ROLE OF PROTEOGLYCANS IN CALCIFICATION OF CARTILAGE. D.S. Howell and J.C. Pita (Miami, Florida, USA)

In our laboratory, physiological events in the processes of calcification have been investigated with special interest in proteoglycans, and over the last decade we have developed a number of ultramicrobiochemical methods to analyze samples of fluid aspirated by renal micropuncture methodology from between rows of hypertrophic cartilage cells at sites of calcification in the upper tibial growth plates of 41-42 day old rats. A partition of electrolytes including "free" calcium (Ca) and phosphate (Pi) as well as a variety of parameters related to calcification have been measured in 20-30 nl of fluid (Cfl) aspirated from the normal or expanded hypertrophic cell zones from growth plates in vivo from the following animal preparations: a) normal rats, b) animals made deficient for three weeks on a rachitogenic diet lacking vitamin D and low in phosphate, c) rats to which had been administered for 10 days sodium etidronate (EHDP) 40 mg per kg per body weight and d) animals b) and c) during recovery. Findings included: 1) in all preparations an alkaline pH 7.6-7.8, which was obliterated in experiments on animals in vivo by treatment with Diamox, a carbonic anhydrase inhibitor, 2) absence of elevation in "free" Ca or Pi that might cause spontaneous precipitation during recovery, 3) an organic acid-resistant agent nucleation for Ca-Pi mineral phase, 4) a proteoglycan "superaggregate" which is polydisperse and approximately has 100-160S sedimentation profile with the subunit weight average value 12S. Removal of this superaggregate by ultracentrifugation (15% of total Cfl proteoglycan) removed the capacity of the total proteoglycans to inhibit mineral growth in an in vitro synthetic lymph seeded with a well-characterized mineral phase. This superaggregate in a crude fraction inhibited mineral phase growth binding up to 400 moles of $CaHPO_4$ for each 10^6 M.W. segment of proteoglycan. There was no evidence in this calcifying system in vitro for the function of Cfl micro inhibitors of mineral growth. Studies with proteoglycan aggregates link protein and subunit from nasal cartilage showed inhibition of mineral growth in vitro only with aggregates. Also, dissociation of the proteoglycan superaggregate with leech hyaluronidase or highly purified rat cartilage lysozyme preparations or alternatively degradation of this superaggregate with cartilage neutral protease or trypsin all destroyed the inhibitory function on mineral growth. Removal of the inhibitor function or disaggregation permitted an organic nucleational agent in rachitic (-DP or phosphonate)$_2$Cfl to generate spontaneously mineral de novo at Ca X Pa products of 2.6 mM^2. An indication that this was not simply an in vitro phenomenon was shown by experiments in which micropuncture fluid from healing -DP rickets revealed spontaneous mineral formation and reduction of aggregate size when incubated in vitro. Similar, but lesser, reductions of S value occurred in the proteoglycan aggregates during healing of -DP rickets in vivo. Although growth and other cartilages have been found to have a neutral protease activity, evidence of this in the healing puncture fluids has been difficult so far to demonstrate. Rather, it appears that the dissociation does not lead to proteoglycan weight average values of less than 12 on prolonged incubation. Furthermore, the subunit can be reaggregated with hyaluronate to large aggregates in a reversible manner. A search, therefore, was made for other factors that might act at this stage of calcification. It was found that cartilage lysozyme activity increased three-fold during healing of -DP rickets. In-

terestingly in phosphonate rickets there is a sharp reduction of proteoglycan synthesis in these rat cartilages accompanied by a total suppression of lysozyme activity in Cfl. Removal of phosphonate treatment promptly restores proteoglycan synthesis and lysozyme reappears. A consistent finding during the period of phosphonate treatment was reduction of total proteoglycan in Cfl, but all of the remaining proteoglycan was in the form of superaggregates. Finally, alkaline phosphatase-6S from gut and other control tissues had an S value of 85 in Cfl. Because of this high value, the alkaline phosphatase is probably present in matrix vesicles or a proteoglycan complex.

Summary: Based on our assumption that the chemical transformations demonstrated in these cartilage fluid samples reflect biological events in vivo proteoglycan superaggregates appear to have an important regulatory role in endochondral calcification as an inhibitor and might be involved in other roles yet to be delineated.

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