The mechanical consequences of vertebral centra

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In spite of their name, the first vertebrates evolved without vertebrae[2]. Both phylogenetic reconstruction and fossil record agree that the hypothetical vertebrate ancestor possessed a continuous, unsegmented notochord as its axial skeleton[8]. Cartilaginous and bony neural arches and spines next appeared as segmental elements in the vertical septum. Only in jawed fishes did vertebral centra make their first appearance, forming as segmental ossifications of the cylindrical walls of the collagenous notochord sheath[4]. With centra, intervertebral joints were created, leaving, between adjacent bony elements, unmineralized connective tissues bearing most of the axial strain during body bending. Thus, from a mechanical perspective, the evolutionary origin of vertebral centra is really the origin of intervertebral joints. Our goal is to develop an experimental system that allows us to examine the biomechanical consequences of creating joints and reducing the axial length of those joints.

The notochord of adult Atlantic hagfish, *Myxine glutinosa*, lacks any cartilaginous or osseous elements[3]. Previously, we have characterized the swimming of hagfish and the dynamic mechanical properties of their notochords, finding strong indirect evidence that the notochord is the primary stiffener of the body and is mechanically tuned to function during undulatory swimming[5]. In this paper, we describe a new method in which we take an excised hagfish notochord and add artificial vertebral centra to it (Fig 1). We can adjust the axial length of the intervertebral joints and hence measure the influence of the amount of joint present on the axial skeleton's mechanical properties.

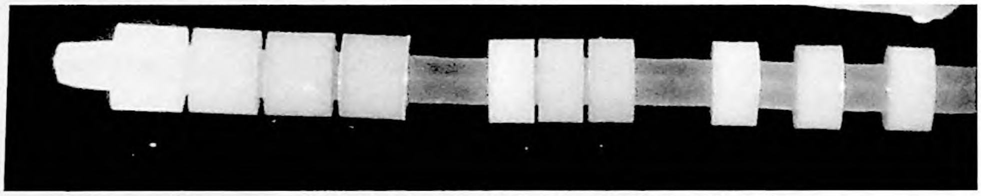

Fig. 1. Artificial vertebral column. Hagfish notochord (inner core) with 'vertebral centra' of rigid nylon. Spacing of centra was adjusted to examine joint length effects.

A hagfish of 46.0 cm total length, 88.52 g total mass was killed with an overdose of propylene phenoxetal. We dissected the intact axial skeleton from the body, and then carefully removed the notochord's outer fibrous sheath with a scalpel. This procedure left the inner core (Fig. 1). The core was bathed continuously in hagfish Ringer solution[7] throughout the experiments.

According to simple beam theory, in pure bending flexural stiffness, EI (Nm^{-2}), is the constant of proportionality between an applied bending moment, M (Nm), and the resulting curvature, κ (m^{-1})[9]:

$$M = EI\kappa \tag{1}$$

where E is the material or shape-independent stiffness (Pa) and I is the second moment of area (m^4), a shape factor that accounts for the cross-sectional distribution and the amount of material. Since, during experiments, the test section was held at constant length and constant overall maximal curvature (108 m^{-1}) in the bending machine (Fig. 2), it follows that the presence of centra will amplify the curvature density, κ^* (m^{-2}), the amount of curvature per unit length of joint. Since the local curvature at the joints increases, we predict, from equation 1, that the moment, M, will increase, causing an

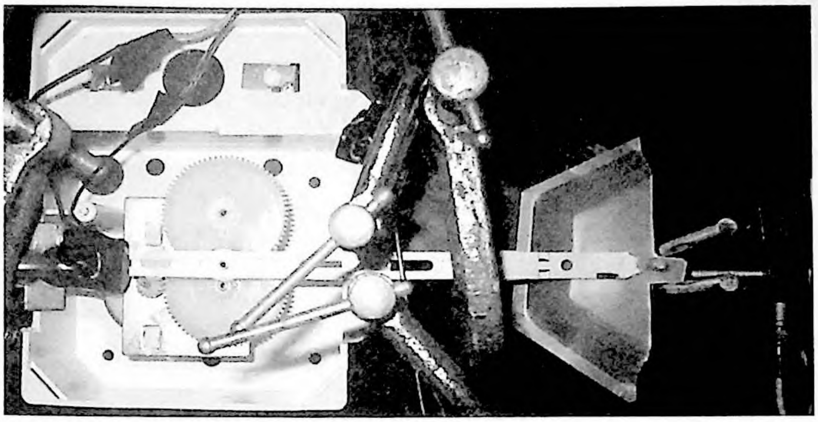

Fig. 2. Dynamic three-point bending machine. Motor-driven gears create a reciprocating translational motion transferred to loading rod (long, white bar). The loading rod presses on the vertebral column (not shown), which rests on two stationary, vertical supports (bars in oblique perspective to the right) that are rigidly attached to a force sensor. Displacement amplitude of the rod is 4.5 mm. Distance between vertical supports is 12.5 mm. Frequency of bending is 1.0 Hz. Artificial vertebral columns had three centra, creating two intervertebral joints that were bent.

increase in the test section's apparent material stiffness, E (note that the overall section curvature, κ, and moment of area, I, in Eq. 1, will remain constant).

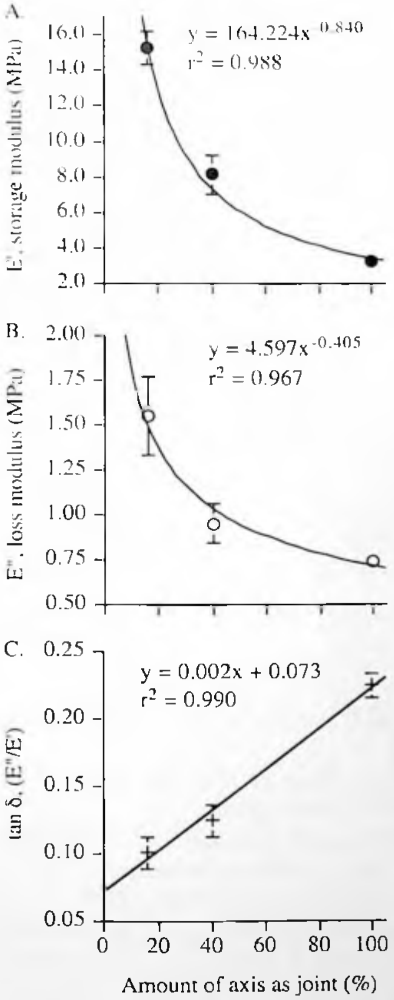

Fig. 3. Mechanical properties of the axial skeleton vary with joint length. 100% = the notochord without centra. Lower percentages indicate the presence of artificial centra with decreasing joint length. Error bars = standard error of mean.

We bent a 12.5 mm segment of the notochord at a sinusoidal frequency of 1.0 Hz with a curvature amplitude, κ_0, of 108 m^{-1}. Artificial centra were nylon spacers (item # RSN-04/02-C, Small Parts, Inc.) of 3.3 mm length, 5.0 mm outer diameter, and 3.0 mm inner diameter. Three centra were added to the 12.5 mm test section of the notochord, which had a diameter of 3.1 to 3.2 mm. Since centra slightly compressed the notochord they did not slip. The gap, or joint length, between centra were of three conditions: (1) notochord or a continuous joint of 12.5 mm length; $\kappa^* = 8.64$), (2) two joints of 2.5 mm length each (40% of section length; $\kappa^* = 21.6$), and (3) two joints of 1.0 mm length each (joint 16% of section length; $\kappa^* = 54.0$). Experiments were monitored to insure that centra never touched and that the two outer centra remained on the vertical support bars. The displacement of the loading bar and the force on the supports were measured continuously at 50 Hz using A-to-D converter (LabPro, Vernier Inc.). From those records, the phase lag, δ, between displacement and force was measured for the five cycles following an initial five used for preconditioning. From the phase lag and the amplitude of the force, the storage, E', and loss, E", moduli (Pa) were calculated according to standard methods[9]. E' and E" represent material (shape-independent) elastic stiffness and material viscous damping, respectively. The ratio of the loss to the storage modulus is tan δ.

The addition of artificial centra increased the storage modulus by a factor of five (Fig. 3A) and increased the loss modulus by a factor of two (Fig. 3B). Power fits show that the effect was dramatic at low amounts of joint, when vertebral centra were present and tightly spaced (1 to 2.5 mm). Over the same interval, tan δ increased, showing that the relative proportion

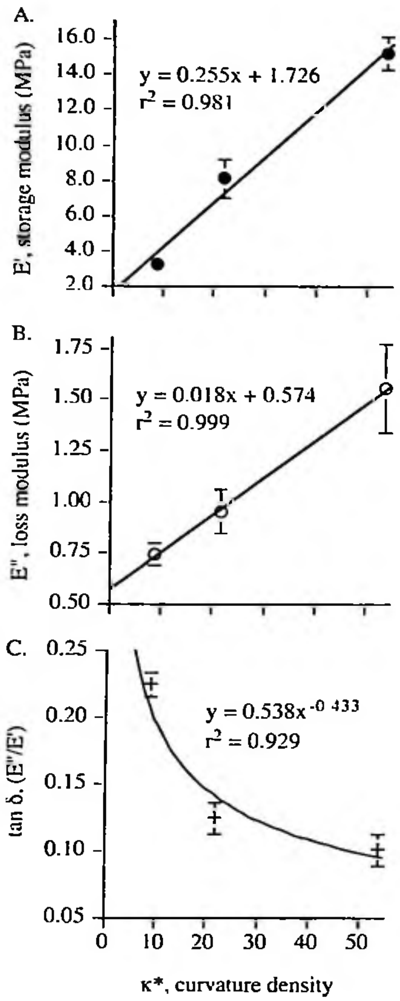

Fig. 4. Mechanical properties of the axial skeleton vary with curvature density. Notochord has a curvature density of 8.64. Higher densities indicate the presence of artificial centra with decreasing joint length. Error bars = standard error of mean.

of damping loss decreased with the addition of centra and the reduction of joint length (Fig. 3C). When the same properties were viewed as a function of curvature density, κ*, both storage and loss moduli increased with greater curvature density, which, in turn, increased when centra were present and joint lengths were reduced (Fig. 4A and B). Note that these relations were linear, whereas tan δ decreased in curvilinear fashion (Fig. 4C).

While care must be taken in interpreting data from a single individual, we can draw two tentative conclusions. First, we have created a viable method for making artificial vertebral columns from hagfish notochords. Artificial vertebral columns have the advantage over real ones of offering morphological variation without the confounding influences of phylogenetic differences. Second, our preliminary data support the prediction that vertebral centra amplify curvature at intervertebral joints and, in so doing, increase the axial skeleton's stiffness and damping. This mechanistic result supports two previous findings: (1) the number of vertebrae is inversely proportional to the magnitude of body curvature in fast-starting fish[2], and (2) the number of vertebrae is inversely proportional to the minimum propulsive wavelength of the undulatory body in steadily-swimming vertebrates[6].

Since mechanical properties control how the body reconfigures under muscular and hydrodynamic loading[6], the evolution of vertebral centra in early jawed vertebrates was likely to have been driven, in part, by selection for different kinds of locomotor behavior.

This research was funded by the National Science Foundation (BCS-0320764 to JHL) and Shriners Hospital for Children (#8610 to TJK).

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