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Enlargement of Growth Plate Chondrocytes Modulated by Sustained Mechanical Loading

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Background: Mechanical compression and distraction forces are known to modulate growth in vertebral growth plates, and they have been implicated in the progression of scoliosis. This study was performed to test the hypothesis that growth differences produced by sustained compression or distraction loading of vertebrae are associated with alterations in the amount of increase in the height of growth plate chondrocytes in the growth direction.

Methods: Compression or distraction force of nominally 60% of body weight was maintained for four weeks on a caudad vertebra of growing rats by an external apparatus attached, by means of transcutaneous pins, to the two vertebrae cephalad and caudad to it. Growth of the loaded and control vertebrae was measured radiographically. After four weeks, the animals were killed and histological sections of the loaded and control vertebrae were prepared to measure the height of the hypertrophic zone (average separation between zonal boundaries), the mean height of hypertrophic chondrocytes, and the amount of increase in cell height in the growth direction.

Results: Over the four weeks of the experiment, the growth rates of the compressed and distracted vertebrae averaged 52% and 113% of the control rates, respectively. The reduction in the growth rate of the compressed vertebrae was significant ($p = 0.002$). In the compressed vertebrae, the height of the hypertrophic zone, the mean chondrocyte height, and the amount of increase in cell height averaged 87%, 85%, and 78% of the control values, respectively, and all were significantly less than the corresponding control values. In the distracted vertebrae, these measurements did not differ significantly from the control values. The height of the hypertrophic zone and the mean chondrocyte height correlated with the growth rate ($r^2 = 0.29$ [$p = 0.03$] and $r^2 = 0.23$ [$p = 0.06$], respectively), when each variable was expressed as a proportion of the control value. The percentage changes in the measurements of the chondrocytic dimensions relative to the control values were smaller than the percentage changes in the growth rates, a finding that suggested that the rate of chondrocytic proliferation was also modulated by the mechanical loading.

Conclusions: Mechanical loading of tail vertebrae in rats modulated their growth rate, which correlated with changes in the height of hypertrophic chondrocytes. The effects of compression were greater than those of distraction.

Clinical Relevance: Information about the growth rate and chondrocytic response to mechanical loads in rat vertebrae undergoing mechanically modulated growth will be helpful in determining how human vertebral growth might respond to altered loading states during progression or treatment of scoliosis and other growth-related angular skeletal deformities.

Mechanical forces modulate the growth of long bones and vertebral growth plates, and they have been implicated in the mode of progression of skeletal deformities such as scoliosis. Progressive deformities in growth plates are commonly thought to be controlled by the Hueter-Volkmann law[1,2], with growth retarded by increased mechanical compression and accelerated by reduced loading of the growth plate in comparison with normal physiological values. However, we are not aware of any studies quantifying this relationship or defining the mechanism of mechanical modulation of endochondral growth.

We investigated the response of rat tail vertebral growth plates to sustained axial mechanical loading. Increases in the lengths of long bones and in the heights of vertebrae are generated by proliferation of the growth plate chondrocytes, their enlargement in the growth direction, and synthesis of matrix that eventually calcifies[3-10]. A several-fold enlargement of hypertrophic chondrocytes has been implicated as a key variable

correlating with differences in the growth rate at different anatomical sites. Investigation of normal and pathological growth states of growth plates in different anatomical locations by Wilsman et al.[11] revealed that 40% to 50% of the difference between the growth rates of different growth plates is explained by differing degrees of hypertrophy. The remaining variability was due to matrix synthesis (explaining about 30% to 40% of the difference) and the rate of chondrocyte proliferation (explaining about 10%). A study by Alberty et al.[12] indicated that mechanical loading of a growth plate probably has a small effect on the rate of chondrocytic proliferation.

The purpose of the present study was to test the hypothesis that growth rate differences produced by sustained mechanical loading of rat tail vertebrae are associated with alterations in the increase in the height of hypertrophic chondrocytes in the direction of growth.

Materials and Methods

With use of a previously described rat tail model[2], the seventh caudad vertebra of Sprague-Dawley rats that weighed about 125 g at the beginning of the experiments was compressed (six animals) or distracted (six animals) with a force of nominally 60% of body weight for four weeks. The loads were applied by an external apparatus that was attached, with the animal under general anesthesia, to vertebrae on either side of the experimental level by means of transfixing pins that were glued to rings placed around the tail (Fig. 1). Postoperative analgesia was administered after the animals had recovered from the general anesthesia. Force was applied by compression of calibrated springs acting on the cephalad side of the caudad ring for distraction and acting on the caudad side for compression. Spring lengths (and hence the forces generated) were set by adjusting the positions of nuts on the threaded rods that were passed through each spring. The spring lengths were adjusted twice each week to maintain the magnitude of the applied load at nominally 60% of body weight. These adjustments were necessary because growth of the loaded vertebrae decreased the spring lengths in the case of compression loading and increased them in the case of distraction loading. Also, corrections of the force magnitude were required to allow for the increasing body weight of the growing animals.

The tail of each animal was radiographed each week with use of fine-resolution film (Microvision; DuPont, Wilmington, Delaware) with controlled positioning of the animal relative to standardized tube and film positions to ensure constant known magnification of the dorsal-ventral images. Animals were fed standard laboratory chow, and they were kept in an artificial-light-cycle environment with twelve hours of light and twelve hours of dark. After four weeks of loading, the animals were killed and the loaded vertebrae and adjacent control vertebrae (the fifth and ninth caudad vertebrae) were excised and fixed immediately. Live-animal procedures were performed at approximately the same time of day on each animal. These procedures were reviewed and approved by the Institutional Animal Care and Use Committee.

The vertebra lying between the two pinned vertebrae was considered to be the loaded vertebra, and the vertebrae cephalad and caudad to the pinned vertebrae were used as controls. The growth rate of the vertebrae was measured from growth

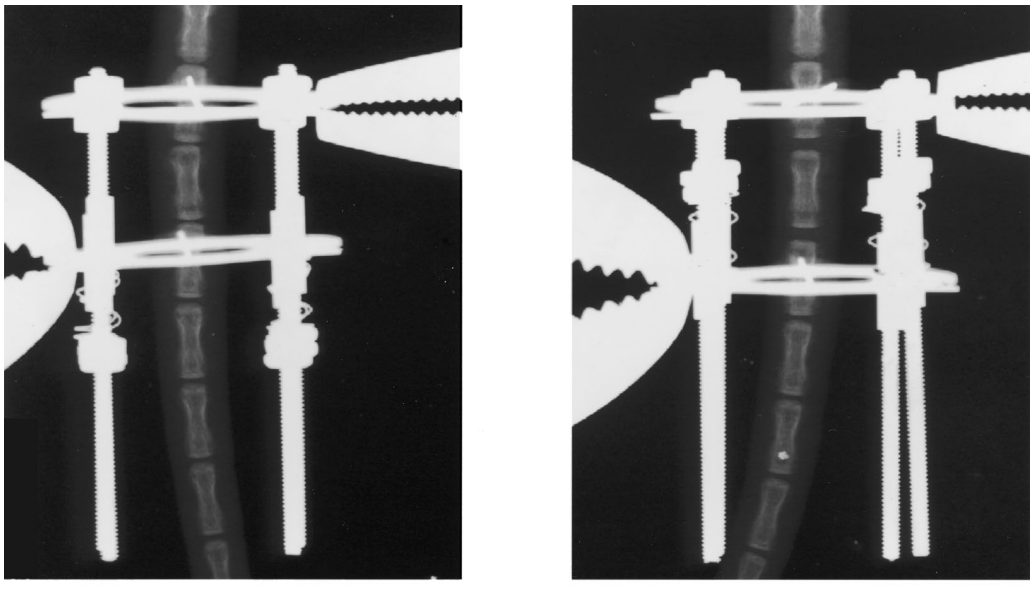

Fig. 1

Radiographs of a rat tail with the apparatus applying compression (a) and distraction (b) to the vertebra that lies between the instrumented vertebrae. The vertebrae caudad and cephalad to the apparatus were used as controls. The clamps were used to prevent motion during radiography.

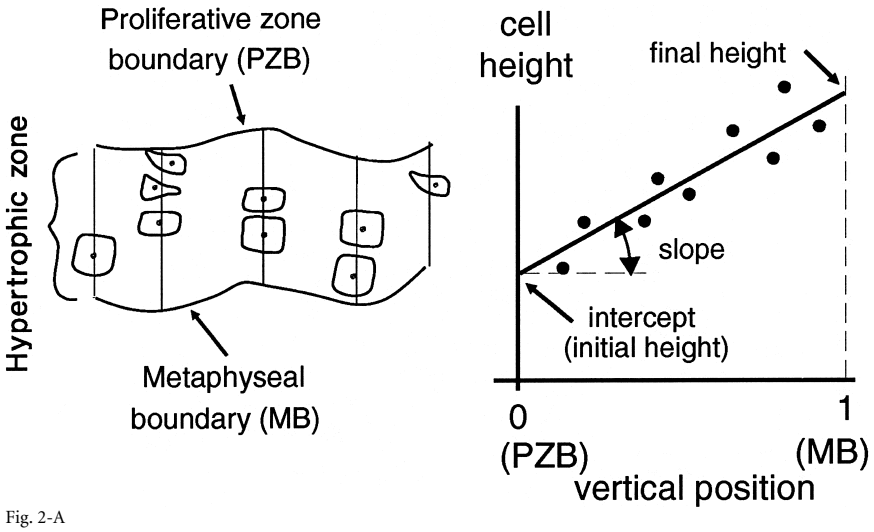

Fig. 2-A

Diagrammatic representation of the measurement of the height of the hypertrophic zone and the heights of chondrocytes. The initial and final chondrocytic heights were estimated from the regression relationship between cell height and normalized vertical cell position at the upper and lower zonal boundaries, respectively.

curves obtained over the duration of the four-week experiment. The positions of the ends of each loaded and control vertebra on the weekly radiographs were determined and recorded with use of a digitizing tablet and custom software[2]. The growth rate was measured as the gradient of the linear regression of the vertebral length with time and was expressed in micrometers per day. This growth occurred at the two growth plates of each vertebra, both of which were loaded by the apparatus in the loaded vertebrae and both of which were not loaded in the control vertebrae.

The vertebrae were fixed, with the method described by Hunziker et al.[13], in 2% glutaraldehyde-2% paraformaldehyde in 0.05M cacodylate buffer (pH 7.3) with 0.7% ruthenium hexamine trichloride for five hours, rinsed in 0.1M cacodylate buffer (pH 7.3), dehydrated in graded alcohols, cleared in propylene oxide, and embedded in Epon-812 (Electron Microscopy Sciences, Fort Washington, Pennsylvania). Parasagittal plane sections (1 to 4 μm thick) in the midsagittal plane region of the vertebrae were cut at 100-μm intervals and stained with methylene blue and basic fuchsin. Four sections of each growth plate were selected for measurement. A digital image of the dorsal and ventral part of each section was captured by the microscope's digital camera, with use of a 4× objective lens, for a total of eight images of each growth plate. Each image was measured with use of custom image-digitizing software written in MATLAB (MathWorks, Natick, Massachusetts), with use of a "mouse" pointing device to select image points. Both the caudad and the cephalad growth plates of each loaded vertebra were measured, along with equal numbers of control growth plates. The controls were the caudad growth plates of the fifth caudad vertebrae and the cephalad growth plates of the ninth vertebrae. In subsequent histological analysis, all of the measurements of the two growth plates in each loaded vertebra were averaged, and the measurements of the cephalad growth plates of the two control vertebrae were averaged. The histological measurements were compared with the average growth rate of the two control vertebrae. This was intended to compensate for the expected lesser growth rate of more caudad vertebrae.

The height of the hypertrophic zone in micrometers was measured as the average separation between the boundaries of the hypertrophic zone. The upper boundary was identified by the upper margins of the first chondrocytes that had increased size relative to the proliferating cells in the proliferative zone. The lower boundary was identified by the lower margins of the terminal intact chondrocytes on the metaphyseal side. Up to thirty points on

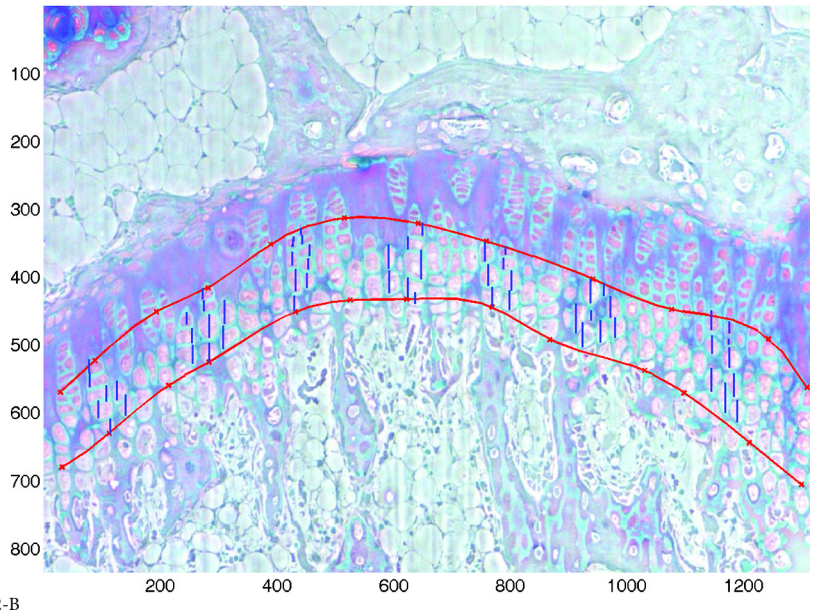

Fig. 2-B

Sample histological image with lines marking the boundaries of the hypertrophic zone as well as the heights of selected hypertrophic chondrocytes. The scale units are pixels, with one pixel equal to 0.49 μm.

TABLE I Vertebral Growth Rate and Four Measures of Growth Plate and Hypertrophic Chondrocytic Dimensions*

Vertebra	Growth Rate	Zonal Height	Increase in Cell Height	Initial Cell Height	Mean Cell Height
Compressed (n = 6)	35.4 ± 14.3 μm/day	73.9 ± 0.6 μm	11.6 ± 3.37 μm	10.6 ± 2.36 μm	15.8 ± 2.08 μm
Control (n = 6)	68.3 ± 24.2 μm/day	85.1 ± 5.2 μm	18.6 ± 2.8 μm	12.0 ± 2.9 μm	18.6 ± 2.8 μm
Compressed as percentage of control	52.1% ± 9.88%	86.9% ± 11.6%	77.7% ± 18.5%	89.7% ± 13.0%	85.4% ± 3.1%
Distracted (n = 6)	74.9 ± 20.5 μm/day	82.7 ± 18.6 μm	14.8 ± 1.64 μm	12.2 ± 2.61 μm	19.0 ± 3.10 μm
Control (n = 6)	66.2 ± 12.4 μm/day	81.5 ± 6.0 μm	19.6 ± 2.0 μm	12.6 ± 2.1 μm	19.6 ± 2.0 μm
Distracted as percentage of control	113% ± 15.9%	102% ± 22.2%	97.4% ± 16.5%	96.6% ± 14.7%	96.7% ± 10.1%

*The values are given as the mean and standard deviation.

each boundary were digitized, and the spline function of MATLAB was used to interpolate a smooth curve through these points. Zonal height was measured as the average separation of these lines in the growth direction. In these and subsequent analyses, the longitudinal axis of the vertebra was considered to be the direction of growth, the epiphyseal side of the growth plate was considered to be the top of the growth plate, and the diaphyseal side was considered to be the bottom of this axis system.

The amount of increase in the chondrocytic height was measured with use of regression analysis of cell heights with respect to their normalized position within the hypertrophic zone (Figs. 2-A and 2-B). A minimum of fifty cells was selected for measurement in each image. The height of each selected cell was measured by digitizing its upper and lower margin. The cell's position as a proportion of the distance between the zonal boundaries was defined by its midpoint relative to the interpolated boundary lines, in the axial direction. The increase in cell height with respect to position was calculated as the slope of the linear regression of cell height versus normalized position in the hypertrophic zone (Figs. 2-A and 2-B). Since the position was normalized, this slope was an estimate of the amount of increase in chondrocytic height, in micrometers, occurring while a chondrocyte was in the hypertrophic zone. The intercept of this regression relationship was used as a measure of the initial height of the hypertrophic chondrocytes. The mean cell height was the average height of all measured cells in the section. The measurements from at least six images of each growth plate from a minimum of three sections separated by at least 100 μm were obtained, and they were averaged for each growth plate. One growth plate from one compressed vertebra was lost in processing.

In the statistical analysis, the growth rates of the loaded vertebrae were first compared with the corresponding within-animal control values with the use of two-sided paired t tests. Subsequently, the differences between the histological measurements for the loaded and corresponding within-animal control growth plates were analyzed for significance within treatment groups (compression or distraction) with use of two-sided paired t tests. Relationships between growth rate and histological measurements were tested for significance by correlation analysis to determine whether differences in growth rates between vertebrae were associated with variations in the histological measurements. In order to separate the effects of the mechanical loading, additional correlation analyses of growth rates and histological measurements, both expressed as a proportion of the corresponding control values, were performed for the loaded vertebrae. In these analyses, it was assumed that much of the between-animal variability was reduced by this normalization, and the effects of mechanical loading were predominant in the residual variation. In all statistical tests, a p value of <0.05 was considered significant.

Results

Radiographic measurements showed that, over the duration of the experiment, the distracted and compressed vertebrae grew at an average (and standard deviation) of 74.9 ± 20.5 and 35.4 ±14.3 μm/day, respectively (Table I). These values corresponded to a mean of 113% and 52%, respectively, of the growth rates of the corresponding control vertebrae (average pooled growth rate, 67.3 ± 18.4 μm/day). The average growth rate of the compressed vertebrae was significantly less than that of the control vertebrae ($p = 0.002$, two-sided paired t test), but that of the distracted vertebrae was not significantly different from the control value ($p = 0.12$, two-sided paired t test). The averaged growth curves of the distracted, compressed, and control vertebrae are shown in Figure 3. Weekly measurements of spring lengths (adjusted with a target level of 60% of body weight) showed that the loading of the compressed vertebrae averaged 60.5% of body weight and the loading of the distracted vertebrae averaged 53.2% of body weight over the duration of the experiment.

In the compressed vertebrae, the average zonal height, increase in cell height, and mean cell height were 87%, 78%, and 85%, respectively, of the control values (Table I); all of these reductions were found to be significant ($p = 0.045$, $p =$

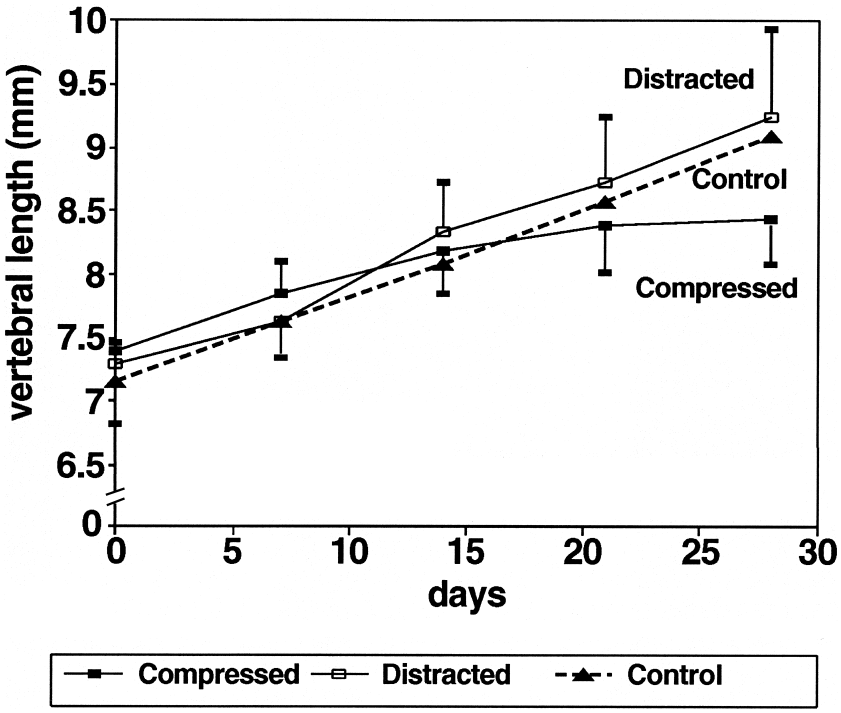

Fig. 3

The mean lengths of the compressed, distracted, and control vertebrae as measured on radiographs made every seven days.

0.032, and $p < 0.001$, respectively). With the numbers available, the values for the histological measurements of the distracted vertebrae were not significantly different from the control values ($p = 0.88$, 0.54, and 0.49, respectively). The initial chondrocytic height did not differ significantly from the control value in either the compressed vertebrae ($p = 0.09$) or the distracted vertebrae ($p = 0.62$). The percentage changes, relative to the controls, in the hypertrophic chondrocyte and growth plate dimensions were smaller than the percentage changes in the growth rates, which were 52% and 113% of the control growth rates.

The zonal height, increase in cell height, and mean cell height all correlated positively with the growth rate ($r^2 = 0.42$, 0.36, and 0.49, respectively). When these variables were expressed as a proportion of the control values, the zonal height and the mean cell height both correlated positively with growth rate ($r^2 = 0.29$ [$p = 0.03$] and $r^2 = 0.23$ [$p = 0.06$], respectively). These correlations suggest that the variation in growth rate that was due to mechanical loading was associated with the alterations in zonal height and chondrocytic height in the zone of hypertrophy.

Discussion

Mechanical loading of rat tail vertebrae modulated their growth rate relative to that of control vertebrae. The longitudinal growth of vertebrae occurs in growth plates at both ends of the vertebral body[14]. Previous experimental studies of rat tail vertebrae showed that their growth was modulated by mechanical loading[2] and that angular deformities could be created and subsequently corrected by combined angulation and compression causing asymmetrical loading[15]. In the latter study[15], fluorochrome labeling confirmed that the wedging was produced by asymmetrical growth in the growth plates, and not by diaphyseal remodeling.

The magnitude of the significant effect of compression on growth rate (a mean of 32.9 μm less growth per day compared with that of the control) was observed to be greater than the nonsignificant complementary effect of distraction (a mean of 8.7 μm more growth per day compared with that of the control). The observed difference in the distracted vertebrae was not large enough to be significantly different from zero. On the basis of the observed variability, a retrospective calculation indicates that differences of >16.56 constitute the range of detectable effect sizes with a power of 80%. The absence of significant histological differences between these growth plates and the controls also suggested that there was minimal response to distraction.

The mean chondrocyte height and the height of the hypertrophic zone correlated with the growth rate of the loaded vertebrae. This finding provides experimental evidence that the amount of enlargement of the hypertrophic cells in the direction of growth together with a change in the height of the hypertrophic zone are important factors in the mechanical modulation of growth. However, the difference in the growth rate was not fully explained by these effects, suggesting that other aspects of growth plate performance were also affected by the mechanical load. The rate of new cell production in the proliferative zone and of matrix production might also have been altered, but they were not directly measured in this study.

Variation in the growth rates of the growth plates included a component due to mechanical modulation of growth as well as variability associated with differences between indi-

vidual animals. The fact that a correlation was found between histological and growth rate measurements that were expressed as a proportion of control values is an indication that the differences in growth rate produced by mechanical loading were caused in part by changes in growth plate activity as reflected by these measures of chrondocytic enlargement.

There have been clinical reports of mechanical influences on vertebral growth. Gooding and Neuhauser[16] reported "tall vertebrae" in patients with paralysis and also in younger patients who had been treated surgically with posterior fusion of the spine. They argued that the relative unloading of the spine increased longitudinal growth. However, this theory was contested by Taylor[17], who found that the vertebral bodies were of normal height in sixty sedentary patients with cerebral palsy who were unable to walk. McCall et al.[18] reported that three patients with idiopathic scoliosis who had been treated with long-term immobilization in a plaster cast had increased vertebral height and thinner discs. Roaf[19] hypothesized that a "vicious circle" develops in the progression of scoliotic deformity. Assuming that the concave side of a curve is subjected to greater compression load, he proposed that this decelerates growth of the vertebral bodies compared with that on the convex side. Growing long bones have been shown to be sensitive to mechanical loading in compression[20,21], distraction[21,22], torsion[23], and bending without evidence of a threshold level of load[20]. However, the relationships between mechanical loading and growth rate are not well quantified. Also, the mechanisms responsible for mechanical modulation of growth are poorly understood and appear to differ from the mechanisms by which mechanical loading influences remodeling of mature bone.

It should be noted that, in the present study, vertebral growth was measured over the course of four weeks but the histological measurements of the growth plate were performed at the termination of the experiment. The data in Figure 3 indicate that growth of the distracted and control vertebrae occurred at an almost constant rate over the four weeks, whereas there was an apparent reduction in the growth rate of the compressed vertebrae. Therefore, we may have overestimated the final growth rate of the compressed vertebrae by averaging the values over the four-week period.

It is unclear why the growth rates of the vertebrae were affected so much less by distraction than by compression. Part of the reason may be that the externally applied compression forces were transmitted more directly to the growth plates, whereas the distraction forces were shared by parallel soft-tissue structures. However, the magnitude of the difference between the responses suggests that the growth plates were inherently more responsive to compression than to distraction forces. Some of the distraction force applied to the tails might have been carried by tension in parallel soft-tissue structures such as tendons. However, the widening of the disc space, such as is evident in Figure 1(b), indicates that the discs and presumably also the growth plates were subjected to tension forces. No qualitative differences among the compressed, distracted, and control growth plates were obvious on subjective examination of the micrographs.

A number of factors must be considered when attempting to infer how human vertebrae might behave under similarly altered loading conditions. In humans, the different forces as well as the different areas on which the forces act would influence the biomechanical stresses. In addition, the anatomical differences in growth plate height and physiological growth rate should be taken into account. Still, this study of tail vertebrae of rapidly growing rats gives some indications as to the variables that determine the response of growth plates to altered sustained loading as well as the relative contributions of these variables. ■

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In support of their research or preparation of this manuscript, one or more of the authors received National Institutes of Health Grants R01 AR 46543, R55 HD 34460, and F32 AR 08453 and a Bristol-Myers Squibb/Zimmer Institutional Excellence Grant. None of the authors received payments or other benefits or a commitment or agreement to provide such benefits from a commercial entity. No commercial entity paid or directed, or agreed to pay or direct, any benefits to any research fund, foundation, educational institution, or other charitable or nonprofit organization with which the authors are affiliated or associated.

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