduction of bone-resorbing surface in microradiographs (62). Inhibition of bone resorption may also be a major cause of the estrogen-induced increase in medullary bone in rats and mice (63). However, these effects are probably indirect, since inhibition of bone resorption in vitro can only be obtained with high doses of these steroids (58).

Effects of sex hormones on bone formation are also contradictory. Estrogens induce medullary bone formation in birds, and increased osteoblast numbers are associated with new medullary bone in estrogen-treated mice (64). However, estrogen can decrease collagen synthesis in rat skin and bone (65). In cartilage, estrogens reduce mucopolysaccharide content (66,

67) and increase calcium. These effects may be related to antagonism of the peripheral actions of growth hormone and promotion of skeletal maturation.

Castration of rats produces a deficiency in skeletal mass relative to body weight (68). It is possible that androgens could increase bone growth by increasing muscle development and thus increase mechanical stress. Testosterone administered to rats results in acceleration of maturation of cartilage cells, increased glycogen reserves, and widening of the calcifying zone of epiphyseal plates (69). Similarly, in culture, increased calcium-to-hydroxyproline ratios have been obtained in androgen-treated chick frontal bones, suggestive of more rapid maturation of the bone matrix (70).

Parathyroid hormone.—It is well established that parathyroid hormone stimulates bone resorption and that this is important not only for calcium regulation but also for bone growth and remodeling (4). The effects of parathyroid hormone on bone formation are less clear. Following large doses of parathyroid hormone in vivo, bone collagen synthesis as measured by incorporation of ^{14}C-proline is initially inhibited (71, 72). However, repeated administration of the hormone leads to increased collagen synthesis (73) and excessive amounts of metaphyseal bone (74). In patients with hyperparathyroidism and overt bone disease, enhanced bone formation was adduced from elevated serum alkaline phosphatase (75) and increased nondialyzable urinary hydroxyproline (76) and has been confirmed by morphological studies (77, 78).

In vitro, parathyroid hormone inhibits glycine and proline incorporation into bone matrix (79, 80). However, amino acid transport in calvaria can be enhanced by the hormone (81). Nucleoside uptake is stimulated in isolated bone cells (82) and cultured calvaria (83), though in vivo incorporation of nucleosides into ribonucleic acid is initially depressed in osteoblasts, the typical bone-forming cells (84). In vivo, parathyroid hormone transiently stimulates the incorporation of hexosamine into bone (71), and in vitro, increases the production of glycosaminoglycans by isolated chondrocytes (85) but reduces their degree of sulfation (86). Thus, the hormone has both stimulatory and inhibitory effects on pathways involved in matrix synthesis.

If the primary effect of parathyroid hormone on bone-forming cells is inhibitory, a subsequent increase in bone could occur because bone formation is tightly coupled to resorption and as resorption increases, formation is stimulated to compensate for bone loss (3). Also, the parathyroid hormone-induced increase in intracellular calcium concentration (87) could act as a nonspecific stimulus for cell division and growth (88, 89).

Calcitonin.—The role of calcitonin as an inhibitor of bone resorption is widely accepted (90), but there is still controversy concerning its effects on bone formation. Increased amounts of bone have been reported in parathyroidectomized (91) and intact rats (92) following repeated administration of calcitonin. However, these effects could be due to inhibition of resorption

as much as to enhanced formation. In thyroparathyroidectomized rats chronically treated with calcitonin, inhibition of matrix formation has been found (93). Both in vivo (94) and in vitro (95) increased numbers of osteoblasts have been reported following calcitonin treatment, but the hormone does not increase collagen synthesis in either situation (96, 97). Calcitonin can stimulate production of glycosaminoglycans by isolated chondrocytes in vitro (98).

Early suggestions that excess calcitonin secretion might be responsible for such diseases as osteopetrosis and pseudohypoparathyroidism have not been confirmed by recent studies, at least in man (90). Patients with medullary carcinoma of the thyroid have high titers of calcitonin both in the thyroid and in the blood. Though some have hypocalcemia and decreased calcium turnover, most patients, even young individuals in whom the epiphyses have not yet closed, do not show any gross abnormalities of bone growth or mineral metabolism (99, 100). This suggests that calcitonin does not play a major role in skeletal development.

Vitamin A.—Vitamin A is essential for normal growth and remodeling of the skeleton. Deficiency of the vitamin is characterized by bony overgrowths, especially the narrowing of foramina of cranial and spinal nerves, leading to pressure damage of the nerves (101). Recent studies confirm that this is due to inhibition of bone resorption rather than stimulation of formation (102). Collagen synthesis and mineral accretion are decreased (103).

Excess vitamin A, in vivo, changes the staining properties and organization of cartilage matrix, decreases linear growth and increases resorption of bone (104, 105). Similar effects can be produced in vitro by the vitamin (106, 107) and these have been attributed to increased synthesis and release of lysosomal enzymes which are directly involved in the breakdown of bone and cartilage matrix (60, 108).

Vitamin C.—The requirement of vitamin C for bone growth has been well established. In vitamin C deficiency, collagen synthesis is impaired both in vitro (109, 110) and in vivo (111). Bone formation is inhibited and osteoporosis may develop (112). Inhibition of collagen synthesis has been attributed to decreased hydroxylation of proline, which prevents completion of the collagen molecule and its extrusion from the cell (113). The vitamin can act as a co-factor in the hydroxylation step, but recent studies of the enzyme protocollagen proline hydroxylase have cast doubt on this role since the requirement for the vitamin decreases as the enzyme is purified (114). The vitamin may also have effects on polysome size in collagen-synthesizing cells (115).

Vitamin D.—The original concept that vitamin D acted mainly to enhance calcium absorption in the intestine (116) must now be reconsidered. Evidence is rapidly accumulating that vitamin D undergoes extensive me-

tabolism in the body to form more polar metabolites (117, 118) and that at least two of these, 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol, have direct effects on bone as well as intestine. Both these metabolites stimulate bone resorption in tissue culture (119, 120) and 25-hydroxycholecalciferol can act synergistically with parathyroid hormone to enhance resorption (4).

Whether or not vitamin D or its metabolites have direct effects on bone formation is still debated. Vitamin D controls bone mineralization by regulating the availability of calcium. In vitamin D deficiency, the (calcium × phosphorus) product is low and there is a delay in the mineralization of newly formed matrix (121), resulting in wide osteoid borders so typical of rickets and osteomalacia. The correlation between low blood calcium concentration and delayed mineralization can be observed even if the supply of vitamin D is adequate (122). Evidence has been presented that serum from normal but not D-deficient animals contains a factor other than vitamin D that stimulates uptake of calcium by bone in tissue culture (123), and it is possible that the vitamin regulates the production of a calcium-binding protein for use in bone, similar to that induced by vitamin D in the intestine (124).

Further characteristic lesions of vitamin D deficiency are wide epiphyseal growth plates with increased numbers of hypertrophic cartilage cells (49, 125), and decreased linear growth. This appears to be related more to defects in mineralization and subsequent failure of resorption of the growth plate than to presence or absence of vitamin D. In rats, the lesions occur only in D-deficient animals on low phosphate diets and can be reversed by phosphate administration alone.

Bone matrix formation may be decreased (121) in vitamin D deficiency. This could represent an intrinsic response of osteoblasts to failure of mineralization or a specific change in cell metabolism due to lack of the vitamin. Data on the latter possibility are conflicting. In rachitic chicks, an increase in incorporation of ^{14}C-proline into collagen has been reported both in vivo and in vitro (126). However, the in vivo observation has been challenged on the basis of errors due to differences in isotope pool size and other in vivo experiments in rats in which collagen synthesis was increased after administration of vitamin D to D-deficient animals before any increase in serum calcium concentration was observed (127).

Ions as Mediators of Hormonal Effects

Many of the effects of humoral agents on bone may be mediated through changes in the ionic composition of the extracellular fluid or of bone cells. In cells, changes are difficult to measure since minerals may be sequestered in subcellular organelles (128, 129) and isolation procedures may themselves change the distribution of ions in these organelles (130). Even more difficult to analyze are ion activities in the space between bone cells and the mineralized matrix. This space is functionally and anatomically isolated

from the extracellular fluid (131) and is a potential site for regulation not only of mineralization (132) but also of matrix deposition and maturation (121). Calcium, phosphate, pyrophosphate, and potassium may all be associated with the effects of hormones on bone growth.

Calcium.—The calcium concentration in plasma is probably maintained by continuous active transport of calcium from bone to blood. This transport involves osteoblasts and osteocytes as well as osteoclasts. In tissue culture, osteoclastic bone resorption once under way is relatively insensitive to medium calcium concentration (133), although the initial steps in the induction of resorption by parathyroid hormone are calcium sensitive (134). Parathyroid hormone stimulation of cell division in rat thymocytes is calcium dependent (89), and calcium loading of rats can simulate the increase in ribonucleic acid synthesis induced in bone by parathyroid hormone (135). Thus it is probable that parathyroid hormone-induced proliferation of mesenchyme to produce bone resorbing cells (136) and stimulation of ribonucleic acid synthesis may be mediated by calcium.

Phosphate.—Raising the phosphate concentration in vitro can inhibit bone resorption (133), but, in vivo, phosphate loading lowers serum calcium concentration, stimulates the secretion of parathyroid hormone (137, 138), and increases resorption. The hypocalcemia with resultant stimulation of parathyroid activity may occur by several mechanisms: high oral loads of phosphate could impair calcium absorption from the gut; both increased formation and decreased resorption of bone would tend to lower serum calcium; phosphate might initially bind to calcium and other divalent cations and result in the formation of complexes that are removed from the circulation (139).

In vitro, increasing phosphate concentration increases mineralization in embryonic chick bone rudiments (140) and stimulates the synthesis of collagen in fetal rat bone (141). In this system phosphate has a greater stimulatory effect on collagen synthesis in bone than in cartilage (141). In young animals there is a positive correlation between growth rate and serum phosphate levels.

The importance of phosphate as a mediator of hormonal effects is not yet known. Growth hormone increases serum phosphate concentration and phosphate could mediate its effects in bone just as sulfation factor does in cartilage. Parathyroid hormone raises urinary phosphate excretion and the resultant lowering of serum phosphate concentration could add to the direct effect of the hormone to inhibit bone formation and increase resorption. Phosphate loading of thyroparathyroidectomized animals markedly enhances the hypocalcemic effect of calcitonin (90). It has been suggested that calcitonin acts through phosphate on the basis of experiments in which changes in serum phosphate concentration occurred without changes in serum calcium (142, 143).

Pyrophosphate.—Pyrophosphate is an inhibitor of apatite crystal growth and dissolution (144), and changes in the local concentration of this ion could mediate hormonal effects on bone mineral. Both parathyroid hormone and calcitonin can alter the activity of pyrophosphatases in vitro (145, 146). Pyrophosphates are poor investigational tools since they are so easily hydrolyzed by tissues, but the diphosphonates (chemically similar but with a P—C—P bond instead of the P—O—P bond of pyrophosphate) are more stable. The success of diphosphonates in inhibiting bone resorption in tissue culture and preventing aortic calcification in rats (144) lends further support to the concept of pyrophosphate as an important factor in regulation of bone mineral turnover.

Potassium.—The total concentration of potassium in bone is considerably higher than can be accounted for by the intracellular potassium content of the associated bone cells (147). The excess potassium is freely exchangeable and presumably is present in the fluid compartment between the bone cells and the mineralized matrix (148). Its high concentration is dependent upon living cells and is positively correlated with growth rate. Bone potassium levels fall with increasing age, after hypophysectomy, and in vitamin D deficiency (147). Assuming that these changes in potassium concentration are the result of active cellular transport, any hormone that affects potassium transport could affect bone growth. These include thyroxine, growth hormone, insulin, and cortisol, and recently both parathyroid hormone (149) and thyrocalcitonin (150) have been shown to affect monovalent cation transport.

Osteoporosis of Aging

Osteoporosis is by far the most prevalent disorder of the skeleton of man. It is not a single disease but a collection of disorders with many different causes (3). Common to these disorders is a decrease in bone mass. The bones are not smaller in outside dimension but have thinner cortices and fewer trabeculae. They are thus less capable of weight-bearing and more prone to distortion and fracture. Osteoporosis may begin as a deficient bone mass early in life (151), and the observation that young women have 20% less cortical area in their metacarpals than young men (152) suggests that there may be a genetic basis for the high incidence of symptomatic osteoporosis in women. The disorder is three times as common in women as in men, over the age of 65 (153)

Whatever the original state of the bones, development of symptomatic osteoporosis depends on the progressive loss of bone mass with age. The loss implies that bone resorption outstrips formation but does not indicate which process, excessive bone resorption or impaired formation, is abnormal. Many factors could induce the imbalance and much more research will be required to elucidate the mechanisms involved.

It has been suggested that increased resorption could be due to increased

secretion of parathyroid hormone or increased end organ responsiveness to the hormone (3). Functional parathyroid and thyroid glands are required for the development of immobilization osteoporosis (154). Much current therapy for osteoporosis is directed toward inhibiting bone resorption. For example, diphosphonates, pharmacological inhibitors of resorption, can prevent immobilization osteoporosis in rats (155). In similar disorders, results with calcitonin, the major physiological inhibitor, have been contradictory (156, 157), and long-term use of calcitonin in generalized osteoporosis in man has not been encouraging, producing some relief from bone pain but no significant improvement in bone structure (158, 159).

The role of calcium in the development of osteoporosis is much debated. The best laboratory model of the generalized disorder is obtained by producing calcium deficiency in adult animals and subsequent secondary hyperparathyroidism (122). Calcium intake or absorption may be impaired in osteoporotics (160). Calcium loading has been used with some success in therapy of rapidly developing idiopathic osteoporosis (161).

The increased osteoporosis of old age, particularly in women, has been attributed to reduction of sex hormone levels. Estrogens can inhibit bone resorption in culture (58) and can reduce bone-resorbing surfaces in osteoporotics (62), but no increase in bone mass is obtained on long-term use of this hormone in man (3). Although several studies have suggested that administration of estrogen may reduce the rate of loss of bone postmenopausally (162, 163), well controlled trials have yet to be carried out to prove the efficacy of estrogen in the prevention and treatment of osteoporosis.

Impairment of collagen synthesis may also be a cause of osteoporosis. "Transparent skin," characterized by low collagen content and small, dispersed collagen fibers, is typical of osteoporotics (164). Radiologically, there is a positive correlation between thin skin and reduced bone mass (165). The fact that skin can be thickened by androgen treatment (166) and that both thin skin and osteoporosis are less prevalent in hirsute women (167) suggests that there is a relationship between androgens, collagen synthesis, and osteoporosis. Androgens may be therapeutic agents worthy of further investigation. Fluoride is the only agent that has been shown to stimulate bone formation in osteoporotics (168). However, the bone formed may be structurally abnormal and poorly mineralized. Kinetic studies reveal no significant increase in total bone calcium in patients treated with fluoride for several months (169).

New Areas for Research

A major difficulty in studying the control of skeletal development has been the lack of accurate methods for measuring changes in bone formation and resorption in vivo. Recent advances in our knowledge of collagen chemistry suggest new approaches to this problem and indicate new sites at which regulation of collagen synthesis by hormones could occur.

Differences in amino acid composition in collagen.—Vertebrate collagens are composed of aggregates of tropocollagen molecules, each a triple helix of amino acid chains. In bone and soluble skin collagen two of these chains are identical, $\alpha 1$ (I), and the third has a slightly different amino acid composition, $\alpha 2$ (170, 171). Insoluble skin collagen from human infants contains a mixture of this $[\alpha 1(I)]_2\alpha 2$ collagen and triple helices of three identical chains of a new type, $\alpha 1$(III) (172). Over 90% of cartilage collagen is composed of triple helices of yet another type of chain, designated $\alpha 1$(II) (173, 174). The synthesis of these different amino acid chains must be under genetic control and each could be regulated independently. This possibility is supported by evidence that phosphate concentrations enhance bone collagen synthesis without having much effect on cartilage collagen (141). Parathyroid hormone inhibits collagen synthesis in bone to a greater extent than in cartilage (80). By isolating, identifying, and quantitating these specific collagens, it should be possible to obtain further information about differential effects of hormones on bone, cartilage, and skin.

Differences in post-translational steps in collagen synthesis.—Collagen chains differ not only in their primary amino acid composition but also in the degree to which they are hydroxylated and glycosylated, obligatory steps prior to their extrusion from the cell (113). Changing the activity of hydroxylases or transferases or availability of cofactors at these stages offers further opportunities for hormonal regulation of collagen synthesis. About 45% of proline residues are hydroxylated in each of the collagen chains (174), but hydroxylation of lysine is more varied, for example, 15% in $\alpha 1$(I), 66% in $\alpha 1$(II) (175). Hydroxylysine residues are used in the formation of cross-links and are also the site of attachment of carbohydrate. Monosaccharide (galactosyl hydroxylysine) and disaccharide (glucosyl galactosyl hydroxylysine derivatives have been isolated. The monosaccharide predominates in bone whereas the disaccharide is commoner in skin and cartilage (176, 177). Thyroxine, cortisol, and growth hormone all inhibit glycosylation of cartilage collagen in vitro (178).

Use of collagen degradation products to measure bone formation and resorption in vivo.—The hydroxyproline that appears in urine is derived from endogenous collagen (179) and 95% is peptide-bound. Most of the peptides are dialyzable, although about 10% are larger, more heterogeneous and nondialyzable (76, 180). An increase in the nondialyzable fraction has been found in bone diseases involving rapid bone turnover (Paget's hyperthyroidism), in patients given growth hormone, and following parathyroidectomy (76, 180). On the basis of these observations it has been suggested that these nondialyzable peptides are derived from newly formed collagen molecules that are not incorporated into bone matrix. These might represent carrier "procollagen" fragments cleaved prior to the formation of tropocollagen (181) or discarded $\alpha 2$ chains (180). Re-

cent evidence shows that $\alpha 1$ and $\alpha 2$ chains are assembled at the same rate (182), although only one $\alpha 2$ chain is used for every two $\alpha 1$ chains in the typical $[\alpha 1(I)]_2\alpha 2$ collagen molecules. Free $\alpha 2$ chains are released into the culture medium by fibroblasts in vitro (181).

Glycosylated hydroxylysines derived from collagen have also been isolated from urine (177). Changes in the ratio of glucosyl galactosyl hydroxylysine to galactosyl hydroxylysine may reflect differences in the rate of collagen breakdown in different tissues (176).

Thus, by studying urine levels of glycosylated hydroxylysines and hydroxyproline-containing peptides, further information may be obtained on collagen synthesis and degradation, in vivo, in different tissues, and as affected by different hormonal agents.

LITERATURE CITED

1. McLean, F. C., Urist, M. R. 1968. *Fundamentals of the Physiology of Skeletal Tissue,* 18–32. Chicago: Univ. Chicago Press. 314 pp.
2. Vaughan, J. M. 1970. *Physiology of Bone,* 200–30. Oxford: Clarendon Press. 323 pp.
3. Harris, W. H., Heaney, R. P. 1969. *N. Engl. J. Med.* 280:193–202, 253–59, 303–11
4. Raisz, L. G. 1970. *N. Engl. J. Med.* 282:909–16
5. Dostal, M., Jelinek, R. 1971. *Nature* 230:464
6. Zimmerman, E. F., Andrew, F., Kalter, M. 1970. *Proc. Nat. Acad. Sci. USA* 67:779–85
7. Abdul-Karim, R. W., Prior, J. T., Nesbitt, R. E., Jr. 1968. *Obstet. Gynecol.* 31:346–53
8. Hannah, R. S., Moore, K. L. 1971. *Teratology* 4:135–40
9. Jones, M. S. 1970. *Am. J. Anat.* 127: 89–100
10. Ornoy, A., Nebel, L., Menczel, Y. 1969. *Arch. Pathol.* 87:563–71
11. Pic, P. 1968. *C. R. Soc. Biol.* 162: 1043–47
12. Garel, J. M. 1971. *Israel J. Med. Sci.* 7:349–50
13. Buckle, R. M. 1971. Proc. 4th Parathyroid Conf. Chapel Hill, N.C. *Excerpta Med. Found.* In press
14. Hamburgh, M. 1968. *Gen. Comp. Endocrinol.* 10:198–213
15. Jost, A., Picon, L. 1970. *Advan. Metab. Disorders* 4:123–84
16. Heins, J. N., Garland, J. T., Daughaday, W. H. 1970. *Endocrinology* 87:688–92
17. Adamson, L. F., Anast, C. S. 1966. *Biochim. Biophys. Acta* 121:10–20
18. Harris, W. H., Heaney, R. 1969. *Nature* 223:403–4
19. Kowalewski, K., Yong, S. 1968. *Can. J. Physiol. Pharmacol.* 46:591–94
20. Daughaday, W. H., Kipnis, D. M. 1966. *Recent Progr. Horm. Res.* 22:49–99
21. Rigal, W. 1964. *Proc. Soc. Exp. Biol. Med.* 117:794–96
22. Daughaday, W. H., Reeder, C. 1966. *J. Lab. Clin. Med.* 68:357–68
23. Salmon, W. D., DuVall, M. R. 1970. *Endocrinology* 86:721–27
24. McConaghey, P., Sledge, C. B. 1970. *Nature* 225:1249–50
25. Liberti, J. P. 1970. *Biochem. Biophys. Res. Commun.* 39:356–62
26. Van Wyck, J. J., Hall, K., Van den Brande, J. L., Weaver, R. P. 1971. *J. Clin. Endocrinol. Metab.* 32:389–403
27. Catt, K. J. 1970. *Lancet* 1:933–39
28. Daughaday, W. H., Laron, Z., Pertzelan, A., Heins, J. N. 1969. *Trans. Assoc. Am. Physicians* 82:129–40
29. Najjar, S. S., Khachadurian, A. K., Ilbawi, M. N., Blizzard, R. M. 1969. *N. Engl. J. Med.* 284:809–13
30. Salmon, W. D., DuVall, M. R. 1970. *Endocrinology* 87:1168–80
31. Stern, P. H. 1971. Abstr. *Fed. Proc.* 30:418
32. White, P. 1960. *Diabetes* 9:345–55
33. Meema, H. E., Meema, S. 1967. *Can. Med. Assoc. J.* 96:132–39

34. Salmon, W. D., DuVall, M. R., Thompson, E. Y. 1968. *Endocrinology* 82:493–99
35. Wettenhall, R. E. H., Schwartz, P. L., Bornstein, J. 1969. *Diabetes* 18:280–84
36. Schwartz, P. L., Wettenhall, R. E. H., Troedel, M. A., Bornstein, J. 1970. *Diabetes* 19:465–66
37. Peck, W. A., Messinger, K. 1970. *J. Biol. Chem.* 245:2722–29
38. Hahn, T. J., Downing, S. J., Phang, J. M. 1969. *Biochim. Biophys. Acta* 184:675–77
39. Yalow, R. S., Berson, S. A. 1960. *J. Clin. Invest.* 39:1157–75
40. Vaughan, J. M. See Ref. 2, 212–15
41. Krane, S. M., Brownell, G. L., Stanbury, J. B., Corrigan, H. 1956. *J. Clin. Invest.* 35:874–87
42. Aubert, J-P., Cherian, A. G., Moukhtar, M. S., Milhaud, G. 1964. *Biochem. Pharmacol.* 13: 31–44
43. Jowsey, J., Detenbeck, L. C. 1969. *Endocrinology* 85:87–95
44. Ingbar, S. H., Woeber, K. A. 1968. *Textbook of Endocrinology,* ed. R. H. Williams, 192. Philadelphia: W. B. Saunders. 1248 pp.
45. Pawelek, J. M. 1969. *Develop. Biol.* 19:52–72
46. Feinblatt, J. D., Raisz, L. G. 1971. *Endocrinology* 88:797–804
47. Raisz, L. G. 1965. *J. Clin. Invest.* 44:103–16
48. Thorn, G. W. 1970. *Schweiz. Med. Wochenschr.* 100:1625–35
49. Kember, N. F., Walker, K. V. R. 1971. *Nature* 229:428–29
50. Simmons, D. J., Kunin, A. S. 1967. *Clin. Orthop.* 55:201–15
51. Kimberg, D. V. 1969. *N. Engl. J. Med.* 280:1396–405
52. Jee, W. S. S., Park, H. Z., Roberts, W. E., Kenner, G. H. 1970. *Am. J. Anat.* 129:477–80
53. Murota, S., Endo, M., Tamaoki, B. 1967. *Biochim. Biophys. Acta* 136:379–85
54. Reynolds, J. J. 1966. *Exp. Cell Res.* 41:174–89
55. Calcagno, M., Goyena, H., Arrambide, E., Arruti de Urse, C. 1970. *Exp. Cell Res.* 63:131–37
56. Peck, W. A., Messenger, K., Brandt, J., Carpenter, J. 1969. *J. Biol. Chem.* 244:4174–84
57. Peck, W. A., Brandt, J., Miller, I. 1967. *Proc. Nat. Acad. Sci. USA* 57:1599–1606
58. Stern, P. H. 1969. *J. Pharmacol. Exp. Ther.* 168:211–7
59. Raisz, L. G., Trummel, C. L., Wener, J. A., Simmons, H. *Endocrinology* In press
60. Reynolds, J. J. 1969. *Lysosomes in Biology and Pathology,* ed. J. T. Dingle, H. B. Fell, 2:163–177. Amsterdam: North-Holland Pub. Co.
61. Katz, F. H., Kappas, A. 1968. *J. Lab. Clin. Med.* 71:65–74
62. Riggs, B. L., Jowsey, J., Kelly, P. J., Jones, J. D., Maher, F. T. 1969. *J. Clin. Invest.* 48:1065–72
63. Vaughan, J. M. See Ref. 2, 220–22
64. Simmons, D. J. 1966. *Proc. Soc. Exp. Biol. Med.* 121:1165–68
65. Henneman, D. H. 1970. *Endocrinology* 87:456–64
66. Minot, A. S., Hillman, J. W. 1967. *Proc. Soc. Exp. Biol. Med.* 126: 60–64
67. Berntsen, E. 1968. *Acta Endocrinol.* 57:69–80
68. Saville, P. D. 1969. *J. Am. Geriatric Soc.* 17:155–66
69. Fahmy, A., Lee, S., Johnson, P. 1971. *Calc. Tiss. Res.* 7:12–22
70. Puche, R. C., Romano, M. C. 1969. *Calc. Tiss. Res.* 4:39–47
71. Johnston, C. C., Deiss, W. P., Miner, E. B. 1962. *J. Biol. Chem.* 237:3560–65
72. Flanagan, B., Nichols, G. 1964. *Endocrinology* 74:180–86
73. Flanagan, B., Nichols, G. 1965. *J. Clin. Invest.* 44:1795–1804
74. Kalu, D. N., Doyle, F. H., Pennock, J., Foster, G. V. 1970. *Lancet* 2: 1363–66
75. Goldsmith, R. S. 1969. *N. Engl. J. Med.* 281:367–74
76. Haddad, J. G., Couranz, S., Avioli, L. V. 1970. *J. Clin. Endocrinol. Metab.* 30:282–87
77. Riggs, B. L., Kelly, P. J., Jowsey, J., Keating, F. R. 1965. *J. Clin. Endocrinol. Metab.* 25:77–83
78. Heath, D. A., Martin, D. J. 1970. *Brit. J. Radiol.* 43:517–21
79. Gaillard, P. J. 1965. *The Parathyroid Glands,* ed. P. J. Gaillard, R. V. Talmage, A. M. Budy, 145–52. Chicago: Univ. Chicago Press. 353 pp.
80. Raisz, L. G. 1970. *Arch. Intern. Med.* 126:887–90

81. Phang, J. M., Downing, S. J., Weiss, I. W. 1970. *Biochim. Biophys. Acta* 211:605–8
82. Peck, W. A. Personal communication
83. Van Wermeskerken, R. K. A. 1968. *Proc. Kon. Ned. Akad. Wet.* (Ser. C) 71:85–98
84. Bingham, P. J., Brazell, I., Owen, M. E. 1969. *J. Endocrinol.* 45: 387–400
85. Martin, T. J., Harris, G. S., Melick, R. A. 1971. *Israel J. Med. Sci.* 7: 356–57
86. Gaillard, P. J., Moskalewski, S., Verhoog, M. J., Wassenaar, A. M. 1969. *Proc. Kon. Ned. Akad. Wet.* (Ser. C) 72:521–36
87. Borle, A. B. 1968. *Endocrinology* 83:1316–22
88. Perris, A. D., Whitfield, J. F., Tolg, P. K. 1968. *Nature* 219:527–29
89. Whitfield, J. F., Perris, A. D., Youdale, T. 1969. *J. Cell Physiol.* 73: 203–12
90. Hirsch, P. F., Munson, P. L. 1969. *Physiol. Rev.* 49:601–4
91. Foster, G. V., Doyle, F. H., Bordier, P., Matrajt, H. 1966. *Lancet* 2: 1428–31
92. Wase, A. W., Solewski, J., Rickes, E., Seidenberg, J. 1967. *Nature* 214:388–89
93. Baylink, D., Morey, E., Rich, C. 1969. *Endocrinology* 84:261–69
94. Matrajt, H. et al. 1968. *Calcitonin: Proceedings of the Symposium on Thyrocalcitonin and the C Cells,* ed. S. Taylor, 338–46 Heinemann. 402 pp.
95. Gaillard, P. J., Thesingh, C. W. See ref. 94, 238–45
96. Flanagan, B., Nichols, G. 1969. *J. Clin. Invest.* 48:595–606
97. Kalu, D. N., Foster, G. V. 1971. *J. Endocrinol.* 49:233–41
98. Martin, T. J., Harris, G. S., Melick, R. A., Frascr, J. R. E. 1969. *Experientia* 25:375–76
99. Melvin, K. E. W., Tashjian, A. H. 1968. *Proc. Nat. Acad. Sci. USA* 59:1216–22
100. Raisz, L. G., Au, W. Y. W., Simmons, H., Mandelstam, P. *Arch. Int. Med.* In press
101. Vaughan, J. M. See Ref. 2, 231
102. Davis, T. E., Krook, L., Warner, R. G. 1969. *Cornell Vet.* 59:90–119
103. Firschein, H. E. 1970. *Am. J. Physiol.* 219:1183–87
104. Clark, I. 1970. *J. Comp. Pathol.* 80: 625–34
105. Jowsey, J., Riggs, B. L. 1968. *J. Clin. Endocrinol. Metab.* 28: 1833–35
106. Fell, H. B., Mellanby, E. 1950. *Brit. Med. J.* 2:235–39
107. Reynolds, J. J. 1968. *Proc. Roy Soc. Ser. B* 170:61–69
108. Vaes, G. See ref. 60, 217–253
109. Jeffrey, J. J., Martin, G. R. 1966. *Biochim. Biophys. Acta* 121:269–80
110. Reynolds, J. J. 1966. *Exp. Cell Res.* 42:178–88
111. Ross, R. 1968. *Biol. Rev.* 43:51–98
112. Wapnick, A. A. et al. 1971. *Brit. J. Nutr.* 25:367–76
113. Rosenbloom, J., Prockop, D. J. 1969. *Repair and Regeneration,* ed. J. E. Dunphy, W. Van Winkle, 117–37. New York: McGraw-Hill. 366 pp.
114. Cardinale, G. J., Rhoads, R. E., Udenfriend, S. 1971. *Biochem. Biophys. Res. Commun.* 43:537–43
115. Fernandez-Madrid, F. 1970. *Clin. Orthop.* 68:163–81
116. Harrison, L. J. 1956. *The Biochemistry and Physiology of Bone,* ed. G. H. Bourne, 581–623. New York: Academic Press. 873 pp.
117. DeLuca, H. F. 1969. *Fed. Proc.* 28: 1678–89
118. Lawson, D. E. M., Fraser, D. R., Kodicek, E., Morris, H. R., Williams, D. H. 1971. *Nature* 230: 228–30
119. Trummel, C. L., Raisz, L. G., Blunt, J. W., DeLuca, H. F. 1969. *Science* 163:1450–51
120. Raisz, L. G., Trummel, C. L. *Science* In press
121. Baylink, D., Stauffer, M., Wergedal, J., Rich, C. 1970. *J. Clin. Invest.* 49:1122–34
122. Jowsey, J., Raisz, L. G. 1968. *Endocrinology* 82:384–96
123. Wells, I. C., Gamal, D. 1968. *Proc. Soc. Exp. Biol. Med.* 127:1006–10
124. Wasserman, R. H. 1970. *The Fat Soluble Vitamins,* ed. M. F. DeLuca, J. W. Suttie. 21–38. Madison: Univ. Wisconsin Press. 531 pp.
125. Simmons, D. J., Kunin, A. S. 1970. *Clin. Orthop.* 68:251–60
126. Paterson, C. R., Fourman, P. 1968. *Biochem. J.* 109:101–6
127. Canas, F., Brand, J. S., Neuman, W. F., Terepka, A. R. 1969. *Am. J.*

Physiol. 216:1092–96
128. Martin, J. H., Matthews, J. L. 1970. *Clin. Orthop.* 6:273–78
129. Nichols, G., Rogers, P. 1971. *Pediatrics* 47:Suppl. 211–28
130. Cohn, D. V., Bawdon, R., Eller, G., Arnold, J., Hamilton, J. W. 1968. *Parathyroid Hormone and Thyrocalcitonin (Calcitonin)* ed. R. V. Talmage, L. F. Belanger, 282–298. Excerpta Med. Found. 553 pp.
131. Neuman, W. F., Ramp, W. K. 1971. *Cellular Mechanisms for Calcium Transfer and Homeostasis,* ed. G. Nichols, R. H. Wasserman, 197–209, New York: Academic Press
132. Howell, D. S. 1971. *J. Bone Jt. Surg.* 53A:250–58
133. Raisz, L. G., Niemann, I. 1969. *Endocrinology* 85:446–52
134. Raisz, L. G., Trummel, C. L., Simmons, H. *Endocrinology* In press
135. Park, H. Z., Talmage, R. V. In ref. 130, 205–15
136. Vaughan, J. M. In ref. 2, 157–59
137. Reiss, E., Canterbury, J. M., Bercovitz, M. A., Kaplan, E. L. 1970. *J. Clin. Invest.* 49:2146–49
138. Slatopolsky, E. et al. 1971. *J. Clin. Invest.* 50:492–500
139. Eisenberg, E. 1970. *N. Engl. J. Med.* 282:889–91
140. Ramp, W. K., Neuman, W. F. 1970. *Am. J. Physiol.* 220:270–74
141. Raisz, L. G. 1970. *Fed. Proc.* 29: 1176–78
142. Robinson, C. J., Martin, T. J., Mathews, E. W., MacIntyre, I. 1967. *J. Endocrinol.* 39:71–79
143. Talmage, R. V., Kennedy, J. W. 1969. *Endocrinol.* 84:1026–37
144. Russell, R. G. G., Fleisch, H. 1970. *Clin. Orthop.* 69:101–17
145. Tenenhouse, A., Rasmussen, H. In ref. 130, 392–402
146. Orimo, M., Ohata, M., Fujita, T. 1970. *Experientia* 26:1378–79
147. Canas, F., Terepka, A. R., Neuman, W. F. 1969. Am. J. Physiol. 217: 117–20
148. Geisler, J., Neuman, W. F. 1969. *Proc. Soc. Exp. Biol. Med.* 130: 608–12
149. Agus, Z. S., Puschett, J. B., Senesky, D., Goldberg, M. 1971. *J. Clin. Invest.* 50:617–26
150. Bijvoet, O.L.M., Veer, T. v.d.s., DeVries, H. R., van Koppen, A. T. J. 1971. *N. Engl. J. Med.* 284: 681–88
151. Rose, G. A. 1970. *Osteoporosis,* ed. U. S. Barzel, 123–32. Grune & Stratton. pp. 290
152. Gryfe, C. I., Exton-Smith, A. N., Payne, P. R., Wheeler, E. F. 1971. *Lancet* 1:523–26
153. Skosey, J. L. 1970. *Med. Clin. N. Am.* 54:141–52
154. Burckhart, J. M., Jowsey, J. 1967. *Endocrinology* 1053–62
155. Fleisch, H., Russell, R. G. G., Simpson, B., Mulbauer, R. C. 1969. *Nature* 223:211–12
156. Fujita, T. et al. 1968. *Endocrinol. Jap.* 15:8–12
157. Singh, M., Jowsey, J. 1970. *Endocrinology* 87:183–86
158. Milhaud, G., Bloch-Michel, H., Coutris, G., Morin, Y., Rouaud, J. P. 1969. *C. R. Acad. Sci. (Ser. D)* 268:3100–2
159. Caniggia, A., Gennari, C., Bencini, M., Cesari, L., Borrello, G. 1970. *Clin. Sci.* 38:397–407
160. Whedon, G. D. 1959. *Fed. Proc.* 18: 1112–18
161. Pak, C.Y.C. et al. 1969. *Am. J. Med.* 47:7–16
162. Henneman, P. H., Wallach, S. 1957. *Arch. Int. Med.* 100:715–23
163. Meema, H. E., Meema, S. 1968. *Can. Med. Assoc. J.* 99:248–51
164. McConkey, B., Walton, K. W., Carney, S. A., Lawrence, J. C., Ricketts, C. R. 1967. *Ann. Rheum. Dis.* 26:219–25
165. Meema, H. E., Reid, D. B. 1969. *J. Gerontol.* 24:28–32
166. Black, M. M., Schuster, S., Bottoms, E. 1970. *Brit. Med. J.* 4:773–74
167. Schuster, S., Black, M. M., Bottoms, E. 1970. *Brit. Med. J.* 4:772
168. Jowsey, J., Schenk, R. K., Reutter, F. W. 1968. *J. Clin. Endocrinol. Metab.* 28:869–74
169. Cohn, S. H., Dombrowski, C. S., Hauser, W., Atkins, H. L. 1971. *Am. J. Clin. Nutr.* 24:20–28
170. Epstein, E. H., Scott, R. D., Miller, E. J., Piez, K. A. 1971. *J. Biol. Chem.* 246:1718–24
171. Nold, J. G., Kang, A. H., Gross, J. 1970. *Science* 170:1096–98
172. Miller, E. J., Epstein, E. H., Piez, K. A. 1971. *Biochem. Biophys. Res. Comm.* 42:1024–29
173. Miller, E. J., Matukas, V. J. 1969. *Biochemistry* 9:1264–68
174. Trelstad, R. L., Kang, A. H., Igarashi, S., Gross, J. 1970. *Biochemistry* 9:4993–98

175. Miller, E. J. 1971. *Biochemistry* 10: 1652–59
176. Pinnell, S. R., Fox, R., Krane, S. M. 1971. *Biochim. Biophys. Acta* 229:119–22
177. Segrest, J. P., Cunningham, L. W. 1970. *J. Clin. Invest.* 49:1497–509
178. Blumenkrantz, N., Prockop, D. J. 1970. *Biochim. Biophys. Acta* 208: 461–66
179. Prockop, D. J., Kivirikko, K. I. 1967. *Ann. Int. Med.* 66:1243–46
180. Krane, S. M., Munoz, A. J., Harris, E. D. 1970. *J. Clin. Invest.* 49: 716–29
181. Layman, D., McGoodwin, E. B., Martin, G. R. 1971. *Proc. Nat. Acad. Sci. USA* 68:454–58
182. Vuust, J., Piez, K. 1970. *J. Biol. Chem.* 245:6201–7