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# Response of Vertebral Cartilage and Bone to Hormonal Imbalances Produced by Anterior Hypophyseal Hormones and Hypothyroidism[1]

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*Abstract.* The response of lumbar vertebrae to hormonal imbalances, caused by administration of somatotrophin, prolactin, propylthiouracil and radioactive iodine $^{131}I$ was investigated in male or female mice of strains $C_{57}Bl$ or Dba. The pituitary hormones given during the growth period and shortly thereafter did not alter the incidence but increased the severity of spondylosis and thus facilitated the tracing of the stepwise evolution of the lesions from residual cartilage of the growth zones. Other lesions arose from calcific foci at the insertion of the annulus at the vertebral margin. Propylthiouracil retarded developmental processes in growth zones and metaphyses, attenuated the aging changes in the intervertebral discs, decreased the incidence of spondylosis, but intensified the development of vertebral epiphyses. Disc prolapse remained unaffected by the hormonal imbalances. The reaction of vertebral cartilage and bone was generally attenuated as compared to the response of the long bones to hormonal stimulation.

*Key Words*
Vertebrae
Spondylosis
Aging
Somatotrophin
Propylthiouracil

Osteoarthrosis of the knee joint of the mouse proved to be a suitable model for the investigation of genetic, hormonal and nutritional influences that play a role in the pathogenesis of the lesions [14]. Genetic and hormonal factors have been demonstrated or suggested in the evolution of spondylosis [2, 3, 6]. Insight into the pathogenesis of these lesions would be considerably advanced if the vertebrae of mice which develop

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spontaneous spondylosis were as responsive to experimental interference as the knee joint. The present investigation was carried out in an attempt to obtain information regarding this point.

## *Material and Methods*

Lumbar vertebrae had been obtained from mice given a variety of treatments and examined originally for the development of aging changes and of osteoarthrosis of the knee joint [14]. At the time of the autopsy, lumbar vertebrae had been removed, freed from the bulk of attached muscle tissue, fixed and decalcified in Bouin's fluid, split sagitally, embedded in paraffin and saved for future use. Semiserial sections, cut at 5 or 6 $\mu$m were stained with hematoxylin and eosin, PAS and toluidine blue. Vertebrae were available from a total of 366 mice treated as follows:

(1) 40–140 $\mu$g somatotrophin (STH) 5 times weekly from the age of 6 weeks to 6 months; 31 males of strain $C_{57}$Bl;

(2) 40–140 $\mu$g prolactin 5 times weekly from the age of 6 weeks to 6 months; 23 males and 25 females of strain $C_{57}$Bl;

(3) 25 untreated males and 25 untreated females of strain $C_{57}$Bl.

Animals of groups 1–3 were killed at the age of 18 months.

(4) Starting at the age of 6 weeks 0.6% propylthiouracil (PT) was added to the diet (a) continuously through life: 18 males of strain Dba and 14 females of strain $C_{57}$Bl; (b) intermittently through life, 1-month periods of feeding PT alternating with 1-month periods of consumption of a regular diet: 26 males of strain Dba; (c) intermittently through life, 3-month periods of feeding PT alternating with 3-month periods of consumption of a regular diet: 29 males of strain Dba.

(5) At the age of 1 year (a) 0.6% PT was added to the diet continuously for the rest of the life span: 9 males of strain Dba; (b) 400 $\mu$Ci radioactive iodine $^{131}$I intraperitoneally: 20 males of strain $C_{57}$Bl.

(6) 60 untreated males and 36 untreated females of strain $C_{57}$Bl and 17 untreated males of strain Dba.

(7) 30 $\mu$g $\alpha$-estradiol benzoate subcutaneously once a week from 1 to 6 months of age; 8 males of strain $C_{57}$Bl, killed at the age of 6 months.

## *Observations*

Sections of vertebrae were examined with special reference to changes in the growth zones, the vertebral bodies, the cartilaginous end-plates, the intervertebral discs and to spondylosis. Corresponding observations in untreated mice have been fully described previously [16].

*Growth zones.* There were no noticeable effects of STH or prolactin on the growth zones of 18-month-old animals. In mice fed PT from the age of 6 weeks

on, there were occasional irregularities in the alignment of the chondrocyte columns associated with retardation of chondrocyte development and of resorption of the primary spongiosa (fig. 1).

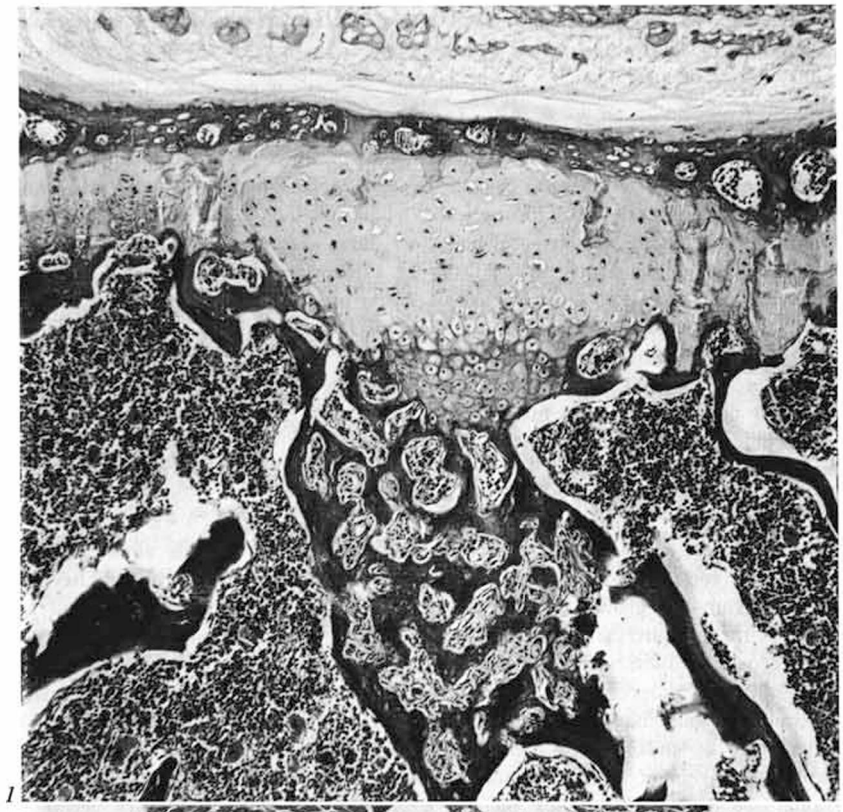
*Vertebral bodies.* As a result of disturbed breakdown of cartilage and primary spongiosa, foci of osteosclerosis had developed at the metaphyseal-cortical angle (fig. 2), but occasionally also through the length of the vertebral body. Multiple osseous bars traversing the vertebral body in a dorso-ventral direction were common in mice treated intermittently with PT (fig. 3). Foci of osteosclerosis were, however, also found in mice treated from the age of 1 year on, long after cessation of growth, and in old untreated mice of both strains $C_{57}Bl$ and Dba.

Estrogenic hormone caused the same type of osteosclerosis as described in long bones [14] (fig. 4). A few incidental findings were made: 1 mouse given prolactin had developed osteitis fibrosa (fig. 5), and in 1 animal treated with STH a pleomorphic sarcoma had replaced the bone marrow, destroyed the cortex and invaded the spinal canal (fig. 6). Tumor was also found in the femoral diaphysis.

*Cartilaginous end-plates.* These plates that overly the growth zones at both ends of the vertebrae differ from the end-plates of human vertebrae. They are separated from the growth zones by a narrow layer of hyaline matrix, and are thicker at the periphery than in the center (fig. 7). With increasing age, they tend to undergo ossification and transformation into true epiphyses, a process that starts peripherally and progresses towards the center. The rate of replacement varies with the strain and may be related to pituitary activity [16]. In the present series, untreated mice of strain $C_{57}Bl$ had about four times as many osseous centers or complete replacement of the end-plates than untreated mice of strain Dba of comparable age. No effect of sex on the development of the epiphyses could be established, but location seemed to have some influence in that the tendency to bony replacement increased in the craniocaudal direction; however, in many vertebrae one end-plate was replaced while the other was still entirely cartilaginous (fig. 13). In strain $C_{57}Bl$, replacement was beginning at 3 months of age; in strain Dba it was not observed until the age of 9 months. The former site of the notochord was recognizable as a hyaline wedge located in the center of the end-plate, projecting from the annulus fibrosus and usually overlying a 'plug' of necrotic or hyalinized cartilage in the growth zone (fig. 7). No effects of STH or prolactin were noted in the appearance or the numbers of epiphyses present: replacement had occurred in 38 and 29 % of the end-plates in untreated control males or females, respectively, and in 29 or 30 % of the end-plates of injected animals.

Radioactive iodine $^{131}I$ administered at 1 year of age had no effect on centers of ossification; however, PT, given at this age as well as earlier in life increased their number 2 to 4 times in individual animals and the number of animals developing the epiphyses $1^1/_2$ to 3 times.

Following administration of large doses of estrogenic hormone for a period of 5 months from the age of 1 month on, 7 of 8 males examined had partly or completely replaced one half of the end-plates present in the sections.



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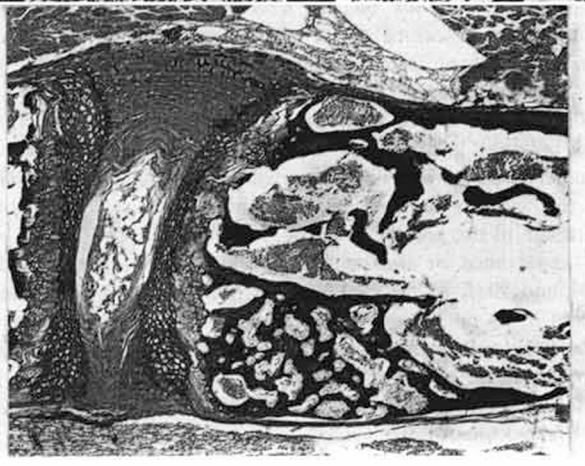

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*Fig. 1 and 2.* Legends see p. 15.

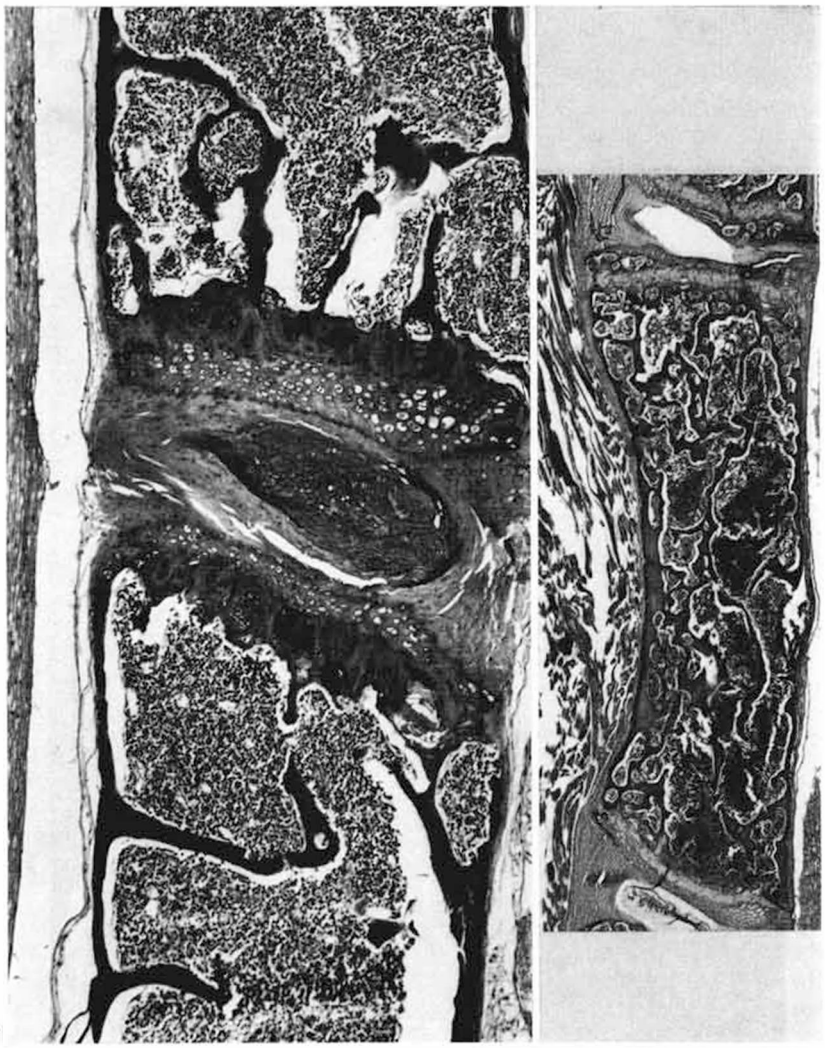


*Fig. 1.* Vertebra of 8-month-old male of strain Dba, given PT at 3-month intervals. Faulty development of chondrocytes in center of growth zone and unresorbed spongiosa in metaphysis. A shallow epiphysis is present. HE. × 105.

*Fig. 2.* Vertebra of 8-month-old male of strain Dba, given PT at 1-month intervals. Osteosclerotic focus in metaphysis. Both end-plates are cartilaginous. HE. × 45.

*Fig. 3.* Portions of two adjoining vertebrae and interposed intervertebral disc of 16-month-old male of strain Dba. The upper vertebra shows one, the lower two transverse bony bars. HE. × 80.

*Fig. 4.* Vertebra of 6-month-old male of strain $C_{57}Bl$ given α-estradiol benzoate from 1 month of age on. Moderate osteosclerosis of vertebral body. HE. × 30.

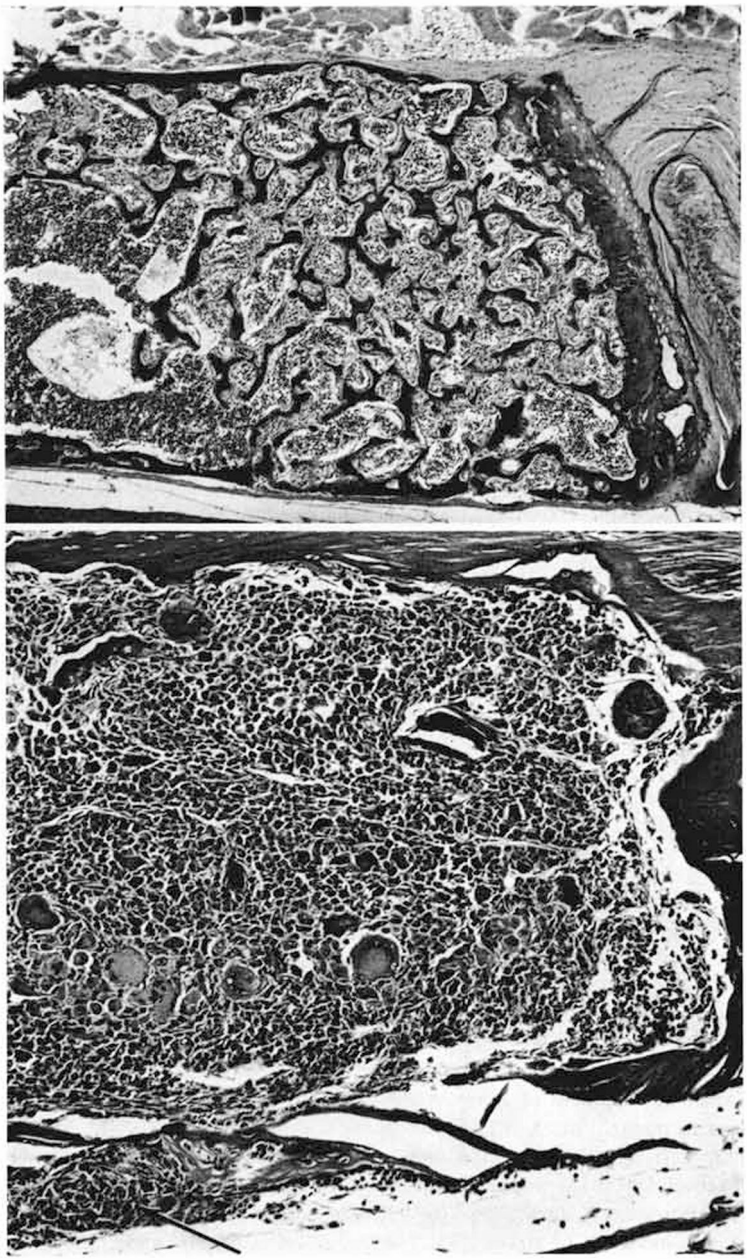


*Fig. 5 and 6.* Legends see p. 17.

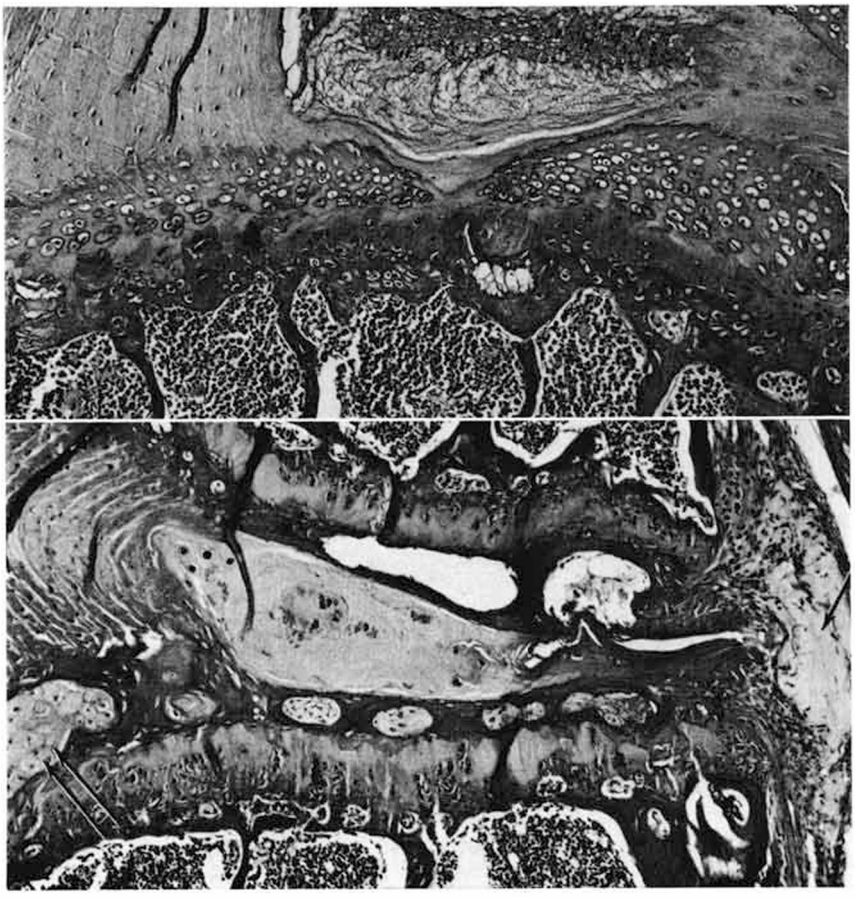


*Fig. 5.* Portion of vertebra of 18-month-old female of strain $C_{57}Bl$, injected with prolactin from 6 weeks to 6 months of age. Osteitis fibrosa, incidental finding. HE. × 55.

*Fig. 6.* Portion of vertebra of 18-month-old female of strain $C_{57}Bl$, injected with STH from 6 weeks to 6 months of age. Pleomorphic sarcoma with many giant cells in diaphysis and invading the spinal canal (arrow). HE. × 130.

*Fig. 7.* Portion of vertebra and intervertebral disc of 15-month-old male of strain Dba, given PT from 1 year of age on. Cartilaginous end-plate overlying the growth zone. A wedge of hyalinized annulus protruding into the end-plate, which is also hyalinized in this area. The underlying part of the growth zone is transformed into a 'plug' of degenerated cartilage. HE. × 115.

*Fig. 8.* Portions of two adjoining vertebrae and interposed intervertebral disc of 18-month-old untreated male of strain $C_{57}Bl$. Prolapse of nucleus pulposus under posterior ligament (arrow) and into the epiphysis (double arrows). Ingrowth of fibroblasts from the perichondrium into the prolapsed hyaline masses at the right. HE. × 87.

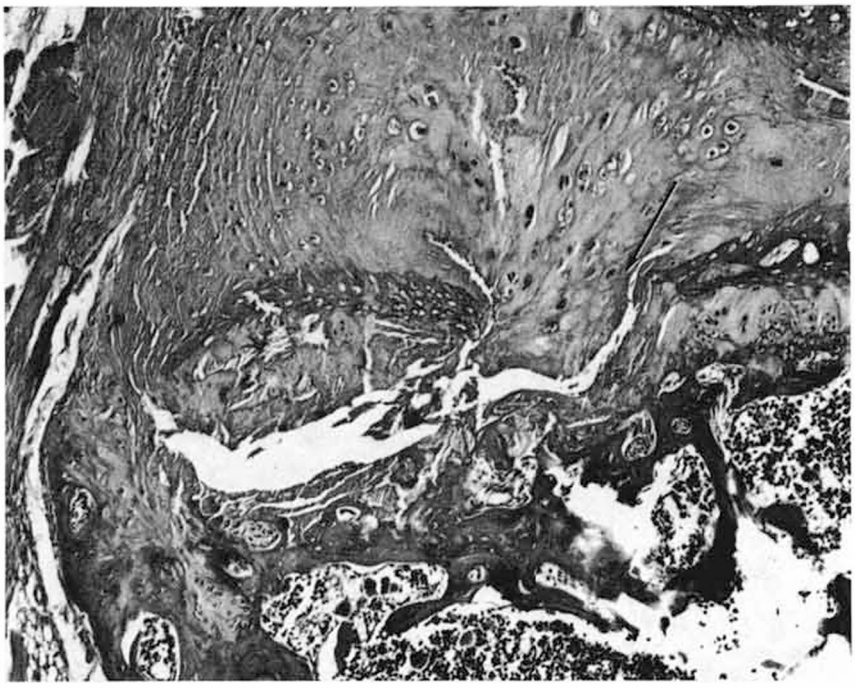


*Fig. 9.* Portion of vertebra and intervertebral disc of 11-month-old untreated female of strain Dba. Prolapse of chondrified disc through the partly ossified end-plate (arrow) into the disrupted growth zone, which shows osteochondrosis-like changes. HE. × 105.

*Changes in nuclei pulposi and annuli fibrosi.* Age-linked hyalinization and chondrification were not conspicuously altered by injections of pituitary hormones, except that mice given prolactin had an unusual number of unaltered discs. A more conclusive retardation of aging changes was noted in discs of mice made hypothyroid by continuous or intermittent consumption of PT from an early age on: these animals had more unchanged discs and relatively more hyalinization and less chondrification than discs of euthyroid controls. Radioactive iodine $^{131}I$ given at 1 year of age did not change the course of the aging of the discs as compared to conditions in euthyroid mice.

Disc prolapse was uncommon, occurring more often in females of strain $C_{57}$-Bl than in any of the other groups. The appearance of prolapsed discs varied considerably: there were well-delimited nodules of nuclear material compressing the spinal cord [16], or poorly defined hyaline masses below the longitudinal ligaments, being organized by surrounding connective tissue (fig. 8) or chondrified material perforating into the epiphyseal marrow space (fig. 8) or disrupting the residual growth zones (fig. 9). The number of prolapsed discs was not altered by any of the experiments.

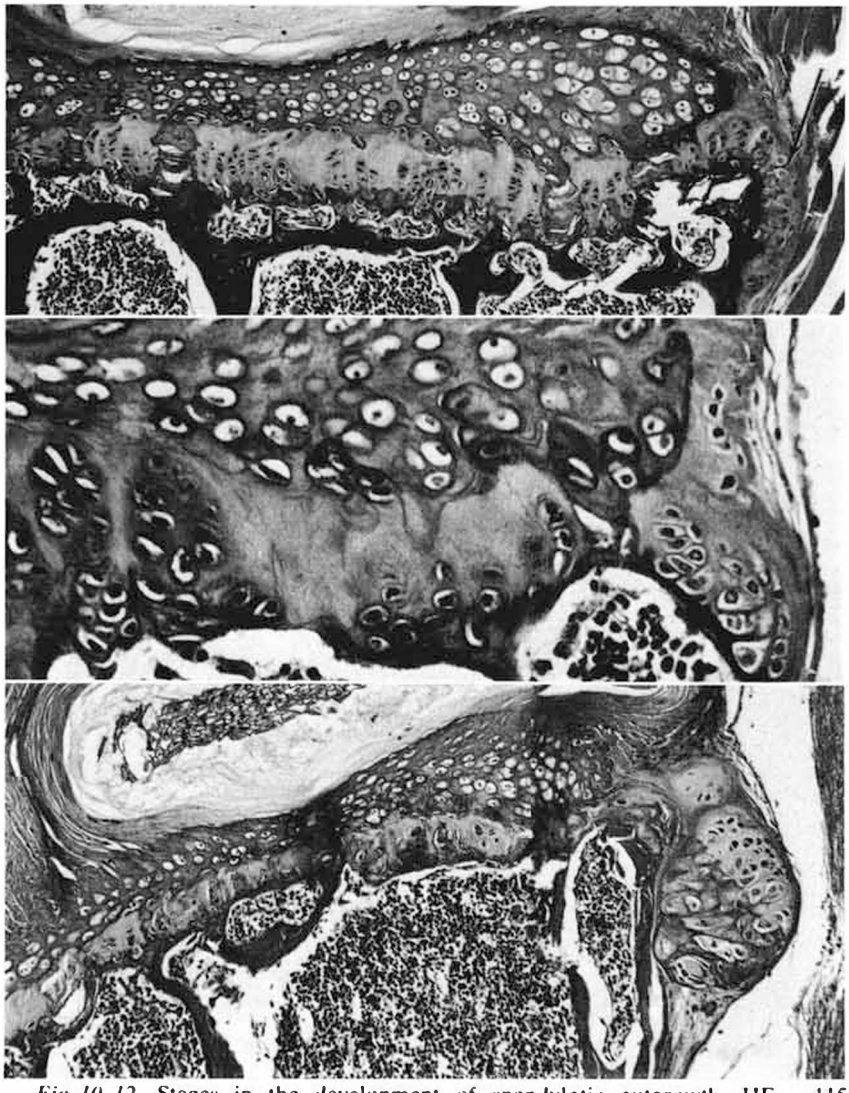


*Fig. 10–12.* Stages in the development of spondylotic outgrowth. HE. × 115. *10* Portion of vertebrae of 18-month-old untreated female of strain $C_{57}$Bl, showing growth zone, end-plate and part of metaphysis. 'Cap-like' extension of the growth zone cartilage over the cortex at the right side of the photograph (arrow). *11* Area similar to that shown in figure 10. 22-month-old male of strain Dba, given PT at 3-month intervals. Beginning proliferation and radial column formation of chondrocytes of 'cap'. *12* Area similar to those shown in figures 10 and 11. 18-month-old female of strain $C_{57}$Bl. Injected with STH from 6 weeks to 6 months of age. Large cartilaginous outgrowth with horizontally oriented columns; slight compression of the spinal cord, a small strip of which appears at the far right of the photograph. Nucleus pulposus is intact with a cluster of chordal cells in the center.

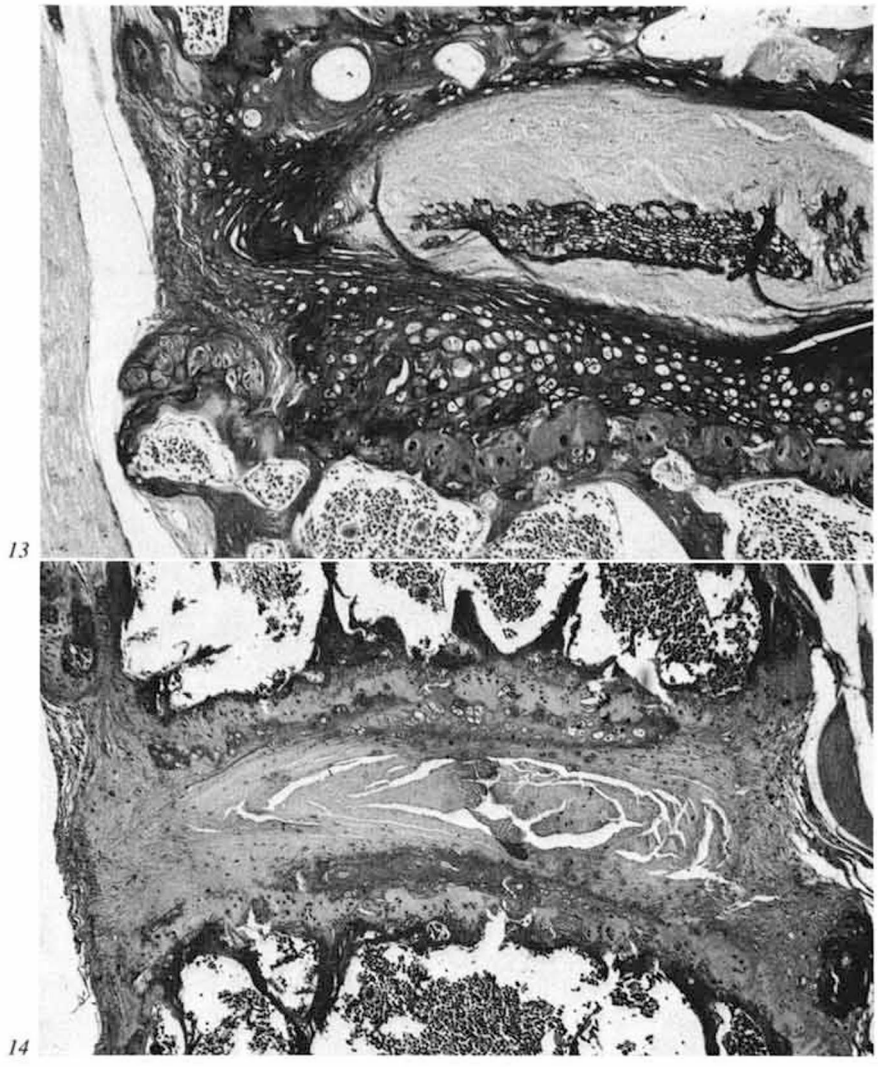
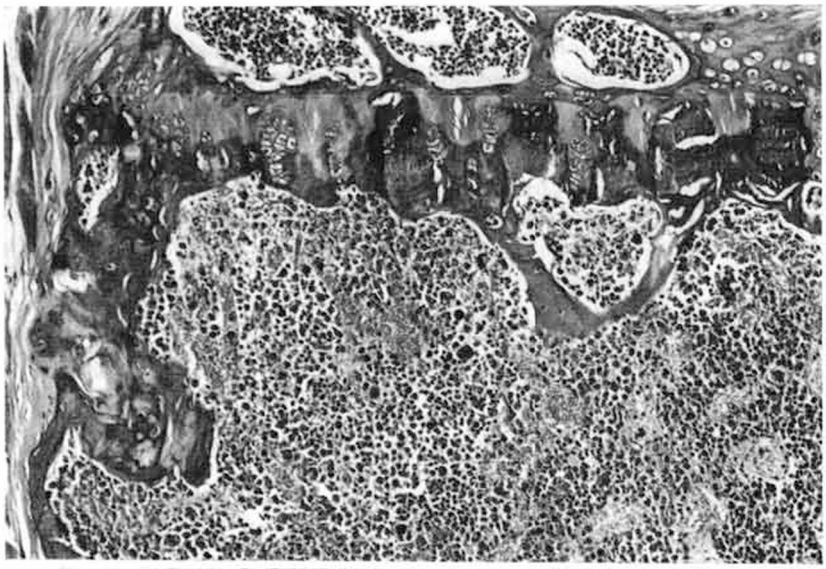


*Fig. 13.* Similar area as in figures 10–12, but including portion of the neighboring vertebra. 18-month-old male of strain $C_{57}Bl$ given prolactin from 6 weeks to 6 months of age. Cartilaginous outgrowth undergoing ossification at the lower vertebral edge, projecting into the spinal canal and slightly compressing the spinal cord, part of which is shown at the far left of the photograph. At the upper left, a smaller cartilaginous outgrowth, also in process of ossification. HE. × 91.

*Fig. 14.* Area similar to those shown in figures 10–13. 20-month-old female of strain $C_{57}Bl$ given PT continuously from 1 year of age on. At the lower right of the photograph, a focus of calcification at the insertion of the annulus at the vertebral margin. At the upper left, a cartilaginous outgrowth undergoing ossification. HE. × 91.



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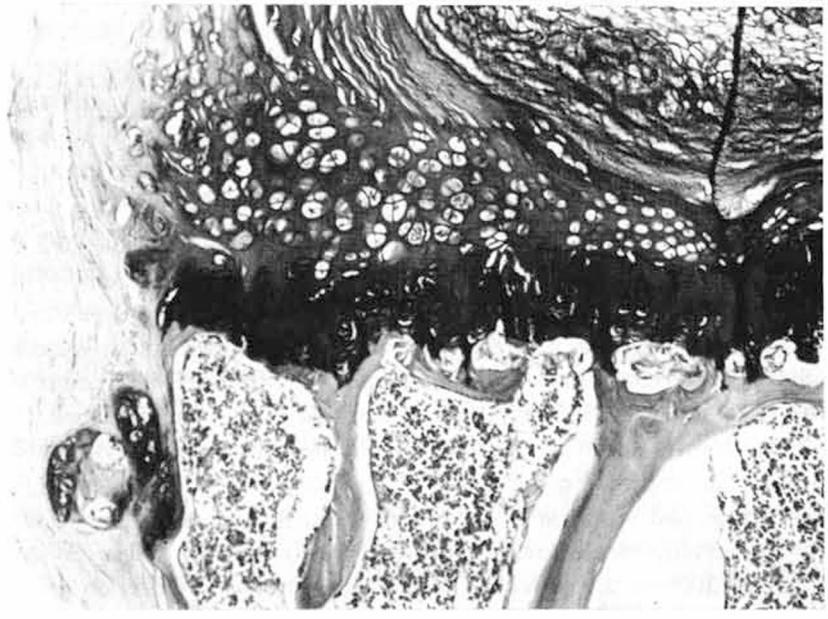

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*Fig. 15.* Portion of vertebra of 15-month-old male of strain Dba, given PT at 3-month intervals. Residual growth zone cartilage in the metaphyseal cortex. HE. × 130.

*Fig. 16.* Area similar to that shown in figure 15. Cartilaginous outgrowth corresponding in location to the residual cartilage shown in figure 15. HE. × 130.

*Spondylosis.* The histogenesis of spondylotic lesions could be traced to several sites and mechanisms. One form, characterized primarily by growth and ossification of cartilage, originated in the cap-like extensions of epiphyseal cartilage along the outside of the cortex of the vertebral body (fig. 10). These cartilage cells may proliferate and align themselves in short columns, which assume radial orientation and are thus set off the vertically oriented columns of the growth zone (fig. 11, 12). In advanced stages, this cartilage undergoes ossification (fig. 13). Residual epiphyseal cartilage is also found at some distance from the growth zone in the metaphyseal cortex (fig. 15) and this cartilage may give rise to nodular outgrowth (fig. 16) into the longitudinal ligaments. In still other instances, spondylotic bars may arise from foci of calcification at the insertion of the annulus fibrosus at the vertebral edge (fig. 14) [15]. Spontaneous spondylosis was first seen in 9-month-old mice of strain Dba and in 18-month-old mice of strain $C_{57}$Bl. In the latter group, spondylosis had proceeded beyond the 'cap' stage only in the presence of a prolapsed disc. Following injections of STH or prolactin, the incidence of the lesions was not significantly changed from that seen in equally old untreated mice; however, the lesions were unusually well-developed even in the absence of nuclear prolapse.

Continuous consumption of PT from 6 weeks of age on, resulted in a decrease of the incidence of spondylosis from 28 to 0% in males of strain Dba. Females of strain $C_{57}$Bl did not survive long enough to reach 'spondylosis age'. Intermittent treatment with PT had a slightly attenuating effect, the decrease in the incidence of spondylosis being from 30 to 15 or 17%, respectively.

Animals treated with estrogenic hormone were killed before they had reached spondylosis age.

## *Discussion*

Lumbar vertebrae of 203 mice of strains $C_{57}$Bl or Dba given STH or prolactin or made hypothyroid with radioactive iodine $^{131}I$ or PT were examined histologically with regard to changes in growth zones, vertebral bodies, intervertebral discs and spondylosis. The findings were compared to those obtained in 163 untreated controls of corresponding ages.

Lesions such as prolapsed discs, osteochondrosis and spondylosis were distinct, but uncommon as they are in other species of mammals [9]. STH, which had significantly increased the incidence, and in male mice also the severity of osteoarthrosis of the knee joint [14] had a less conspicuous effect on the vertebrae of the same animals. Effects on growth zones and epiphyses that might have been present during or soon after treatment were no longer noticeable at 18 months of age, when the animals were killed. At this age, spontaneous aging changes might have caught up with any premature aging produced by the hormone. A slight stimulating effect of STH was noted in the degree of spondylosis, which

was characterized by marked proliferation of chondrocytes originating in residual cartilage near or below the periphery of the growth zones, even in the absence of disc prolapse. The evolution of cartilaginous outgrowths which later on undergo ossification gives a satisfactory explanation for the subepiphyseal insertion of osteophytes, which has been the object of much discussion in the older literature [11, 12]. The present findings give qualified support to the view stressing the cartilaginous origin of the osteophytes [7, 12] as opposed to the hypothesis attributing them to membranous ossification at the fibrous insertion of the annulus fibrosus at the vertebral margin [12]. Proliferation of chondrocytes may be triggered by mechanical irritation following nuclear prolapse and subsequent malalignment of the opposing vertebrae. In the absence of damage to the disc, systemic influences may come into play, as suggested by SCHMORL and JUNGHANNS [12] and GLOOBE and NATHAN [8] and demonstrated in cases of acromegaly [6], and by the attenuation of spondylosis in hypopituitary dwarf mice [17].

In other instances, the spondylotic process seemed to arise in foci of calcification and ossification within the annulus fibrosus, sometimes, but not always close to its insertion at the vertebral margin. This type of outgrowth is thus not endochondral in origin, but still may lead to the formation of conspicuous longitudinal bars and bridges, resembling lesions described in the cervical spine of cats [5]. With respect to location, these lesions then correspond to the view of SCHMORL and JUNGHANNS [12], although histogenetically they are not chondrogenic. PT acting during the growth period and afterwards, exerted more conspicuous effects on the vertebrae than STH. The irregular growth in the growth zones and the retardation of resorptive processes are consistent with the effects of hypothyroidism in the long bones [15]. However, the consistently increased number of complete or incomplete epiphyses in all groups given PT was unexpected and in apparent contradiction to the retardation of development elsewhere in the skeleton. The fact that radioactive iodine injected at 1 year of age had no effect on the epiphyses, whereas PT given from that age on was effective, indicates that the observed increase in epiphyses was not directly or indirectly related to hypothyroidism. Whether or not PT has any extrathyroidal action on the skeleton is unknown; furthermore, as long as the causes for the development of vertebral epiphyses in quadrupeds as opposed to men and hominid apes are unknown [1, 10, 12], it is impossible to offer a well-founded explanation for these observations.

The number of prolapsed discs was not altered in hypothyroid animals, although a change in incidence had to be considered as a possibility in view of the changes in hexosamine levels of cartilage produced by thyroid-stimulating hormone [4].

Lesions corresponding to 'athyroid joint disease' of the knee joint [13] were not observed in vertebrae. Such lesions might still occur in the small vertebral joints, which were not examined during the present investigation. The inhibition of spondylosis by PT is in keeping with a similar but more conspicuous effect on the incidence of osteoarthrosis of the knee joint [14].

The stimulating action of estrogen on skeletal development manifested itself in its large numbers of epiphyses present or forming in 6-month-old animals. Similarly, vertebral osteosclerosis seen after treatment with estradiol corresponds to the reaction of the long bones to this type of treatment.

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