INTRODUCTION: Periosteum contains undifferentiated mesenchymal stem cells that have the potential for cartilage formation. This is an important factor in the repair of damaged articular surfaces and in fracture healing. Periosteum regenerates both cartilage and bone. A limiting factor in chondrogenesis is the age of the subject. There are indications that the chondrogenic potential of periosteum is qualitatively and quantitatively inferior in mature and older rabbits versus immature ones (6). This correlates with the observation that the biological activity of periosteum diminishes with age following the completion of skeletal growth (1, 4, 6) and the observation that its chondrogenic potential decreases dramatically with age (5). The goal of this study was to elucidate the cause of the age-related decrease in periosteal chondrogenic potential by examining how it correlates with age-related changes in periosteal morphology.

METHODS: All work in this study was conducted with the approval of the Mayo Clinic Institutional Animal Care and Use Committee. 256 periosteal explants were examined from the medial side of the proximal tibia of 44 male New Zealand White rabbits aged 2, 6, 12 or 24 months. The explants harvested using sharp subperiosteal dissection and were cultured in agarose and DMEM as previously described (7). TGF-β1 was added for the first 2 days of culture. Skeletal maturity was evaluated by examining the distal femoral and proximal tibial growth plates grossly and histologically at the time of periosteal harvesting. Whole joints including the distal femora and proximal tibiae, were decalcified and sectioned for histological examination, except for the twenty-four month-old rabbits, whose skeletal maturity was certain. Safranin O and H&E stains were obtained.

Autoradiography: To determine whether the decline in proliferative activity with age reflected a reduction in the number of cells in the periosteum or a decrease in the percentage of cells undergoing proliferation (or both), explants from all age groups were cultured for 1 to 14 days, and labeled with 3H-thymidine for 24 hours before being taken form culture. The explants (5 per age per day) were then sectioned subjected to autoradiography. To determine the percentage of cells undergoing proliferation, a 'labeling index' was measured. The labeling index was defined as the number of labeled cells divided by the total number of cells multiplied by 100. Data Analyses: Statistical analyses were performed using an ANOVA with Duncan Multiple Range post hoc testing. Data are represented as means ± 1 standard deviation unless otherwise stated.

RESULTS: Rabbit Weights & Skeletal Maturity - the weights of the rabbits increased steadily until six months of age, at which time they reached a plateau around four kilograms. Gross and histological examinations at the time of sacrificing the animals for periosteal harvesting, revealed that the growth plates in all of the two month-old rabbits were open, while those in the six and twelve month-old rabbits were closed. These observations are consistent with published data, which have shown that skeletal maturation occurs by the age of 6 months (3). Periosteal Morphology - changed significantly with age (Fig. 1 & 2). Most notably, the cambium layer became thinner with age, although changes were seen in the fibrous layer as well. At two months the thickness of the cambium layer of the periosteum, was intact on the bone, was 118 ± 43 µm. This diminished significantly (p < .0001) in the 6-month rabbits to 26 ± 9.6 µm and 15 ± 2.6 µm at 12 months and 8.3 ± 3 µm at 24 months respectively. Cell density was three times as high in the cambium layer as in the fibrous layer, but did not change significantly with age (Fig. 2C, p > .8). The normalized total cell number, or cellularity, in the cambium layer decreased from 393 ± 106 at 2 months to 80 ± 27 at 6 months, 50 ± 10 at 12 months 29 ± 5 cells per mm of length of periosteum (Fig. 2A, p < .0001). The 2 and 6 month groups were significantly different from each other and from the 12 and 24 month groups. This indicates that the total number of available stem cells decreases with age. Changes in the fibrous layer are also evident with age, but less dramatically than the cambium layer. The fibrous layer diminished significantly in thickness and total cell number (p < .0001). The thickness declined from 172 ± 42 µm at 2 months to 68 ± 21 µm at 24 months. As the cell density did not change significantly with age, the decline in total cell number reflected the decline in fibrous layer thickness.

POSTER SESSION - CARTILAGE CELL BIOLOGY - VALENCIA D

ACKNOWLEDGEMENTS: This work was funded by NIH Grant AR43890.