HYDROSTATIC PRESSURE VARIATIONS WITHIN THE HAGFISH (*MYXINE GLUTINOSA)* NOTOCHORD

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The goal of our work on *Myxine glutinosa* has been to determine the biomaterial and biomechanical behaviors of the notochord and to ascertain the biochemical basis of these properties (Koob T.J., et al., *Bull. MDIBL* 33: 5-8, 1994; Kielstein, J.T. et al., *Bull. MDIBL* 35: 105-107, 1996; Long, J.L., Jr., et al., *Bull. MDIBL* 37: 114-116, 1998; Sinwell, B.J., et al., *Bull. MDIBL* 38: 94-96, 1999). Previously, we identified a functional connection between the notochord's biomaterial and biomechanical behaviors and the osmolarity of the fluid in which the notochord is bathed. The core contents of the notochord are osmotically active (Koob, T.J., et al., op. cit.), and volumetric changes of isolated, intact notochord are inversely proportional to external salt concentration (Kielstein, J.T., et al., op. cit). Also, the notochord's flexural stiffness, EI (Nm^2), apparent material stiffness, E (Nm^{-2}), curvature-specific bending work, $W\kappa^{-1}$ (J·m), and resilience (% energy return) in lateral bending all change in inverse proportion to the external salt concentration (Sinwell, B.J., et al., op. cit). Missing, however, is an understanding of how osmotic changes influence the hydrostatic pressure within the core of the notochord. Since hydrostatic pressure determines, in part, the flexural stiffness of notochords in frog embryos (Adams & Koehl, *Development* 110: 131-139, 1990) and sturgeon (Long, *Environ. Biol. Fishes* 44: 199-211, 1995), we sought, in hagfish, answers to the following questions — what is the notochord's internal hydrostatic pressure, and does that hydrostatic pressure vary with ges in the external osmotic environment?

Hydrostatic pressure measurements were obtained via polyethylene 90 (ID = 0.86mm, OD = 1.27mm; Beckton Dickinson) tubing connected to a physiological pressure transducer (Gould, model T-P231D). The pressure cannula (tubing) was filled with commercially available vegetable oil that had been flushed free of air bubbles. The transducer was attached to the input of a bridge amplifier (Omega Engineering, model DMD 520 MD, full-bridge configuration). The amplifier output signal was transferred simultaneously to a digital oscilloscope (Tektronix, model 2201) and a data acquisition board (National Instruments, model SCB 68), connected to a G3 Macintosh computer, that was controlled using LabView software (National Instruments, ver. 5.0). The pressure transducer was calibrated using a standing column of water of varying heights. The product of water height, water density and gravitational acceleration yields a known hydrostatic pressure.

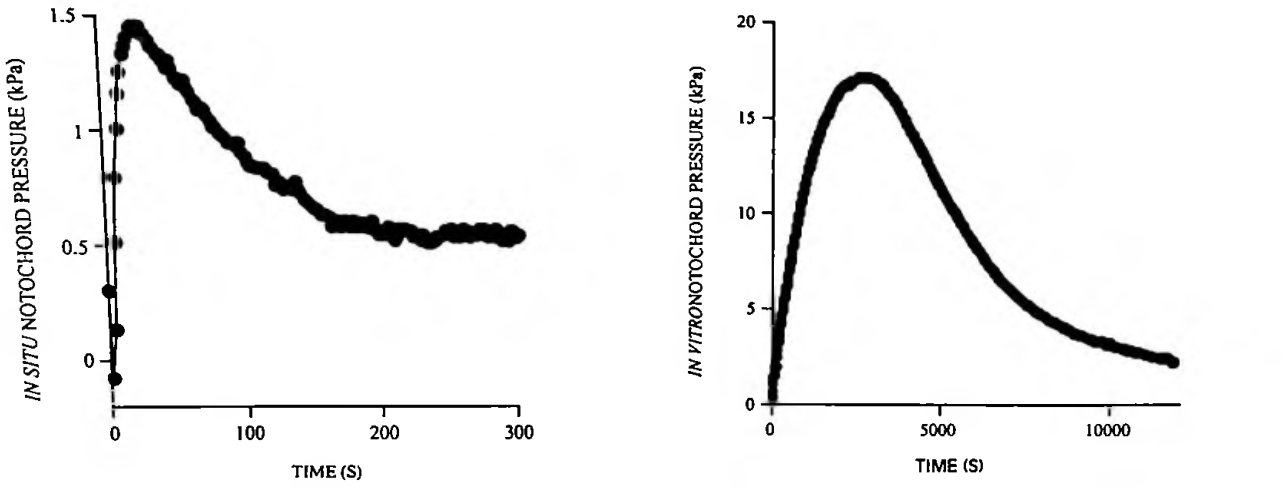
For *in situ* pressure measurements, four hagfish (mean weight 42.0 ± 5.03 g; mean length 36.4 ± 1.09 cm) were euthanized with an overdose of propylene phenoxetal. To visualize the notochord for cannula insertion, a ventral incision was made along the entire length of the hagfish; the thoracic and abdominal contents were removed without violating the integrity of structures dorsal to the retro-peritoneum. In order to reduce temperature effects within the specimen during the experiment from overhead lighting, the specimen was secured to a two-foot long plastic ruler that was floated on a 15 cm deep bath of iced-seawater within a polystyrene cooler. Internal notochord pressure was measured every 2 cm along the length of the exposed

notochord by inserting a 26-gauge hypodermic needle attached to the pressure cannula; measurements were obtained at a sample rate of 5 Hz.

Fresh notochord specimens were excised from four euthanized hagfish (mean weight 41.7 ± 7.47 g; mean length 34.10 ± 2.24 cm). Segments for osmotic experiments were obtained by ligating the notochord with 00-silk sutures at 2 cm intervals allowing for a 0.5 cm segment between successive test segments along the length. Every odd numbered segment was used as a control to determine curvature or deformation due to swelling while every even numbered segment (caudal to the adjacent odd numbered segment) was cannulated for pressure measurements (only pressure measurements reported). After ligating the rostral end of the even numbered test segment, the caudal end was transected using a surgical blade. The pressure cannula was then introduced and secured to the segment using two 00-silk sutures. Both the odd and even numbered adjacent test segments were then immediately placed into a 2 cm deep distilled water bath and internal pressure was recorded at 0.2 Hz. Previous analyses have shown that ligated notochord specimens swell when placed in distilled water (Kielstein, J.T., et al., op. cit). The position of each pressure measurement was taken as a function of overall body length and ANOVA was performed at the 95% level to test the null hypotheses that (1) the mean peak pressures, (2) times to peak pressure, and (3) overall pressure ranges do not vary along the length of the notochord.

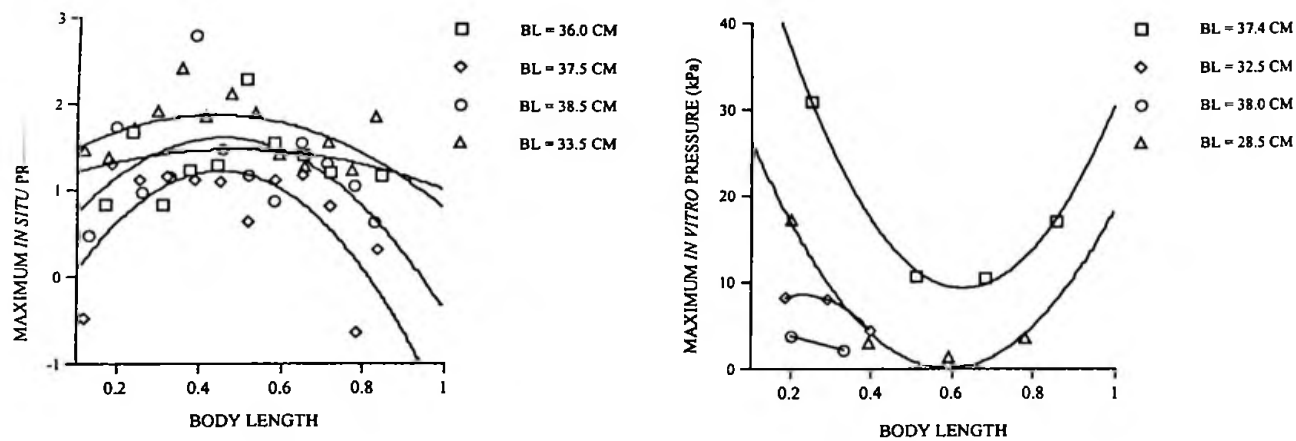
In situ pressure measurements were characterized by a rapid rise to peak pressure followed by a slower decay to equilibrium (Fig. 1a). *In situ* mean peak pressures for the rostral, midldle, and caudal regions measured 1.17 ± 0.15 kPa (n=16), 1.51 ± 0.12 kPa (n=21), and 0.95 ± 0.2 kPa (n=11), respectively (Figs. 2a and 3a). In the *in vitro* experiments in which ligated notochord segments were placed in water, time to peak pressure was significantly longer than that observed in the *in situ* measurements (Fig. 1b). This difference resulted from the diffusion time necessary to eliminate mobile ions from the notochord core. Mean peak pressures in the notochord segments in water were significantly higher than those *in situ*. Peak pressure within the same regions measured 11.64 ± 4.41 kPa (n=6), 4.79 ± 2.07 kPa (n=4), and 10.34 ± 3.7 kPa (n=3), respectively (Figs. 2b and 3b).

ANOVA provided statistical evidence to suggest the *in situ* mean peak pressures within the notochord may not be equal ($p<0.05$); confidence intervals revealed mean peak pressures between the rostral and middle, and between the rostral and caudal, regions were not different. The mean peak pressure within the caudal region, however, may be significantly lower than the mean peak pressure within the middle region. Furthermore, both the mean pressure ranges (mean, +/- 1 sem) of 1.31 ± 0.16 kPa, 1.56 ± 0.21 kPa, and 1.26 ± 0.14 kPa, and the mean times to reach peak pressure of 15.6 ± 1.6 s, 17.3 ± 1.5 s, and 17.3 ± 1.4 s were not statistically different within the three body regions *in situ* ($p>0.05$).



(a) (b)

Figure 1. Representative plots of (a) the *in situ* pressure versus time of a hagfish notochord (hagfish BL = 33.5 cm, rostral region), and (b) the *in vitro* pressure versus time of a hagfish notochord segment (hagfish BL = 28.5 cm, rostral region) placed in a distilled water bath. BL = body length.



(a) (b)

Figure 2. Peak pressures within notochords measured as a function of body length. (a) the *in situ* pressures, and (b) the *in vitro* pressures; the midpoint of the segment length was used to determine the pressure measurement location with respect to body length. Note there were no data > 0.5 BL for hagfish BL=32.5 & 38.0 cm due to violating sheath integrity during harvest.

The *in situ* pressure of the hagfish notochord measured here is lower than that measured in the sturgeon notochord (60 kPa, Long, op. cit) and significantly less than the calculated pressure in the notochord of elongating frog embryos (2.4 MPa, Adams and Koehl et al., op. cit). Whether this difference reflects a fundamental divergence in the function of the notochord in these diverse species, or rather indicates that the level of notochord pressurization is adapted to the specific mechanical requirements for specific functions is presently unknown. Notochords in

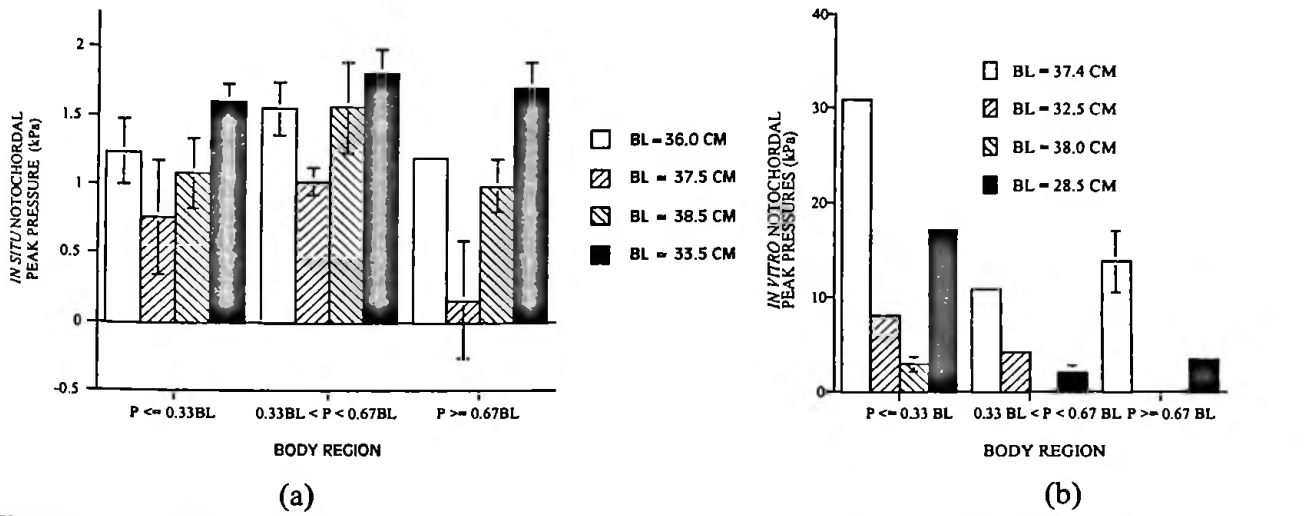


Figure 3. Peak pressures within a given body region of notochords (a) *In situ*, and (b) *In vitro*. (P = pressure reading). Error bars indicate ± sem.

hagfish and sturgeon, which have more similar *in situ* pressures, contribute to locomotor function, whereas pressurization of the notochord in the developing frog embryo has a distinct function related to straightening the axis. Unfortunately, too little is known about notochords in other species to support further speculation.

Removal of mobile counter-ions by incubating ligated specimens in water resulted in an approximate 10-fold increase in internal pressure in the *in vitro* experiments. This observation supports our earlier conclusions that the notochord is osmotically active and explains the changes in flexural stiffness, apparent material stiffness, curvature-specific bending work, $W\kappa^{-1}$ (J·m), and resilience in lateral bending. As internal pressure increases, the notochord segments "swell" (producing an increase in its cross-sectional radius), and exhibit a curvilinear shape. This swelling may produce changes in the fiber orientation angle which become more parallel to the long axis of the fiber helix — the longitudinal axis of the notochord. This straightening of the fibers during pressurization may be exhibited as an increase in the apparent material stiffness (Young's modulus) of the notochord, and the increase in radius produces an increase in the cross-sectional area moment of inertia (I in m^4). Since a decrease in external osmolarity increases both the flexural stiffness and internal pressure of notochord segments, these results are consistent with the observed changes in biomaterial properties and biomechanical behaviors due to notochord pressure variations induced by external osmolarity. However, whether the fiber angle (or the number of turns per unit length) may vary along the notochord is unknown, and may be of interest in determining *in situ* pressure differences between the middle and caudal regions.

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