GEORGE H. SMITH
Founder, 1928, *The Yale Journal of Biology and Medicine;*
Editor-in-Chief, 1928-49; Editor-in-Chief Emeritus, 1949-.
(From the portrait by Deane Keller)

SOME ENDOCRINE INFLUENCES ON SKELETAL GROWTH AND DIFFERENTIATION*

MIRIAM E. SIMPSON, C. WILLET ASLING, AND HERBERT M. EVANS

Introduction

In the skeleton, growth is measured by increase in size and particularly by increase in length of the axial and appendicular skeleton, whereas differentiation or maturation is measured by the establishment of ossification centers and their subsequent fusion. In normal development these processes of increase in size and differentiation proceed concurrently. The smoothly coordinated mechanism may, however, become deranged. In man dwarfism may occur, although the open epiphyses would indicate further capacity for growth, yet continued open epiphyses may also be associated with gigantism. An understanding of the hormones controlling skeletal growth therefore necessitates an analysis of the factors, or balance of factors, responsible for each of these phases of growth—increase in size, and differentiation.

As dwarfism results from removal of the pituitary, attention is immediately focused on the role of the hormones of the pituitary and of its target organs. Dwarfism and retarded skeletal maturation also accompany thyroid deficiency; it is therefore evident that among the target organs the thyroid must receive prominent attention. One pituitary hormone, the growth

* From the Institute of Experimental Biology and the Division of Anatomy, University of California, Berkeley, California. Aided by grants from the U. S. Public Health Service (RG - 409); the Research Board of the University of California; the American Cancer Society, Inc., N. Y.; and the University of California Cancer Grant. Thirteenth Harry Burr Ferris Lecture presented by Miriam E. Simpson at the Yale University School of Medicine April 3, 1950.

Received for publication May 20, 1950.

hormone, is unique in that its effects are demonstrable in the absence of all the target organs.

Requirements for skeletal studies

Techniques used. Increase in size of the skeleton may be determined by direct measurements of the total length of the animal, usually under anaesthesia or at autopsy. Skeletal growth of small experimental animals, such as the rat, can also be followed at suitable intervals during life, and without anaesthesia, by direct measurement of the tail. The size of individual bones can be determined by measurement after dissection, or in roentgenograms. These methods of measuring skeletal growth in the rat have a maximal error of 2%.

In the study of differentiation of the skeleton, roentgenograms are of great service in tracing both the appearance and disappearance of centers of ossification. Confirmation of the accuracy of roentgenographic determinations has been derived from parallel histological studies, particularly in the determination of epiphyseal fusion.*

Histological sections also allow analysis of the state of activity, i.e., the processes which lead to the formation of the epiphyseal disc and its removal. Briefly, some of these characteristics are: number, size and vacuolation of cartilage cells, orderliness of alignment, amount and quality of matrix, number of capillaries impinging upon cartilage, the number and size of osteoblasts and osteoclasts, also the number, size, and arrangement of primary and secondary bony trabeculae.

The rat as an experimental animal. As some question has been raised as to the suitability of the rat as an experimental animal in studies of skeletal growth, a defense of the use of this animal will be made. A common opinion (as expressed by Washburn[71]) is that inasmuch as the rat does not show maturity by epiphyseal closure and is hence capable of continued growth far past the period of sexual maturity, deductions of general import cannot be made from the reactions of its skeleton. The position taken here is that in spite of certain peculiarities in the growth of the skeleton, the rat is useful in a study of the hormonal factors controlling skeletal growth. As a matter of fact, the majority of epiphyses in the rat do close, and the times of closure of these epiphyses are accurately known. Further, most of them close in early adult life (80 to 120 days). For example, all epiphyses of the paws close and, in addition, at least one of the two epiphyses possessed by the other long bones of the extremities. (The ulna and femur are exceptions.) The epiphysis of the metacarpal closing at 110 days exemplifies this group.[14]

Those epiphyses which remain open are in the minority. They occur notably in the limbs and tail. Each of the major long bones has one epiphysis which remains open into senescence. In the ulna and the femur both epiphyseal discs remain. Patency of the epiphyses of caudal vertebrae can also be recognized histologically even in old age.

---

* For accuracy in analysis of roentgenograms of bones of small animals a fine grain emulsion film is essential; for accuracy in histological deduction greatest care must be taken in determining the plane of section.

The proximal epiphysis of the tibia is one of the carefully studied epiphyses of the type which does not close until late in life. Even though such an epiphyseal cartilage remains, the conclusion is unwarranted that continued growth is occurring in this bone. Abrupt narrowing of the plate has been noted in early adult life at the time of closure of the majority of epiphyses. Such persisting epiphyseal discs become dormant, sealed by bony plates from the marrow. The growth curve of the tibia (Fig. 1) shows that its length does not increase over long periods although the epiphysis remains patent into senility. It will be noted that the leveling off of the growth curve coincides with the abrupt narrowing of the epiphyseal disc.

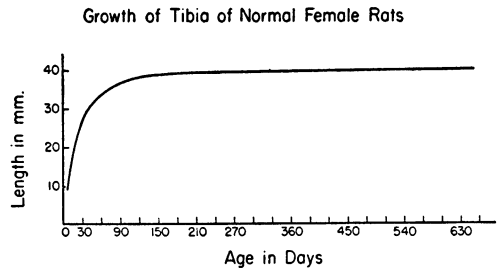


FIG. 1. Curve showing growth of the tibia in normal female rats of the Long-Evans strain.

Determination of skeletal age. The epiphyseal closures around the 110th day of life occur with such reliability within a limited period characteristic for each bone that they can be used as an index of skeletal ageing. By comparison of the skeletal differentiation in an experimental animal with that in normal rats of known chronological ages, it is possible to assign the "skeletal age." For determination of skeletal ages at less than 80 days, the time of appearance of new epiphyseal ossification centers is more important than the time of epiphyseal closure. Table 1 shows the times of appearance of centers which are important in determining skeletal ages in the period between 12 and 60 days of age. It will be noted that this table is devoted exclusively to times of appearance and disappearance of the secondary ossification centers, all primary centers having been established in the rat by birth or shortly thereafter.*

Although the closure of some epiphyses is greatly delayed in the rat, the sequence of closure follows the general mammalian pattern (Todd, as cited by Krogman[38]). In Table 2 the order of closure in rat and man is compared.

In order to use the rat in experimental studies on osteogenesis it is only necessary to know the periods during which secondary centers appear or

* Additional criteria are available for the earlier group (12 to 60 days) and appear in the publications from which the table has been adapted (Scow *et al.*[55]; Ray *et al.*[48]). These criteria are chiefly determined from roentgenograms, though histological confirmation has been obtained for the proximal tibia, metacarpals, and distal humerus (Becks *et al.*[9, 12, 14]). The criteria for determination of skeletal ages between 80 and 120 days are chiefly histological. It will be noted that no satisfactory criteria are presented for skeletal ageing between 60 and 80 days, or between 120 and 940 days.

TABLE 1

DETERMINATION OF SKELETAL AGES

Ossification center	*Chronological age in days*														
	12	15	18	21	25	30	40	50	60	80	100	120	Latent period	940	1006-1091	1135+
Humerus, proximal	P												.....			A
distal	P							A								
medial epicondyle				P								A				
Radius, proximal			P								A					
distal	P												.....			A
Ulna, proximal			P										.....		A	
distal	P												.....			A
Metacarpal, distal	P										A					
Phalanges			PI		PII						A					
row I, II, III					PIII											
Femur, proximal								P					.....		A	
trochanter						P							.....		A	
distal	P												.....			A
Patella						P										
Tibia, proximal	P												.....			A
distal	P										A					
Metatarsal, distal	P3	P2	P4	P1							A					
				P5												
Pelvis, acetabulum	lip						P	—A								
triradiate								A								

"P" represents present; "A" represents absent.
Adapted from Scow *et al.*,[55] Ray *et al.*,[48] and Dawson.[19]

TABLE 2

SEQUENCE OF EPIPHYSEAL CLOSURE

*Rat** *Age in days*	*Epiphyses*	*Man†* *Age in years*
40	Humerus, distal (capitulum-trochlea)	14-15
85	Radius, proximal	16-17
90	Tibia, distal	16-17
100	Metacarpal, distal	16-17
120	Humerus, distal (medial epicondyle)	16-17
1135	Tibia, proximal	17-18
1135	Femur, distal	17-18
1135+	Radius, distal	18-19
1135-1270	Humerus, proximal	19-20

* Histological studies of the authors supplemented in later chronological ages by data from Dawson.[19]

† Todd (1933),[64] modified by Krogman (1941).[30]

become fused.* During these critical periods experimental contrivances which significantly hasten or retard differentiation should be as significant as when applied to other adequately studied mammalian forms.

Dietary and environmental factors. In studies of the effects of hormones on skeletal growth particular care must be taken to control dietary and other environmental conditions. The diets must be adequate in amount and complete in all known components. It has been shown that dietary restriction can cause changes in the skeleton similar to hypophysectomy. In starved animals bone growth ceases, epiphyseal plates are narrower and less active than normal.[7, 32] In experiments where the hormone administered affects the appetite, it may be necessary to do paired-feeding in order to get significant results. Even in the presence of adequate caloric intake, vitamin deficiencies can cause similar effects.[27] Diminished food consumption such as occurs after hypophysectomy may lead to deficient vitamin intake. On the other hand, vitamin requirements may be increased by hormone therapy. Thyroxin increases the need for practically all vitamins.[22] This is especially noticeable in the B vitamins.[20]

The salt content of the diet must be adequate, since it is known that mineral deficiency (e.g., magnesium deficiency) will produce effects with some resemblances to hypophysectomy.[4]

The amount of protein in the diet is also significant. Low protein diets will produce changes in bone not unlike hypophysectomy (Nelson, Sulon, and Becks, unpublished). It has been claimed repeatedly that high protein diets will promote growth in excess of normal, but in many of these cases the stock diet with which comparisons were made were low in protein.[45]

Forced feeding has been reported to lead to growth of hypophysectomized rats.[40, 52] Such increase in weight is, however, largely due to accumulation of fat; true skeletal growth has not been proven.

In all studies on bone growth from the Institute of Experimental Biology which are reported here greatest care has been taken to maintain a satisfactory environment and to supply an adequate diet. The hypophysectomized rats, which form the basis of much of the experimental work, have been given a modified MacCollum's diet (Diet I). (It is true that very old hypophysectomized rats maintained on this diet do accumulate some fat so that the body weight increases slowly in old age; however, this weight gain does not indicate growth.) Survival of hypophysectomized animals, under the favorable conditions specified, is adequate for chronic experimental procedures; considerable numbers have been maintained for more than two years, some for three-year periods.

---

* A useful summary of the roentgenographic and histological standards for the rat will soon be published in atlas form by Becks *et al.*

Effects of endocrine gland ablations

Effects of hypophysectomy. The results of hypophysectomy on skeletal growth will first be described for a rat of an age and sex commonly used in experimental studies, the 28-day female. Virtually all primary and secondary centers of ossification are present at this age. After the operation, such an animal ceases growth almost at once, both in weight and length. The changes in the osseous system will be described first as seen in the tibia, a bone which has been used so commonly in testing effects of experimental procedures. This bone normally retains an epiphyseal disc at its proximal end until late in life. Following hypophysectomy, the epiphyseal disc soon shows evidence of inactivity. Its width decreases and by two weeks it is far below normal. The cartilage cells of the disc decrease in size; the vacuolated juxta-medullary zone disappears; osteoblasts decrease in size and lose their epithelioid character. Fine trabecular bone of the primary spongiosa disappears and only a few coarse secondary trabeculae remain. The epiphyseal cartilage plate finally becomes sealed from the marrow by a lamina of bone, but persists even into old age. As a result of this premature arrest of growth, the plate resembles that of the senescent normal rat. Other epiphyses, which, like the tibia, do not close until late in life, likewise become sealed and persist.

It is interesting that the sealing lamina of bone found in the rat after hypophysectomy resembles closely the condition described by Erdheim[21] in a case of human pituitary dwarfism (Figs. 2-3).

Epiphyseal plates which normally disappear between 80 and 100 days of age (proximal radius, distal tibia, the metacarpals, and the medial epicondyle of the humerus) also fail to fuse after hypophysectomy. They become sealed by bone and have been observed to persist for one to two years. The failure of such epiphyses to close in rats hypophysectomized at 28 days is evidence that deprival of pituitary hormones gravely disturbs normal osseous differentiation. (This effect of hypophysectomy in delaying epiphyseal closure was first shown by Dandy and Reichert, in 1938, by roentgenographic study of hypophysectomized dogs.[18])

The epiphysis at the distal end of the humerus which normally fuses between 31 and 42 days of age continues differentiation and closes after hypophysectomy at 28 days of age. Therefore hypophysectomy does not immediately arrest the normal progress of differentiation, and epiphyseal centers whose fusion is imminent progress to closure. After hypophysectomy at 28 days of age the advance in skeletal differentiation leads to a bone age of 47 days. The effect of hypophysectomy at 28 days on epiphyseal closure is summarized in Table 3.

If it is a general principle that hypophysectomy delays closure of epiphyses, it should be possible by earlier hypophysectomy so to check skeletal differentiation that the earliest closing epiphyseal plates would

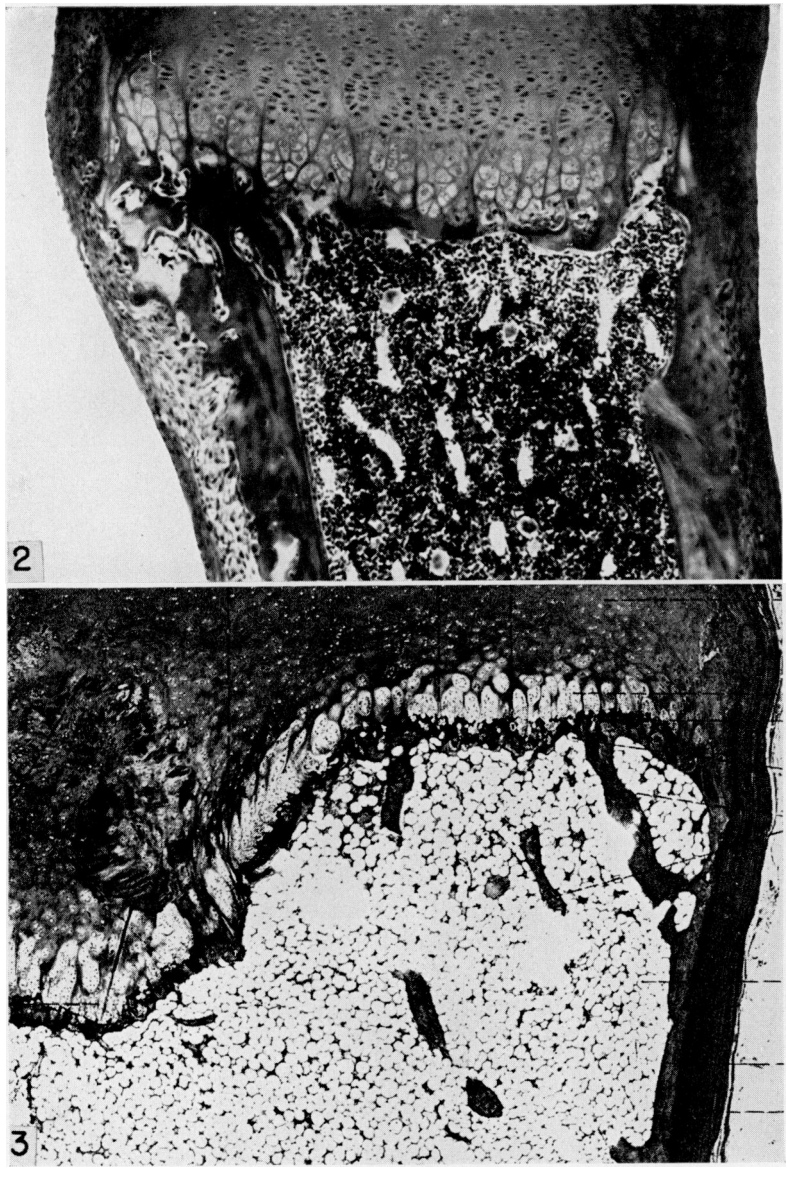
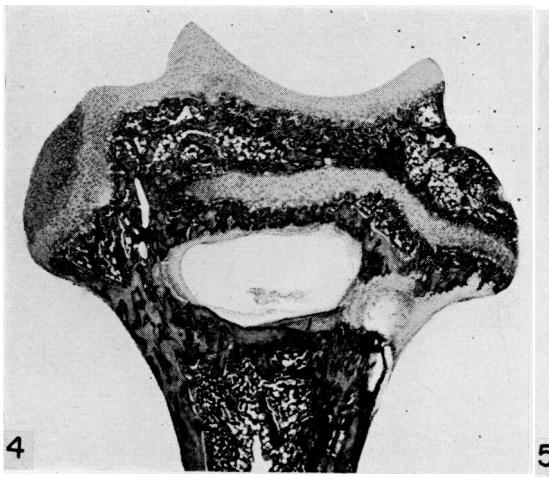


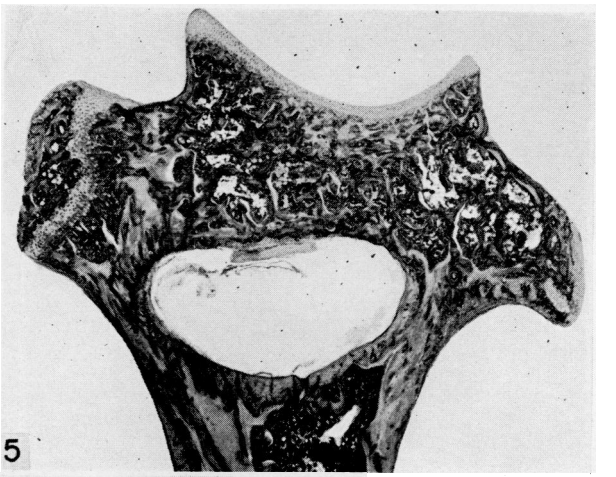
PLATE I

Comparison of experimental hypophysectomy with a case of human pituitary dwarfism.

FIG. 2. Costochondral junction of hypophysectomized 60-day-old female rat, 39 days postoperative. (H & E stain, x106.) (After Ray *et al.*[47])

FIG. 3. Costochondral junction from human male, 38 years of age; diagnosis nanosomia pituitaria. (After Erdheim.[21])





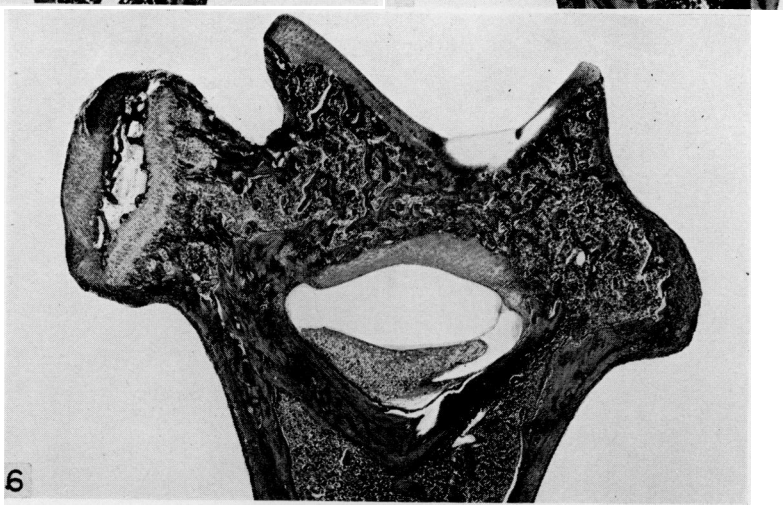


PLATE II

remain intact, for instance that in the distal humerus. It was found that if hypophysectomy was performed at 13 days,[3,70] arrest of differentiation occurred much sooner and only partial closure occurred in the distal humerus. In assigning to these rats a skeletal age of 33 days, complete dependence was not placed on epiphyseal closure. The appearance of new secondary centers was even more helpful. (By again consulting Table 1, showing the differentiation of the skeleton at progressive chronological

TABLE 3

EPIPHYSEAL STATUS OF RATS HYPOPHYSECTOMIZED AT 28 DAYS OF AGE AFTER A POSTOPERATIVE PERIOD OF 480 DAYS

Epiphyseal center	*Normal rats Age of epiphyseal closure in days*	*Hypophysectomized rats Epiphyseal status*
Humerus, distal	40	—
Radius, proximal	85	+
Tibia, distal	90	+
Metacarpal, third	100	+
Humerus, medial epicondyle	120	+
Ulna, olecranon	940	+
Humerus, proximal	1135	+
Radius, distal	1135	+
Ulna, distal	1135	+
Fibula, proximal	1135	+
Tibia, proximal	1135	+

Modified from Evans *et al.*[71]
Epiphyses separate are marked +; those fused are marked —.

ages, it will be seen how many secondary centers appear during this early period, 13-33 days). Rats hypophysectomized at day 6 showed an advance in skeletal age to 24 days. The operation at this very early age delayed markedly both the fusion of established centers of ossification and

PLATE II

Effects of hypophysectomy at different ages on the degree of differentiation attained by the humerus of the rat at 60 days of age. (H & E stain, x14.)

FIG. 4. Rat hypophysectomized at 6 days of age. The main epiphyseal plate has persisted. Establishment of the medial epicondylar ossification center has not occurred. Bone age 20 days.

FIG. 5. Rat hypophysectomized at 28 days of age. Epiphyseo-diaphyseal union at the main plate has occurred in spite of the operation. The plate in the medial epicondyle remains. Bone age 40 days.

FIG. 6. Normal 60-day-old rat. The main epiphyseal plate has disappeared at the normal time (40 days), leaving only that in the medial epicondyle. (After Asling *et al.*[3])

the acquirement of new secondary centers. All centers present were found to be sealed from marrow by bone. The much greater retardation in skeletal differentiation occasioned by hypophysectomy at day 6 than at day 28 is illustrated in Figures 4-6 which show the degrees of development attained at the distal end of the humerus.

Table 4 also shows the extent of differentiation of the skeleton which had taken place by the time a chronological age of 60 days was reached following hypophysectomy at progressively earlier ages. Further, it will be noted here that the advance in skeletal age was constant, being 18 to 21 days, regardless of whether the hypophysectomy was performed at 28, 21, 13, or 6 days of age.* It appears then that there is no more potentiality for skeletal differentiation independent of the pituitary at earlier ages than at older ages.

TABLE 4

ESTIMATED SKELETAL AGE IN DAYS AT 60 DAYS CHRONOLOGICAL AGE FOLLOWING HYPOPHYSECTOMY AT PROGRESSIVELY YOUNGER AGES

Age at hypophysectomy	*Skeletal age at 60 days*	*Advance in skeletal age*
28	47	19
21	42	21
13	33	20
6	24	18

Adapted from Asling *et al.*[3]

On the other hand increase in body size (weight and length) following hypophysectomy at these progressively younger ages was arrested at a constant chronological age, 30 days. As the daily increment was approximately the same in all groups, the total weight and length gain between the day of operation and 30 days chronological age was greater in the younger of these groups (Figs. 7 and 8). It appears, therefore, that whereas maturation continues for a constant interval after operation, 20 days, increase in size persists to a fixed set chronological age (30 days). The evidence from hypophysectomy thus indicates that skeletal differentiation is under a control different from increase in size.

In seeking an explanation for the persistence of osseous differentiation during the definite period after hypophysectomy, attention was directed to

* It may be possible to show that this constancy of the progress in differentiation following hypophysectomy applies to animals older than those cited, provided hypophysectomy is performed in periods where adequate criteria are available for determination of skeletal age. Asling *et al.*[2] have noted progress for 20 days in the differentiation of the metacarpal in rats hypophysectomized at 75 days.

the thyroid hormone due to the well-known effect of this hormone on skeletal maturation. This hormone is known to continue to give effects for a considerable period after administration.[33] An effect of thyroid hormone on differentiation after hypophysectomy could be due to continued low-grade activity of the thyroid, or to persisting action of thyroid hormone already present in the body at the time of operation. (The persistence of

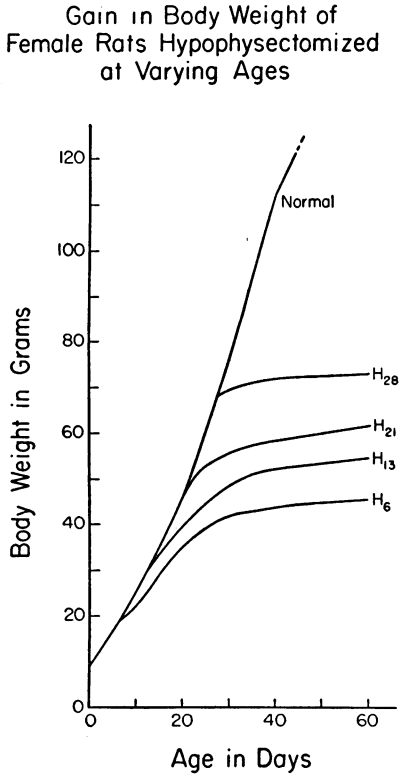


FIG. 7. Curves showing gain in body weight of rats hypophysectomized at 6, 13, 21, or 28 days of age. (After Asling *et al.*[3])

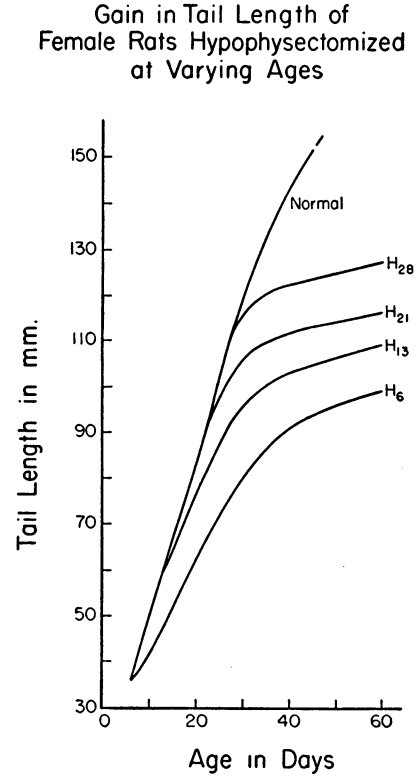


FIG. 8. Curves showing increase in length of the tail of rats hypophysectomized at 6, 13, 21, or 28 days of age. (After Asling *et al.*[3])

action of pituitary hormones, especially growth hormone, would not be probable, as pituitary hormones are characterized by short survival of their effects (Van Dyke *et al.*, unpublished).

Effects of thyroidectomy. In considering the role of the thyroid hormone in osseous growth and differentiation, the effects of thyroid ablation will first be described. As in the case of pituitary deprivation, it was desirable to study the effects of thyroidectomy at as early an age as possible. Salmon[50, 51] had shown that thyroidectomy could be performed on the first day of life, and through the collaboration of Scow[53, 54, 55] and Ray[46, 47] we have

been able to study the effect of thyroid deprival at this early age.* It was found that skeletal age advanced to 18 days by a chronological age of 60 days. The differentiation undertaken by thyroidectomized rats was then of the same order as was achieved by hypophysectomized rats by the same chronological age, though the actual skeletal age attained was less, as the thyroidectomy was performed earlier than the earliest hypophysectomy.

Although growth was markedly retarded and the skeletons of thyroidectomized rats resembled those of hypophysectomized rats in their small size, and in the unexpanded epiphyseal centers, the bones differed in several important respects. The epiphyseal cartilages did not become sealed by bony plates, either on the epiphyseal or diaphyseal sides, even after prolonged postoperative periods.†

Some degree of proliferative activity persisted in the cartilage plates, and some erosion was still occurring. However, the plate was not reduced in width normally with increasing chronological age; by 56 days of age the plates were found to be actually wider than normal. This condition contrasts with that in hypophysectomized rats where the plate shows almost no activity, is reduced in width at once, and where no erosion can occur as the cartilage is sealed from marrow by bone.

When the thyroidectomized rats were allowed to survive for long periods, some very slow continued differentiation of the skeleton was detected. Scow and Simpson[53] noted that in animals which survived for 120

* The parathyroids were removed with the thyroid. Rats are known to be very resistant to parathyroidectomy, rarely developing tetany, especially if on a high calcium diet. As these thyro-parathyroidectomized rats were on a diet high in calcium and as tetany was observed only in a few instances, and then only transiently, and as chemical analysis showed complete mineralization of the bones of such animals when placed under the influence of thyroxin, it was considered justifiable to omit consideration of the parathyroids for the present.

† Becks *et al.*[5] had noted in rats thyroidectomized at somewhat greater age that sealing of cartilage plates by bone did not occur; persisting unsealed plates were observed in the proximal tibia 17 months after operation. In the rats thyroidectomized at day 1, persisting unsealed plates have been observed in the tibia, caudal vertebrae, and metacarpal.

PLATE III

Comparison of the effects of thyroidectomy and of hypophysectomy on the humerus of the rat. All specimens 60 days of age. (H & E stain, x14.)

FIG. 9. Thyroidectomized day 1. The main epiphyseal ossification center has appeared but is unexpanded. There is no ossification center in the medial epicondyle.

FIG. 10. Hypophysectomized day 20. The main epiphyseal cartilage plate has been removed except for a few fragments in the lateral epicondyle. The medial epicondylar ossification center and its cartilage plate are well established.

FIG. 11. Thyroidectomized day 1, subsequently hypophysectomized day 20. Only the main epiphyseal ossification center has appeared. Note that in the absence of the thyroid the advance of ossification which follows hypophysectomy has not occurred.

FIG. 12. Normal control of the same chronological age. Complete fusion has occurred at the main epiphyseal plate. Note the active epiphyseal plate in the medial epicondyle and the resulting prominence of the epicondyle.

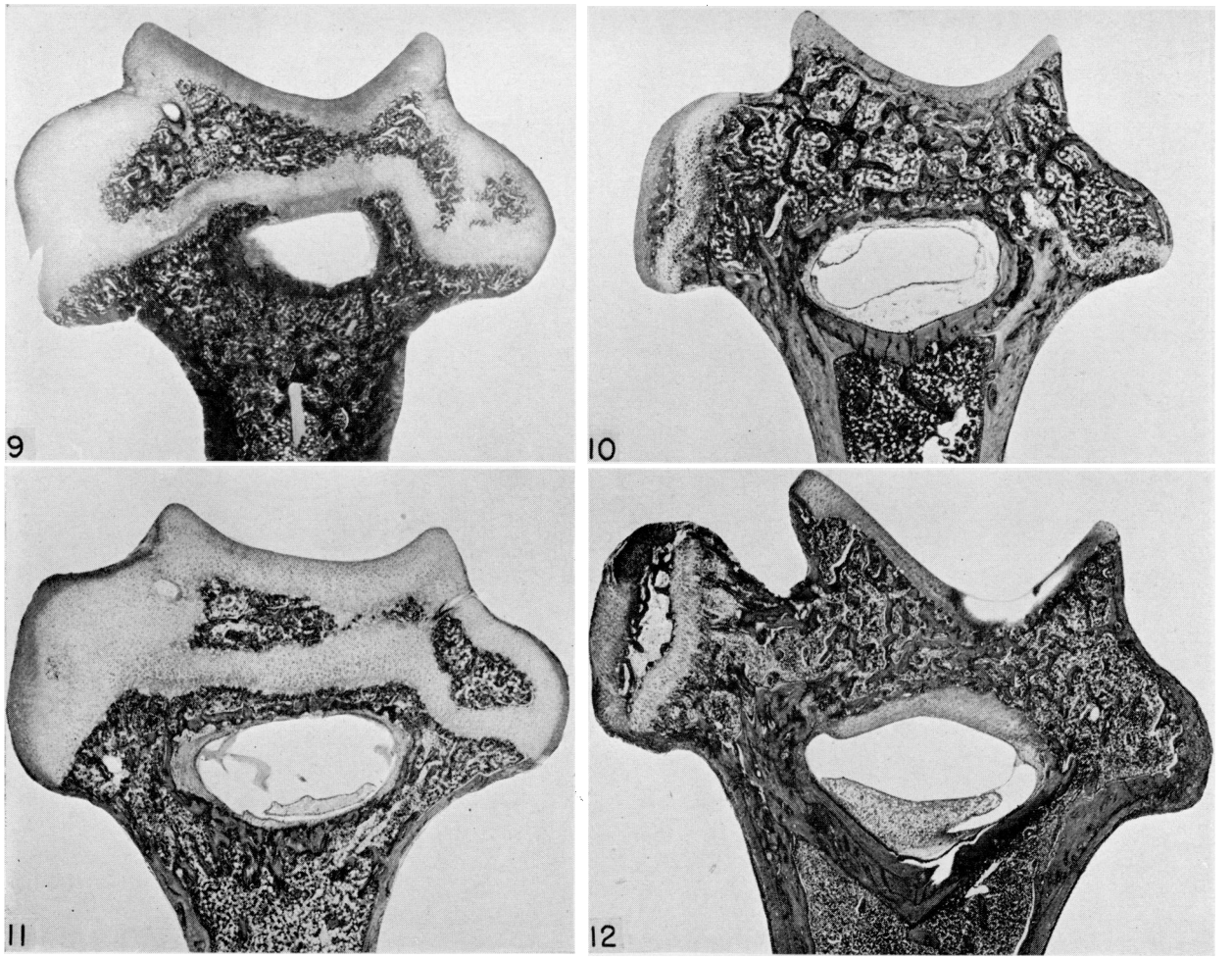


PLATE III

to 140 days, the bone age had advanced to 20 or 24 days.* It will be remembered that in the hypophysectomized rats differentiation ceased completely after a three-week advance; no further differentiation occurred even though the animals survived for over a year.

The bones of the thyroidectomized rats also differed from those of hypophysectomized rats in that the primary trabeculae remained delicate. In hypophysectomized rats the fine trabeculae were soon transformed, and only a few coarse trabeculae remained continuous with the sealing lamina of bone.

TABLE 5

COMPARISON OF THE EFFECTS OF THYROIDECTOMY AND HYPOPHYSECTOMY ON SKELETAL MATURATION IN THE RAT

Operation	*Age at operation* Days	*Skeletal age at 60 days* Days	*Observer*	*Advance in skeletal age after operation* Days
	1	15	Ziskin *et al.* (1940)	14
	1	18	Scow *et al.* (1948)	17
Thyroidectomy	1	23	Ray *et al.* (1950)	22
	7	23	Ziskin *et al.* (1940)	16
	6	24	Walker *et al.* (1950)	18
	13	33	Walker *et al.* (1950)	20
Hypophysectomy	21	42	Ray *et al.* (1950)	21
	28	47	Walker *et al.* (1950)	19
	75	95	Asling *et al.* (1949)	20
Thyroidectomy and hypophysectomy	1 20	18	Walker *et al.* (unpubl.)	17

Since slow growth as well as slow differentiation characterizes the thyroidectomized rat, some continued pituitary function, such as the secretion of small amounts of growth hormone, might be assumed. It is known that the pituitary is badly deranged after thyroidectomy; the alpha cells (which are supposed to produce growth hormone) are recognizable in only very small numbers, but it is possible that some activity persists in them. Although this might account for the growth, it is improbable, as will be seen later, that growth hormone would influence the slow matur-

* The differentiation in the skeleton of the thyroidectomized rats was already very slow by 30 days of age. At this time a bone age of 15 days had been attained and the bone age only advanced 3 days in the ensuing 30 days.

ation. If one assumes that the slow but sustained skeletal growth following thyroidectomy is due to continued production of small amounts of growth hormone, and that the limited but definite advance in skeletal differentiation following hypophysectomy is due to continued action of thyroxin, or to low-grade thyroid activity, then simultaneous removal of the two organs should give more complete stasis than either operation alone. The earlier the two operations could be performed, the more striking the retardation should be.

Effects of combined thyroidectomy and hypophysectomy. It has been possible[70] to combine thyroidectomy on day 1 with hypophysectomy on day 20. It was found that the bones of the doubly operated animals resembled those of thyroidectomized rats in that the cartilage plates were not sealed by bone. The skeletal age was the same as after thyroidectomy alone, a skeletal age of 18 days being attained by 60 days. A comparison at a chronological age of 60 days of the skeletal ages of hypophysectomized, thyroidectomized, and hypophysectomized-thyroidectomized rats is shown in Table 5. The estimates of skeletal age of several workers who have studied the effects of one or more of these operations are included, and it is interesting to note the close agreement in data obtained.

The histological findings in rats subjected to the three types of operations may be compared from sections of the humerus (Figs. 9-12).

Effects of hormone administration on skeletal development— Growth hormone

The pituitary growth hormone has been tested for its capacity to restore growth and cause differentiation in the skeletal system of deficient rats (hypophysectomized, thyroidectomized, and doubly operated animals) and has been tested for its effects when given in excess to normal rats.

Growth hormone in normal rats. When normal adult female rats which have reached the growth "plateau" are injected with growth hormone, growth in weight and length is resumed and gigantism eventually results. All ossification centers present respond. It is to be remembered that only epiphyseal plates belonging to the group characterized by greatly delayed closure are still present in such mature rats.

If, however, growth hormone administration is begun at earlier ages, great differences become evident in the responsiveness of different parts of the skeletal system. These variations in response seem to be related to the periods of closure of epiphyses characterizing individual bones. In general it may be said that all epiphyses respond to growth hormone unless at the onset of injection the epiphyses are too near the time of normal closure. For example, in animals in which injections were begun at 81 days of age the epiphysis of the metacarpal continued differentiation and closed at the normal time (110 days) without elongation of the bone.[1]

This was equally true of the distal epiphysis of the tibia (Asling *et al.*, in press*). In the same animals the proximal tibial epiphysis responded and in the 30-day injection period the tibia achieved its definite length, that usually attained by 250 days. Even after chronic administration, when the tibia had exceeded normal limits by 15%, its epiphysis still remained open and capable of response.[27] The evidence is, therefore, that the response of different bones of the normal rat to growth hormone is conditioned by the physiology of the individual bone and that growth hormone does not accelerate epiphyseal closure, any more than it prevents closure at the normal time.

The skeletons of giants produced by chronic administration of growth hormone are characterized by many disproportions due to this fact that some of their epiphyses close without the bones' exceeding normal dimensions, whereas others remain open and the bones continue to grow beyond normal dimensions. The possibility that these disproportions may be interpreted as similar to acromegalic changes in man needs further analysis and will be discussed in relation to the response of hypophysectomized rats to growth hormone.†

Growth hormone in hypophysectomized rats. On injection of growth hormone into hypophysectomized rats, increase both in weight and skeletal dimensions occurs promptly. Hypophysectomized rats are more sensitive to growth hormone than are normal rats; smaller doses are required to produce a given increment than are required in the normal. All epiphyseal cartilages present respond, becoming more cellular and wider. The increases in epiphyseal cartilage width, within certain limits of dose and time, are proportional to the dose. The reliability of this response has furnished the basis of a very satisfactory method of assay of the hormone.[25, 31, 36] The test is specific in the sense that the growth hormone is the only hormone which widens the plate in proportion to dosage.‡

The resumption of growth of epiphyseal cartilages is accompanied by removal of the sealing lamina of bone, the reëstablishment of mechanisms of erosion, the removal of old, heavy trabeculae, and deposition of new bone in delicate trabeculae. Reëstablishment of the youthful picture thus occurs, replacing the aged appearance characteristic of the bone of hypophysectomized rats.[11]

It will be recalled that in normal rats growth hormone neither delayed nor accelerated epiphyseal closure. In hypophysectomized rats epiphyseal

* To appear in Anat. Rec., 1950, *107*, no. 4.

† The most noteworthy change simulating acromegaly was the excessive growth of the mandible which resulted in dental malocclusion and marked distortion of condylar form.

‡ Small increases in cartilage width, below the unit defined for growth hormone, can be produced by other hormones. None produces continued growth, or growth proportional to dose. (Marx *et al.*[42]).

closure is already postponed indefinitely by the operation and therefore no question arises of a further delay with growth hormone. Whether growth hormone hastens epiphyseal closure is very doubtful. As increased dimensions result from injection of growth hormone, this is a part of the general problem as to whether epiphyseal closure occurs when bones reach their definitive length, or when a characteristic chronological age is reached, or after a definite period of activity has elapsed. Silberberg and Silberberg[67] have taken the position that epiphyses close when, as a result of endogenous or exogenous stimuli, the bones have reached full size.

In order that experiments in which growth hormone is administered may contribute to the solution of this problem, it is extremely important that growth hormone preparations should be free of extraneous biologically active substances. Traces of thyrotropic hormone in such preparations would undoubtedly have a differentiating effect on the bones. In the studies reported here, growth hormone has been used which was pure by physico-chemical standards, and known to be free of thyrotropic effects at many multiples of the minimally effective dose.

It would appear that growth hormone does not hasten closure, but that the time of closure, as well as the responsiveness of bones to growth hormone, is dependent on, or related to, the normal physiology of the bone as reflected in normal time of closure. Although all epiphyses present at hypophysectomy were reactivated by growth hormone, the continuance of the response of each bone followed the same pattern as the epiphyseal closure. Epiphyseal discs which persisted until late in life—such as that at the proximal end of the tibia—continued to respond as long as the hormone was administered, and the bones reached a size in excess of normal, whereas epiphyses which normally close early responded only until the normal size of bone was attained.

The latter situation may be illustrated by the reaction of the metacarpal of the hypophysectomized rat during the chronic injection of growth hormone (Becks *et al.*,[16] p. 182, footnote). Epiphyseal closure in the metacarpal was delayed, as in the uninjected control, far beyond the normal age of closure (110 days) though the sealing lamina of bone was absent and the epiphysis responded to the hormone until normal length was attained. Nor did the epiphysis close promptly once the definitive bone length was achieved, 180 days after the normal time of closure, when rats were 285 days old. Instead, this epiphyseal disc remained for some time without further contributing to the length. (The epiphysis did eventually close, by 450 days, which it did not do in the uninjected controls.)

Hypophysectomized rats becoming gigantic as a result of injection of growth hormone (i.e., exceeding normal weight and length) developed many disproportions in the skeleton. These were even more marked than in the intact injected rats.[27] Part of these disproportions are certainly

attributable to the differences in responsiveness of the various epiphyseal centers. The relatively small paws of the growth hormone induced giant rats are one of the outstanding disproportions noted which can be attributed to this differential responsiveness of various epiphyses.*

Aside from the disproportion in the paws, other outstanding disproportions were present in the skull and pelvis. In the skull the disproportions were chiefly in the parts dependent on membranous bone growth. In the pelvis the sustained growth was not attributable to an epiphysis. In the scapula also, disproportions must have been due to bony additions from periosteal growth. The tuberosity of the humerus, which might be considered a periosteal excresence, surpassed in size anything seen in normal giants.

Growth hormone in thyroidectomized rats. That growth can be elicited in thyroidectomized rats by injection of growth hormone has been reported many times.[24, 28] Salmon[50, 51] questioned whether rats thyroidectomized on the first day of life could respond. Scow and Marx,[54] Scow *et al.*,[55] and Ray *et al.*[48] have found, however, that rats thyroidectomized at this early age responded by an increase both in body weight and length; bones throughout the body were stimulated. It is true these animals were relatively insensitive to the hormone, 25 times as much being required as is necessary to induce similar growth in hypophysectomized rats. However, no maturation of the skeleton resulted from the injection of growth hormone into thyroidectomized rats. This could be shown to optimal advantage in those rats thyroidectomized on the first day of life, as both growth and osseous maturation were so markedly retarded by the operation.

Growth hormone in thyroidectomized-hypophysectomized rats. Rats in which both thyroid and pituitary were removed responded to growth hormone like thyroidectomized rats. They increased in weight and length, but were relatively insensitive to the hormone. Furthermore, no maturation of bone occurred; bone age remained at 18 days as in the doubly operated controls.

---

* Bones with one epiphysis, and therefore dependent for growth only on one locus which normally fuses early, grew to normal dimensions and then ceased to grow. The paws, which contain many bones of this type (metacarpals, phalanges, and at least some of the tarsals, e.g., the calcaneous) behaved in this way, with the result that the paws were never enlarged in hypophysectomized giants. The other long bones have two foci of growth. Usually one focus (such as that at the distal tibia and proximal radius) closes early. Such epiphyseal discs ceased to respond to growth hormone during chronic injection. The second epiphysis of such long bones, characterized by delayed closure, remained responsive to growth hormone and therefore growth continued to excess in such bones.

It is puzzling to reconcile the occurrence of large feet and hands in human giants with the unenlarged paws of chronically injected rats (normal or hypophysectomized). It cannot be said that the difference is due to lack of time for influencing the bones of the paws because of early closure of epiphyses. The metacarpals attain the same length whether chronic injections are started at 21 days or later. In this sense the rat is a more highly specialized animal than man.

Growth hormone is effective in the absence of the target organs. The response to growth hormone of rats deprived of other endocrine organs has also been tested. Hypophysectomized-adrenalectomized rats were found to respond to growth hormone with the same sensitivity as hypophysectomized rats.[59] Hypophysectomized-adrenalectomized-thyroidectomized rats also responded well to growth hormone (Simpson, Li, and Evans unpublished). Hypophysectomized-thymectomized rats were injected with growth hormone and were found to be as sensitive in their response as hypophysectomized rats.[49] This was of interest as it was known that the thymus enlarged in either normal or hypophysectomized rats in-

TABLE 6

SKELETAL AGE OF FEMALE RATS HYPOPHYSECTOMIZED AT 21 DAYS OF AGE AND INJECTED WITH THYROXIN BETWEEN 30 TO 60 DAYS OF AGE

Treatment	*Skeletal age*		*Advance in skeletal age*
	At 30 days	*At 60 days*	
Hypophysectomized untreated	29	42	13
Hypophysectomized + Thyroxin 2.5 μg per day	29	55	26
Normal untreated	30	60	30

After Ray *et al.*[47]

jected with this hormone. Gonadectomized rats also respond well to growth hormone.[23] It may then be safely said that growth hormone does not act through any of the known target organs.

Thyroxin therapy

Thyroxin in normal rats. Thyroxin injected into normal rats (at 5μg doses) caused no increase in body length, but increased even further the growth resulting from growth hormone.[24] Osseous differentiation has also been shown to be accelerated by thyroid hormone and premature aging of the skeleton results. Smith and McLean[61] demonstrated this for the tibia in the rat. Premature closure of the epiphysis of the intact rat's metacarpal has been observed in this laboratory; the disc disappeared at 82 days, the average time of disappearance being 110 days (H. V. Christensen and I. M. Carlson, unpublished). Silberberg and Silberberg[56] showed premature epiphyseal closure in adult mice. Noback *et al.*[44] were able to show that thyroxin advanced osteogenesis in newborn rats.

Thyroxin in hypophysectomized rats. Thyroxin injected into hypophysectomized rats caused barely detectable growth.* The outstanding result of thyroxin injection was increased differentiation of bone. Maturation occurred up to normal for the age, even though the animal remained dwarfed.[2, 47] The advance in skeletal age is shown in Table 6.

Thyroxin reduced the width of the epiphyseal plates so that they were even narrower than in hypophysectomized controls; erosion of cartilage dominated, leading to the increased bone age. The formation of a lamina of bone sealing the cartilage was prevented (Ray *et al.*,[47] also C. P. Williams, unpublished), and resorption of such laminae occurred if already formed—even though the sealing bone had been present for long periods.[11]

Thyroxin with growth hormone

When thyroxin was injected into hypophysectomized rats in combination with growth hormone, both growth and maturation occurred. A very delicate balance of dosages is necessary, otherwise one or the other effect predominates. Smith[62] first described this synergic action between thyroid hormone and growth hormone and it has been confirmed many times (e.g., Evans *et al.*[24]). Histological analyses of the skeletons showed that greater increases in dimensions of the bones resulted than after either treatment alone, and that the combination also caused a more marked increase in chondrogenesis and osteogenesis.[6] Thyrotropic hormone from the pituitary showed the same synergic action with growth hormone in hypophysectomized rats.[41]

Thyroxin, and thyroxin with growth hormone, in thyroidectomized rats. Thyroxin injected into thyroidectomized rats led to spectacular resumption of growth (increase in size)—a growth as marked as that caused by growth hormone in hypophysectomized rats. Differentiation of the osseous system also occurred, leading to normal rate of increment of skeletal age. In rats thyroidectomized on the day of birth a dose of thyroxin of only 2.5 to 3μg caused an advance in differentiation so that at 60 days of age, the bone age attained was 56 days even though therapy had not been instituted until 30 days of age.[17, 48, 55]

As thyroxin therapy is accompanied by repair of the abnormal pituitary of the thyroidectomized rat, it may be assumed that the increase in size was caused by resumption of secretion of pituitary growth hormone.

Thyroxin, and thyroxin with growth hormone, in thyroidectomized-hypophysectomized rats. In rats both thyroidectomized and hypophysect-

* Doses of thyroxin of 5 to 7 μg, doses which are marginally toxic, caused no growth. Doses of 2 to 3 μg caused a small amount of growth. As usual, in interpreting these minimal growth effects one must distinguish between barely detectable and transient increases and growth which is proportional to dose and which continues as long as the substance is administered.

omized, on days 1 and 20 respectively, thyroxin administered from day 30 caused slight but significant increases in body weight and length, also increase in the length of individual bones. Differentiation of the skeleton was resumed at a normal or greater than normal rate.* The body growth

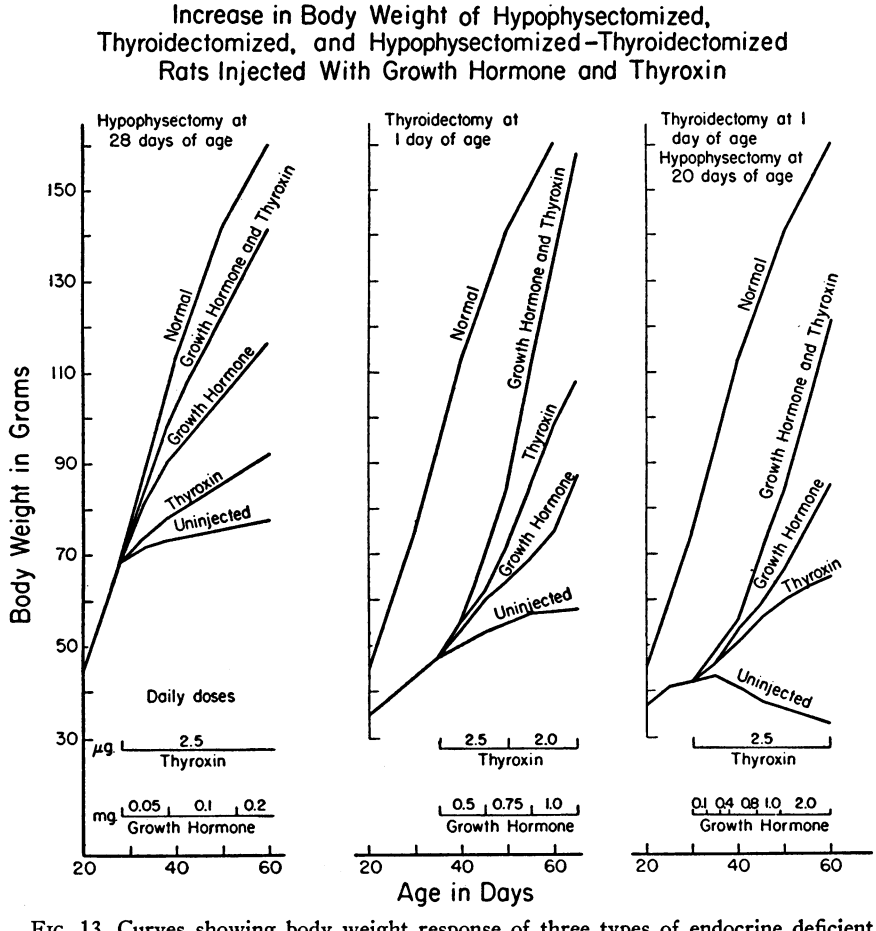


FIG. 13. Curves showing body weight response of three types of endocrine deficient rats to growth hormone, thyroxin, and the combined treatment.

resulting from injection of growth hormone was greater than that from thyroxin, but growth hormone caused no skeletal differentiation. The combination of growth hormone with thyroxin was more efficient than either hormone alone, causing a normal or greater than normal rate of growth with maturation.[70] Both the hormones necessary for coordinated growth with maturation had to be supplied in this type of animal to elicit the

* Skeletal maturation may even occur at a greater than normal rate as indicated by the closure of the medial epicondyle of the humerus at 60 days though the normal age is 100 days.

complete response, since, unlike the thyroidectomized animal, the thyroid-ectomized-hypophysectomized rats could not react under the stimulus of thyroxin to produce endogenous growth hormone.

The graphs of Figs. 13 and 14 serve to illustrate the relative effectiveness of thyroxin, growth hormone, and the combination of thyroxin and growth

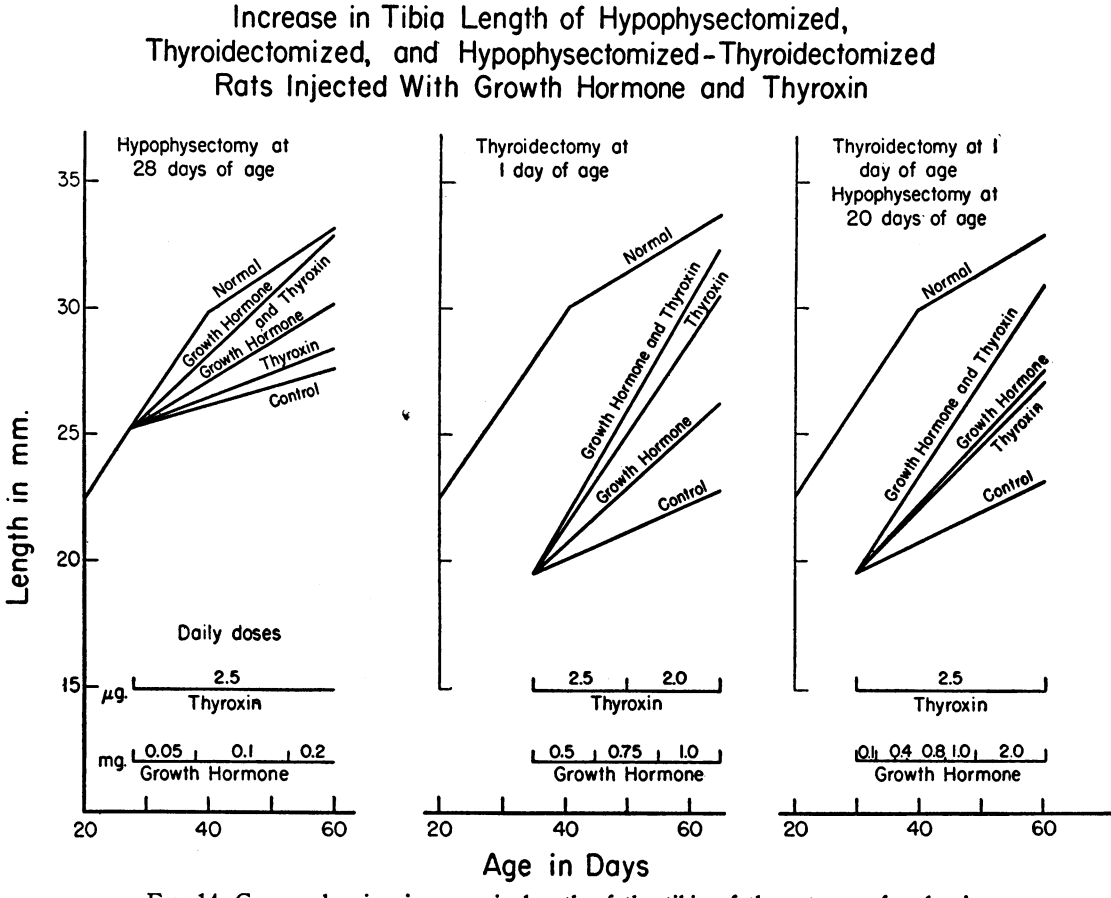


FIG. 14. Curves showing increase in length of the tibia of three types of endocrine deficient rats to growth hormone, thyroxin and the combined treatment.

hormone in thyroidectomized, hypophysectomized, and doubly operated rats. The first chart (Fig. 13) shows the increase in body weight, the second the increase in bone length (tibia, Fig. 14).

Figures 15-20 show the differential histological response of the humerus of the thyroidectomized-hypophysectomized rat to injection of thyroxin, growth hormone, and the combination.

Emphasis has been placed in this paper on the roles of growth hormone and thyroxin on growth of the skeleton—i.e., their effects on increase in

size and maturation—and no complete discussion has been attempted of the action of other pituitary hormones or of their target organ hormones. Nevertheless, a few other outstanding hormonal effects on growth must be mentioned, as the basic action of growth hormone and thyroxin is markedly modified by other hormones. Even though many of these interrelations are still obscure, it would be a mistake not to touch on some of the better established ones.

Effect of adrenocorticotropic hormone and cortical steroids. In normal rats adrenocorticotropic hormone (ACTH) caused retarded body growth.[7, 48] Retardation of both chondrogenesis and osteogenesis occurred, the epiphyseal plates were narrower than is normal, though not as narrow as in hypophysectomized rats. This antagonism to growth was shown to be effective through the cortex of the adrenal as it was not given in the absence of the cortex.[36] Cortical steroids have been shown to have a similar antagonistic action on growth.[34]

In hypophysectomized rats ACTH did not further decrease chondrogenesis or osteogenesis when given alone, but when injected simultaneously with growth hormone it was antagonistic to the growth promoting effects.[5] The body weight increase and epiphyseal disc widening anticipated on injection of growth hormone did not occur (Becks *et al.*, 1944).† No attention was devoted to the effect of ACTH or cortical steroids on maturation of the skeleton.*

† Since writing this, roentgenographic studies in this laboratory of the skeletons of intact and of hypophysectomized rats treated from 22 to 60 days of age with doses of ACTH which produced growth stasis have shown no appreciable effect of this hormone on skeletal maturation.

* This antagonism of ACTH to growth hormone may be associated with opposite and opposing action on nitrogen metabolism, ACTH wasting nitrogen and growth hormone conserving it.

PLATE IV

Response of the humerus of thyroidectomized-hypophysectomized rats to thyroxin, growth hormone, and the combination. Specimens taken at 60 days of age except as noted. (H & E stain, x9.)

FIG. 15. Normal rat 18 days of age, "bone age" control. The main epiphyseal ossification center is present.

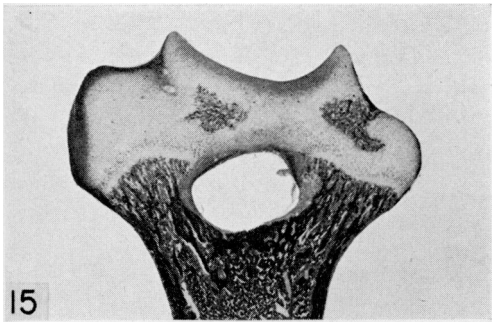
FIG. 16. Thyroidectomized day 1, subsequently hypophysectomized day 20. Only the main epiphyseal ossification center is present.

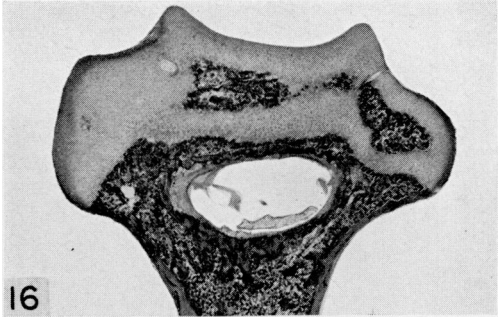
FIG. 17. Both operations as described above, treated with thyroxin, 2.5 μg daily, day 30-60. The main epiphyseal cartilage plate and the plate in the medial epicondyle are both perforated.

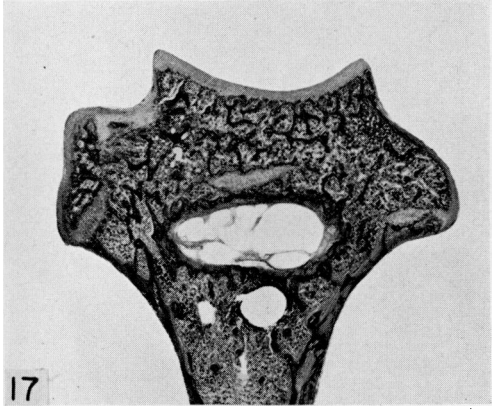
FIG. 18. Both operations. Growth hormone, dose increasing from 0.1 to 2.0 mg daily, day 30-60. Increase in size of the bone, but no further advance in ossification beyond the doubly operated control.

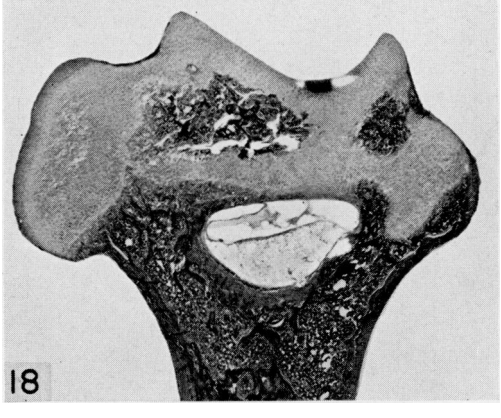
FIG. 19. Both operations. Growth hormone and thyroxin (both as above). The main epiphyseal cartilage plate has disappeared. That in the medial epicondyle is intact and active.

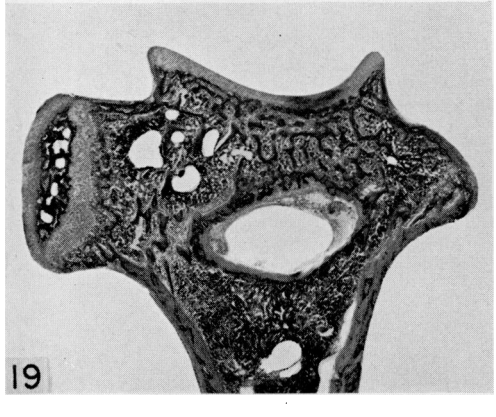
FIG. 20. Normal rat 60 days of age, chronological age control. The main epiphyseal cartilage plate has disappeared. The plate in the medial epicondyle is still active.











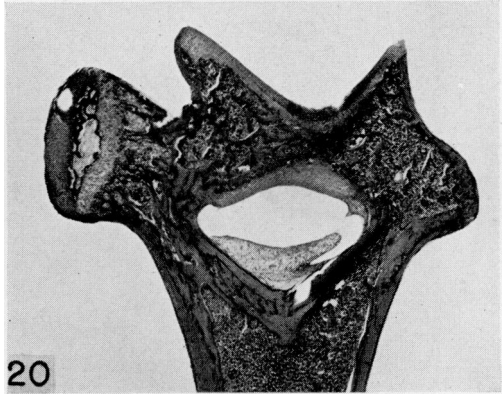
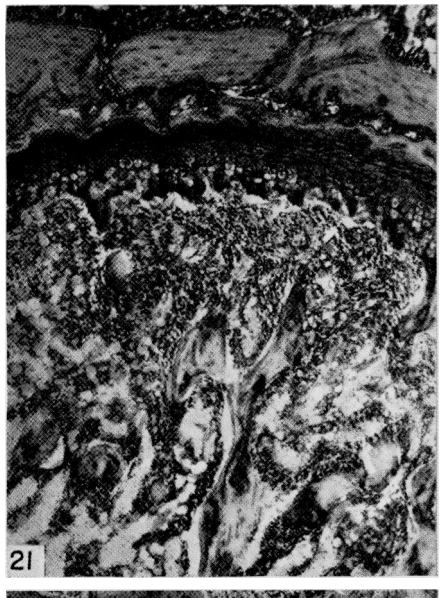
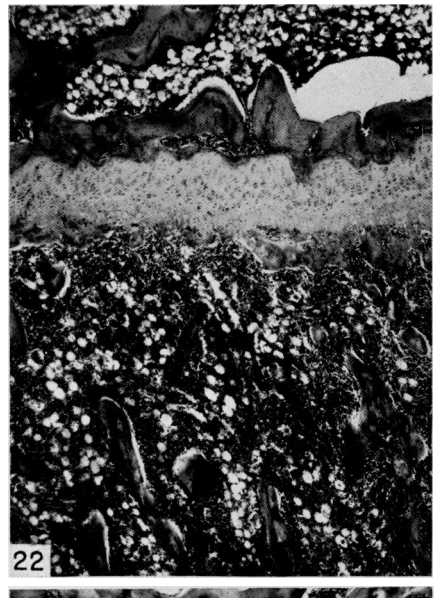
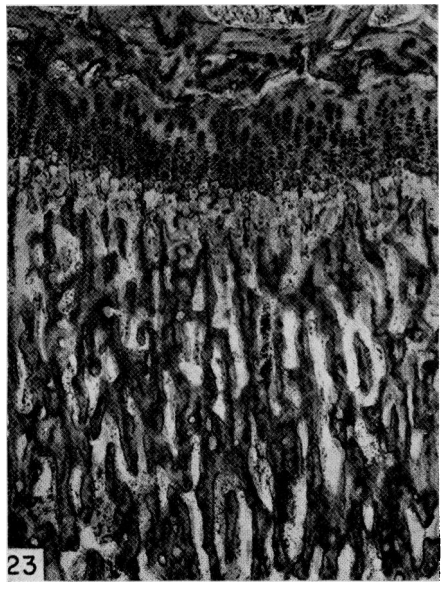


PLATE IV







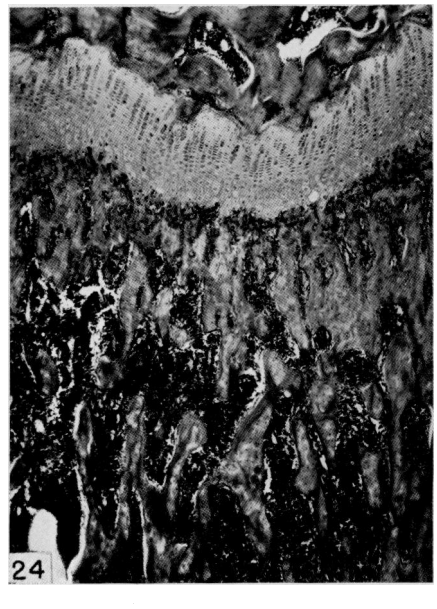


PLATE V

Effect of testosterone. Testosterone has been shown to stimulate growth in normal animals of several species, including the human being. In the absence of the pituitary the rat gave no significant increase in weight or length when injected with testosterone (Simpson *et al.*[60]; also G. G. Cayler, unpublished, and C. P. Williams, unpublished). No significant stimulation of the growth of bones has been noted, with the exception of the os penis which has been shown by Thyberg and Lyons[88] to respond to testosterone rather than growth hormone. Testosterone caused differentiation as well as growth of this sexually dimorphic bone.†

Some augmentation of the effects of growth hormone by testosterone has been reported to occur in hypophysectomized rats.[60]

Testosterone combined with thyroxin therapy in the hypophysectomized rat did not give any effects other than those anticipated from thyroxin alone (C. P. Williams, unpublished).

From these results it might be inferred that the growth-promoting effects of testosterone observed in normal animals, but not in the absence of the pituitary, are due to stimulation of the pituitary, or perhaps to synergism with pituitary growth hormone. The expected synergism with thyroxin was not observed, and the synergism with growth hormone under the conditions so far tested was slight. The conservation of nitrogen by testosterone occurs in the absence of the pituitary, so that nitrogen conservation cannot be the only factor in the growth which it initiates.[80]

Effect of estrogen. The smaller size of the normal female rat than the castrate female may be interpreted as due to the antagonistic action of endogenous estrogen on growth. So also the smaller size of growth-hor-

† Turner *et al.*[85] showed that castration of day-old male rats was without effect on their skeletal age (except the os priapi and ischial epiphysis). They showed roentgenographically that testosterone did not increase skeletal age of normal or castrate males but that it did accelerate development of the os priapi. G. G. Cayler in this laboratory (unpublished) has found that the pelvis of the rat similarly increases in size and differentiates with testosterone.

PLATE V

Comparison of the effects of lactogenic hormone with those of estrogen. Proximal epiphyseal cartilage plate of the tibia (H. & E. stain, x38.)

FIG. 21. Hypophysectomized 54-day-old female rat treated with estradiol dipropionate for 18 days (10 mg pellet implanted subcutaneously at weekly intervals). No difference from the control is to be noted.

FIG. 22. Untreated hypophysectomized 40-day-old female rat, 21 days postoperative.

FIG. 23. Normal female rat treated with estradiol dipropionate for 18 days. (10 mg pellet implanted subcutaneously at weekly intervals). Trabeculae are numerous and sturdy.

FIG. 24. Hypophysectomized 40-day-old female rat injected with lactogenic hormone for 10 days (day 11-21 postoperative). Note resemblance to the normal estrogen treated.

mone-injected normal females compared with injected castrate females may be interpreted as due to antagonism of estrogen to growth hormone.[23]

The effects of injected estrogen on bone are very much more marked in some species of animals than in others. In mice (and pigeons) estrogen causes complete filling of the medullary cavity with bone (Gardner and Pfeiffer[29]). In rats, injected estrogen has less effect on bone structure than in mice. There is, however, an increase in amount of trabecular bone and a decrease in cartilage width.[58] Urist, Budy, and McLean[66] consider that the mechanism in the rat is inhibition of resorption of bone, while in the mouse estrogen both stimulates endosteal ossification and inhibits resorption. In hypophysectomized rats no response of bone has been observed following treatment with doses of estrogen known to be effective in the normal.[37] The estrogen was administered immediately after operation and the resorption of trabecular bone characteristic of the immediate post-hypophysectomy period was not prevented. From this one can infer that in the rat, at least, the response to estrogen is mediated through the pituitary.

The effect of estrogen on skeletal ageing in the absence of the pituitary has not been adequately studied.

Effect of lactogenic hormone. Lactogenic hormone does have a marked effect on bone structure in hypophysectomized rats. The bony trabeculae found in the medullary cavity not only are not resorbed as usual after operation but are actually heavier than normal (Figs. 21-24) (Simpson, Asling, Becks, Evans, unpublished). The changes were quite comparable to those observed in normal rats injected with estrogen, suggesting that the mechanism for estrogen action in normal rats is through the pituitary, the estrogen stimulating the pituitary to produce more lactogenic hormone.*

While assuming that the growth and differentiation of the skeleton is primarily under the control of growth hormone and thyroxin, due care has been taken to stress the importance of many other factors which influence growth. Diet and other environmental factors have been mentioned, and lastly, the other endocrine influences. Though the parathyroids have been neglected, there has been no intent to minimize the importance of the hormone from this organ on mineral metabolism and hence on bone formation and resorption. The role of ACTH and cortical steroids, and of testosterone, in influencing nitrogen metabolism and hence the formation

* This interpretation is not without supporting evidence. A higher content of lactogenic hormone was shown in the pituitaries of estrogen-treated rats when tested by implanting the pituitary tissue in the crop wall of pigeons. Furthermore, the large persistent functioning corpora lutea of estrogen-treated normal rats may be interpreted as due to increased output of lactogenic hormone—through its luteotrophic action (Simpson and Evans, unpublished). Work with parabionts has given further convincing evidence of increased pituitary production of lactogenic hormone under the influence of estrogen.[85]

of bone protein matrix is equally inescapable. No adequate investigation has been made of these accessory endocrine factors to understand which of these influence only bone size, and which influence maturation, i.e., which of them act with or against growth hormone on the one hand or thyroxin on the other. Nor do we understand how they relate to proportionate or disproportionate growth of the various parts of the osseous system or to the deceleration or cessation of growth.

Summary

This account has largely been confined to analysis of the growth and differentiation of the osseous system in the rat under the influence of pituitary hormones and pituitary target organ hormones, stress being laid on studies to which our group has contributed. No attempt has been made to give a general review of osteogenesis.

As all the pituitary hormones, directly, or indirectly through their target organs, influence bone growth, it is important to stress the necessity of using pure hormones in any such study designed to disentangle the effects of individual hormones. It should be stressed particularly that the growth hormone preparations whose effects are here reported were pure, judged by physico-chemical criteria, and were free of biologically active contaminants at many multiples of the levels necessary for stimulation of growth.

The clearest evidence of dissociation of the phenomena of increase in size from differentiation in the skeletal system has been obtained from the study of osteogenesis in the animals deprived of the pituitary or thyroid, and from replacement studies with pituitary growth hormone and thyroid hormone.

After hypophysectomy in the young rat, growth and differentiation of the skeleton soon ceased. The skeletal age attained varied with the age at operation, being progressively less as the operation was performed in younger and younger animals. The period during which maturation continued after operation was, however, constant, being about 3 weeks, regardless of whether the rats were hypophysectomized at 28 days or 6 days of age. The capacity to increase in size was lost at a constant age, approximately 30 days chronological age.

No endocrine deficiency was produced in which growth hormone was not effective in promoting increase in size (hypophysectomy, thyroidectomy, adrenalectomy, the three operations concurrently; hypophysectomy-thymectomy or gonadectomy), i.e., growth hormone does not act through known endocrine organs.

The effect of growth hormone on the skeleton was chiefly to increase its size. Bones increased in length and diameter. All epiphyseal centers of ossification responded to growth hormone unless at the onset of therapy

they were too near their normal time of closure. Growth hormone in normal rats neither hastened epiphyseal closure nor delayed it. In hypophysectomized rats where epiphyseal closure was already postponed, growth hormone did not cause further delay. In fact, closure took place during its administration, though tardily.

The gigantism induced by growth hormone in normal, and especially in hypophysectomized rats, was characterized by many disproportions which appeared to be related to differences in responsiveness among epiphyses, which in turn was related to normal closure time of these epiphyses. Bones with early closing epiphyses closed without undergoing excessive growth, whereas bones with late closing epiphyses continued to grow as long as growth hormone was administered. The distortions characterizing growth hormone induced gigantism were also due to continuation of membranous or periosteal bone formation.

Thyroxin accelerated maturation of skeleton in normal rats; closure of epiphyseal plates occurred before adult size was reached. In the hypophysectomized rat also the effect was chiefly to promote differentiation or maturation. The most complete replacement in this deficient animal was therefore obtained by combinations of the two hormones, growth hormone and thyroxin.

Thyroidectomized rats reacted to growth hormone by increase in skeletal size, but showed no acceleration of maturation. Thyroid hormone in the thyroidectomized rat was, however, almost as effective in causing growth with maturation as the combination of growth hormone and thyroxin was in hypophysectomized animals.

No other hormones tested, alone or in combination, have stimulated significant amounts of growth, although other hormones are known to have important effects on skeletal growth—adrenocorticotropic hormone (or cortical steroids) and estrogens inhibiting growth and being antagonistic to growth hormone, and testosterone accelerating growth in the presence of the pituitary.

References

1 Asling, C. W., Becks, Hermann, Simpson, M.E., Li, C. H., and Evans, H. M.: The effect of anterior hypophyseal growth hormone on epiphyseal closure in the third metacarpal of normal female rats. Anat. Rec., 1948, *101,* 23.

2 Asling, C. W., Becks, Hermann, Simpson, M. E., and Evans, H. M.: The effect of thyroxin injections on growth and epiphyseal closure of the third metacarpal bone in hypophysectomized female rats. Anat. Rec., 1949, *104,* 255.

3 Asling, C. W., Walker, D. G., Simpson, M. E., and Evans, H. M.: Differences in the skeletal development attained at the sixtieth day by female rats hypophysectomized at ages varying from 6 to 28 days. Anat. Rec., 1950 (in press).

4 Becks, Hermann and Furuta, W. J.: Effects of magnesium deficient diets on oral and dental tissues; changes in enamel epithelium. J. Am. Dent. Ass., 1939, *26,* 883.

5 Becks, Hermann, Kibrick, E. A., and Evans, H. M.: The bone histology of adult male rats thyro-parathyroidectomized when one month of age. J. Exp. Zool., 1942, *89,* 297.

6 Becks, Hermann, Ray, R. D., Simpson, M. E., and Evans, H. M.: Effect of thyroxin and the anterior pituitary growth hormone on endochondral ossification. Arch. Path., Chic., 1942, *34,* 334.
7 Becks, Hermann, Simpson, M. E., Li, C. H., and Evans, H. M.: Effects of adrenocorticotropic hormone (ACTH) on the osseous system in normal rats. Endocrinology, 1944, *34,* 305.
8 Becks, Hermann, Simpson, M. E., Marx, Walter, Li, C. H., and Evans, H. M.: Antagonism of pituitary adrenocorticotropic hormone (ACTH) to the action of growth hormone on the osseous system of hypophysectomized rats. Endocrinology, 1944, *34,* 311.
9 Becks, Hermann, Simpson, M. E., and Evans, H. M.: Ossification at the proximal tibial epiphysis in the rat. I. Changes in females with increasing age. Anat. Rec., 1945, *92,* 109.
10 Becks, Hermann, Simpson, M. E., and Evans, H. M.: Ossification at the proximal tibial epiphysis in the rat. II. Changes in females at progressively longer intervals following hypophysectomy. Anat. Rec., 1945, *92,* 121.
11 Becks, Hermann, Simpson, M. E., Evans, H. M., Ray, R. D., Li, C. H., and Asling, C. W.: Response to pituitary growth hormone and thyroxin of the tibias of hypophysectomized rats after long postoperative intervals. Anat. Rec., 1946, *94,* 631.
12 Becks, Hermann, Asling, C. W., Simpson, M. E., Evans, H. M., and Li, C. H.: Ossification at the distal end of the humerus in the female rat. Am. J. Anat., 1948, *82,* 203.
13 Becks, Hermann, Simpson, M. E., Scow, R. O., Asling, C. W., and Evans, H. M.: Skeletal changes in rats thyroidectomized on the day of birth and the effects of growth hormone in such animals. Tibia, metacarpal and caudal vertebrae. Anat. Rec., 1948, *100,* 561.
14 Becks, Hermann, Asling, C. W., Collins, D. A., Simpson, M. E., and Evans, H. M.: Changes with increasing age in the ossification of the third metacarpal of the female rat. Anat. Rec., 1948, *100,* 577.
15 Becks, Hermann, Collins, D. A., Asling, C. W., Simpson, M. E., Li, C. H., and Evans, H. M.: The gigantism produced in normal rats by injection of the pituitary growth hormone. V. Skeletal changes: skull and dentition. Growth, Ithaca, 1948, *12,* 55.
16 Becks, Hermann, Asling, C. W., Simpson, M. E., Li, C. H., and Evans, H. M.: The growth of hypophysectomized female rats following chronic treatment with pure pituitary growth hormone. III. Skeletal changes: Tibia, metacarpal, costochondral junction and caudal vertebrae. Growth, Ithaca, 1949, *13,* 175.
17 Becks, Hermann, Scow, R. O., Simpson, M. E., Asling, C. W., Li, C. H., and Evans, H. M.: Response by the rat thyroidectomized at birth to growth hormone and to thyroxin given separately or in combination. Anat. Rec., 1950, *107,* (July).
18 Dandy, W. E. and Reichert, F. L.: Studies on experimental hypophysectomy in dogs. III. Somatic, mental and glandular effects. Bull. Johns Hopkins Hosp., 1938, *62,* 122.
19 Dawson, A. B.: The age order of epiphyseal union in the long bones of the albino rat. Anat. Rec., 1925, *31,* 1.
20 Drill, V. A.: Interrelations between thyroid function and vitamin metabolism. Physiol. Rev., 1943, *23,* 355.
21 Erdheim, J.: Nanosomia pituitaria. Beitr. path. Anat., 1916, *62,* 302.
22 Ershoff, B. H.: Conditioning factors in nutritional disease. Physiol. Rev., 1948, *28,* 107.
23 Evans, H. M. and Simpson, M. E.: Hormones of the anterior hypophysis. Am. J. Physiol., 1931, *98,* 511.
24 Evans, H. M., Simpson, M. E., Pencharz, R. I.: Relation between the growth promoting effects of the pituitary and the thyroid hormone. Endocrinology, 1939, *25,* 175.
25 Evans, H. M., Simpson, M. E., Marx, Walter, and Kibrick, E. A.: Bioassay of the pituitary growth hormone. Width of the proximal epiphyseal cartilage of the tibia in hypophysectomized rats. Endocrinology, 1943, *32,* 13.
26 Evans, H. M., Li, C. H., and Simpson, M. E.: Inhibiting effect of adrenocorticotropic hormone on the growth of male rats. Endocrinology, 1943, *33,* 237.
27 Evans, H. M., Asling, C. W., Simpson, M. E., and Becks, Hermann: The growth of hypophysectomized female rats following chronic treatment with pure pituitary growth hormone. IV. Skeletal changes: differences in response from that of intact rats. Growth, Ithaca, 1949, *13,* 191.

28 Flower, C. F., and Evans, H. M.: The repair of dwarfism following thyroidectomy by the administration of anterior hypophyseal fluid. Anat. Rec., 1924, *29,* 383.
29 Gardner, W. U. and Pfeiffer, C. A.: Influence of estrogens and androgens on the skeletal system. Physiol. Rev., 1943, *23,* 139.
30 Gordan, G. S., Evans, H. M., and Simpson, M. E.: Effects of testosterone propionate on body weight and urinary nitrogen excretion of normal and hypophysectomized rats. Endocrinology, 1947, *40,* 375.
31 Greenspan, F. S., Li, C. H., Simpson, M. E., and Evans, H. M.: Bioassay of hypophyseal growth hormone: The tibia test. Endocrinology, 1949, *45,* 455.
32 Handler, Philip, Baylin, G. J., and Follis, R. H., Jr.: The effects of caloric restriction on skeletal growth. J. Nutrit., 1947, *34,* 677.
33 Harington, C. R.: *The thyroid gland, its chemistry and physiology.* London, H. Milford, Oxford University Press, 1933.
34 Ingle, D. J., Higgins, S. M., and Kendall, E. C.: Atrophy of the adrenal cortex in the rat produced by administration of large amounts of cortin. Anat. Rec., 1938, *71,* 363.
35 Jacobsohn, Dora: The action of corticotropic and adrenal cortex hormones on the mammary gland. Acta. physiol. scand., 1949, *17,* 423.
36 Kibrick, E. A., Becks, Hermann, Marx, Walter, and Evans, H. M.: The effect of different dose levels of growth hormone on the tibia of young hypophysectomized female rats. Growth, Ithaca, 1941, *5,* 437.
37 Kibrick, E. A., Simpson, M. E., Becks, Hermann, and Evans, H. M.: Effects of crystalline estrin implants on the tibia of young hypophysectomized female rats. Endocrinology, 1942, *31,* 93.
38 Krogman, W. M.: Contributions of T. Wingate Todd to anatomy and physical anthropology. Am. J. Phys. Anthrop., 1939, *25,* 145.
39 Krogman, W. M.: Tabulae Biologicae, vol. XX (*Growth of Man*), den Haag, 1941.
40 Levin, Louis: The relation of food consumption, hypophysis and adrenal cortex to serum albumin metabolism in the rat. Am. J. Physiol., 1943, *138,* 258.
41 Marx, Walter, Simpson, M. E., and Evans, H. M.: Synergism between thyrotropic and growth hormones of pituitary. Body weight increase in hypophysectomized rat. Proc. Soc. Exp. Biol., N. Y., 1942, *49,* 594.
42 Marx, Walter, Simpson, M. E., and Evans, H. M.: Specificity of the epiphyseal cartilage test for the pituitary growth hormone. Proc. Soc. Exp. Biol., N. Y., 1944, *55,* 250.
43 Moon, H. D.: Inhibition of somatic growth in castrate rats with pituitary extracts. Proc. Soc. Exp. Biol., N. Y., 1937, *37,* 34.
44 Noback, C. R., Barnett, J. C., and Kupperman, H. S.: The time of appearance of ossification centers in the rat as influenced by injections of thyroxin, thiouracil, estradiol and testosterone propionate. Anat. Rec., 1949, *103,* 49.
45 Osborne, T. B. and Mendel, L. B.: The relation of the rate of growth to diet. I. J. Biol. Chem., 1926, *69,* 661.
46 Ray, R. D.: Effects of the pituitary growth hormone and of thyroxin on growth and differentiation of the skeleton of the rat. Doctoral thesis, University of California, 1948.
47 Ray, R. D., Asling, C. W., Simpson, M. E., and Evans, H. M.: Effects of thyroxin injections on growth and differentiation of the skeleton of hypophysectomized female rats. Anat. Rec., 1950, *107* (July).
48 Ray, R. D., Simpson, M. E., Li, C. H., Asling, C. W., and Evans, H. M.: Effects of the pituitary growth hormone on growth and differentiation of the skeleton of the rat thyroidectomized at birth. Am. J. Anat., 1950, *86,* 479.
49 Reinhardt, W. O., Marx, Walter, and Evans, H. M.: Effect of pituitary growth hormone on the thymectomized rat. Proc. Soc. Exp. Biol., N. Y., 1941, *46,* 411.
50 Salmon, T. N.: The effect on the growth rate of thyro-parathyroidectomy in newborn rats and of the subsequent administration of thyroid, parathyroid and anterior hypophysis. Endocrinology, 1938, *23,* 446.
51 Salmon, T. N.: Effect of pituitary growth substance on the development of rats thyroidectomized at birth. Endocrinology, 1941, *29,* 291.
52 Samuels, L. T., Reinecke, R. M., and Bauman, K. L.: Growth and metabolism of young hypophysectomized rats fed by stomach tube. Endocrinology, 1943, *33,* 87.
53 Scow, R. O. and Simpson, M. E.: Thyroidectomy in the newborn rat. Anat. Rec., 1945, *91,* 209.
54 Scow, R. O. and Marx, Walter: Response to pituitary growth hormone of rats thyroidectomized on the day of birth. Anat. Rec., 1945, *91,* 227.

55 Scow, R. O., Simpson, M. E., Asling, C. W., Li, C. H., and Evans, H. M.: Response by the rat thyro-parathyroidectomized at birth to growth hormone and to thyroxin given separately or in combination. I. General growth and organ changes. Anat. Rec., 1949, *104,* 445.
56 Silberberg, Martin and Silberberg, Ruth: Changes in the skeletal tissues of mice following the administration of thyroxin. Growth, Ithaca, 1940, *4,* 305.
57 Silberberg, Martin and Silberberg, Ruth: Influence of the endocrine glands on growth and aging of the skeleton. Arch. Path., Chic., 1943, *36,* 512.
58 Simpson, M. E., Kibrick, E. A., Becks, Hermann, and Evans, H. M.: Effect of crystalline estrin implants on the proximal tibia and costochondral junction of young female rats. Endocrinology, 1942, *30,* 286.
59 Simpson, M. E., Marx, Walter, Becks, Hermann, and Evans, H. M.: Response of adrenalectomized-hypophysectomized rats to the pituitary growth hormone. Endocrinology, 1944, *35,* 234.
60 Simpson, M. E., Marx, Walter, Becks, Hermann, and Evans, H. M.: Effect of testosterone propionate on the body weight and skeletal system of hypophysectomized rats. Synergism with pituitary growth hormone. Endocrinology, 1944, *35,* 309.
61 Smith, E. E. and McLean, F. C.: Effect of hyperthyroidism upon growth and chemical composition of bone. Endocrinology, 1938, *23,* 546.
62 Smith, P. E.: Increased skeletal effects in A.P. growth-hormone injections by administration of thyroid in hypophysectomized, thyro-parathyroidectomized rats. Proc. Soc. Exp. Biol., N. Y., 1933, *30,* 1252.
63 Thyberg, W. G. and Lyons, W. R.: Androgen-induced growth of the os penis of hypophysectomized-gonadectomized rats. Proc. Soc. Exp. Biol., N. Y., 1948, *69,* 158.
64 Todd, T. W.: Growth and development of the child. Part II. Anatomy and Physiology (White House Conference on Child Health and Protection). New York, Appleton Century, 1933.
65 Turner, H. H., Lachmann, Ernest, and Hellbaum, A. A.: Effect of testosterone propionate on bone growth and skeletal maturation of normal and castrated male rats. Endocrinology, 1941, *29,* 425.
66 Urist, M. R., Budy, A. M., and McLean, F. C.: Species differences in the reaction of the mammalian skeleton to estrogens. Proc. Soc. Exp. Biol., N. Y., 1948, *68,* 324.
67 Walker, D. G.: The effects of hypophysectomy on the rat when performed at 28 days of age compared with the effects when performed at 6 days of age. Master's thesis, University of California, 1949.
68 Walker, D. [G.], Simpson, M. E., and Asling, C. W.: Differences in the effects of hypophysectomy on the rat when performed at 6 and at 28 days of age. Anat. Rec., 1949, *103* (suppl.), 100.
69 Walker, D. G., Simpson, M. E., Asling, C. W., and Evans, H. M.: Growth and differentiation in the rat following hypophysectomy at six days of age. Anat. Rec., 1950, *106,* 539.
70 Walker, D. G. *et al.*: Growth and differentiation of the skeleton of thyroidectomized-hypophysectomized rats treated with thyroxin, growth hormone and the combination. (unpublished)
71 Washburn, S. L.: The sequence of epiphyseal union in the opossum. Anat. Rec., 1946, *95,* 353.
72 Wolbach, S. B.: Vitamin A deficiency and excess in relation to skeletal growth. Proc. Inst. M. Chicago, 1946, *16,* 118.
73 Ziskin, D. E., Salmon, T. N., and Applebaum, E.: The effect of thyroparathyroidectomy at birth and at 7 days on dental and skeletal development of rats. J. Dent. Res., 1940, *19,* 93.