

CHAPTER 4

Growth Hormone and Skeletal Tissue Metabolism

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I. Introduction

A large part of present knowledge of growth hormone comes directly from observations on the skeletal system. For historical background,

the reader is referred to epochal experiments on skeletal development recalled in Chapter 21 by Asling and Evans (1956) of the first edition of this book. The painstaking studies of Asling and Evans and their associates at the University of California, affirmed by many research workers of the past fifteen years, are an important source of stimulation for a large group of new investigators. To make space for a review of literature on biochemical and metabolic studies of growth hormones and bone of recent years, permission has been granted by the editor to exclude morphological descriptions of skeletal maturation, body and bone disproportions, and craniofacial abnormalities. Important articles of the literature of the period from 1925 to 1950 and many recent papers expertly covered in review articles by Catt (1970), Asling (1965), McGarry *et al.* (1964), Li (1968), Theoleyre (1970), Wilhelmi (1955), and McLean and Urist (1968) are also excluded from the bibliography of this edition. This chapter summarizes selected contributions to experimental and clinical knowledge of growth hormone in hard tissue metabolism published in the period from 1950 to 1970.

Growth hormone (GH), also called somatotropin (STH), is the pituitary factor which promotes overall growth of the body, even when acting in the absence of other pituitary hormones (e.g., when injected into hypophysectomized animals). Growth hormone is the only one of the six anterior pituitary hormones that does not depend directly upon another endocrine gland for its action. All others—follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PL), adrenocorticotropic hormone (ACTH), and thyrotropic hormone (TH)—depend on a second endocrine organ to secrete another hormone for the physiologic effect. However, growth hormone has synergistic effects with other hormones and enhances their effects on many different target glands and tissues.

Growth is a highly complicated phenomenon. By definition, growth involves the laying down of new tissue. Measuring growth by weight increase alone is misleading since a weight increment may result from water retention, fat deposition, and a variety of other factors, as well as addition of new tissue. As growth is associated with protein anabolism, GH always induces nitrogen retention. Nitrogen retention is actually a result of protein synthesis and generally is associated with a decrease in the plasma NPN, urea nitrogen, amino acid nitrogen, and an increase in the tissue protein nitrogen. Since the hypophysectomized animal has a poor appetite and suffers at the same time from other hormonal deficiencies, the optimum effect of the growth hormone can be demonstrated in hypophysectomized animals only under certain well-defined circumstances. For example, nitrogen retention and deposition of body

protein must be studied under conditions of paired feeding in order to control factors related to nonhormonal nutritional conditions of the body before and after treatment.

Not all growth phenomena are under strict hypophyseal control. Mitotic activity and regeneration of liver following partial hepatectomy, for example, show only slight impairment in hypophysectomized animals as compared to the intact ones. The same is true of fracture healing. However, the size of the liver increases disproportionately in relation to the rest of the body in animals treated with growth promoting pituitary extracts. In some species, at least, growth and development of newborns continue for several weeks in the absence of pituitary and only from the fourth week on does its deficiency manifest itself.

The mode of action of GH is not known. As a rule, the concept of growth implies permanent addition of differentiated tissues and excludes temporary additions to bulk and weight such as would result from seasonal development of the gonads or accumulation of fat or glycogen. Thus, increase in linear dimensions offers the safest criteria for detection of body growth, particularly over a specified interval of time for an experimental bioassay study. And inasmuch as the growth in length of a bone takes place in the epiphyseal cartilage, roentgenologic, histologic, and radiochemical measurements on its proximal epiphyses of the tibia of a rat is the standard area for measurement of growth hormone. Of the three measurements, radiosulfate incorporation is the most precise, sensitive, and specific (Collins *et al.*, 1961).

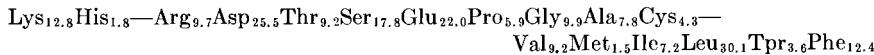
II. Chemical Properties, Including Species Differences

Growth hormone is a single polypeptide chemical entity of high activity. The isoelectric point is estimated to be at pH 6.8 and the molecular weight has been found to be 44,250 (Li, 1957). When growth hormone is subjected to enzymic hydrolysis under strictly controlled conditions, smaller molecules are obtained which still have the same activity as the starting material; this suggests that the activity does not depend upon the integrity of the growth hormone protein but resides in only a portion of the whole molecule.

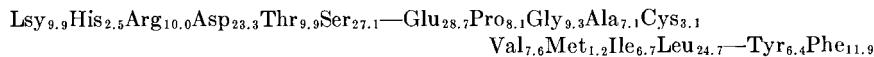
Li (1968) reviewed research establishing a primary sequence of amino acids of human growth hormone (HGH). Smaller in size than growth hormones of any other species, HGH is composed of a single chain of 188 amino acids without carbohydrate substituents. In man, the amino acid chain probably exists with a large and small loop formed from intramolecular disulfide bonds. Unlike the disulfide bonds of insulin

and the neurohypophyseal hormones, the disulfide bonds of HGH are not essential for biologic activity.

Using a strategy employed for synthesis of ribonuclease by the solid phase method, Li and Yamashiro (1970) recently synthesized HGH. Spectrophotometric measurements on the synthetic protein indicated a tyrosine-tryptophan ratio of 7.5 as compared to the known value of 8. Amino acid analysis of an acid hydrolysate gave:



These values were comparable with the analysis of HGH treated with HF and Na-NH₃:



A few months after the Li and Yamashiro (1970) synthesis of HGH, Niall (1971) proposed that because the structure of HGH is about the same as that of human prolactin (HPL), the number of amino acids should be increased from 188 to 190. Leucine and arginine should be added at the end of the aberrant sequence. The facts, however, are that even with two amino acids missing, the biologic activity is present, and that assuming HGH and HPL are identical in structure, the action of HGH does not depend upon the integrity of the total sequence of the HGH molecule.

Growth hormone from other primates resembles HGH in many physical properties but immunologic differences increase with the degree of phylogenetic separation. These immunologic differences indicate differences in amino acid sequence similar to those that have been described for insulin and ACTH from various species. Growth hormones from pig, whale, sheep, and cow pituitaries are believed to have significantly larger molecular weight than HGH. Bovine hormone has one amino end group and two carboxyl end groups which suggest that this hormone exists as a branched chain. These hormones show little or no immunologic cross-reactivity when tested in most immune systems. The larger size of most of the nonprimate GH has suggested to Li (1968) that the inactivity in human beings and the immunologic unrelatedness is dependent upon a core structure that is enclosed in a cloak of amino acids which does not contribute to the biologic activity of the core. When bovine growth hormone is subjected to various enzymic digestions, as much as 24% of the weight of growth hormone may be removed before there is loss in biologic activity in rats. The residual core material

has biologic activity in man and cross-reacts with antibodies reactive with HGH.

Briefly stated, monkey pituitary growth hormone is effective in both monkey and man, but human growth hormone pituitary is only effective in man. Monkey and human growth hormone differ chemically from beef growth hormone. Beef, sheep, horse, and pig hormone differ chemically but all appear to be active in the rat, the most commonly used test animal. Knobil *et al.* (1956) injected hypophysectomized monkeys with allogeneic GH and reinstated epiphyseal growth not obtainable with beef GH. Growth hormone prepared from fish pituitaries is inactive in the rat whereas beef growth hormone is active in fish. These studies are interpreted as suggestive of a common nucleus responsible for growth hormone activity (Knobil and Greep, 1959). There is some suggestion that there are important structural differences that do not pertain to the active core. The outer structure of the growth hormone molecule would be responsible for many of the species-specific characteristics (Wilhelmi, 1955). Synthetic HGH reacts immunologically with rabbit antiserum to HGH as revealed by the agar diffusion test. When synthetic HGH is assayed by the rat tibia test for growth promoting activity and pigeon crop sac test for lactogenic activity, the levels are 10 and 5%, respectively, in comparison with native hormone. Now that HGH has been synthesized (Li and Yamashiro, 1970), the core-activity hypothesis can be subjected to rigorous experimental tests. The possibility still exists that the effector sites in the epiphyseal plate are more selective in the primates than in the rat in terms of a molecular lock and key mechanism (Wilhelmi, 1955).

Human pituitary is particularly rich in growth hormones and is resistant to autolysis after death. The yield is 4–10 mg of hormone per gram, and between 4 and 10% of the dry weight of human pituitaries is HGH. Octogenarians have nearly as much HGH in the pituitary as a rapidly growing child.

III. Assay Methods and Plasma Levels

Radioimmunoassay for growth hormone is remarkably accurate because ^{131}I -labeled growth hormone has high specific activity and minimal denaturation. The validity of the method is supported by finding no detectable hormone after total hypophysectomy and the complete identity of the slope of the reaction of plasma extracts and purified hormone in test systems. With radioimmunoassay, the level of circulating HGH in a well-rested adult, prior to breakfast, is less than 3 m $\mu\text{g}/\text{ml}$ of plasma.

With moderate exercise, higher values are found. The tendency of plasma HGH level to rise after exercise is greater in women than in men, and a rise occurs in plasma HGH in many individuals 2-4 hours after a meal and during prolonged fasting beyond one night.

In the first days of life very high levels of HGH may be observed, but there is great variability among individual infants. After 2 weeks of age, lower mean levels are found. In older children the circulating fasting plasma concentration of HGH is not greatly different from that reported for adults. Information obtained from isolated measurements tells little about total secretion of hormones during a day. Turnover of growth hormone in the plasma is rapid; the half-life is only about 25 minutes. Hypoglycemia induces prompt secretion of growth hormone.

In normal individuals, GH influences the level of the blood sugar and vice versa. A fall in blood sugar as small as 10 mg % was sufficient to activate growth hormone secretion. Agents which inhibit the utilization of glucose by tissues stimulate the secretion of growth hormone. Hypothyroidism in the rat greatly reduces growth hormone content of the pituitary and the concentration of the hormone in the plasma (Glick *et al.*, 1965). Control of GH secretion is a highly active field of chemical research. In sleep, when plasma glucose is not fluctuating, and insulin has fallen to a very low level, GH is released (Quabbe *et al.* 1971). About two hours later, plasma fatty acids may rise but insulin does not rise in response to nocturnal GH release. Protein depletion initiates compensatory elevation in plasma GH. Vander Laan (1971) regards acromegaly as a disease in which the brain perceives the individual as malnourished. Levodopa, a drug used in patients with parkinsonism, stimulates GH release (Boyd *et al.*, 1970).

A. AMINO ACID INFUSION TEST

Simulating the effects of ingestion of a large quantity of protein, intravenous infusion of amino acid stimulates release of HGH. The level of plasma HGH rises promptly to levels comparable to those observed after insulin hypoglycemia. The response is less consistent in males than in females. A fasting-resting woman is particularly responsive, but high levels of plasma amino acid produced by intravenous infusion of a dose of 0.5 mg/kilo of neutralized arginine within 30 minutes is essential. The blood is collected at 30 minute intervals for 2 hours. A rise, greater than 10 m μ g of HGH per milliliter of plasma, is normal. Pretreatment with 3 mg of stilbestrol per day for 2 days will heighten the response. Measurement of HGH is done only by radioimmunoassay, using anti-serum to HGH and radioiodinated GH tracer to establish the saturation

analysis system. In general, differences in GH titer in response to arginine infusion must be interpreted with caution and are of diagnostic value chiefly in patients with acromegaly (Catt, 1970).

B. OBSERVATIONS ON SITES OF ACTION OF GROWTH HORMONE

Although the response of the epiphyseal cartilage to hypophysectomy in the rat is consistent and sensitive enough to use for assay work, the site of action of GH on epiphyseal cartilage cells is not known (Daughaday and Mariz, 1962; Daughaday and Reeder, 1966). Sulfate incorporation by epiphyseal cartilage is reduced 30–40% of normal by hypophysectomy, but it is restored within 24 hours by administration of pituitary growth hormone. Addition of growth hormone directly to an *in vitro* incubation of a hypophysectomized rat cartilage, has little stimulating effect. Normal serum is capable of stimulating cartilage directly, whereas serum from hypophysectomized rats is inactive. Still, growth hormone may not act directly on cartilage but may exert its effect through a sulfation factor which is directly active. Tritiated thymidine uptake is also stimulated by a factor contained in normal rat serum but absent in the serum of hypophysectomized rats. This factor is probably identical to the sulfation factor (Daughaday and Kipnis, 1966).

The stimulation of mitogenesis in epiphyseal cartilage under influence of GH is accompanied by similar responses in pancreas, intestinal mucosa, adrenal cortex, liver, and adipose tissues (Murakawa and Raben, 1968). ^3H -thymidine uptake during DNA synthesis is quantitatively accelerated in growth cartilages even *in vitro* and is a reaction that is reliable in HGH assay work. Human growth hormone produces a four-to fivefold increase in ^3H -thymidine uptake above values obtained in hypophysectomized rats (Breuer, 1968). *In vivo*, the response is low in fetal and weanling rats, high in the premature growing rats, and again low in rats in the long postmature periods of life. Provided that serum containing the sulfation factor of Daughday and Kipnis (1966) is used for the incubating medium, these levels of responsiveness are the same in systems *in vitro* as in the living rat. If the sulfation factor, an intermediary agent in the local action on cartilage, is absent, *in vitro* assay methods are unsatisfactory (Heins *et al.*, 1970). Weltenhall *et al.* (1969), using labeled precursors, chemically defined media, and postnatal bone tissue, demonstrated stimulation of growth by insulin but not by GH (as measured by collagen and chondroitin sulfate synthesis) and concluded that the reaction to GH may be mediated by insulin and other agents as well as by a sulfation factor. Nevertheless, this factor mediates both the action of GH on muscle as well as cartilage metab-

olism (Salmon and DuVall, 1970). Van Wyk *et al.* (1971), making important progress on chemical identification of the sulfation factor (SF) and other constituents of acromegalic plasma, suggest SF is protein with a molecular weight of 8000, bound to a larger carrier protein.

Work-induced growth of muscle occurs from both synthesis of protein and decrease in the average rate of protein catabolism. In GH-treated hypophysectomized rat, muscle mass, as measured by uptake of ^3H -leucine, increases owing to increase in the rate of synthesis, but there is no change in the normal rate of protein catabolism (Goldberg, 1969). This balance in protein metabolism is lost only by muscles atrophied from cortisone treatment or degeneration, or similar disorders, and would not be expected to be restored by GH.

Little is known of the effect of GH on the biochemical reactions of collagen synthesis. Growth hormone increases the rate of synthesis of soluble collagen, determined by total activity of ^{14}C -hydroxyproline in skin and bones following injections of ^{14}C proline into rats. The uptake is followed by increased urinary excretion of hydroxyproline, presumably reflecting the increase in rate of catabolism of soluble collagen. Growth hormone does not change the soluble to insoluble collagen when expressed per unit of soluble collagen (Aer *et al.*, 1969).

As a rule, hormones interact with a component of the cell membrane and initiate a chain of reactions, the products of early events acting as inducers of subsequent events. A hormone thus activates a built-in chain of reacting substances, and at any one time only a part of the whole system may be in motion. Adenosine-3',5'-phosphate (cyclic AMP) seems not to act as an intracellular mediator for GH (Butcher *et al.*, 1970), but other systems must be present in cells to amplify its effects. Growth hormone can produce a general effect on protein synthesis within 15 minutes, presumably without intervention of gene mechanism of cell regulation. Korner (1970) observed that an effect of GH on the rate of synthesis or degradation of messenger RNA or of ribosomes is unlikely, although it does enhance the activity of isolated ribosomes *in vitro*. Talwar *et al.* (1970) noted that GH influences the rate of RNA synthesis, and the main locus of its action is on the membranous structures of the cell. When tissue is incubated with GH for 2–10 minutes and examined by the fluorescent antibody technique using rabbit antigrowth hormone plus goat antirabbit globulin serum, the hormone appears in the membranous structures of adipose or endothelial cells. Glucose and amino acids are incorporated rapidly in cell suspensions and homogenates exposed to GH for only a few minutes.

The epiphyseal plate of the hypophysectomized rat is sensitive enough to respond to GH-like substances as well as GH itself. Spargana, a tape-

worm, secretes GH-like substance and restores epiphyseal growth of infested hypophysectomized rats (Mueller, 1968; Mueller and Reed, 1968). Injections of the plasma of infested hypophysectomized rats stimulates uptake of radiosulfate by costal cartilage. The GH equivalents are as high as 100 $\mu\text{g}/\text{ml}$ of plasma, far greater than the quantity in normal rat plasma and about the same as obtained from a single pituitary gland of a rat (Steelman *et al.*, 1970). A worm factor stimulates the hypophysectomized rat to produce the sulfation factor in the absence of GH (Garland *et al.*, 1971).

IV. Experimental Models, Target Tissues, and Deficiency of Growth Hormone

Nearly every tissue in the body may be considered a target tissue for the effect of GH, but some tissues are more responsive than others. One of the prime targets for GH is the *bone growth apparatus*, the epiphyseal plate, and particularly the zone of cell proliferation adjacent to the layer of rows of mother cells (Ross and McLean, 1940). During the period of growth, the epiphyseal plate maintains a constant thickness and maintains an equilibrium between proliferating cartilage, hypertrophic cartilage, calcified cartilage, and endochondral bone. This structure is extremely sensitive to the level or titer of growth hormone in the circulating plasma. When the titer falls from hypophysectomy and deficiency of growth hormone, the epiphyseal plates decrease in thickness and become sealed by a plate of lamellar bone. When growth hormone therapy is instituted in the rat, a species that grows slowly throughout adult life, the growth apparatus is restored by proliferation of cartilage, increase of thickness of the epiphyseal plates, resorption of the lamellar bone-seal, and continuation of growth of the diaphyses in length.

A. HYPOPHYSECTOMY

The classic experimental model on growth hormone is the hypophysectomized rat. With the technique devised by P. E. Smith (1930) and special measures to feed a diet high in protein from natural sources and supplements of the essential vitamins and minerals, the rat will survive hypophysectomy very well. Paired feeding of measured quantities of the diet to normal and hypophysectomized rats is essential for low morbidity and mortality and for a consistently reproducible standard animal model. Replacement therapy with thyroxin and sex hormones

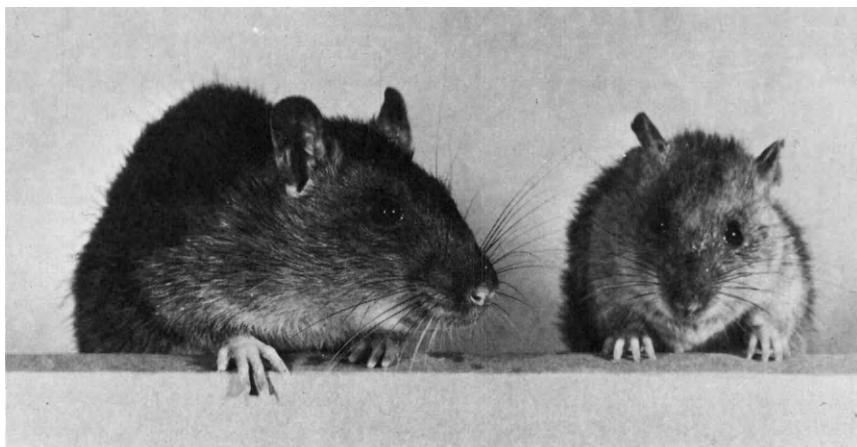


FIG. 1. Photograph of normal (left) and hypophysectomized (right) rat of the Long-Evans strain at 3 months of age. Note the markedly smaller size of the head and paws of the GH-deficient rat. Reproduced from the *Atlas of Skeletal Development of the Rat* with permission of the American Institute of Dental Medicine, San Francisco, Calif.

to compensate for the loss of thyrotropic hormone and gonadotropins is necessary in any experimental design which requires a high level of precision. Two easily detectable responses to deficiency of GH in immature rats are (1) decline in the rate of increase in body weight with growth and (2) decrease in thickness of the epiphyseal plates (Figs. 1 and 2).

B. ENDOCHONDRAL OSSIFICATION IN HYPOPHYSECTOMIZED RATS

The abnormalities of endochondral ossification in the hypophysectomized rat are illustrated in Figs. 3 and 4A and 4B. The plates are from copy No. 112 of a limited edition of Becks and Evans' (1953) atlas of skeletal development of the Long-Evans rat of the Institute of Experimental Biology, University of California. Hypophysectomy causes cessation of proliferation and maturation of the growth cartilages, and as a result the epiphyseal plates decrease in thickness. The vascular tufts from the marrow which normally invades the hypertrophic vacuolated cells disappear and the metaphyseal trabecular bone is resorbed without replacement or reconstruction. Eventually, calcification of the juxta-medullary portion of the cartilage ensues and deposition of lamellar bone plates finally seals epiphyseal plate off from the marrow. This lamellar bone-seal closely resembles that observed in growth stasis from

other causes. Among the other conditions which similarly seal the epiphyses are normal aging, phosphorus poisoning, thyroidectomy, vitamin A deficiency, riboflavin deficiency, protein deficiency, caloric restriction, and radiation injury. The radiographic "lines of arrested growth," observed in children recovering from an acute infectious disease, is indicative of a similar abnormality in the pattern of epiphyseal bone growth.

In the hypophysectomized rat, internal remodeling of bone is much less affected by GH deficiency than epiphyseal endochondral ossification. Anderson and McKeen (1969) injected ^{85}Sr into GH-treated hypophysectomized, untreated hypophysectomized, and normal rats, and measured whole body radioactivity over an interval of 6 weeks. No significant difference in ^{85}Sr retention or weight of the whole tibias was noted among groups of animals, but the net gain in weight was always significantly greater in normal and GH-treated hypophysectomized rats than in untreated hypophysectomized rats. Hence, the increase in weight from treatment with GH was chiefly in nonosseous tissues and not associated with measurable alterations in rate of turnover of mineralized bone tissue. These seemingly negative data testify to the fact that the layer of interdigitating calcifying cartilage and primary spongiosa is a specific target while bone remodeling sites are unspecific targets of action of GH. Similar data obtained on patients treated with HGH will be discussed in Section V.

C. REPLACEMENT THERAPY

Growth hormone replacement therapy must be evaluated with the aid of a preparation of animal GH or HGH of maximum physicochemical and biologic purity. For a valid test, growth hormone must be of a single molecular species as demonstrated by electrophoresis and free from contamination with other pituitary hormones. Evans *et al.* (1943) considered a minimal effective dose of the order of magnitude of 10 μg of GH (free from any detectable inclusion of other anterior pituitary hormones), but at least 5 μg of GH administered daily over a period of 4 days is necessary for a valid test. Evidence of the effects on the skeleton is obtained from gross measurements of the length of the bone, radiographic measurements of the thickness of the epiphyseal plate, and histologically detectable resorption of the bone-seal between metaphysis and epiphysis.

Administration of the GH of the anterior lobe of the pituitary promptly repairs the defect in growth of hypophysectomized rats. The animals are responsive to the effect of GH whether the injections are commenced

Normal - 71 days

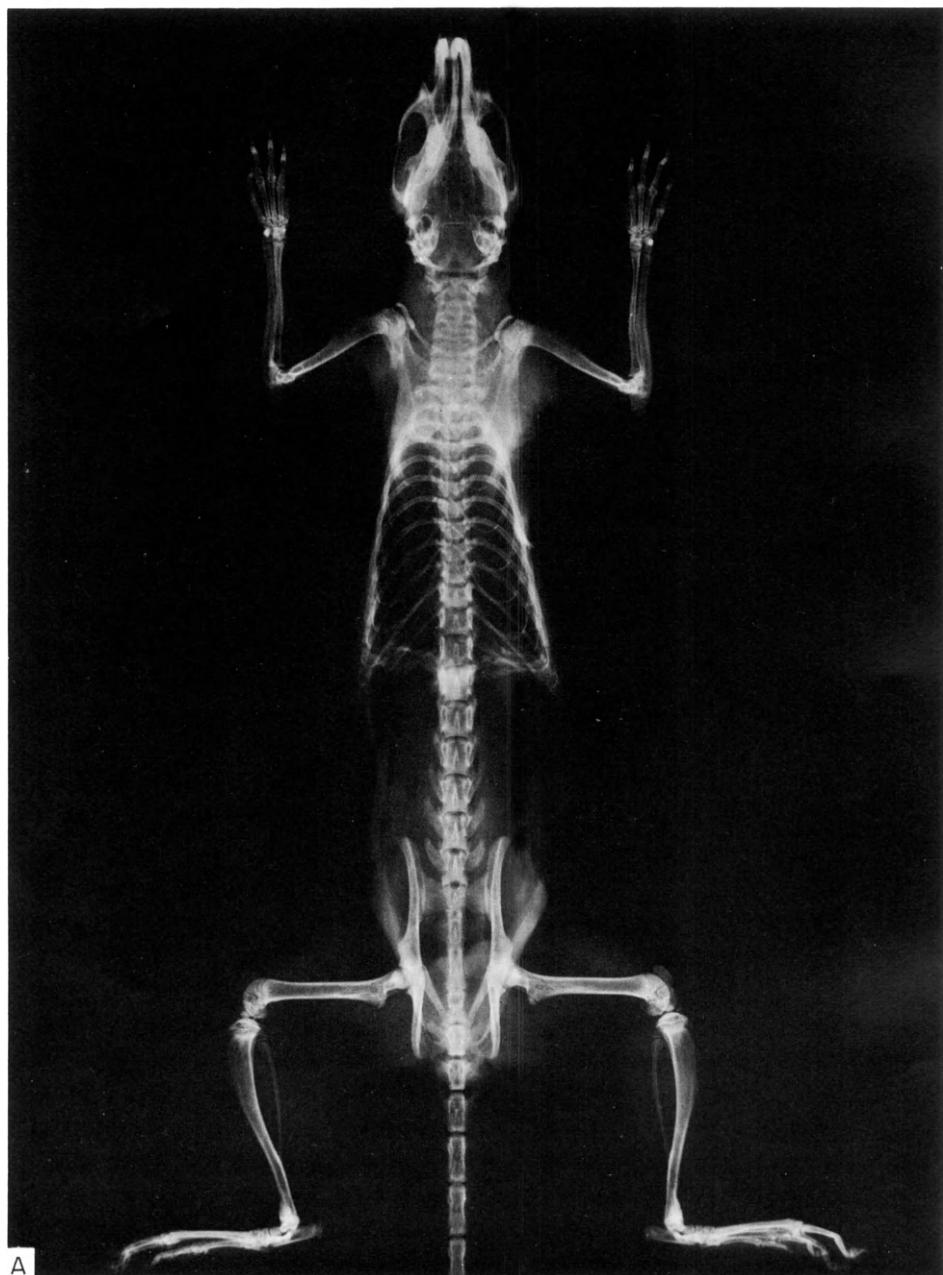


FIG. 2A.

Hypophysectomized
77 days postoperative



FIG. 2B.

FIG. 2. (A) Roentgenogram of the whole skeleton of the normal individual at about 10 weeks of age, when the growth of the Long-Evans rat begins to decrease in rate. (B) Roentgenogram of the skeleton of a hypophysectomized rat at about 10 weeks of age. Note the 30% smaller diameter and length of the bones; the metaphyses are 50% as long as normal; the epiphyseal lines are barely perceptible; and the overall radiodensity of both flat and long bone structure is low. Reproduced by permission of the American Institute of Dental Medicine, San Francisco, Calif.

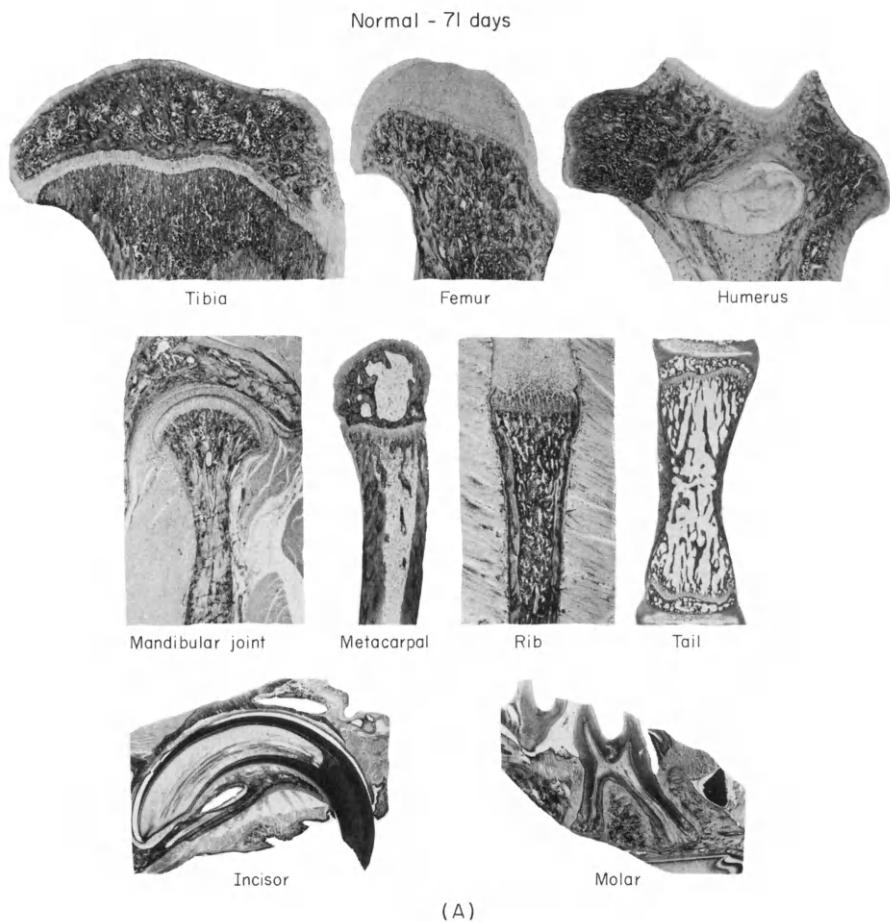


FIG. 3. (A) Photomicrographs in low power magnification showing bones and teeth of a normal rat at 10 weeks of age. Note the thickness of the epiphyseal plates of the long bones and quantity of trabecular bone in ribs and tail vertebra.

shortly after the operation or whether the delay is a year or longer (Becks *et al.*, 1946). Although the majority of experiments have been made on rats hypophysectomized when 28 days old, age at the time of hypophysectomy is not a factor; rats hypophysectomized when as young as 6 days of age and as old as 6-7 months have been shown to be responsive to administration of the growth hormone.

Growth hormone administration restores the histologic appearance of epiphyseal cartilage plates to that characterizing young, actively growing bones. Promptly (in 5 days or less), chondrogenesis is reawakened,

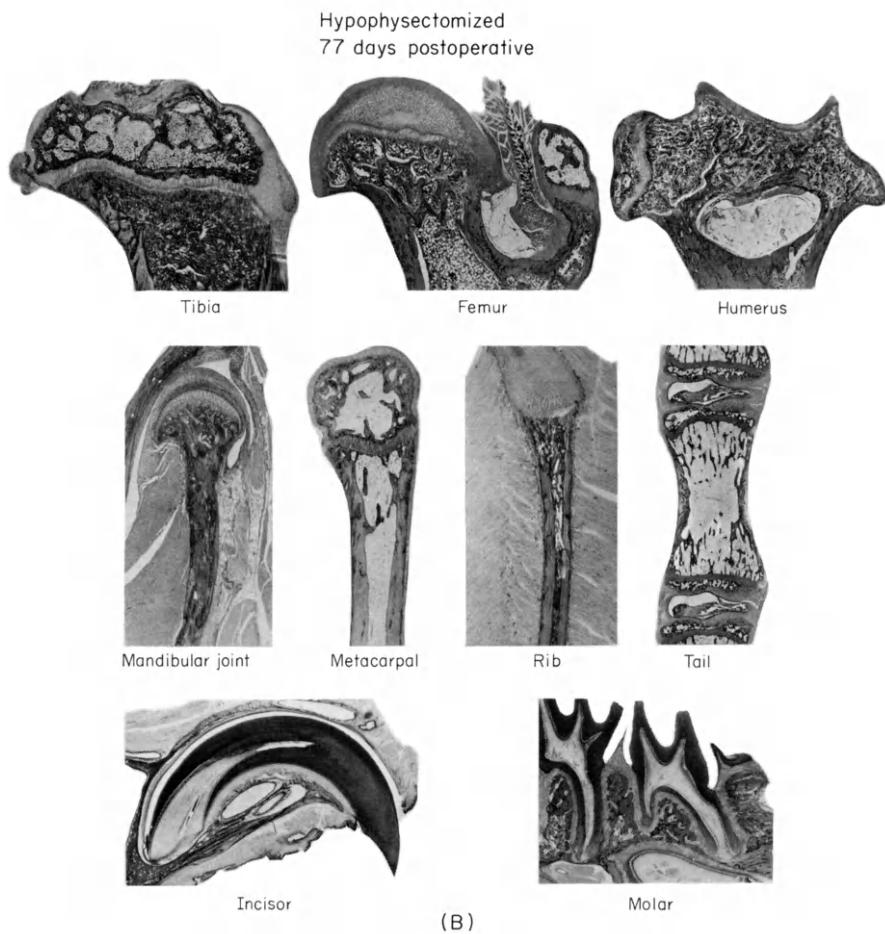


FIG. 3. (B) Photomicrograph of bones and teeth from a hypophysectomized rat of about 10 weeks of age. Compared with the specimens from the rat shown in (A), the overall dimensions of the epiphyses are smaller, the epiphyseal plates are thinner, the trabecular bone tissues are attenuated, while the ratio of diameter of shaft to thickness of cortical bone is not significantly different. Figure 3 reproduced by permission of the American Institute of Dental Medicine, San Francisco, Calif.

vascular tufts from the marrow again invade the vacuolated cells and even resorb the lamellar bone-seal if one was present. The trabecular bone structure of the metaphysis is redeposited by palisades of newly differentiated osteoblasts. The cortical bone, as one may eventually see by differential staining, develops layers of metachromatic-formed new bone concentrically round layers of old eosinophilic bone. The widening

of the epiphyseal cartilage plate is, within a broad range of dosages, proportional to the quantity of hormone administered (Becks *et al.*, 1941). The epiphyseal plate method of assay of the potency of hormone

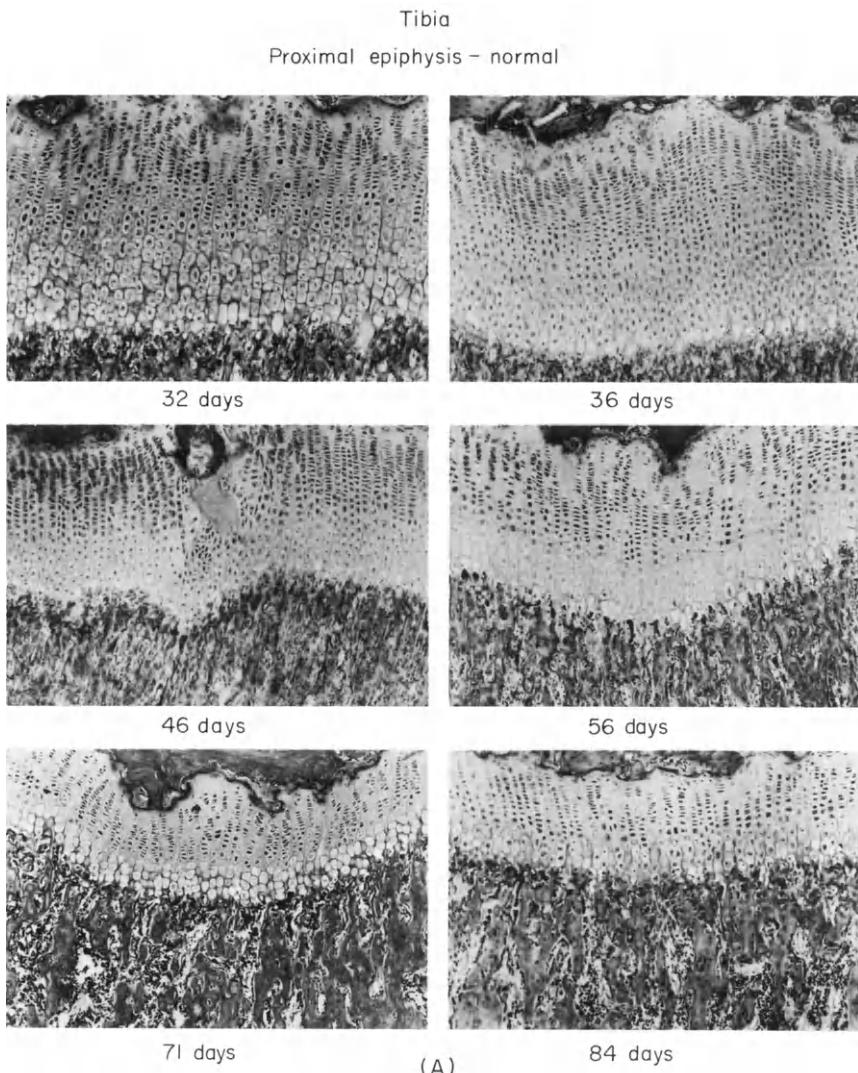


FIG. 4. (A) Photomicrographs in higher power magnification showing the normal structure of the epiphyseal plate of the proximal end of the tibia at successive intervals of time between 32 and 84 days of age in the Long-Evans strain of rats. Note the progressive decrease in thickness of the zones of hypertrophy and vesiculation of chondrocytes.

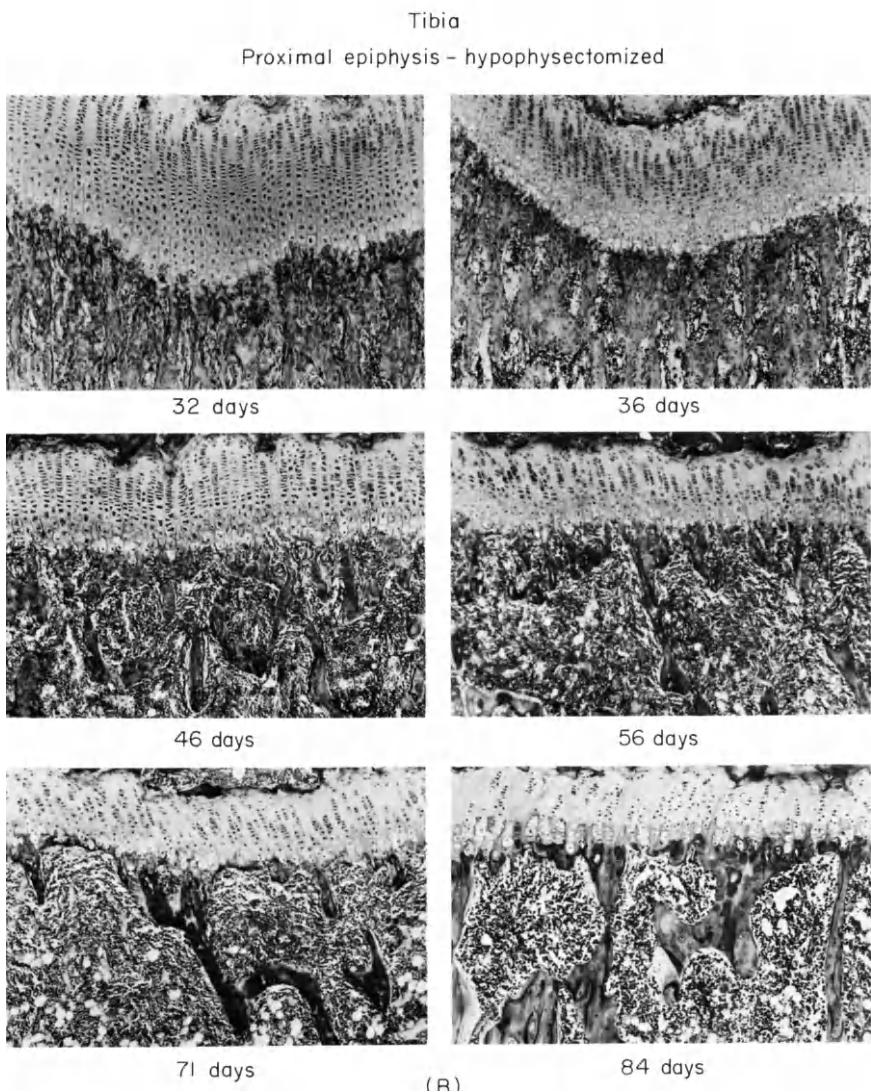


FIG. 4. (B) Photomicrographs of a series of sections of the proximal end of the tibia of hypophysectomized rats, at the same intervals of time as shown in (A). Note the pronounced decrease in the zone of cell hypertrophy within a 4-day period between 32 and 36 days, the loss of about 30% of the thickness of the epiphyseal plate within the 24-day interval between 32 and 56 days of age, the epiphyseal plate sealed off from the metaphysis at 71 days of age, and the thick malformed bone trabeculae of the metaphysis at 84 days.

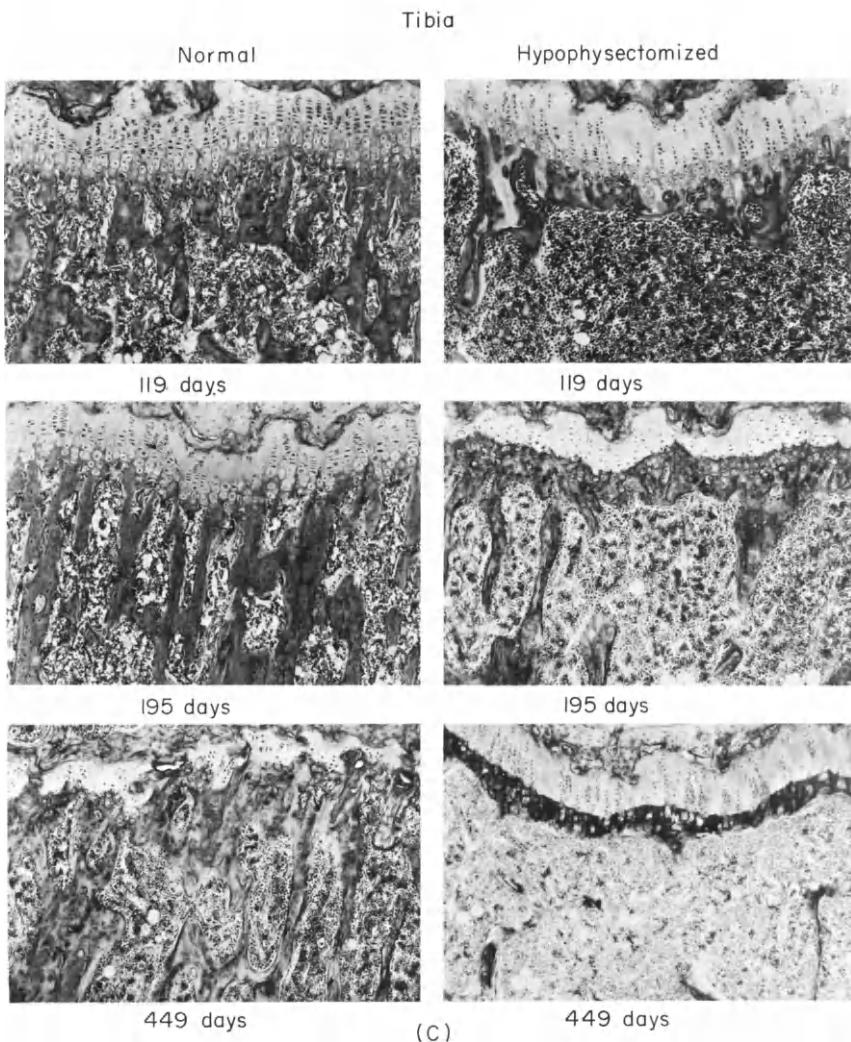


FIG. 4. (C) Photomicrographs of the normal (left) and hypophysectomized (right) rats at intervals of 119, 195, and 449 days. Note the progress of replacement of trabecular bone with bone marrow and development of the characteristic lamellar bone seal of the epiphyseal plate of the hypophysectomized rats. Normally, the rat tibia continues to grow slowly in length long after sexual maturity; parts of the epiphyseal plate are resorbed and bars of bone bridge the gap between metaphysis and epiphysis at about 400 days of age. In the hypophysectomized rat, the epiphyseal plate is not resorbed or replaced by bone but simply enclosed in a dense mass of avascular lamellar bone. Figure 4 reproduced by permission of the American Institute of Dental Medicine, San Francisco, Calif.

preparations is sensitive to as little as 5 μg of hormone given over a 4-day period (Greenspan *et al.*, 1949).

The synergistic effect on the thyroid gland and thyroxin augments the action of GH. Accordingly, thyrotropic hormone acts synergistically with GH (Marx *et al.*, 1943). Although appreciable (but subnormal) growth may be maintained when thyroxin is administered immediately after hypophysectomy (at 28 days of age), this growth does not continue very long (Asling *et al.*, 1954). Histologically, after 14 months the proximal tibial epiphyseal cartilage plates show the same inactivity and atrophy as does untreated hypophysectomized controls. Thyroxin is not a growth-promoting hormone *per se*, since one of the established criteria for such a hormone is that administration over a prolonged period should result in sustained growth of the hypophysectomized rat.

In hypophysectomized animals, ^3H -thymidine labeling experiments demonstrate that a number of labeled cells are reduced to about 20% of normal for animals of the same age. Although the cells are dividing more infrequently, the proliferation zone in the columns of cartilage cells is reduced to only about $\frac{3}{4}$ of the usual length. After administration of growth hormone there is a delay of as much as 16 hours, and the maximum response may occur as late as 48 hours. The cause of the lag is not known, but it generally occurs in nonskeletal tissue or in any site in which a stimulus is necessary to initiate cell division. In hypophysectomized animals both before and after growth hormone is administered, there is no change in the labeling profile; the distribution of cells along the columns of proliferating chondrocytes is the same. Growth hormone, or its active derivative, affects all cells of the epiphyseal plate, and not just the row mother cells preferentially (Kember, 1971; Asling and Nelson, 1962). However, Rigal (1964) noted that *in vitro* labeling with tritiated thymidine is confined to the row mother cell or proliferating zones of epiphyseal cartilage excised from a rabbit injected with growth hormone. Additional studies are necessary to characterize the factors controlling interaction between endothelial cells and epiphyseal cartilage under the influence of growth hormone. Hansson and Thorngren (1971) are increasing the precision of the epiphyseal plate bioassay method with the use of an oxytetracycline labelling technique.

Labeling experiments do not explain how hypophysectomy reduces the rate of cell division in the cartilage plate with little change in the length of the proliferation zone or how rickets produces little decrease in the length of the proliferation zone but a large increase in the cell hypertrophy zone. Some of the changes in the epiphyseal plate produced by hypophysectomy resemble those seen in aging rats; the intercolumnar

matrix increases in quantity along with a corresponding reduction in the number of columns of cells. Hypophysectomy produced a marked decrease in the incorporation of proline-¹⁴C and ³⁵SO₄ into costal cartilage (Daughaday, 1968). Cartilage slices from hypophysectomized rats take up little or no ³⁵SO₄, but growth hormone reconstitutes sulfate metabolism *in vitro*. The very presence of growth hormone in rat serum can stimulate uptake of ³⁵SO₄, presumably by increasing the rate of sulfate incorporation into chondroitin sulfate in cartilage. Growth hormone also enhances the conversion of proline to hydroxyproline in surviving tissues *in vitro*. Growth hormone increases the rate of DNA synthesis (Daughaday and Reeder, 1966) and enhances RNA synthesis and increased formation of polysomes (Korner, 1965). Other hormones share with GH the capacity to accelerate protein synthesis, as, for example, thyroid, adrenal cortical steroid, insulin, and sex hormones. Because of great differences in organ structure and variable rates of growth of the individual bones, experiments on growth-promoting endocrine agents must be very carefully planned to be conclusive (Cheek and Hill, 1970).

D. HUMAN DWARFISM AND HUMAN GROWTH HORMONE REPLACEMENT THERAPY

Deficiency of HGH in childhood produces pituitary dwarfism. Before birth and in early infancy, deficiency of pituitary function does not impair growth (Grunt and Reynolds, 1970). Without any brain or pituitary, anencephalic monsters can grow to very large or normal size (Cheek and Hill, 1970; Naeye and Blanc, 1971). In early childhood and before the end of the fourth year, HGH deficiency becomes apparent by the grossly visible immature facial features and body proportions. Facial wrinkles give the appearance of presenility mixed with immaturity. Low fasting blood sugar and abnormally high glucose tolerance may occur. The bone age, as determined by roentgenograms of the wrist, is always less than the chronological age. The primary teeth appear on time, but eruption of secondary teeth is delayed. In most cases of pituitary dwarfism, the etiology is either entirely obscure or inherited by an autosomal recessive mechanism, or by some acquired defect in a hypothalamic center regulating HGH secretion such as Hand Schüller-Christian disease and brain tumors. The diagnosis is unequivocally established only by radioimmunoassay and amino acid infusion tests. True, pituitary dwarfism is rare. The differential diagnosis must include Russel dwarfs, premordial dwarfs

(Lorrain-Livi), hypothyroidism, gonadal aplasia, malnutrition, mongolism, constitutional idiopathic retardation of growth, achondroplasia, Laurence-Moon-Biedl syndrome, neurofibromatosis, congenital heart disease, congenital hemolytic anemias, and progeria (Daughaday, 1968).

After injection of HGH into a pituitary dwarf, as differentiated from any other kind of dwarf, nitrogen balance becomes strongly positive; plasma urea and urine urea values promptly fall. Although the levels of daily nitrogen retention gradually decrease with continuous growth hormone treatment, it is still adequate to maintain greatly increased growth rate. Plasma levels of hydroxyproline rise, suggesting increased rate of conversion of proline to hydroxyproline and deposition of new collagen (Daughaday and Mariz, 1962). Sodium, chloride, potassium, magnesium, and phosphorus are retained concomitant with positive nitrogen balance. Calcium may be retained by the body despite increased calciuria. This is explained by increased gastrointestinal absorption of calcium. Plasma phosphorus levels may or may not rise after growth hormone administration in dwarfism. When HGH raises plasma phosphorus levels, the effect is to increase kidney tubular reabsorption of phosphate ions (Corvalain *et al.*, 1962). The urinary hydroxyproline excretion also rises sharply (Jason *et al.*, 1962). Serum alkaline phosphatase is little changed. The long-term administration of growth hormone does not produce diabetes unless very large doses are administered. Infusion of HGH very promptly blocks the ability of insulin to increase glucose removal by muscle tissue. Human growth hormone possesses an intermediate blood sugar lowering action; it alters the central nervous system metabolism directly or indirectly in such a way as to cause pituitary dwarfs to become much more active and alert.

African pygmies are genetic dwarfs with no deficiency in immunologically active HGH, but they show less response to the hormone than either normal or hormone-deficient subjects. Low levels of response of end-organ biosynthetic mechanisms may be responsible for genetic dwarfism. Antigen antibody and other blocking reactions are other possibilities for investigation in genetic forms of dwarfism (Merimee *et al.*, 1968).

E. REGENERATION OF BONE

Hypophysectomy retards the healing of ordinary undisplaced long bone fractures. Apituitary dwarf mice produce only unmineralized soft callus and delayed union of fractures of the tibia (Hsu and Robinson, 1969). Large defects in the calvarium heal in normal weanling rats

but not hypophysectomized rats; nearly normal repair occurs in hypophysectomized rats treated with growth hormone (Simpson *et al.*, 1953). Large diaphyseal defects not repaired by normal mature dogs are repaired by mature dogs treated with bovine growth hormone (Zadek and Robinson, 1961).

Shepanek (1953) treated normal mice with 5.0 mg of GH daily and noted enlargement of callus trabecular bone mass; union was inhibited rather than hastened by treatment with GH. Koskinen (1959) measured fracture healing in normal rats by correlative roentgenographic, ^{32}P autoradiographic, and line diagram histologic techniques and concluded that procine GH alone increased the volume of callus while GH plus thyroxin produced union 2 weeks earlier than in untreated controls. Udupa (1966) and Lindohm *et al.* (1967) corroborated Koskinen's observations on rats. Koskinen (1963, 1967) also treated 64 patients with thyroxin and procine GH for anticipated delay or nonunion and reported beneficial results in some instances. Misol *et al.* (1971) reported delayed union in a fracture of the femur in a 26-year-old man who had subnormal levels of plasma growth hormone, and they suggested that HGH therapy is justifiable in such cases. Thus, experimental and clinical studies suggest that the availability of synthetic HGH might bring important benefits to patients with bone defects from injury or disease.

Anterior pituitary secretions, as noted previously, are essential for regeneration of whole limbs, including bone in adult urodeles. Hypophysectomy inhibits regeneration in the adult newt but not in the larval amphibian. The whole process of limb regeneration is restored by whole gland implants, crude extracts, and purified GH and ACTH hormones (Schmidt, 1960). Regeneration of the deer's antler is profoundly influenced by sex hormones (Goss, 1969) and as such is prevented by hypophysectomy and withdrawal of FSH or by castration. The effects of hypophysectomy in the buck, under adequate testosterone replacement therapy, require further study.

F. LOWER VERTEBRATES

Cells comparable to GH hormone secreting cells in the pituitary glands of mammals are present in the pituitary of fish. Accordingly, it is reasonable to suppose that the same cells secrete GH or prolactin-like GH in all species from cyclostomes to man.

In fishes, gill, kidney, and integumentary membranes regulate calcium total ion concentrations and osmotic equilibrium. Pituitary hormones, possibly prolactin or GH, influence ion regulatory mechanism. Parathyroids (which are absent) or ultimobranchial bodies—calcitonin-secreting

glands—(which are present) are not involved. Pang *et al.* (1971) described hypocalcemic tetany in the hypophysectomized teleost in calcium-free, but not calcium-rich, sea water. Thus, gill membranes regulate calcium fluxes in sea water (in which the levels of calcium are normally 10 mM/l) but need the pituitary hormones to defend the organism against hypocalcemia in calcium-free sea water or fresh water (in which the levels of calcium are normally only 0.7 mM/l). Which pituitary hormone is responsible for control of calcium ion concentrations is not clear. The possibility that a pituitary hormone, as yet undiscovered, may regulate calcium separately from sodium to maintain calcium homeostasis when the fish is a fresh water habitat, warrants further investigation.

Hypophysectomy prevents limb regeneration in fish, but neither the character of the hormones involved nor their sites of action has been elucidated (Goss, 1969). In reptiles and birds, prolactin has growth-promoting properties. In teleost fish, as noted above, prolactin is identified more easily by its effects on osmoregulation and resistance to environmental stress than by promotion of growth (Bern and Nicol, 1968). In urodeles, prolactin stimulates limb regeneration (Waterman, 1965). In hypophysectomized newts, in which administration of ACTH or cortisone can restore regeneration of limbs, it appears that the pituitary is required only insofar as it stimulates secretion of glucocorticoids from adrenals (Schmidt, 1960). These observations point out the difficulty of separating unspecific from specific effects of pituitary hormones in lower vertebrates.

V. Excessive Action of Growth Hormone

Excessive secretion of growth hormone is frequently associated with eosinophilic adenoma, a tumor of the pituitary gland. In immature individuals this tumor produced gigantism, in adults acromegaly. The characteristic feature of acromegaly is enlargement of the terminal parts of the body—nose, mandible, fingers, toes—with an increase in diameter of the long bones. Overgrowth of the skeleton is a constant reaction to administration of growth hormone in excess of normal requirements for long periods of time. In a short-term (30 days) experiment, in which the hormone was administered to normal growing, female rats, starting at 81 days of age, 1 mg of GH per day stimulated growth in body and tibia length in excess of normal, and widened the epiphyseal cartilage plate (Asling *et al.*, 1948, 1950). Evans and Simpson (1931) produced gigantism in gonadectomized as well as intact rats. The intact

males receiving the hormone exceeded their controls in body length by over 9%; the corresponding females exceeded their controls by 15%. Gonadectomized rats were surprisingly responsive; in fact, the response seemed even greater in ovariectomized rats.

Gigantism generally can be produced to the degree that most of the skeletal dimensions exceed normal by 12–16% in immature as well as mature hypophysectomized female rats. In immature rats, in which the pituitary is removed at 28 days of age, the hormone can be injected for 14½ months starting 12 days after hypophysectomy (Simpson *et al.*, 1949) (as little as one-fifth to one-tenth the dose given to adult rats) and still produces gigantism. Such rats, while exceeding normality in weight, maintain normality in length and in dimensions of the individual bones. In mature hypophysectomized rats, the dose of hormone given over a period of 13 months may closely approximate that given the intact rats and still produce gigantism (Moon *et al.*, 1951). Hypophysectomized GH-treated rats often exceed in some skeletal dimensions even the intact giant rats (Asling *et al.*, 1955).

Harris and Heaney (1969) treated adult dogs of both sexes with bovine GH, 0.5 mg/kg/day for 84 days, and increased the mass of skeletal tissue without inducing acromegaly or diabetes. The increase was measured by tetracycline labeling and mineral accretion using ^{45}Ca kinetic techniques. Formation, not resorption, occupied almost every endosteal bone surface, and the estimated increase in skeletal mass was 63% compared with less than 5% in control periods in corresponding bones. Absorption of calcium from the diet was more than doubled under the influence of GH. Previously, Marx and Rheinhardt (1942), using radiostrontium uptake measurements, were unable to detect a stimulating effect of GH on accretion of bone. On the assumption that endocrine deficiencies cause osteoporosis and that postmenopausal women might have lower than usual output of growth hormone (either resting and after hypoglycemia), Harris and Heaney (1969) suggested that HGH might be an effective therapeutic agent for postmenopausal osteoporosis. A therapeutic trial of HGH in a patient with senile osteoporosis produced only negative results (Urist *et al.*, 1963). More recently, five patients with severe senile osteoporosis showed no depression of the GH titer (Urist and Heuser, 1971). Inasmuch as senility alters the molecular conformation of intracellular enzymes (Root and Oski, 1969), there is the possibility that bone cells have a low level of end organ response to normal HGH secretion in idiopathic or senile osteoporosis. Aging also reduces the response of cells to the sulfation factor, which mediates the effect of GH (Heins *et al.* 1970).

Reinhardt and Li (1953) produced chronic arthritis in knees and

ankles of gonadectomized-adrenalectomized rats under prolonged treatment with growth hormone. Joint deformities developed in both intact and hypophysectomized rats, and most constantly in the vertebral column, knee, and ankle joints. The incidence and the degree of deformity was much greater in hypophysectomized rats than in intact rats similarly treated. M. Silberberg *et al.* (1964) described the ultrastructure of GH-induced arthropathy in mice; GH increases the rate of development of coarse endoplasmic reticulum, Golgi vesicles, glycogen bodies, and other organelles of the chondrocyte, but it breaks the cell down after about 4 weeks. The intercellular collagen fibers become disoriented and frayed, producing the so-called asbestos transformation characteristic of acromegalic arthropathy and osteoarthritis of advanced age. Through these changes, GH treatment produces arthrosis within 2 weeks in adult animals (R. Silberberg and Hasler, 1971). Grossly, these deformities are comparable to those found in human acromegalic joint disease (Waine *et al.*, 1945; Kellgren *et al.*, 1952; Bluestone *et al.*, 1971).

The kyphosis and the characteristic overgrowth of bone on the anterior aspect of the vertebral bodies, described by Erdheim (1931), are characteristic features of human acromegalic osteoarthropathy. These features of acromegaly in the human are similar but not exactly the same as in the skeleton of the rat. The paws become thickened both laterally and dorsoventrally in mature individuals treated chronically with growth hormone but the phalanges do not elongate beyond normal. The thickening seems chiefly in skin and subcutaneous connective tissues. Epiphyseal fusion occurs during the treatment if it has not already taken place before the hormonal injections began. The incisor malocclusion of mandibular prognathism is not demonstrable in the rat, perhaps because of the special features of incisal occlusion in rodents, whose incisors form and erode continuously throughout life. However, there is overgrowth and deformity of the mandibular zone of endochondral ossification (that cartilage of the condyle subjacent to the temporomandibular joint), actually the squamosomandibular articular cartilage. Some malocclusion of the molar teeth, by antero-displacement of the mandibular molars as a result of the excessive condylar growth, is demonstrable. The bony attachments of the temporal and suboccipital muscles are unusually large. Kurtz *et al.* (1970) treated rats with 1 IU/day for 10 days and noted reactivation of the cartilage in the palatal suture and mandibular symphysis. The chondrocytes increased in number and hypertrophied osteoclasts destroyed the zone of calcified cartilage; no changes were visible in the adjacent bone structure. Thus, GH produced an easily detectable growth-promoting effect on cartilaginous closing sutures and symphyses. Prominences of the appendicular skeleton,

such as the deltoid tuberosity of the humerus, are markedly overgrown in GH-treated rats.

VI. Interaction with Other Hormones

A. THYROIDECTOMY AND THYROID REPLACEMENT THERAPY

The hypophysectomized animal has a low level of oxidative processes owing to secondary hypothyroidism. Administration of thyroid hormone improves the growth-promoting effect of growth hormone both morphologically and metabolically. Thyroid alone, however, does not induce growth in the hypophysectomized animals. The response of an hypophysectomized rat's epiphyseal growth zone is somewhat misleading in this respect inasmuch as a widening obtained by treatment with thyroxin is similar, albeit quantitatively inferior, to that obtained with growth hormone. Removal of thyroid hormone also results in a deficient pituitary function, one of the first signs of which is degranulation of the acidophils, the presumptive source of growth hormone. Evans *et al.* (1939) described growth retardation following thyroidectomy at 35–45 days of age much like that which follows hypophysectomy. Thyroidectomized-hypophysectomized rats had body lengths still shorter than those only thyroidectomized. The epiphyseal cartilage plate regression after thyroidectomy corresponds in all essential features to those observed after hypophysectomy (Becks *et al.*, 1942a,b).

Thyroidectomized growing rats gain about 140% over their original body length in 2 months, while intact rats gain 300%. The greatest part of these gains is achieved before the rats are a month old (Ray *et al.*, 1950). Some excess of growth of thyroidectomized rats over that of rats hypophysectomized and observed at comparable ages is to be expected. Thyroid hormone is known to be relatively long lasting, and the full effect of its deprivation (including pituitary hypofunction) might not be developed immediately after thyroidectomy, whereas growth hormone has a very short survival time and would be expected to disappear promptly after hypophysectomy. With the above reservations, the effect of the two deficiencies on skeletal growth is quite similar. The similarity is reflected in a stereotyped atrophy of histologic structure of the proximal tibial epiphyseal cartilage plate when rats operated upon at an early age are compared. In both GH and thyroid hormone deficiencies the plates remain wider and the abnormalities of the epiphyseal ossification center are greater in young than in older rats (Asling and Evans, 1956).

Reinstating growth after thyroidectomy differs in some respects from that after hypophysectomy; thyroxin will stimulate active growth in thyroidectomized rats. Concurrently, pituitary function is restored. As attested by evidence from gonads and adrenals, gonadotropic and adrenocorticotropic hormones again come into good supply from endogenous sources. The repair of pituitary structure, and particularly the regranulation of acidophils (Koneff *et al.*, 1949), make it likely that the growth from administration of thyroxin can be attributable to an endogenous supply of growth hormone. Thus, many of the conditions necessary for a growth hormone-thyroxin synergism are restored simply by the administration of thyroxin.

The response of rats to hypophysectomy and thyroidectomy combined, and to replacement therapy, has been studied by Evans *et al.* (1939) in rats approximately 40 days of age when operated upon. It has also been studied by Ray *et al.* (1954) who performed thyroidectomy on the first day of life and hypophysectomy on the twentieth day of age. The presence of each gland complicated the analysis of results obtained when administering products of the other. Thyroxin administration has negligible effects on growth in a hypophysectomized rat. Still, analysis of the results of growth hormone administration is complicated by variability in response and the delicate condition of these test rats. Fortunately, some individuals tolerate the experiment well enough to demonstrate that concurrent administration of growth hormone and thyroxin stimulates vigorous growth and even surpasses the growth rate of intact rats.

B. PANCREAS AND INSULIN THERAPY

Insulin is another factor influencing the protein-anabolic action of growth hormone. In the completely depancreatectomized dog receiving no insulin, the growth hormone fails to induce nitrogen retention. If such a dog is treated with small amounts of insulin (not even enough to control diabetes completely), growth hormone produces its characteristic nitrogen sparing protein-anabolic effect. Such findings suggest that some insulin must be present to permit growth hormone to act as a protein anabolizer. In the Houssay depancreatectomized-hypophysectomized cat, growth hormone fails to induce nitrogen retention in the absence of insulin. Optimum nitrogen retention is reached only when the insulin does not induce true growth in cat, but the results in rodents are contradictory even with the use of the tibia growth effect. Antenatally, insulin acts in the absence of GH (Naeye and Blanc, 1971). Human growth hormone has an insulin-like effect immediately and an

anti-insulin effect late after administration by injection (Goodman and MacDonald, 1969). A synergistic effect similar to that of insulin has been reported with glucagon.

In hypophysectomized rats, as noted previously, administration of GH increases the total body protein and decreases the total body fat. Some of these effects of GH on skeletal tissue can also be reproduced with insulin, but the major increase in body constituents with insulin takes place in the body fat rather than the protein. The primary metabolic action of growth hormone has not yet been definitely localized. The rate of conversion of amino acids to blood urea and to urinary urea is decreased. Entrance of amino acids into the cell is accelerated by growth hormone because there is a prompt fall in the plasma level of amino acids. Hypophysectomy leads to a fall of the hepatic RNA synthesis with a decrease not only of the number of ribosomes but also in the amount of messenger and soluble RNA. When growth hormone is given to the hypophysectomized rats there is a stimulation of all RNA synthesis. While these results have established definite effects of growth hormone on the subcellular protein synthesizing machinery, they fail to pinpoint the specific locus of growth hormone action (Daughaday, 1968).

C. ADRENAL GLANDS AND ACTH THERAPY

In castrated rats, adrenocorticotropic hormone (ACTH) depressed growth of body weight (Moon, 1937). As demonstrated later in intact male rats, ACTH impairs bony growth markedly (Evans *et al.*, 1943; Becks *et al.*, 1944). In hypophysectomized rats, a direct antagonism to the effects of pituitary growth hormone is shown by injecting ACTH concurrently with a potent growth hormone preparation (Marx *et al.*, 1943). In the tibia, the proximal epiphyseal cartilage plate becomes narrow with many irregularities in cell columns. Osteogenesis is slow (Becks *et al.*, 1942b). These effects are not attributable to reduced food intake because the rats treated with both hormones actually consume more food than those given only growth hormone. In spite of marked growth inhibition, skeletal maturation is not delayed by ACTH treatment.

The extent to which ACTH participates in the pituitary gland's regulation of normal skeletal development through its opposition to the stimulating effect of growth hormone requires further investigation. Adrenal corticosteroid therapy retards growth in children by suppression of the hypothalamic pituitary-adrenal axis, but the effect is not entirely attributable to insufficient secretion of HGH. The deleterious effects on

growth of children with Still's disease can be mitigated by intermittent administration of cortisone (Sturge *et al.*, 1970). In the future, supplements of synthetic HGH may become available to counteract some of the side effects of cortisone on bone.

D. SEX GLANDS, SEX HORMONE THERAPY, AND OSTEOPOROSIS

Pituitary control of skeletal development through the action of gonadotropic hormones was presented by Asling and Evans (1956) and might be brought up to date by a few brief statements. Sex hormones do not initiate skeletal growth but only augment the action of growth hormone in hypophysectomized rats. Species differences are very important in the pituitary gland-sex hormone influence upon formation and development. Gardner and Pfeiffer (1943) observed no inhibition of estrogen-induced endosteal bone formation in hypophysectomized mice, while Kibrick *et al.* (1942) observed that in rats hypophysectomy inhibited the intramedullary ossification normally induced by estrogen. In birds, estrogen produces still another reaction in which the intramedullary ossification of selected long bones occurs even after hypophysectomy. In human beings, estrogen produces neither endosteal nor intramedullary bone. These seemingly diverse results demonstrate that the action of sex hormones on osteogenesis in the rat at least depends upon the cellular activity in growth apparatus. Factors responsible for inhibition of bone resorption and growth by estrogen in the rat but not in the mouse, or bird (Urist *et al.*, 1948), are not known.

In human beings, sex hormones stimulate secretion of HGH. Franz and Robkin (1965), Merimee *et al.* (1966), Buckler (1969), and Bacon *et al.* (1969) observed release of HGH in response to estrogen treatment, and Martin *et al.* (1968) reported a similar release in response to androgen treatment (1968). Sperling *et al.* (1970) viewed the arginine infusion test as a dependable means of stimulation of HGH release and confirm the above-noted effects of estrogen but not androgen. In response to the amino acid infusion, pubertal girls secrete more HGH than prepubertal children of both sexes, and even pubertal boys.

Attempts to apply the amino acid infusion test to disorders of bone are now in progress by several research groups. R. W. Smith (1967) supplied the impetus with an observation that the postmenopausal female has a reduced HGH response to hypoglycemia. Henneman *et al.* (1960) reported increases in absorption of calcium and net positive calcium balance following injections of GH.

In an investigation supported by the Endocrine Study Section of the National Institutes of Health, U. S. Public Health Service, human growth

hormone became available in 1961 for treatment of patients with severe osteoporosis. Urist and associates (1963) administered by intramuscular injection 2 mg of HGH per day to a patient under intensive investigation in the hospital for a period of 75 days for: (1) metabolic balance studies for calcium, phosphorus, and nitrogen; (2) radioisotope kinetic measurements with ^{47}Ca -, ^{85}Sr -, and ^{131}I -labeled serum albumin; and (3) bone biopsy. The patient was 71 years of age and able to walk only with the aid of two canes because of muscle atrophy secondary to multiple compression fractures of the dorsal and lumbar vertebrae. The diagnosis of osteoporosis was established by clinical and laboratory studies which were negative for osteomalacia, malabsorption syndrome, hyperparathyroidism, Paget's disease, multiple myeloma, and malignancy. In this patient, exhibiting the most common form of pathologic osteoporosis in the United States, HGH produced 2 g of positive nitrogen balance but the calcium and phosphorus balances were either unchanged or slightly negative (Fig. 5A). Radioisotope osteograms, urinary excretion, and whole body counting revealed that the rate of accumulation of mineral was the same as in normal subjects (Figs. 5B-5D). The rate of resorption was slightly higher than the rate of accretion. Biopsies after tetracycline labeling of the bone, before and following administration of growth hormone, showed the same quantity of lamellar new bone as in non-osteoporotic untreated controls.

Table I summarizes the results of studies with injections of HGH compared with injections of chondroitin sulfates A and C (Osseofac, Squibb), extracted from bovine bone matrix, but without any growth stimulating properties. High growth hormone evokes a consistently reproducible increase in nitrogen retention in aged individuals with osteoporosis, but the effect is the same as in normal aged individuals.

An intravenous injection containing 5 μCi of ^{47}Ca -, 5 μCi of ^{85}Sr -, and 1 μCi of ^{131}I -labeled serum albumin was administered during the period of treatment with daily injection of polysaccharides and again during the period of treatment with HGH. Osteograms of the midshaft of the tibia showed a slow change in rate of uptake, rising over a period of more than 1 hour; on treatment with human growth hormone, the rate of accumulation was the same as in nonosteoporotic normal subjects, where the accumulation of radioisotopes reaches a plateau in 15 minutes. Other areas of the skeleton responded in the same way as the tibia. The daily loss of ^{85}Sr in the urine during treatment with polysaccharide, especially during the first 3 days, was less than with human growth hormone. One-half of the ^{85}Sr was excreted in 5 days on polysaccharide treatment; on human growth hormone, the same percentage of the dose was excreted in only 2 days; ^{47}Ca was always much better retained

in the skeleton than ^{85}Sr . The 50% excretion point was reached after 18 days on placebo treatment but only 5 days on human growth hormone; strontium is not reabsorbed as efficiently as calcium by the kidney tubules.

When relative values of the exchangeable pool and calcium excretion rate are calculated on the basis of urinary clearance of strontium, the values are not absolute but can be used to compare one case with another (Table I). The size of the exchangeable calcium pool (on the basis of the osteogram) was the same during the entire period of treatment, but the bone accretion rate was not greater than in normal subjects with human growth hormone. Because calcium balance was unchanged, it is necessary to assume that the higher rate of accretion may be associated with a higher rate of resorption. This is reflected in the urinary excretion curves (Fig. 5C). The daily loss of ^{85}Sr in the urine on polysaccharide treatment was slightly less during the first 10 days than with the growth hormone. Thereafter, more ^{85}Sr was lost per day on polysaccharides, suggesting a higher rate of resorption of bone which had been labeled with the isotope. Thus, the ^{85}Sr excreted during the early days comes mainly from soft tissue and from ^{85}Sr deposited on preexisting bone salt crystals by exchange rather than by incorporation into newly forming crystals (accretion). Only at later times could the daily loss be derived from slow resorption of mineral crystals which contained ^{85}Sr laid down earlier by accretion. Although difficult to interpret, radioisotope turnover studies show no reason to assume HGH will increase skeletal accretion in patients with senile osteoporosis.

The major effect of HGH upon the normal nonosteoporotic skeleton is to increase the rate of bone formation which also leads to increased rate of bone remodeling. Fraser and Harrison (1960) regarded these effects as secondary to increased activity of parathyroid glands because growth hormone alters the renal and intestinal transport of calcium and phosphate in addition to having direct effects on the skeleton. The renal effects of growth hormone are opposite to those of parathyroid hormone (PTH), causing increased retention of phosphate. This makes for tendency toward hypocalcemia which is corrected by increased secretion of parathyroid hormone. Increased secretion of parathyroid hormone promotes increased calcium absorption from intestine. In this respect the effect of PTH is synergistic to that of the growth hormone because positive calcium balance would increase bone turnover. Slightly elevated plasma phosphate levels are also characteristic of the state of a high level production of growth hormone. Nordin (1966) reviewed and evaluated the conflicting data in the clinical literature and summarized present knowledge as follows: GH raises plasma calcium, reduces parathyroid

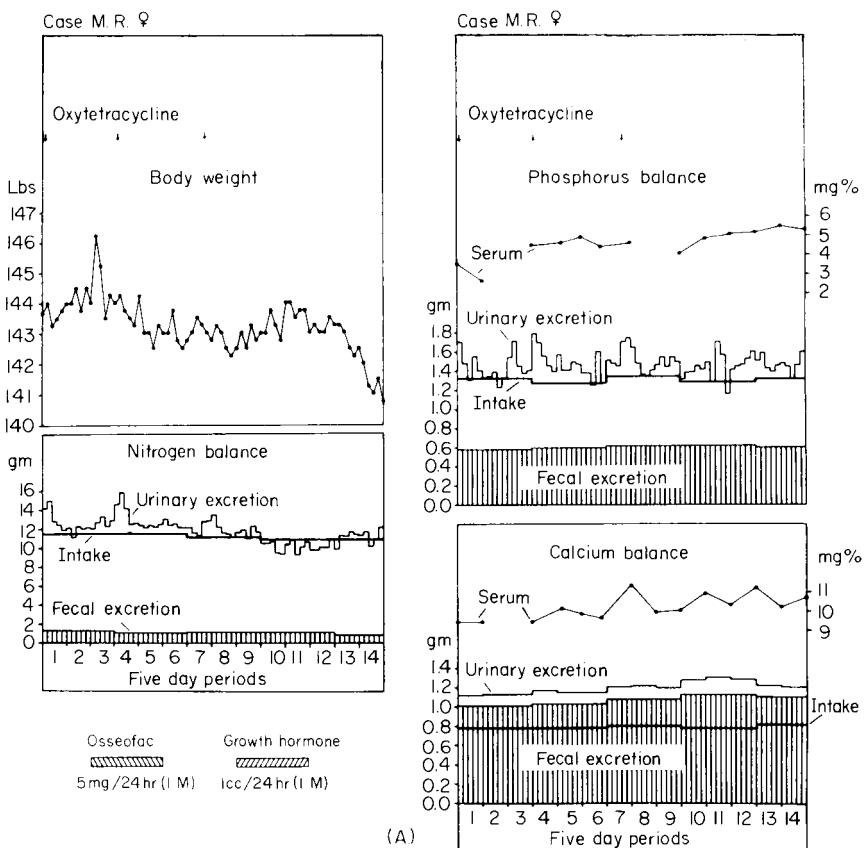


FIG. 5. (A) Charts showing metabolic balance studies in a 71-year-old woman with senile osteoporosis. The patient lost weight and was in continuous negative calcium, phosphorus, and nitrogen balance. Placebo treatment with injections of bone polysaccharides (Osseofac, Squibb) did not restore equilibrium. Human growth hormone produced nitrogen retention but did not correct negative calcium and phosphorus balances. (B) Radioisotope osteogram showing the changes in rate of uptake of an intravenous injection of ^{85}Sr and ^{47}Ca in the patient referred to in (A). The upper curve (first study) represents the reaction of the tibia during a period of treatment with intramuscular injections of polysaccharides. The lower curve (second study) represents the response to treatment with human growth hormone. These results with HGH are the same as obtained in untreated nonosteoporotic subjects and corroborate results with bovine GH in experimental animals reported by Anderson and McKeen (1969). (C) Graph showing urinary excretion of ^{85}Sr and ^{47}Ca in patient referred to in (A) and (B). The total loss of ^{85}Sr isotope during the first 3 days was only 23% on placebo polysaccharide therapy (first study). The net effect of the treatment on the loss of calcium in the urine over a period of 3 weeks is about the same on placebo and HGH. (D) Charts illustrating the retained amounts of ^{85}Sr and ^{47}Ca as measured by whole body counting in the patient referred to in (A) to (C). At all times, the amounts of both isotopes retained by the skeleton were slightly greater during treatment with the placebo (first study) than with human growth hormone (second study). The time required to lose 40% of the ^{85}Sr was 5 days for the first study as compared with 2 days for the second. These observations reveal no preferential effect of growth hormone on deposition of new tissue in the skeleton, and, if anything, less effect than an unspecific placebo. Figures (A) to (D) reproduced with permission of Urist *et al.* (1963) and Amer. Ass. Advance Sci., Washington, D.C.

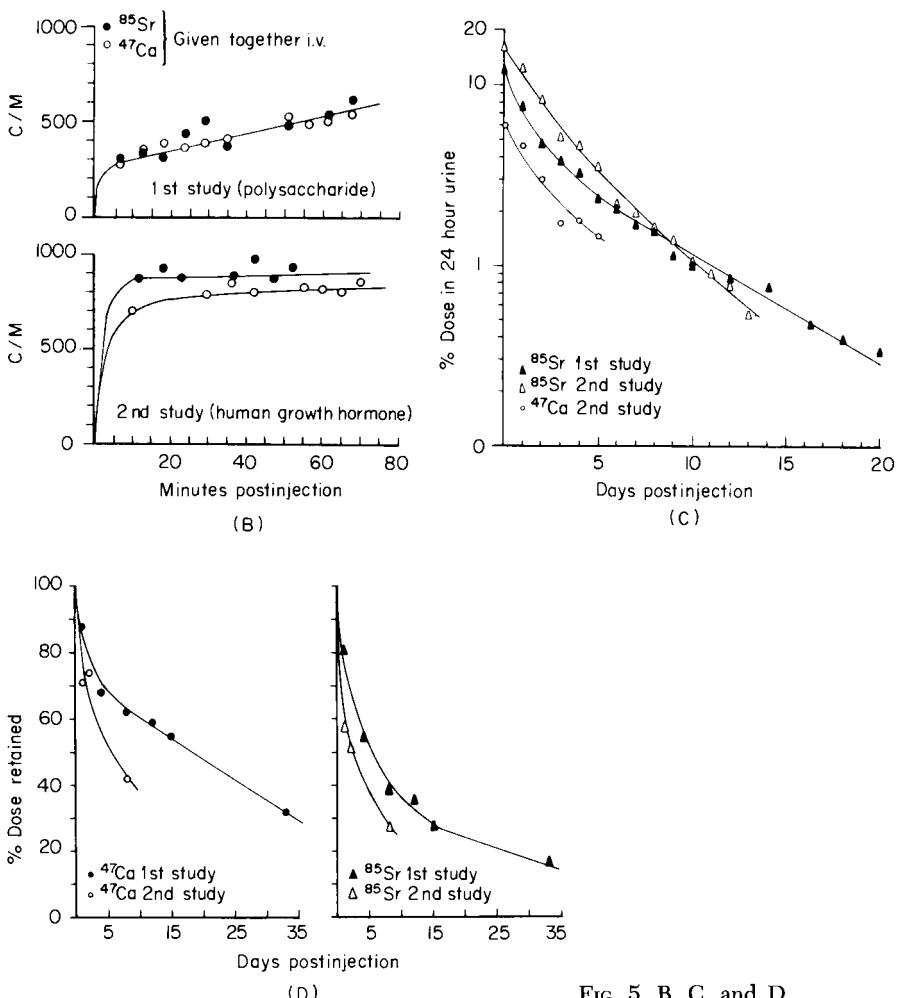


FIG. 5. B, C, and D.

activity, raises plasma phosphate, increases urinary calcium. Hypercalciuria would cause negative calcium balance and possibly lead to osteoporosis.

Inhibition of bone resorption (as determined by positive calcium balance) conceivably could be produced in patients with osteoporosis by administration of estrogens. The question arises whether this response is secondary to effects upon secretion of growth hormone. According to Buckler (1969), both men and women of all ages show an increase in GH production in response to large doses of estrogen. Other mechanisms not dependent upon GH production may also explain the action of estrogen on patients with osteoporosis, as, for example: (1) suppression of parathyroid hormone production, (2) decrease in sensitivity of bone tissue and/or kidney to the adverse effects of parathyroid hormone (Young and Nordin, 1967), (3) decrease in the tendency to bone loss

TABLE I
SUMMARY OF RESULTS OF INTRAVENOUS INJECTIONS OF A MIXTURE OF
 ^{85}Sr **AND** ^{47}Ca **IN A 71-YEAR-OLD OSTEOPOROTIC TREATED WITH**
PLACEBO (POLYSACCHARIDES) AND HGH

Determinations	Treatment	
	Placebo	Human growth hormone
Tibia osteogram	Both ^{47}Ca and ^{85}Sr still rising after 70 minutes	Both ^{47}Ca and ^{85}Sr leveled off after 15 minutes
Knee osteogram	Both ^{47}Ca and ^{85}Sr still rising after 70 minutes	Both rising after 70 minutes but less steeply
Urinary excretion (3 days)	Sr, 23%; Ca, 15%	Sr, 37%; Ca, 14%
Total excretion (total body counting): time to lose first 50% of dose	Sr, 5 days; Ca, 18 days	Sr, 2 days; Ca, 5 days
Exchangeable calcium pool ^a		
From ^{85}Sr data	1.9 gm	1.5 gm
From ^{47}Ca data	—	5.9 gm
Accretion rate for calcium ^a		
From ^{85}Sr data	0.06 gm/day	0.04 gm/day
From ^{47}Ca data	—	0.22 gm/day

^a Calculated by the method of Bauer *et al.* (1955).

from the acidosis which accompanies physiologic secondary hyperparathyroidism, (4) restored response of bone to balanced calcitonin-parathyroid hormone production following the menopause, and (5) retardation of the adverse effects of parathyroid hormone on bone marrow mastocytosis (Frame and Nixon, 1968). The level of response of the adult human skeleton either to HGH or estrogen is too small to restore bone mass in osteoporotics to a degree demonstrable by roentgenography. Since the cause of pathologic osteoporosis in human beings is not known, and since excessive HGH may aggravate rather than retard the disorder, HGH must be considered another unspecific modality and therefore indicated only in selected patients under investigation in metabolic wards (Urist, 1970).

The osteoporosis of acromegaly is an inconstant and puzzling entity. Some observers suggest the cause is hypersecretion of both ACTH and GH (Snapper, 1957). Chronic suppression of calcitonin production is a possibility. Some writers suggest the cause is premature aging (M. Silberberg and Silberberg, 1942; Fourman, 1955).

E. CALCITONIN

The rate of secretion of calcitonin is influenced only indirectly but not necessarily insignificantly by GH. According to Milhaud and Mouk-

thar (1965) the calcitonin content of the C cells of the thyroid is comparable in hypophysectomized and normal rats. Zileli *et al.* (1968) observed secretion of calcitonin in the urine of rats injected with guinea pig GH; thyro-parathyroidectomy prevented this effect of GH on production of calcitonin. Care *et al.* (1969) perfused the thyroid glands of pigs with hypercalcemic blood and noted secretion of calcitonin for 4 hours in intact pigs but only 1.3 hours in hypophysectomized pigs. Salzer and Lischka (1970) contended that hypophysectomy, first reduces the size of the nuclei of C cells of the thyroid in rats, and second, produces slower and weaker defense against hypercalcemia (produced by injections of calcium chloride). Whether GH increases secretion of calcitonin and inhibits bone resorption, or decreases secretion of calcitonin and increases bone resorption (as in acromegaly) or has no effect is not altogether clear, but a GH effect on resorption seems essential for longitudinal growth of bones. Indeed, GH may be necessary to maintain the proper balance of thyroid, parathyroid, and calcitonin-hormone activity that accounts for internal remodeling (resorption and reformation) of bone.

VII. Tissue Culture

Growth hormone is secreted into media by tissue organ cultures of pituitaries from human (Thompson *et al.*, 1959) and rodents (Smolders, 1939; Gaillard, 1942; Verdam, 1946). Explanted rat (Verdam, 1946) and chick (Hay, 1958) bones do not elongate beyond control bones when supplements of bovine GH are added to the media. Embryonic chick cartilage anlage show uptake of ^{35}S -labeled chondroitin sulfate in greater quantities and grow longer in the presence of supplements of GH in the media (Ito *et al.*, 1959, 1960a,b). Embryonic chick somites also synthesize increased quantities of polysaccharides when GH is added to a standard culture media (Lash and Woodhouse, 1960). Nogami and Urist (1971), using chemically defined media containing both GH and thyroxin, described differentiation of mesenchymal cells into chondro-osseous tissues but not bone or bone marrow. These observations demonstrate that GH acts on skeletal tissues and sustains growth of cartilage *in vitro*. In view of the species specificity of the chemical structure of GH, autologous and allogeneic GH might produce responses different from those obtained with bovine xenogeneic GH, particularly in systems reported to yield negative results.

VIII. Summary

From the demonstrations of growth hormone (GH) by Evans and associates (reviewed by Asling and Evans, 1956) in the third decade of

this century, there emerged a twenty-year period of intensive research on the physiology of GH deficiency and replacement therapy. In the fifth and sixth decades, the mainstream of research effort was directed toward the biochemistry, bioassay, mode of action, and metabolism of GH. The beginning of the seventh decade was heralded by the synthesis of HGH by Li and Yamashiro (1970).

Human growth hormone is a chain of 188 to 190 amino acids, with a molecular weight of about 44,000. Beef, sheep, horse, and pig GH differ from HGH and from each other chemically, but all may have a common core structure, and all are active in the rat, the most commonly used test animal. Octogenarians have almost as much HGH in the pituitary as rapidly growing individuals, but the tissues of immature individuals respond more rapidly and completely.

Hypophysectomy of immature animals retards chondrogenesis; proliferation and maturation of chondrocytes ceases and the thickness of the epiphyseal plates diminishes precipitously; vascular loops are unable to perforate the zone of preparatory calcification, osteoblasts differentiate slowly, and the quantity of metaphyseal bone declines. The normal proportions of calcified cartilage to primary spongiosa to metaphysis is maintained only when both GH and thyroid hormones are present. While the effects of these two hormones are difficult to separate, growth hormone sustains the rate of growth of the bones in length while thyroxin controls differentiation and maturation of chondrogenetic and osteogenic tissues. Treatment of hypophysectomized rats with thyroxin alone produces rapid premature closure of the epiphyseal lines. Treatment of thyroidectomized rats with growth hormone produces rapid proliferation of epiphyseal cartilage, but the cells fail to hypertrophy or invite ingrowth of osteoprogenitor cells for differentiation of osteoblasts. Immature hypophysectomized rats fail to develop normal ossification centers.

Growth hormone accelerates the rate of proliferation of cartilage in the epiphyseal plates. Skeletal growth can be restored to normal in hypophysectomized rats (or even stimulated to excess) by injection of growth hormone in adequate amounts. The extent of skeletal retardation is independent of the age at the time of hypophysectomy. The equivalent of 2-3 weeks of further long bone growth occurs in immature rats even after hypophysectomy or thyroidectomy; maturation but not growth can be restored to normal by administration of thyroid hormone. No other hormone overcomes the effects of GH deficiency. In thyroidectomized rats, thyroxin also stimulates growth, but as much by its action of restoring pituitary function as by its own action. Unlike thyroid hormone, GH does not control skeletal maturation.

The mode of action is not known but GH appears to interact with constituents of cell membrane and activate a chain of reactions in pre-existing cell machinery. Growth hormone may increase mitogenesis four-fold and accelerate RNA synthesis without any effect on the rate of protein catabolism. A sulfation factor, insulin, and presumably other factors in serum are intermediary agents in the action of GH on growth cartilages *in vitro*.

Growth hormone is essential for the normal rate of regeneration. The treatment of delayed and nonunion of fractures with HGH is open to question. The effects of HGH on patients with osteoporosis are not clear. Restoration of growth in pituitary dwarfism is well established by clinical and metabolic balance studies. The development of sensitive methods of measurement of HGH in plasma, and the availability of synthetic HGH, provide the momentum for important progress in this decade of research on GH in health and disease in man and other animals.

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