DEVELOPMENT AND DISEASES OF CARTILAGE AND BONE MATRIX

Arup Sen and Thomas Thornhill, Organizers

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Plenary Sessions

Poster Sessions

Collagens

H1 COORDINATE REGULATION OF CARTILAGE COLLAGEN AND PROTEOGLYCAN SYNTHESIS IN SCORBUTIC GUINEA PIGS, Beverly Peterkofsky, Robert G. Spanheimer, and Timothy A. Bird, Laboratory of Biochemistry, National Cancer Institute, Bethesda, MD 20892

Ascorbic acid deficiency, or scurvy, occurs in species that cannot synthesize the vitamin, including primates and guinea pigs. It is known that the disease is associated with defects in connective tissue, particularly in wound healing, but there are still a number of questions regarding its pathogenesis. The discovery that ascorbate was required for prolyl hydroxylase, and consequently stimulated procollagen secretion, led to the assumption that defects in these processes were responsible for the effects of scurvy. Our studies suggest, however, that this is not the case. Tissues from normal and scorbutic guinea pigs were labeled in short-term cultures with [^{3}H] and [^{14}C]proline and proteins were digested with bacterial collagenase, allowing us to measure collagen and noncollagen protein synthesis and proline hydroxylation simultaneously. Collagen synthesis decreased by 80% in scorbutic calvarial bone and there was a direct correlation between the extent of this effect and the loss of body weight induced by scurvy, rather than with decreased proline hydroxylation (1). The same results have been found in articular and costal cartilage. The effect on collagen synthesis could be reproduced in a number of tissues from guinea pigs that were acutely fasted for 96 h but received vitamin C, so that proline hydroxylation was normal (2). Decreased collagen synthesis resulting from scurvy or fasting was associated with decreased levels of procollagen mRNAs. There was relatively little effect on non-collagen protein synthesis in either scurvy or acute fasting, but in both cases cartilage proteoglycan synthesis, as measured by $^{35}SO_4$ incorporation, was inhibited. In scurvy, the inhibition occurred coordinately with the effects on weight loss and collagen synthesis. The defect in proteoglycan synthesis occurred beyond the stage of core protein synthesis and coincided with a decrease in proteoglycan galactosyl transferase activity. We propose that the anorexia characteristic of ascorbate deficiency leads to a chronic fasting state, which in turn results in changes in levels of hormones involved in regulation of extracellular matrix components. This concept is supported by our recent observation that the level of growth factors in serum was reduced during scurvy.

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H2 COLLAGEN TYPES: A REVIEW, Karl A. Piez, Connective Tissue Research Laboratories, Collagen Corporation, 2500 Faber Place, Palo Alto, CA 94303.

The collagen triple helix seems particularly suited to form long (150-450 nm) connecting segments between associating nonhelical domains. In some cases the helical segments are interrupted as well as terminated by nonhelical domains. These associations are commonly stabilized by lysine-derived cross-links of several types and sometimes by disulfide bonds or both. The resulting aggregates play critical structural roles. In addition, specific interactions with cell surface macromolecules or intermediary attachment factors are an important element in modulation of cellular function. We are just beginning to recognize the magnitude of the problem of cell-matrix interactions. Types I, II and III collagen, 300 nm long, are the major interstitial collagens, all present as banded fibrils with a 67 nm periodicity. Type II collagen is specific to hyaline cartilage. Types I and III collagen are usually found together, but bone and tendon contain largely type I collagen. Type V collagen probably belongs in this group since it has a similar helical domain and forms fibrils that are widely distributed. Type IV collagen, 400 nm long, is quite different in its aggregate form. It makes a mat-like structure with proteoglycans and glycoproteins in a manner specific to basement membranes. Type VI collagen, 105 nm long, is widely distributed like types I and III but forms quite different fibrils with a 100 nm repeat. Its function is unknown. Type VII collagen is unique in that its aggregate form is apparently two molecules long (900 nm) to form a strap-like structure connecting basement membranes to interstitium. Type VIII collagen is an endothelial cell product of unknown function. It contains triple helical domains about 150 nm long connected by nonhelical regions. Whether the monomer contains one or several helical domains is yet uncertain. Types IX and X collagen, like type II, are unique to cartilage. Type IX has a uniform distribution like type II and the structure of one of its chains determined at the DNA level predicts interrupted helices with a monomer length of about 200 nm. The aggregate structure and function are unknown. Type X collagen is also unique in that it appears only in association with hypertrophying cartilage during endochondral bone formation. At that time it is a major biosynthetic product. Its function is unknown. As the smallest (150 nm long) of the collagens, type X may not be a structural component. Still another collagen, with chains designated 1α, 2α and 3α, is also unique to cartilage. It has not yet been given a type number since its molecular form is unknown. The size of the chains predicts a helical segment about the same size as type I collagen. Thus, cartilage demonstrates its individuality in that it contains four unique collagens. Bone on the other hand contains only type I collagen except for traces of other types. The type I collagen in other tissues is the same gene product as in bone but there may be tissue-specific posttranslational differences.

H3 MUTATIONS IN GENES FOR TYPE I PROCOLLAGEN AND THEIR CONSEQUENCES IN MAN, Darwin J. Prockop, Department of Biochemistry, University of Medicine and Dentistry of New Jersey-Rutgers Medical School, Piscataway, NJ 08854

Type I collagen is the major constituent of connective tissues such as skin, ligaments, tendons, and bone. The fibrils found in these tissues vary widely in both diameter and in 3-dimensional configuration, but all are products of the two structural genes for the proα1(I) and proα2(I) chain of type I procollagen. Expression of the two structural genes, however, requires coordinated expression of genes for at least 11 post-translational enzymes. Over the past several years, we and others have tried to obtain further information about this gene-protein system by examining mutations of the structural genes for type I procollagen in patients with either osteogenesis imperfecta (OI) or Ehlers-Danlos syndrome (EDS). In one variant of OI studied by us and others, the mutation is a sporadic deletion of 3 exons of the pro α1(I) chain. Because the deletion is in-phase, expression of the gene gives rise to proα1(I) chains that are 84 amino acids shorter than normal proα1(I) chains. Because of the structure of the carboxy-terminal propeptide is normal, the shortened proα1 chains associate with and become disulfide-bonded to normal proα1(I) and proα2(I) chains synthesized by the same fibroblasts. Trimers containing the shortened chains, however, are unstable at 37°C and are rapidly degraded in a process referred to as "protein suicide". The biological inactivation of normal proα chains in this process probably explains why the mutation was lethal. In a variant of EDS, the mutation is either splicing mutation or a gene deletion that gives rise to a shortened proα2(I) chain. The shortening is a deletion of about 20 amino acids near the N-terminal region of the chain. The major consequence of the deletion is that the conformation of the N-terminal region of procollagen is altered. As a result, N-proteinase does not cleave the protein and the N-propeptide persists. In another variant of OI, the mutation is a deletion of 4 bp coding for part of the carboxy-terminus of the proα2(I) chain. Because the deletion causes a frameshift, the last 33 amino acids of the proα2 chains have an abnormal sequence, and the proα2 chains do not associate with proα1 chains. As a result, only trimers of pro α1 chains are synthesized by the patient's fibroblasts. The trimers of proα1 chains are secreted by fibroblasts but the carboxy-terminus of the molecules unforlds at 36°C. The partial unfolding probably explains why the patient had moderately severe form of OI. The result suggest several general conclusions: (1) the tissue at risk from these mutations varies; (2) a mutation that causes the synthesis of a structurally abnormal proα chain is more deleterious than a mutation that makes an allele non-functional; (3) mutations giving rise to shortened proα chains may be unusually common; and (4) heterozygous carriers for some of the mutations may be prone to more common diseases of connective tissue.

H4 THE MECHANISM OF SYNTHESIS AND ASSEMBLY OF THE TYPE I PROCOLLAGEN MOLECULE, Arthur Veis, Theresa Z. Kirk and John S. Evans, Department of Biological Science, Northwestern University, 303 E. Chicago Ave, Chicago, Ill. 60611.

The pathway by which the type I procollagen molecule assembles poses many problems. It is known that each proα(I) chain is translated from an individual mRNA and is, after synthesis of the signal peptide, inserted and elongated into the cisternal space of the endoplasmic reticulum (ER). Triple-helix formation finally proceeds in the direction carboxyl-propeptide to amino-terminus. Thus, the chain registration event has been assigned to carboxyl-propeptide interactions among completed, released molecules. There is also evidence that the three chains of a single molecule are synthesized within the same time period, suggesting coordinate synthesis. The problems come in assembling these data into an acceptable mechanism. How are the chains (or ER surface) addressed so that the correct chains are inserted into the correct ER compartment in the correct ratio? How does the cell coordinate chain elongation? How do the proper newly synthesized chains find each other in the registration process? Recent electron microscopic evidence (1) shows that molecular organization may begin prior to or at the time of attachment of the mRNA-ribosomal complex to the ER surface. There are two directions of attack on this problem: one can look for interactions between mRNA's, cytosolic proteins, and membrane proteins to examine the mRNA complex formation and membrane attachment; and, one can examine the nature of the chains in the ribosomal complexes. We report here studies on the latter problem. Chick tendon fibroblasts from ^{14}C-or ^{3}H-proline labeled 17 day old embryos, were lysed and the collagen-containing polysomes collected by sucrose gradient centrifugation. The polysomes were passed over QAE-Sephadex columns under conditions in which nascent t-RNA associated chains are bound but completed molecules are not. The two fractions were collected and analyzed by gel electrophoresis, slicing and counting. The unbound fraction yielded 25% of the collagenase sensitive counts and was shown to consist of completely elongated but not fully hydroxylated proα1(I) and proα2 (I) chains in a 2 to 1 ratio. The nascent chains did not produce a continuum of chain sizes, but rather a characteristic, uneven molecular weight distribution pattern. These data suggest that there are significant pauses in chain elongation, with a final pause following chain completion, but prior to completion of proline hydroxylation. These underhydroxylated, polysome-bound, complete collagens are resistant to pepsin and chymotrypsin but not to a mixture of chymotrypsin and trypsin. It is clear, however, that chain selection and registration have already taken place while the chains are still attached to the mRNA polysome complex. Supported by NIH Grant AM 13921.

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Noncollagenous Proteins of Bone and Cartilage

H5 CELLULAR REPLACEMENT AND SYNTHETIC EVENTS OF THE CARTILAGE/BONE TRANSITION

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An intimate inter-relationship exists between the embryonic cartilage core model and the newly forming bone. For example, we will show that in the embryonic tibia, chondrocalcin, a protein associated with mineralization, is made and stored by the developing chondrocytes which eventually release this 35,000 dalton protein to be concentrated in the adjacent mineralizing bone matrix. However, even though this cooperative interaction exists, embryonic long bone cartilage does not serve as a scaffolding or template for the newly forming bone. In the chick embryonic tibia, over 90% of the length of the day 16 bone forms completely outside the cartilage model or core. In fact, the cartilage is completely replaced by the marrow cavity and precisely defines the limits of this space. Thus, cartilage is not replaced by bone; it is replaced by marrow and vasculature. The first bone forms as a periosteal collar of tissue outside of the cartilage core and becomes vascularized before the eroding vasculature enters the cartilage core. The osteoblasts of this first bone differentiate directly from mesenchymal progenitor cells which can be morphologically delineated from non-osteogenic cells before the core chondrocytes fully differentiate. Taken together, these observations suggest the hypothesis that the mesenchymal cells commit to an osteogenic lineage prior to the cyto-differentiation of the cartilage core. This hypothesis could infer that it is the osteogenic commitment which determines the shape and orientation of the cartilage core. Biochemical and immunocytological analysis of newly synthesized proteoglycans, the deposition of collagens and localization of chondrocalcin provide support for this hypothesis. Proteoglycans of distinctive structure and different collagen types can be used as markers for mesenchymal cells, chondrocytes, hypertrophic chondrocytes, muscle and osteoblasts. The exact morphological localization of these molecules defines the tissue's limits and provides data to support the view that cartilage may not provide the biochemical scaffolding for bone, but rather delineates the marrow space. This prediction is also realized upon re-evaluation of the morphological events associated with ectopic formation of bone as stimulated by demineralized bone particles in histological preparations kindly supplied to us by Dr. Hari Reddi of NIDR. Analysis of these molecular, cellular and tissue replacement events provides a detailed understanding of the dynamics of long bone formation. Supported by grants for N.I.H.

H6 NONCOLLAGENOUS PROTEINS OF BONE, Kenneth G. Mann, Debra Stenner, Robert W. Romberg and B. Lawrence Riggs, Department of Biochemistry, The University of Vermont, Burlington, VT 05405 and The Mayo Clinic/Foundation, Rochester, MN 55901.

The underlying hypothesis for studying the noncollagenous proteins of bone can be divided into three subtheses: 1) the noncollagenous proteins will serve as useful markers to evaluate bone physiology in vivo; 2) the noncollagenous proteins will provide immunochemical markers for the identification of bone cells; 3) the noncollagenous proteins are implicitly associated with the structure, formation and remodelling of bone. The practical approacheswe have chosen to evaluate these hypotheses have involved the identification and isolation of these proteins; preparation of immunochemical reagents directed against them; and, the evaluation of the physical structure of these proteins and their interactions with other constituents of bone. We have constructed, using two-dimensional electrophoretic techniques, a catalogue of the major noncollagenous proteins in bone and have proceeded to conduct isolation studies on these proteins using both conventional and immunochemical techniques. Our principal efforts have been aimed at bone Gla-protein (BGP) and at osteonectin. We have developed both monoclonal and polyclonal antibodies to each of these proteins, which have been applied to assays aimed at evaluating physiologic fluid levels of these proteins, and the association of blood levels with normal and abnormal bone metabolism. BGP decreases with aging in normal individuals, and appears to be a marker for osteoblast function. Data indicate increased osteoblast function with aging and a lack of impairment of osteoblast function in postmenopausal osteoporosis. We have evaluated conformational transitions in BGP which are associated with its interaction with calcium ion using both physical and immunochemical approaches. We have also evaluated, using quantitative approaches, the interaction of osteonectin, with collagen, calcium and hydroxyapatite. One promising line of research involves the interactions of bone proteins in the inhibition of hydroxyapatite crystal growth, which potentially implicates these proteins in some aspects of the regulation of the mineralizing surface in bone synthesis. We are presently further evaluating the structure of osteonectin and validating immunochemical analyses of blood fluid levels of osteonectin in aging and disease. At present, our studies must be regarded as the preparation of a secure foundation for the tests of our underlying hypotheses. Our presentation will thus be a progress report, rather than a definitive description of the function of the noncollagenous proteins in bone.

H7 THE ROLES OF CHONDROCALCIN AND PROTEOGLYCAN IN THE CALIFICATION OF CARTILAGE MATRIX, A. Robin Poole, Joint Diseases Laboratory, Shriners Hospital, McGill University, Montreal, Quebec, H3G 1A6. Canada.

The calcification of cartilage matrix involves a complex series of events. Prior to calcification an extracellular matrix is synthesized made up primarily of type II collagen and large chondroitin sulfate proteoglycans which aggregate with hyaluronic acid. When cartilage calcifies these proteoglycans undergo an organizational change whereby they are concentrated in discrete foci in the longitudinal septa of primary growth plates: these foci have sizes of 300-500 nm in diameter (1). They can be artificially created prior to calcification by extraction of cartilage with EDTA (A.R. Poole, I Pidoux and L.C. Rosenberg, submitted). Their formation may naturally be mediated by cleavage of hyaluronic acid leading to a focal collapse of proteoglycan aggregates in calcifying sites. When mineral is deposited in these sites a second molecule appears in these focal deposits which is a calcium binding protein which we have called chondrocalcin (2,3). Sequence analyses have revealed that this is the same as or very similar to the C-propeptide of type II collagen (M. van der Rest, B. Olsen, L.C. Rosenberg and A.R. Poole, submitted). Chondrocalcin is thought to be concerned with the accumulation of mineral in these focal sites since this only occurs in an extensive manner when chondrocalcin is present in high concentrations in these sites. Hypertrophic chondrocytes synthesize increased amounts of this molecule compared with those from non-calcifying cartilages. The synthesis and secretion of chondrocalcin is regulated by metabolites of vitamin D. (A. Hinek, A. Reiner, L.C. Rosenberg and A.R. Poole, submitted). These observations indicate that aggregating cartilage proteoglycans and chondrocalcin (in the C-propeptide of type II collagen) are involved in the calcification of cartilage matrix (4).

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H8 MOLECULAR VARIANCE IN THE PHENOTYPIC EXPRESSION OF THE NONCOLLAGENOUS PROTEINS OF BONE, John D. Termine, Bone Research Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892

Two key molecules synthesized by the bone forming cell and secreted to the extracellular matrix (1) are osteonectin and the small bone proteoglycan, PG II. Osteonectin is a phosphorylated glycoprotein of apparent M_r = 40-45,000 on SDS gels, 32,000 on guanidine HCl gel filtration and 29,000 on sedimentation equilibrium (2,3). This molecule has saturable binding domains for both collagen and hydroxyapatite, inhibits hydroxyapatite crystal growth strongly in the absence of collagen, but promotes calcium phosphate formation when combined with collagen in heteromolecular complexes (2,3). Osteonectin is localized to the bone matrix in vivo with less present in woven than in lamellar bone (4) and only negligible amounts detected in non-mineralizing connective tissues (2). Recent data (P. Gehron Robey and M.F. Young) indicate that osteonectin mRNA is present and an osteonectin-like molecule synthesized in fibroblast cultures in vitro. However, preliminary experiments suggest that this fibroblast form of osteonectin may not be completely identical to that produced by the normal bone osteoblast. Osteonectin message is sensitive to viral transformation and expression of the protein may be transcriptionally and translationally regulated. The bone proteoglycan, PG II, consists of a 45,000 M_r core protein to which are attached several oligosaccharides and a single chondroitin sulfate chain of average $M_r \sim$ 40-50,000(5). Similar molecules are found in tendon, skin, sclera and articular cartilage. It is thought that these small proteoglycans interact specifically with collagen fibrils in composite connective tissue matrices (6). Preliminary data (L.W. Fisher and K.G. Vogel) suggest that the various tissue forms of PG II may differ either by specific post-translational modifications or actual peptide sequence variation. PG II mRNA appears as a doublet in the different connective tissues in which its core protein is expressed (M.F. Young, and A.A. Day). Finally, osteopenic bone is often deficient in osteonectin and/or PG II. For example, several human (progressively deforming) and two bovine variants of osteogenesis imperfecta present bones practically devoid of one or both proteins. Bone cells isolated from affected individuals generally synthesize the missing protein but display altered incorporation of the molecules(s) in question to the extracellular matrix space (P. Gehron Robey). Consequently, the regulation of expression for these noncollagenous bone proteins would appear critical to the maintenance of healthy skeletal tissue.

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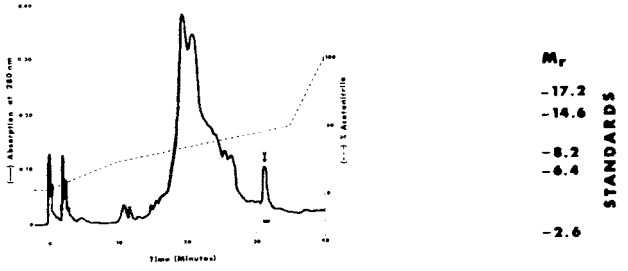
Bone Regeneration

H9 CARTILAGE INDUCTION AND DIFFERENTIATION: THE ROLE OF BONE DERIVED CARTILAGE INDUCING FACTOR (CIF), Saeid M. Seyedin, Andrea Y. Thompson, Hanne Bentz, David M. Rosen and Karl A. Piez, Connective Tissue Research Laboratories, Collagen Corporation, 2500 Faber Place, Palo Alto, CA 94303; Ned R. Siegel and Gerald R. Galluppi, Department of Biological Sciences, Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198
Subcutaneous implantation of demineralized bone matrix or guanidine-HCl extracted proteins from demineralized bone induces chondrogenesis in rats. Two naturally occurring peptides that induce chondrogenesis in culture have been purified from bovine demineralized bone and termed cartilage-inducing factors (CIF-A and CIF-B). CIF-A and CIF-B at concentrations of 1-10 ng/ml each induce embryonic rat mesenchymal cells in agarose gels to assume chondrocyte morphology and synthesize cartilage-specific macromolecules. Both factors have an apparent Mr of 26,000, as determined by SDS-polyacrylamide gel electrophoresis, and each appears to be a dimer of identical or very similar chains. In addition to their chondrogenic properties, we have found that CIFs are capable of stimulating normal rat kidney (NRK) fibroblasts to form colonies in soft agar, in the presence of epidermal growth factor (EGF). Anchorage-independent growth of NRK cells in soft agar is a function that is described for transforming growth factor-β (TGF-β). Colony forming activity in response to CIF-A or B was dose-dependent with half-maximal response similar to that observed for purified TGF-β. CIF-A, the major species in bone was purified and partially sequenced. Comparison of the sequence of the N-terminal 30 amino acids of TGF-β with the corresponding sequence of CIF-A revealed 100% identity. TGF-β has been purified from a variety of tissues. If CIF-A and TGF-β are identical molecules, then bone appears to be a major storage site for this activity (∿200 μg/kg tissue). The abundance of CIF-A in bone suggests that cartilage induction may be a normal function of TGF-β like factors.

H10 BOVINE BONE MORPHOGENETIC POLYPEPTIDE GENERATED BY LIMITED PEPSIN PROTEOLYSIS, Marshall R. Urist, Robert J. Delange, Jin-Jyi Chang, Yong Kang Huo, William Hirota, Arthur Lietze, Anna G. Brownell, G.A.M. Finerman, UCLA Bone Research Laboratory Department of Surgery, Division of Orthopedics, and Department of Physiological Chemistry, University of California, Los Angeles, CA 90024

The non-collagenous proteins extractable from bovine bone with 4 M to 1.5 M GuHCl form water insoluble aggregates, which include a bone morphogenetic protein (BMP). Limited pepsin proteolysis of this protein aggregate generated a group of low MW bone morphogenetic polypeptides which, like BMP, induced differentiation of cartilage and bone in the mouse thigh. The term limited was applied because the morphogenetic products of proteolysis were stable and identified by the capacity to induce bone formation. The osteoinductive polypeptides were isolated from the products of limited proteolysis by sequential hydroxyapatite chromatography, Sephadex G-50 gel filtration, and hydrophobic reversed phase HPLC. Figs. 1 and 2 show HPLC elution profiles and SDS PAGE patterns. The osteoinductive polypeptides stained intensely with Coomassie Blue but produced a negative image with silver staining. The M_r of these polypeptides was 5 ± 1k with a pI of 5.8. HPLC provided only 200 μg quantities which were assayed in tisssue culture, and produced differentiation of neonatal muscle connective tissue outgrowths into cartilage. HPLC fractions with M_r 10,8,7, and 1k polypeptides were used as controls. The amino acid composition of HPLC isolated polypeptides was slightly acidic and consisted of approximately 40 residues. Limited proteolysis of a partially purified BMP with an M_r of 18.5k generated osteoinductive polypeptides with M_r of 4.1 and 4.7k. The 4.7k polypeptides reacted with both monoclonal and polyclonal mouse anti-bovine BMP.



Bone Resorption

H11 ASSESSMENT OF METABOLIC BONE DISEASE WITH BONE-SPECIFIC MARKERS, B. L. Riggs, R.J. Duda, and K. G. Mann, Endocrine Research Unit, Mayo Clinic and Foundation, Rochester, MN 55905; Department of Biochemistry, University of Vermont, Burlington, Vermont 05405

There is an urgent need to develop simple, noninvasive methods for determining bone turnover in metabolic bone disease that are well-characterized, specific, and accurate. This talk will discuss the possibility that this can be accomplished by measurement of one or more bone specific biochemical markers and will focus the measurement of serum bone Gla-protein (BGP), also called osteocalcin, as an example of this approach. Serum BGP in these studies was measured by radioimmunoassay as previously described (J. Clin. Invest. 71:1316, 1985). In young adult women, mean ± SD is 4.4 ± 1.6 ng/ ml and this increases two-fold by extreme old age. Serum BGP is increased in hyperparathyroidism and decreased in hypoparathyroidism which demonstrates a large dynamic range for this measurement. In these conditions, serum iPTH and BGP correlated directly ($r = 0.91$ $P < 0.001$). Although some BGP diffuses the circulation after synthesis by osteoblast, most is incorporated into bone matrix where it remains until bone is resorbed. Thus, serum BGP could reflect bone formation, bone resorption, or a combination of both. The relationship of serum BGP to the components of bone turnover was evaluated in 18 normal women who received a continuous 24-h intravenous infusion of the 1-34 synthetic fragment of human parathyroid hormone. We compared baseline and post-treatment values for 24-h excretion of urinary hydroxyproline, an index of bone resorption; fasting levels of serum alkaline phosphatase, an index of bone formation; and fasting levels of serum BGP. Urinary hydroxyproline increased by 69% ($P < 0.001$) while serum alkaline phosphatase was unchanged, demonstrating that as expected PTH infusion increased bone resorption without increasing bone formation. Despite the increase in urinary hydroxyproline, serum BGP decreased by 23% ($P < 0.01$). This divergence suggests that serum BGP is a measure of bone formation not of bone resorption. The abnormality of bone turnover responsible for osteoporosis is controversial. We found that serum BGP was increased ($P < 0.002$) in 62 women with postmenopausal osteoporosis as compared with age and sex matched normals. After 6-days administration of calcitriol (a possible BGP secretogogue), the increase in serum BGP in 14 osteoporotic women was twice as large as in 10 premenopausal and 9 postmenopausal normals. These results provide further evidence that defective osteoblast function is not a major cause of overall skeletal loss in postmenopausal osteoporosis.

Cell Biology of Bone and Cartilage

H12 THE CELL SURFACE AND OSTEOCLAST DEVELOPMENT, P. Osdoby, Department of Cell Biology, Washington University School of Dental Medicine, St. Louis, Missouri 63110.

Osteoclasts are multinucleated cells which are developmentally and functionally related to cells of the mononuclear-phagocyte system. It is postulated that during bone development and remodelling osteoclasts precursors are recruited (via chemotaxis) to bone, fuse, attach, and resorb the mineralized matrix. Humoral, cell-cell, and cell-matrix interactions apparently mediate these processes. This regulation in part is initiated at specific membrane receptors. Skeletal pathologies may arise from qualitative and or quantitative cell surface modifications or pathological microenvironmental signals acting through the osteoclast plasma membrane. To gain insight into these events isolated chick osteoclasts were used to develop a monoclonal antibody library containing 30 osteoclast-specific hybridoma clones (Oursler et. al. 1985). Immunohistochemical, ELISA, RIA, Western blot analysis and EM immunocytochemical methods were employed to confirm the antibody specificity for osteoclasts. Osteoclast-specific antibodies reacted with osteoclasts but not with monocyte or marrow derived giant cells. Antibody bound specifically to osteoclasts in tissue sections of bone further confirming specificity. These antibodies have been used to purify and biochemically characterize osteoclast-specific cell surface components. Studies are in progress to elucidate the function of the osteoclast-antigens. Moreover, experiments have been undertaken to determine if osteoblast expression is a prerequisite for osteoclast cytodifferentiation. Monocytes and or marrow cells were isolated from chicks, cultured alone, with osteoblasts, or with skin fibroblasts. At select intervals after co-plating cultured were analyzed morphologically and immunochemically (RIA) for the appearance of osteoclast-specific antigens. Monocytes or marrow cells cultured alone or with fibroblasts form giant cells that do not express osteoclast-specific antigens. Immunohistochemical and RIA data demonstrate that giant cells formed in the presence of osteoblasts develop osteoclast antigens. Cell-contact may be necessary for this induction. Monoclonal antibody technology has been used to identify and purify osteoclast-specific cell surface molecules. These antibodies have been used to begin to understand the role of cell interactions in bone development. These reagents should help clarify factors involved in normal and pathological bone remodelling.

H13 PROSTAGLANDIN AND BONE REMODELING, Gideon A. Rodan, Sevgi B. Rodan, Chi K. Yeh* and David D. Thompson, Department of Bone Biology and Osteoporosis, Merck Sharp and Dohme Research Laboratories, West Point, PA 19486 and *Department of OralBiology, University of Connecticut, Farmington, CT 06032

Several years ago we proposed that cells of osteoblastic lineage may be involved in the regulation of bone resorption since they possess receptors for bone resorbing hormones [parathyroid hormone (PTH), $1,25(OH)_2D_3$, PGE_2, hypercalcemia factors], which are apparently absent in osteoclasts. To test this hypothesis we looked for bone resorbing activity in conditioned media of osteoblastic cells. We found that most of that activity was accounted for by prostaglandin E_2 (PGE_2). PGE_2 and PGI_2 are the major products of arachidonic acid in osteoblastic cells. We found that among osteosarcoma cell lines, only those with osteoblastic properties produced PGE_2, the non-osteoblastic cells apparently lacking the enzymes for conversion of arachidonic acid to PGE. Among bone-resorbing stimuli PTH and $1,25(OH)_2D_3$ did not enhance PGE production, whereas thrombin, bradykinin and rabbit serum enhanced prostaglandin synthesis in osteoblastic cells by about 30-50%. A much larger effect was produced by mechanical stimulation. One-minute stretching and relaxation (0.2-0.5 Hz) of collagen ribbons on which calvaria cells had been grown for two weeks enhanced prostaglandin production over 2-fold one hour later. This effect was mimicked by microtubule disrupting agents. Colchicine, vinblastine or nacodazole enhanced prostaglandin production 3-4-fold. At least two-hour exposure to the drug was needed to produce that effect, which peaked between 8 and 24 hours. The rise in PGE production could be attributed to an increase in cyclooxygenase activity which was not dependent on de novo protein synthesis, suggesting compartmentalization of the PGE producing enzymes. To test in vivo the potential role of prostaglandins in mediating mechanical effects on bone remodeling, knee tenotomy was produced in rats and the resulting changes in the tibia metaphysis were followed histomorphometrically. This procedure caused a 2-fold increase in osteoclast numbers at 30 hours, a 2-fold increase in resorption surface at 72 hours and a 50% reduction in trabecular bone volume at 10 days, accompanied by a 15% reduction in the dry weight of the tibia, by comparison to the contralateral control. The increase in resorption surface was fully blocked by treating the animals with the prostaglandin synthase inhibitor, indomethacin.

These and other recent findings, showing PGE enhancement of bone formation, further implicate prostaglandin E as possible physiological mediators of bone remodeling.

H14 BONE RESORPTION AND THE IMMUNE SYSTEM, Steven L. Teitelbaum, Zvi Bar-Shavit, David L. Lacey, and Arnold J. Kahn, Washington University Medical Center, and Pediatric Research Institute, Cardinal Glennon Hospital, St. Louis University, St. Louis, MO 63110

Bone resorption is an ever occurring process in man and other vertebrates and is essential in these species for maintenance of mineral homeostasis. While it has been known for some time that a lymphocyte-produced factor is capable of stimulating the activity of the principal bone resorptive cell, the osteoclast, the relationship of the immune system to bone resorption has until recently, remained largely unexplored. The realization, however, that the osteoclast is derived from a member of the monocyte/ macrophage family has underscored the possibility that bone resorption may be immunoregulated.

Our interest in this area was engendered by the role of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) as an inducer of macrophage differentiation. We found that this steroid, which is the bioactive form of vitamin and a pivotal factor in maintenance of skeletal homeostasis, also promotes the human promyelocytic leukemia cell line, HL-60, and authentic bone marrow cells, to undergo monocytic differentiation. In so doing, these $1,25(OH)_2D$-induced cells not only acquire the biochemical markers of macrophages, but express Class II antigens. Most importantly, like osteoclasts, $1,25(OH)_2D$-treated HL-60 cells acquire the capacity to resorb bone.

Recently, our interest has also focused on the capacity of $1,25(OH)_2D$ to regulate helper-T cell proliferation. We found that this calcium-regulating steroid stimulates the DNA synthesizing by the non-transformed helper-T cell line D10, up to fifty fold in the presence of lectin or specific antigen. In contrast, however, the vitamin metabolite suppresses D10 proliferation when enhanced by Interleukin-1. Hence, $1,25(OH)_2D$ presents itself as a broad-based and bipotential regulator of the immune system.

Hormones, Growth Factors and Other Metabolites

H15 TRANSFORMING GROWTH FACTORS AND BONE, K. J. Ibbotson, S. M. D'Souza and G. R. Mundy, Dept. of Medicine, Univ. of Texas Health Science Ctr, San Antonio, TX 78284.

It has become apparent that transforming growth factors (TGFs) probably have a role in bone destruction associated with the humoral hypercalcemia of malignancy (HHM). The best evidence that TGF's is associated with tumor-mediated bone destruction comes from the rat D6 Leydig cell tumor. We found that the bone resorbing activity produced by these tumor cells co-eluted with activities identical to those of a high molecular weight (30 kd) TGF's, and the bone resorbing activity was inhibited by antisera to the epidermal growth factor (EGF) receptor which inhibits the biological activity of both EGF and TGFα. Northern blot analysis of this tumor showed excessive transcription of TGFα mRNA. We also found excessive TGF mRNA production in a human squamous cell carcinoma of the lung associated with hypercalcemia when examined by Northern blot analysis. The bone resorbing activity in extracts of this tumor was also inhibited by the EGF receptor antisera. In further support for a role for TGFα in this type of bone destruction, we found that both rat synthetic and human recombinant TGF$\alpha$are potent bone resorbing agents in vitro. Since there is such a strong link between TGFα production and hypercalcemia, we examined two tumors which had not been associated with hypercalcemia but which produce large amounts of TGFα. Both tumors, a virus transformed rat fibroblast line, and a human melanoma line, caused hypercalcemia when carried in rats or nude mice respectively. Both tumors released bone resorbing activity into that cell culture medium and this bone resorbing activity was again inhibited by the EGF receptor antisera, suggesting that TGFα mediated the hypercalcemia. In HHM there appears to be inhibition of new bone formation in addition to increased osteoclastic bone resorption. We examined the effects of human recombinant TGF on two parameters of bone formation in vitro, alkaline phosphatase activity in rat osteosarcoma cells, and collagen synthesis in organ cultures, of fetal rat calvariae. Alkaline phosphatase is thought to be important in bone matrix mineralization and collagen synthesis represents the first step in the formation of the bone matrix. We found that TGFα was a potent inhibitor of both alkaline phosphatase activity and collagen synthesis, providing further evidence that TGFα mediates abnormalities in bone metabolism which occur in HHM. TGFα however is clearly not the sole agent responsible for HHM. In a second rat model of HHM, the Walker 256 breast tumor, we have found that the bone resorbing activity produced by the tumor cells co-eluted with TGFβ-like activity and was not inhibited by antisera to the EGF receptor. We and others have since shown that TGFβ stimulates bone resorption in vitro. Thus, it is possible that TGFβ may be responsible for some cases of HHM. Interestingly when we examined the effects of TGFβ on bone formation we found that TGFβ stimulated alkaline phosphatase activity and also enhanced collagen synthesis. TGFβ has been previously implicated in wound healing and it may therefore play a role in fracture repair where its actions may be modulated by other growth factors. In conclusion it is clear that TGF's may not only have an important role in HHM but may also be important in the normal regulation of bone cell function and bone turnover.

H16 BIOSYNTHESIS OF CALCIUM-REGULATING HORMONES, John W. Jacobs, Biological Chemistry, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486

The synthesis of two calcium-regulating hormones, calcitonin and a bone growth factor, was examined in human tumors associated with altered skeletal metabolism. In a squamous cell lung carcinoma associated with malignant hypercalcemia, large molecular weight forms of calcitonin (MW=3,500) were secreted by cultured tumor cells. To establish the molecular basis for the production of these aberrant forms of calcitonin, mRNA was isolated from tumor cells and examined by cell-free translation assays and Northern blot analyses. Results indicated similar, if not identical, size calcitonin-related gene products were synthesized in the tumor cells compared to the thyroid, the normal site of synthesis of calcitonin. Partial DNA sequence analyses of tumor-derived calcitonin cDNAs indicated no alterations in the primary structure of calcitonin mRNA which could account for the presence of larger molecular weight forms of the hormone in tumor cells. Further studies were undertaken to examine alterations, if any, in the calcitonin gene locus in normal and tumor cells. Southern blot analyses indicated no gross rearrangements of the calcitonin gene in lung tumor cells versus normal leukocytes. Furthermore, chromosomal localization studies indicated a single locus for the calcitonin gene on human chromosome 11. Taken together, these results indicate that lung tumor cells are synthesizing calcitonin-gene products identical to that expressed in the thyroid. Larger molecular-weight forms of the hormone present in tumor cells result, in all probability, from differences in post-translational processing, i.e. calcitonin precursor cleavage and glycosylation, between tumor and thyroidal C-cells.

In a second series of studies, the biosynthesis of a bone growth factor was examined in tumors associated with ectopic bone formation in vivo. Partial purification by gel filtration chromatography indicated a molecular weight of 20,000 for the factor. Microinjection of tumor-derived mRNA into Xenopus oocytes gave rise to translation products with biological activities associated with bone growth, namely, stimulation of cell division and alkaline phosphatase activity in bone forming cells (osteoblasts). Fractionation of mRNA from the tumor cells indicated a discrete mRNA fraction of approximately 2000 bases which, following microinjection into oocytes, contained all the bone-growth promoting activities. Further characterization of this factor indicates the tumor cells are synthesizing a heretofore unrecognized stimulator of osteoblastic-like cells.

H17 PROSTAGLANDINS AND OTHER LOCAL FACTORS REGULATING BONE METABOLISM, Lawrence G. Raisz, M.D., Dept. of Medicine, University of Connecticut School of Medicine, Farmington, CT 06032

Local regulators of bone formation and resorption may not only be important in the remodeling of the skeleton in response to stress and other local needs, but may also mediate the response to calcium regulating hormones. Abnormal production of local regulators is likely to be responsible for bone loss in inflammation and malignancy and might play a role in the pathogenesis of osteoporosis. Among the local regulators thus far identified, prostaglandins, particularly PGE_2 and PGI_2, have been most extensively studied because they are produced in bone and clearly affect its metabolism. PGE_2 differs from other stimulators of bone resorption in its ability to stimulate bone formation. PGE_2 production is increased by parathyroid hormone, decreased by glucocorticoids and epinephrine and increased by a large number of pathologic factors such as thrombin, bradykinin, transforming growth factors (TGF) and interleukin-1 (IL-1), as well as epidermal (EGF) and other growth factors. TGF, EGF and IL-1 can also stimulate bone resorption independent of prostaglandin production. Tumor necrosis factor (TNF) and lymphotoxin (LT) are also potent stimulators of bone resorption. Although local bone-derived growth factors have been identified, they have not been fully characterized and none of the agents listed above other than PGE_2 have clearly been shown to stimulate bone growth. Indeed, several have been shown to inhibit osteoclast function. There are remarkably few local inhibitors of bone resorption. Interferon-gamma can inhibit bone resorption both directly and by blocking prostaglandin synthesis. A factor, which competitively inhibits PTH-stimulated bone resorption, has been found in rat bone culture medium but not yet characterized. The physiologic and pathologic importance of the many factors which influence bone metabolism is little understood. Specific identification of their roles may lead to a better understanding of the pathogenesis and treatment of metabolic bone disease and treatment of metabolic bone disease.

Molecular Biology of Bone and Cartilage Development

H18 GENE EXPRESSION IN A MODEL SYSTEM FOR MONOCYTE-OSTEOCLAST DEVELOPMENT. P.H. Reitsma, S.L. Teitelbaum, T. Hilliard and A.J. Kahn, Pediatric Research Institute, St. Louis U. and the Washington U. Medical Center, St. Louis, Missouri 63110.

Data obtained by a variety of different methods and from a number of independent laboratories indicate that osteoclasts are closely related to and probably descendent from a monocytic precursor cell. The human promyelocytic leukemic cell line, HL-60, possesses many of the characteristics of such precursor cells including the ability to respond to exogenous inducers (e.g. the active metabolites of vitamin D_3) by differentiating into cells with monocytic and osteoclastic characteristics, including the ability to bind and resorb mineralized bone matrix. In the present study, a cDNA library prepared from differentiated HL-60 cells was screened with hybrid selected and nonselected cDNA probes generated from HL-60 mRNA in order to identify clones representing genes active during the differentiation process. These clones were then used in Northern blot analysis to determine the nature and extent of change in the mRNA pool subsequent to induction. The data show that (i) ~10% of the induced HL-60 cDNA library contains sequences that are more highly expressed in treated than in untreated cells, (ii) >90% of the latter clones contain repeats of the ALU-1 type, (iii) repeat-associated mRNA's are most abundant ~12-24 hours after the addition of inducer and (iv) ALU-1 sequences appear to be covalently linked to otherwise unique mRNAs. Thus, as has been reported for many other developing systems, differentiation in HL-60 is accompanied by specific changes in the mRNA population, particularly among those members containing ALU-1 sequences. However, whether such sequences play an active role in regulating transcription and/or translation in HL-60 remains to be determined.

Bone Matrix Proteins

H19 TYPE X COLLAGEN IS SYNTHESIZED DURING ENDOCHONDRAL OSSIFICATION IN FRACTURE REPAIR, W.T. Grant, G.J. Wang and G. Balian, University of Virginia, Charlottesville, VA 22908

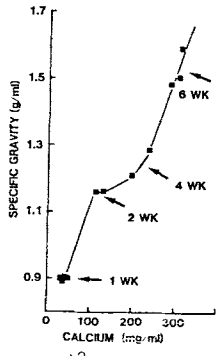
During fracture healing, the histologic changes in the cartilaginous callus are similar to other forms of endochondral ossification. Collagen synthesis was studied in fractures created in the humerus of skeletally mature, White-Leghorn chicken. At 8-10 days the callus was dissected into subgroups according to color, consistency and vascularity and these areas were examined histologically and by metabolic labeling with ^{14}C-proline followed by SDS-PAGE and fluorography. Samples comprising fibrous tissue synthesized primarily type I collagen whereas type II synthesis was increased in the areas of the callus containing chondroid tissue. A 55Kd polypeptide was synthesized, initially as a minor component, by the cartilaginous areas of the callus. This 55Kd became a major form in association with areas of hypertrophic chondrocytes and mineralized matrix. Identification of this protein as type X collagen was achieved by its sensitivity to bacterial collagenase, conversion to 40Kd by limited digestion with pepsin and by comparison of peptides obtained by cyanogen bromide or S. aureus V8 protease digestion with type X prepared from chick sternal chondrocyte cultures. Thus far type X collagen synthesis has been associated with embryonic endochondral bone formation, longitudinal bone growth and, in this study, with fracture repair.

H20 CYSTEINE IN α1 CHAINS OF TYPE I COLLAGEN PRODUCES A CLINICALLY HETEROGENOUS FORM OF OSTEOGENESIS IMPERFECTA, W. Nancy de Vries and Wouter J. de Wet, Department of Biochemistry, Potchefstroom University, 2520 Potchefstroom, South Africa.

Extensive radiological examination of five individuals representing three generations of a large family with an autosomal dominant form of OI showed a remarkable variation in phenotype, ranging from multiple fractures, blue sclera and severe skeletal deformities, to no fractures, white sclera and mild truncal shortening. Synthesis of procollagen was examined in skin fibroblasts. Proteolytic removal of the propeptide regions of newly synthesized procollagen, followed by two-dimensional SDS-PAGE, revealed the presence of type I collagen in which two α1(I) chains were linked through interchain disulfide bonds. Fragmentation of the disulfide linked α1(I)-dimers with vertebrate collagenase and cyanogen bromide demonstrated the presence of a cysteine residue in α1(I)-CB8. The patients' fibroblasts also synthesized normal α1(I) chains. The presence of the mutant α1(I) chains in trimers of type I procollagen was consistently found to reduce the thermal stability of the protein as judged by resistance to digestion by trypsin and chymotrypsin. Molecules containing disulfide linked α1(I) chains has a Tm of about 39°C, whereas the Tm of type I collagen from control fibroblasts was 40.5°C. The most likely explanation for the disruptive change in the physical stability of the mutant collagen is that a cysteine residue is substituted for a glycine in fragment α1(I)-CB8. Our observations also indicate that the mutant procollagen was cleared from the cells with no significant delay in secretion. Also, the cleared extracellular cysteine-containing type I procollagen molecules were cleaved by procollagen N-proteinase, suggesting that the OI phenotype is due to the incorporation of cysteine-containing type I trimers into collagen fibrils.

H21 MINERALIZATION OF HEALING CANINE TIBIAL DEFECTS, J. P. Gorski, J. T. Bronk and T. P. Moyer, Mayo Clinic, Rochester, MN 55905

As part of a study of the effect of matrix proteins on mineralization, we measured calcium content per time in a fracture healing model. Similar results were obtained with six mongrel dogs in two age groups, 1-2 yrs and >8 yrs. After anesthesia, four 0.9 cm holes were drilled through one cortex of each tibia and defects allowed to heal for 1, 2, 4 or 6 weeks. Vascular volume of defects was determined with ^{99}Tc-labeled red blood cells and tibiae removed 4h later; ingrowth tissue was removed from each hole for independent analysis. Ingrowth tissue was devoid of cartilage by histologic analysis and suggests repair proceeds via an intramembranous mechanism. Vascularization of defects peaked at 2 wks after surgery. Water content of ingrowth tissue was 74% at 1 and 2 wks and then dropped to 40% at 6 wks. The calcium/phosphorous molar ratio of ingrowth tissue averaged 1.827 at 1 and 2 wks, and 1.594 at 4 and 6 wks. A plot of calcium content per volume vs. specific gravity is shown and indicates that calcium uptake is triphasic. The data suggest phase one (40-100 mg calcium/ml) may represent accretion by proliferating mesenchymal tissue. Uptake during the second phase (100-200 mg/ml) is consistent with substitution of Ca^{+2} for another metal ion bound to anionic matrix sites. The third phase (>200 mg/ml) appears to involve deposition of calcium hydroxyappatite at the expense of tissue water.



H22 SPECIES SPECIFIC DIFFERENCES IN THE ARTHRITOGENIC EPITOPES EXPRESSED BY MAMMALIAN AND AVIAN TYPE II COLLAGENS. Marie M. Griffiths, University of Utah, Salt Lake City UT 84132.

Type II Collagen-Induced Arthritis (CIA) is an experimentally-induced, autoimmune model of chronic, peripheral arthritis that is under multiple gene control in rats. A panel of 12 inbred prototype and congenic rat strains have been tested for susceptibility to CIA as induced by native type II collagen extracted from the articular cartilage of several mammalian (deer, rat, calf, pig) and avian (chick) species. In a strain analysis 1) Porcine (PII) type II collagen was the most arthritogenic overall and chick (CII) type II collagen was the least arthritogenic of the collagens using a standard immunization protocol. 2) A general pattern of DA,WF>LEW>BN.1A, BN.1L, AUG > ACI, M520>BN, WF.1N, F344 as based on incidences of CIA was observed. However, under strong immunization conditions, reciprocal, RT1-controlled, differences in recognition of species specific arthritogenic determinants present in PII and CII were found with the WF.1N (MAXX) and BN.1L (LEW) strains. Susceptibility vs resistance to CIA (as induced by PII or CII) was paralleled by high or low cross-reactive, IgG anti-rat type II collagen antibody titers in the two strains 28 days after immunization.

H23 **CHARACTERIZATION OF GROWTH FACTORS DERIVED FROM BONE MATRIX,** **P. Hauschka, S. Doleman, M. Iafrati, J. Sasse, M. Klagsbrun. Children's Hospital, Boston, Massachusetts 02115.**

Bone matrix contains a variety of polypeptide growth factors (GF) which are extractable by demineralization (EDTA or HCl). Quantitation of these activities involves mitogenic assay on quiescent BALB/c 3T3 fibroblasts, where 1 GF unit/0.2 ml medium causes half-maximal stimulation of [^{3}H]-thymidine incorporation into DNA. Up to 600 units of total GF activity is present in 1 g of dry bone powder. Resolution by affinity chromatography on heparin-Sepharose reveals up to six peaks of bone derived GF activity (BDGF), provisionally named by the NaCl molarity at which they elute. For fetal bovine mandible (cartilage-free) these peaks include: BDGF-0.1 (<10% of total activity), BDGF-0.45 (23%), BDGF-1.1 (10%), BDGF-1.5 (50%), BDGF-1.7 (7%), BDGF-2.0 (7%). In adult bovine femur the proportion of BDGF-1.5 (20%) and BDGF-1.7 (30%) is shifted, possibly because of endochondral origin. BDGF-1.1, -1.5, and -1.7 share certain chemical properties with fibroblast GF-like mitogens from brain, pituitary, cartilage, and tumors, and are potent mitogens for capillary endothelial (CE) cells. BDGF-0.45 is stable to boiling, 0.1N HCl, and 4M guanidine, inactivated by DTT and trypsin, and inactive on CE cells, thus resembling platelet derived growth factor (PDGF). Characterization of BDGF's by antisera to other known polypeptide GF's is in progress.

H24 Monoclonal Antibodies to Human Chondronectin V.J. Horn, H.H. Varner, J. Dromsky, G.R. Martin, and H.K. Kleinman, NIH, NIDR, LDBA, Bethesda Md.

Chondronectin is a glycoprotein found in cartilage and in serum which mediates the attachment of chondrocytes to type II collagen (Hewitt et al, PNAS 77:385;1980). It has a molecular weight of 180,000 before reduction of disulfide bonds and 77,000 after reduction. We have partially purified human chondronectin from serum using affinity and adsorption chromatography. We have raised monoclonal antibodies to human chondronectin by immunizing Balb-c mice with native partially-pure human chondronectin. The immunized spleen cells were fused to Ag8 myeloma cells. The resulting colonies were screened by ELISA against pure chondronectin obtained by electroelution from SDS-PAGE gels. Two positive clones were obtained after growth and repeated subcloning. Using Western blot techniques, the antibodies have been shown to recognize both the reduced and unreduced chondronectin in whole human serum and in human cartilage extracts. The antibody does not cross react with other serum or cartilage proteins, and is species specific. Indirect immunofluorescence of fetal human sternal and articular cartilage localized the antibody pericellularly around chondrocytes. A competitive ELISA is being developed to detect variations in serum chondronectin concentration in patients with bone and cartilage disorders. Differences are expected as chondronectin serves an important role in cartilage, and is cartilage-specific.

H25 MEASUREMENT OF BONE MORPHOGENIC PROTEIN AND PROSTAGLANDINS IN PATIENTS WITH FIBRODYSPLASIA OSSIFICANS PROGRESSIVA, Joan C. Marini, Robert T. Hudak, James Renig, Marshall R. Urist and Michael A. Zasloff. National Institutes of Health and University of California, Los Angeles.

Fibrodysplasia Ossificans Progressiva (FOP) is a heritable disorder of connective tissue which is characterized by the progressive appearance of heterotopic enchondral bone. Lesions arise as sarcoma-like soft tissue swellings. By CT studies, ossification initiates at random points in fascial planes surrounding muscles. The lesion progresses through a cartilage stage and ends as histologically normal bone with cnetral marrow cavity. The basic lesion is unknown and there have been no abnormal laboratory tests by which the disease may be followed.

We have discovered that patients with active lesions have elevated levels by RIA of bone morphogenic protein in serum and a PGE-like compound in plasma. Levels in these patients are up to ten times the normal value. The ectopic ossification in these pateints follows a pattern that is strikingly similar to the ossification induced by injection of bone morphogenic protein into rat muscle, suggesting that these values provide information on the basic biology of FOP. Measurement of these compounds on additional patients (with active vs. inactive, treated vs. untreated disease) will be presented to provide information on their usefullness as disease markers. Further, we will present the results of selective venous catherization of active lesions and their implications for the biology of heterotopic bone induction.

H26 Specific Binding Sites for Epidermal Growth Factor (EGF) in Bone and Incisor Enamel Organ of the Rat. B. Martineau-Doizé, W. Lai, H. Warshawsky and J.J.M. Bergeron. An in vivo binding assay using radioautography was employed to visualize EGF receptors in rat calcified tissues. Experimental rats were injected intravenously with ^{125}I-EGF (207 μCi/μg). Control rats received the same quantity of ^{125}I-EGF together with 100 μg of unlabeled EGF. Two and 30 minutes later, free hormone was washed out by perfusion with Ringer's solution followed by glutaraldehyde, which fixed the bound hormone in situ. Silver grains indicated sites of hormone binding in tissues of experimental rats. These sites were defined as specific binding (receptors) when similar sites in control rats showed a significantly reduced reaction. Specific binding sites were observed on undifferentiated cells in the zone of vascular invasion at the metaphyseal surface of the epiphyseal plate. These mononucleated cells had elongated processes located between the calcified cartilage and the capillary endothelium. Similar cells containing receptors for EGF were also observed between endothelial cells and bone in the zone of mixed spicules. In enamel organ, receptors were present on the outer dental epithelium, stellate reticulum, stratum intermedium and papillary layer cells of the presecretory, secretory and maturation zones and the ruffle-ended ameloblasts in maturation. Within thirty minutes, the labeled receptors on ruffle-ended ameloblasts were internalized to endosomes in a region within the Golgi zone. EGF has been implicated in cell division, differentiation and invasiveness. The demonstration of EGF receptors in bone and enamel organ provides the first evidence for a direct role of this hormone in bone and enamel formation. Supported by MRC of Canada.

H27 A POTENTIAL MOLECULAR MARKER FOR OSTEOARTHRITIC CARTILAGE, Cahir A. McDevitt*, Stephen P. Arnoczky and Mark Mitchell, *Cleveland Clinic Foundation, Cleveland, OH 44106; Hospital for Special Surgery, New York, NY 10021; State University of New York, NY 11794.

Specific molecular markers for degradation products of articular cartilage would be invaluable in diagnosis, classification and management of the sub-types of osteoarthritis. The soluble, non-proteoglycan proteins were systematically investigated in experimental canine and naturally acquired human (OA) with the aim of identifying possible markers for the disease. Experimental OA was induced for 3 to 7 months by section of the anterior cruciate ligament in 10 dogs; human osteoarthritic patellae were obtained at autopsy.

Proteins were extracted with 4M GcHCl with inhibitors, purified by density graduate centrifugation and analyzed by gradient gel electrophoresis and silver periodic acid staining. The results were notable: all of the pathological canine and human sepcimens contained a protein (M.W: 135kDa) that was absent or markedly reduced in concentration in controls. Preliminary characterization studies suggest the protein is an early degradation product of cartilage that could be employed as a marker for the disease.

H28 Metalloproteinases in Endochondral Bone Formation, Yuko Mikuni-Takagaki, The Children's Hospital and Harvard Medical School, Boston, MA 02115

During endochondral bone development, explants of embryonic chick limbs release neutral metalloproteinases capable of degradaing cartilage proteoglycan and gelatin. The enzyme(s) which is secreted from explants of the region adjacent to the bone marrow cavity degrades proteoglycan as well as gelatin and is unique in being in an active form in the medium and resistant to the serum proteinase inhibitor, α_2-macroglobulin and cartilage tissue proteinase inhibitors including TIMP (tissue inhibitor of metalloproteinases). The other enzymes present in media conditioned by tarsus and bone marrow explants are in latent forms. Although the activated tarsus enzyme(s) is capable of degrading proteoglycan, it is blocked by the above inhibitors. Marrow enzyme does not seem to degrade proteoglycan effectively, but is resistant to these inhibitors. Since it has been suggested that cartilage proteoglycan inhibits mineralization perhaps by sequestering calcium at its onset, our results suggest that the "cavity-surround" tissue produces proteinase(s) which is essential to cartilage removal and mineralization during endochondral bone formation.

H29 OSTEOINDUCTION IN RATS WITH A XENOGENEIC COMPOSITE, Ranga M. Nathan, Rosa M. Armstrong, Hanne Bentz, Andrea Y. Thompson, Karl A. Piez and Saeid M. Seyedin, Connective Tissue Research Laboratories, Collagen Corporation, 2500 Faber Place, Palo Alto, CA 94303

Subcutaneous implantation of allogeneic demineralized bone matrix (DBM) in rats results in endochondral bone formation. In contrast, implants of bovine DBM in rat subcutaneous tissue show inconsistent cartilage and bone formation, along with an intense inflammatory reaction at the implant site. The inflammatory response appears to be due to species-specific immunogens present in bovine implants. To overcome this type of a response, partially purified bone-inducing factor(s) was prepared from bovine bone through a series of steps that included guanidine HCl extraction of the DBM followed by gel filtration and CM-cellulose chromatography of the proteins. Embryonic rat muscle cells embedded in agarose gel culture were used to assay for chondrogenesis. Only cells treated with the CM-cellulose bound (CM-B) fraction differentiated and synthesized cartilage specific macromolecules. To develop an in vivo delivery system for CM-B, the inactive guanidine HCl-extracted bone residue was trypsinized and extensively washed to remove the antigenic components yielding a predominantly insoluble collagenous matrix. Xenogeneic composites were prepared by combining different amounts of CM-B with a carrier system that consisted of 90% trypsinized insoluble bone collagen and about 10% purified soluble bovine dermal collagen. Osteoinductive activity of materials implanted in rats was measured by determining cartilage proteoglycan levels, alkaline phosphatase activity and histological methods. Subcutaneous implantation of the composite materials resulted in dose- dependent endochondral bone formation in rats, with CM-B. Osteogenic activity was detectable in a mixture of 1 part CM-B to 200 parts of the carrier. At higher doses, 1:100 and 1:50, biological activity was comparable to allogeneic implants of rat DBM, with little or no inflammatory response. The results, therefore, show that the osteoinductive activity of a bovine DBM is enhanced upon minimizing the immunogenic components in the xenogeneic bone matrix.

H30 CARTILAGE-DERIVED ENDOTHELIAL CELL GROWTH FACTOR IS STRUCTURALLY RELATED TO TUMOR AND PITUITARY DERIVED GROWTH FACTORS. Joachim Sasse, John A. Smith*, Robert Sullivan and Michael Klagsbrun. Department of Surgery, Children's Hospital, Boston, MA 02115 and Department of Molecular Biology*, Massachusetts General Hospital, Boston, MA 02114.

Cartilage-Derived Growth Factor (CDGF) has been extracted from human cartilage with 1 M NaCl and purified to homogeneity by cation-exchange chromatography on BioRex-70 and affinity chromatography on Heparin-Sepharose. The strong affinity for heparin of CDGF suggested to us a possible homology to tumor and brain derived growth factors. Accordingly, two synthetic peptides were synthesized corresponding to two sequences present in both human hepatoma-derived and bovine pituitary-derived growth factor. Peptide 1 is the 15-mer amino-terminal region of brain and pituitary FGF (Ala-Leu-Pro-Glu-Asp-Gly-Gly-Ser-Gly-Ala-Phe-Pro-Pro-Gly; Boehlen et al., P.N.A.S., 81: 5364, 1984) and peptide 2 is a 10-mer sequence close to the bovine FGF C-terminus (Ala-Ile-Leu-Phe-Leu-Pro-Met-Ser-Ala-Lys; Esch et al., P.N.A.S., 82: 6507, 1985). High titer antisera raised against these synthetic peptides strongly cross-reacted with CDGF in Western-Blot and Immuno-Slot-Assays. These results combined suggest that CDGF and tumor- and brain-derived growth factors are structurally related members of a class of cationic growth factors characterized by their strong affinity for heparin.

H31 ISOLATION AND CHARACTERIZATION OF PROTEOGLYCANS FROM THE MINERALIZED COMPARTMENT OF RAT CALVARIA, K. Shimizu and J. P. Gorski, Mayo Clinic, Rochester, MN 55905

Recent studies have described the isolation and analysis of a small proteoglycan (PG) from bovine bone. In view of the potential usefulness of a rodent model in metabolic studies, it was of interest to purify and characterize the PG fraction from rat calvaria. Young rat calvaria were excised, parietal and frontal bones freed of sutures, and bone was minced and lyophilized. The tissue was first extracted with 4M guanidine-HCl (pH 7.4) containing inhibitors for 72h at 4°C and then with 4M guanidine-HCl (pH 7.4) containing 0.5M EDTA and inhibitors. The latter extract was subjected to DEAE-Sephacel chromatography in 6M urea; PG were eluted with a linear salt gradient at about 2.0M NaAc. This fraction was purified further on hydroxyappatite in the presence of 7M urea where PG eluted as a single peak during a linear phosphate gradient. Subsequent SDS-PAGE under reducing conditions on gradient gels showed "Stains-All" bands at 295K, 180K, 130K and 35K. Additional Sepharaose CL4B chromatography separated the 35K species (K_{av} 0.7) from the PG peak (K_{av} 0.35). Treatment of the purified PG pool with proteinase K followed by SDS-PAGE resulted in a wide glycosaminoglycan band of apparent M_r=55K. Incubation with chondroitinase AC or ABC indicated that the 180K and 130K species were chondroitin sulfate proteoglycans with core proteins of M_r=42K. The 295K PG form was only partially digested by AC, however, ABC digestion also yielded a 42K core protein. These data suggest that the proteoglycans of the mineralized compartment of young rat calvaria can be purified by the above scheme, are heterogeneous with respect to hydrodynamic size and possibly glycosaminoglycan composition, and yet exhibit a common core protein size.

Bone and Cartilage Cells

H32 INDUCED MINERALIZATION OF CHONDROCYTE CULTURES INCREASES SYNTHESIS OF TYPE X COLLAGEN, Jenny Morris, William T. Grant and Gary Balian, University of Virginia, Charlottesville, VA 22908

Synthesis of type X (short chain) collagen is restricted to the region of transition between cartilage and bone. To determine if type X is associated with matrix mineralization, cells were cultured in the presence of β-glycerophosphate (BGP). Osteoblasts isolated from chick embryo calvaria and chondrocytes prepared from "calcifying" or "permanent" cartilage regions of sterna were cultured with or without 10mM BGP. Both osteoblasts and calcifying chondrocytes cultured with BGP demonstrated areas of mineralization beginning on day 10 whereas permanent chondrocytes did not mineralize. BGP induced a 25 fold increase in calcium concentration and an 8 fold increase in phosphorus in the cell layers of "calcifying" chondrocytes. While no type X collagen synthesis could be detected in osteoblasts or "permanent" chondrocyte cultures with or without BGP, treatment of "calcifying" chondrocytes with BGP significantly increased incorporation of ^{14}C-proline into type X in both cell layer and media compartments. Stimulation of type X collagen synthesis by BGP in association with the induced mineralization of calcifying chondrocyte matrix suggests a role for this collagen in the process of cartilage mineralization.

H33 TYPE X COLLAGEN BIOSYNTHESIS BY CULTURED RABBIT GROWTH PLATE CARTILAGE CHONDROCYTES Bashey, R.I., Campo,R., Rao, V., Jimenez, S.A. University of Pennsylvania 19104

During the last few years it has been shown that hyaline cartilage contains in addition to Type II collagen, several minor species which include 1α, 2α, 3α and Type IX collagens. In addition it has been shown that avian chondrocytes synthesize a short-chain collagen which is now classified as Type X collagen. We reported the synthesis of Type X collagen by rabbit and bovine growth plate cartilage in organ cultures. In the present study, we developed methods to isolate chondrocytes from the different zones of rabbit growth plate and successfully established primary cultures of these cells.

Chondrocytes were isolated from four sequential sections of costochondral growth plate representing hypertrophic, reserve, proliferative and structural cartilage. When the chondrocytes reached confluency they were metabolically labeled with ^{14}C-proline. Analysis of the labeled proteins by SDS-polyacrylamide gel electrophoresis showed that type II procollagen was the major collagen species synthesized by the chondrocytes from the four separated regions of the growth plate. In contrast, Type X collagen which represented about 20% of total collagen was exclusively synthesized by the condrocytes cultured from the hypertrophic zone. The newly synthesized Type X collagen was only visualized after pepsinzation of the media suggesting that it is probably present in a higher molecular weight form. Further work is in progress to elucidate the nature of the Type X aggregate.

H34 EFFECT OF HEPARIN ON COLLAGEN SYNTHESIS BY CHONDROCYTES, Cameron C. Brown and Gary Balian, Univ. of Virginia, Charlottesville, VA 22908

Chondrocytes isolated from either "permanent" or "calcifying" regions of embryonic chick sternae were grown to confluence and treated with heparin for 48h. One group was incubated with ^{14}C-proline and another allowed to grow an additional 72h from the time heparin treatment was stopped. There were no changes in cell morphology due to heparin. Radiolabeled proteins studied on SDS-PAGE showed a marked increase in bands that were pepsin-sensitive, corresponding to the region of precursor molecules of type II collagen as well as a decrease in relative synthesis of type II and type X collagens. No type X collagen was detected in the "permanent" cartilage cells with or without heparin. Bacterial collagenase assay identified a significant reduction in collagenase sensitive material predominantly in the media compartment. All inhibitory effects were reversed upon removal of heparin. Heparin appears to inhibit collagen processing demonstrated by the accumulation of a precursor in the cell layer and a corresponding decrease in collagenase sensitive material in the media. This effect may correlate with the clinically known induction of osteoporosis following long term heparin therapy. Our results are consistent with reports that exogenous proteoglycan inhibits collagen synthesis by chondrocytes.

H35 CHARACTERIZATION OF HUMAN OSTEOBLAST LIKE CELLS CULTIVATED "IN VITRO"
Annette Chamson, Aline Rattner, Nadine Raby and Jacques Frey, Medical School of Saint-Etienne, 42023 Saint-Etienne, France.

Osteoblast like cells were cultivated by enzyme digestion (trypsin - collagenase) from spongious bone. They were propagated by trypsinization through serial subcultures.

The subcultures were characterized by their biochemical properties. The cells contain alkaline phosphatases, and it was possible to determine that it was the bone isoenzyme by electrophoresis and inhibition at 56°C. The presence of osteocalcin was inconstant.

The collagen biosynthesis was studied after labelling with ^{14}C proline. It was observed that type III collagen was two to three times less than in fibroblast cultures and type I trimer was twice greater than in fibroblast cultures.

H36 MODULATION OF CHONDROCYTE METABOLISM BY CYTOKINES PRODUCED BY A SYNOVIAL CELL LINE, C.H. Evans, H.I. Georgescu, D. Mendelow, K. Sung, M. Tsao and S. Watanabe, Orthopaedic Research Laboratory, University of Pittsburgh, Pittsburgh, PA 15261

HIG-82 is an established line of lapine, synovial fibroblasts. Following activation by phorbol myristate acetate (0.1 µg/ml), HIG-82 cells secrete cytokines which have several effects upon the metabolism of primary, monolayer cultures of lapine articular chondrocytes. The synovial factor suppresses both the incorporation of $^{35}SO_4^{2-}$ into GAG and the incorporation of ^{3}H-proline into collagen, without drastically reducing the synthesis of non-collagenous protein. Chondrocytes increase their synthesis of PGE_2 within one hour of being exposed to HIG-82 cell products; measurable increments in the secretion of collagenase, gelatinase and caseinase follow 8-11 hours later. The synovial activator is heat stable, precipitable by 60-95% saturated $(NH_4)_2 SO_4$ and of M_r between 10,000 and 25,000, properties consistent with those of the catabolin/interleukin-1 family.

H37 CONTRACTION OF COLLAGEN LATTICE BY OSTEOBLAST LIKE CELLS, Jacques Frey and Annette Chamson, Medical School of Saint-Etienne, 42023 Saint-Etienne, France.

Human osteoblast like cells were studied after propagation through serial subcultures. They were characterized by their biochemical properties.

These cells were able to condense and contracte an acid soluble collagen gel. A collagen lattice was obtained and it looked like a tissue. Electron microscopy showed microfibrills and fibrills of collagen with normal cross-striation. Some of these fibrills seemed to have a beginning of calcification.

H38 EFFECT OF STAPHYLOCOCCUS AUREUS CELL WALL COMPONENTS ON BONE CELLS IN CULTURE, Elliot Jacob, Aram Y. Balekjian, Robert E. Carson and Heraline E. Hicks, Naval Medical Research Institute, Bethesda, MD 20814-5055

Although S.aureus is the most frequently isolated organism in osteomyelitis, the factor(s) that enable it to initiate and propogate osseous infections have not been identified. This study evaluated the effects of staphylococcal cell walls(CW), peptidoglycan(PG), and an ammonium sulfate fraction precipitated from the culture supernatant(CS) on bone cell growth in vitro. Bone cells were isolated from fetal rat calcariae and pooled to contain either osteoclast-rich(OC) or osteoblast-rich(OB) populations. Staphylococcal components(CW, PG, CS) were added at a concentration of 0.1 mg/ml to cultures of either OC or OB cells and incubated at 37°C for 24h. The cells were pulsed with ^{3}H-thymidine for 72 h, and the degree of isotope incorporation was measured. S.aureus CW and PG caused a greater inhibition of ^{3}H-thymidine uptake in OC cells (17.0% and 19.4%, respectively) than in OB cells (6.7% and 9.4% respectively as compared to control values. In contrast, the extracellular fraction caused a greater reduction of DNA synthesis in OB cells (13%) than in OC cells (7.7%). E. coli lipopolysaccharide (0.1 mg/ml) did not cause a significant reduction of ^{3}H-thymidine uptake in either OC or OB cells. This study demonstrates that S.aureus possesses cell-associated as well as extracellular components that interfere with bone cell growth in vitro and that these components may play an important role in the pathogenesis of osseous infections.

H39 PLURIPOTENT HEMOPOIETIC STEM CELLS GIVE RISE TO OSTEOCLASTS, Gary B. Schneider, John Nicolas and Melanie Relfson, Loyola University, Maywood, IL 60153

Osteopetrosis in the ia/ia rat is the result of reduced bone resorption due to abnormal osteoclasts. Studies in the ia mutant have shown that hemopoietic stem cells from normal littermates could cure the skeletal sclerosis and result in the formation of normal osteoclasts when transplanted into ia rats. The following studies were conducted to determine the mechanism of the cure, i.e., do the stem cells provide a helper function to transform pre-existing mutant osteoclasts, or do they differentiate into osteoclasts themselves. Goldschneider et al. (J. Exp. Med. 152:419) demonstrated that stem cells from rat bone marrow are relatively large mononuclear cells that are strongly positive for Thy 1.1 antigen. Using the parameters described by Goldschneider, and fluorescence-activated cell sorting, the pluripotent hemopoietic stem cells were isolated from normal rat bone marrow, labeled with saturated FITC and injected intraveneously into irradiated (600 rads) ia rats. After 48 hrs, the recipient tibiae and femora were removed, split longitudinally and the endosteal surface scraped with a sharp forceps. After the bone fragments were allowed to settle, the resulting cellular suspension was examined by phase contrast and fluorescence microscopy. Fluorescing mononuclear cells of donor origin that had homed to the bone marrow demonstrated moderate cytoplasmic fluorescence. 30% of the osteoclasts observed demonstrated light cytoplasmic fluorescence. When cellular pools that are incapable of curing osteopetrosis (thymocytes) were FITC-labeled and injected into ia recipients, no labeled osteoclasts were observed. These studies indicate that pluripotent hemopoietic stem cells, when transplanted into ia hosts, become osteoclasts.

H40 CHARACTERIZATION OF NEW CELL LINES ESTABLISHED FROM SPONTANEOUS MOUSE OSTEOSARCOMAS, Natalie M. Teich, Maureen A. Harrison, and Janice Rowe, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, England

We have recently established cell lines from spontaneously arising osteosarcomas of C57BL mice. First we determined that there were no transforming retroviruses produced by these lines, in contrast to lines derived from tumors induced by the FBJ and FBR strains of mouse sarcoma virus. Next we examined the expression of cellular oncogenes in these lines; of 18 oncogenes examined, 5 were expressed in all lines (but the sis oncogene was not expressed as has been reported for the human osteosarcoma cell line U2-OS). Interestingly, one cell line only contained fos gene expression. A molecular analysis showed that this property was due to insertion of apparently unique cell DNA into the 3' coding region of the cell fos gene. The different cell lines and subclones derived therefrom show specific patterns of mineralization in vitro influenced by cell density, organic phosphate, vitamin D_3, dexamethasone, and parathyroid hormone. In collaboration with others, we have detected growth factor activities produced by the cells for fibroblast mitogenicity, chondrogenesis, osteogenesis, and osteoclast resorption; purification of these activities is in progress.

H41 SPECIFIC INHIBITION OF H^+-K^+-ATPase INHIBITS BONE RESORPTION IN VITRO, Kalervo Väänänen and Juha Tuukkanen, University of Oulu, Department of Pathology, SF-90220 Oulu

Recent immunohistochemical studies have suggested the presence of H^+-K^+-ATPase in osteoclast ruffled border area (Baron et al., J. Cell. Biol. 97,416a,1983). Omeprazole, a specific inhibitor of H^+-K^+-ATPase has been shown to be a potent inhibitor of acid production in gastric parietal cells (Fellenius et al., Nature 290,159,1981). We have recently reported that omeprazole inhibits PGE_2 stimulated bone resorption in vitro (Tuukkanen and Väänänen, In: Cell Mediated Calcification and Matrix Vesicles, Cambridge 1985). Now we have extended these studies to other bone stimulating factors: PTH and $1,25(OH)_2D_3$. Both these agents cause stimulation of $^{45}Ca^{++}$ liberation from prelabeled mouse calvaria in vitro. This stimulation could be inhibited by omeprazole. Resorption could be further restimulated by PTH or PGE_2 even after 24 hours incubation with 10^{-4} M omeprazole, which indicates that omeprazole has no toxic effects to calvaria cultures.

These results strongly support the role of H^+-K^+-ATPase mediated acid production by osteoclasts as a primary mechanism of bone resorption.

H42 A COMPARISON OF THE MORPHOLOGICAL AND HISTOCHEMICAL PROPERTIES OF THE OSTEOCLAST, MACROPHAGE POLYKARYON, AND BONE-INDUCED MULTINUCLEATED CELL, Linda M. Walters and Gary B. Schneider, Loyola University Medical Center, Maywood, IL 60153

Based on functional similarities, macrophages and macrophage polykaryons (MK's) have been proposed as investigational surrogates for osteoclasts. We examined this premise by comparing the histochemical and morphological characteristics of multinucleated cells (MC's) found adjacent to subcutaneously implanted bone with those of suture-elicted MK's and tibial osteoclasts. Functional studies involving the use of ^{45}Ca labelled long bone chips indicated that significant resorption had occured over the implant period. Devitalized trabecular bone chips and silk suture were implanted subcutaneously into 4 week old rats. After 2 weeks the implants were recovered along with portions of proximal tibia from implant recipients. The recovered material was bisected; one half was processed for light and electron microscopy and the other for tartrate resistant acid phosphatase (TR), a marker for osteoclasts. Ultrastructurally, the osteoclasts exhibited typical clear zones and ruffled borders at the bone-cell interface. The MK's and bone-induced MC's possessed occasional clear zone-like areas, but at no time demonstrated ruffled borders. Histochemically, osteoclasts were strongly positive for TR while MK's and MC's showed little or no reaction product. The results of this study indicate that osteoclasts exhibit significant structural and enzymatic differences from MK's, implying that MK's and osteoclasts may not be equivalent cells. We have also shown that bone-induced MC's are probably MK's and that the ^{45}Ca release which occured was due to the action of members of the mononuclear phagocyte system rather than by true osteoclastic resorption.

H43 MOLECULAR APPROACHES TO THE CHARACTERISATION OF OSTEOCLASTS. Julia Warwick*, Timothy Chambers and Michael Horton*, Depts. of Haematology and Histopathology, St. Bartholomew's* and St. George's Hospitals, London, U.K.

A traditionally held view is that the osteoclast (OC), arises from mononuclear phagocytes. Whilst there is support for this contention, evidence has accumulated that they develop from a separate cell lineage. Interpretation of existing experimental data is hampered by the possible confusion of OCs with macrophages. In order to develop convenient tools for dissection of OC, in contrast to macrophage, ontogeny, monoclonal antibodies (mabs) have been raised against functionally active-osteoclasts from osteoclastoma tumours (Horton et al, Cancer Research, in press). 8 mabs were found which differentiate between human OCs and macrophages in foetal bone and other tissues. As an alternative approach to this problem we are seeking to identify OC-specific mRNA sequences using a cDNA library in λgt-11,prepared from human osteoclastoma tumours. The library will be screened with osteoclast -derived cDNA depleted of sequences complementary to mRNA from monocytes,macrophages and osteoclastoma-derived stromal cell lines. Following Northern blot analyses,protein produced by the putative specific recombinants will be used to generate antisera for tissue screening. A subset of our OC-specific mabs (13C2,17D1,17B5) mimic the inhibitory effect of calcitonin on OC bone resorption and motility; a further mab (23C6) is without effect but reacts with OCs from a wide range of species other than man. We hope to isolate the corresponding mRNA sequence from osteoclastoma library by screening for antigen expression. Otherwise it may be possible to derive a cDNA probe enriched for antigen-specific sequences from human renal cells which also bind 13C2 and 23C6 mabs.

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H44 TRANSLATIONAL DISCRIMINATION BETWEEN TYPE I AND TYPE II COLLAGEN RNAs IN CHONDROBLASTS. Sherrill L. Adams, Vickie D. Bennett and Ingrid M. Weiss. University of Pennsylvania, Philadelphia, PA 19104-6072.

Chick vertebral chondroblasts grown in suspension culture synthesize a large amount of type II collagen; they also contain moderate amounts of type I collagen RNAs, although they synthesize no detectable type I collagen protein. We have examined the basis for this apparent translational discrimination between types I and II collagen RNAs by analyzing the association of these RNAs with polysomes. Addition of polysomes from these cells to a rabbit reticulocyte lysate in the presence of the translation initiation inhibitor aurintricarboxylic acid results in the efficient synthesis of type II, but not type I, collagen. However, hybridization of cloned α2(I) collagen DNA to RNA isolated from sucrose gradient fractionated chondroblast polysomes shows that the α2(I) collagen RNA is in fact associated with small polysomes (3-4 ribosomes per RNA), implying inefficient translation initiation. In contrast, the type II collagen RNA is associated with large polysomes. These results suggest that the chondroblast ribosomes have the ability to discriminate between the types I and II collagen RNAs at the level of translation initiation. Furthermore, they suggest that there must be specific sequences in those RNAs which permit this discrimination. Nuclease mapping experiments show that the 5' untranslated region of the α2(I) collagen RNA in chondroblasts differs from that in other cell types, which may alter its translational efficiency. We suggest that this discriminatory ability may be an important control mechanism mediating the transition from type I to type II collagen synthesis during chondrogenesis.

H45 INDUCTION OF CHONDROGENESIS IN VITRO: CHANGES IN PROTEINS AND IN LEVELS OF SPECIFIC mRNAs, Marlissa A. Campbell and Kurt J. Doege, LDBA/NIDR, NIH, Bethesda, Md, 20892

Demineralized bone matrix induces bone formation in vivo, and cartilage formation in vitro. We have used this phenomenon to study the molecular events of chondrogenesis. Outgrowth cells from explants of fetal mouse muscle were exposed to demineralized bone matrix (DBM) (the DEAE-cellulose nonbound fraction of Gu-HCl extracted bovine bone; a gift from Collagen Corp., Palo Alto, Ca.) at a concentration of 50ug/ml, and 3uM dihydrocytochalasin B. After 1 week of culture, the treated cells had become elongated and were surrounded by a fibrous-appearing extracellular matrix. Pulse-labeling with ^{35}S-methionine, followed by polyacrylamide gel electrophoresis of the cell lysates, revealed a series of proteins which were strongly induced by DBM. Several of these proteins were of the appropriate size and fulfilled other criteria permitting identification as cartilage-specific markers. Furthermore, northern hybridization of RNA prepared from these cells to cDNA probes specific for link protein and for type II collagen, confirmed that DBM induced expression of these genes. It is proposed that DBM-mediated chondrogenesis of mesenchymal cells in culture provides an ideal model system for studies on mechanisms of coordinated gene expression during cartilage differentiation.

H46 EFFECT OF 2,2'-DIPYRIDYL ON THE PROPERTIES OF NEWLY SYNTHESIZED CARTILAGE COLLAGENS, C. C. Clark and C. F. Richards, University of Pennsylvania, Philadelphia, PA 19104

Cartilage cells isolated from 17-day chick embryo sterna were incubated in suspension culture with [^{14}C]proline in either the absence or presence of 0.5 mM 2,2'-dipyridyl (DP) for up to 2 h. Under these conditions total incorporation of ^{14}C was inhibited by only 9%, and incorporation into collagenase-susceptible protein was inhibited by 22% in the presence as compared to the absence of DP. In time course experiments in the absence of DP, all cartilage collagen precursors (types II, IX and $1\alpha,2\alpha,3\alpha$) were secreted after a 30-40 min lag period; in the presence of DP, the lag period for all collagen precursors increased to 75-80 min and total collagen secreted was only 21% of control after 2 h. Analysis of the collagens by SDS-polyacrylamide gel electrophoresis showed that the mobility of all unhydroxylated species was significantly increased compared to their hydroxylated counterparts. Surprisingly, protease digestion at temperatures as low as 0° C and for times as little as 15 min showed that the newly synthesized unhydroxylated type IX and $1\alpha,2\alpha,3\alpha$ collagen precursors were extensively degraded whereas the unhydroxylated type II procollagen was only partially susceptible and was converted to unhydroxylated collagen. These results suggest that the minor cartilage collagens (type IX and $1\alpha,2\alpha,3\alpha$) failed to form a stable triple helix under conditions where type II was successful. Since there appeared to be no significant effect of DP on disulfide bond formation, the reason for this failure is unknown at this time. (Supported by NIH Grant AM-32481).

H47 ANTIBODIES TO CARTILAGE-INDUCING FACTOR-A, Larry R. Ellingsworth*, Jane E. Brennan*, Karl A. Piez*, and Kam Fok**, *Connective Tissue Research Laboratories, Collagen Corporation, 2500 Faber Place, Palo Alto, CA 94303, **Department of Biological Sciences, Monsanto Corporation, 700 Chesterfield Village Parkway, St. Louis, MO 63198

Antibodies were produced to an amino terminal amino acid sequence shared by cartilage-inducing factor-A (CIF-A) and transforming growth factor-beta (TGF-β) using a synthetic polypeptide derived from amino acid residues 1 through 30. The monospecificity of the antibodies was determined by immunoblotting, ELISA, and antibody competition assays. These results show that antibodies to the polypeptide were immunoreactive with both nonreduced (26 Kd) and β-mercaptoethanol reduced (12 Kd) CIF-A, as well as human platelet-derived TGF-β. The antibodies, however, were not reactive with the related polypeptide CIF-B, suggesting that the primary structures of CIF-A and CIF-B are significantly different. Having established the specificity of the antibodies, immunohistochemical staining methods were used to localize CIF-A in fetal (6 month gestation) bovine bone. The results show specific staining of osteocytes in cancellous and cortical bone as well as articular chondrocytes. Epiphyseal chondrocytes were not labeled by the antibodies. Results presented here suggest that bone is a source of CIF-A (or TGF-β) synthesis <u>in vivo</u>.

H48 FORMATION OF 1,25-DIHYDROXYVITAMIN D_3 BY ACTIVATED NORMAL HUMAN MACROPHAGES: AN EXPRESSION OF THE VITAMIN D PARACRINE SYSTEM, A. W. Norman, H. R. Reichel and H. P. Koeffler, University of California, Riverside, CA 92521

Cultures of normal human macrophages were examined for their capacity to metabolize [^{3}H]-25-hydroxyvitamin D_3 [25(OH)D_3]. Macrophages which were exposed to γ-interferon (γ-IFN) or lipopolysaccharides (LPS) converted [^{3}H]-25(OH)D_3 to a more polar vitamin D metabolite in a dose- and time-dependent fashion. In contrast untreated macrophages did not metabolize ^{3}H-25(OH)D_3 in significant amounts. Subsequently, the polar metabolite was identified as 1,25-dihydroxyvitamin D_3 {1,25(OH)$_2$D$_3$} on the basis of (i) its migration on four different HPLC solvent systems, (ii) by its affinity for specific chick intestinal 1,25(OH)$_2$D$_3$ receptors and (iii) by its ability <u>in vivo</u> to stimulate characteristic vitamin D responses in the intestine and bone of rachitic chicks. Pulmonary alveolar macrophages (PAM) which had the highest 25(OH)D_3 metabolizing activity as compared to peripheral blood monocytes and bone marrow derived macrophages, were utilized to study the regulation of γ-IFN or LPS-stimulated 1,25(OH)$_2$D$_3$ formation in macrophages. The results indicate that 1,25(OH)$_2$D$_3$ production by macrophages is inhibited by dexamethasone and 1,25(OH)$_2$D$_3$, but not by other vitamin D_3 metabolites. Parathyroid hormone did not influence vitamin D metabolism in macrophages. Our studies suggest that the 1,25(OH)$_2$D$_3$ which is secreted by activated macrophages may participate in a paracrine system during immune response. Potential effects of 1,25(OH)$_2$D$_3$ include the modulation of T-lymphocyte function (e.g. suppression of interleukin-2 release) and induction of myeloid progenitor cells to preferentially differentiate towards mature monocytes/macrophages.

H49 VARIABLE METHYLATION PATTERNS OF THE HUMAN TYPE II COLLAGEN GENE

Eero Vuorio, Andrea Superti-Furga and Beat Steinmann, University of Turku, Finland, and Kinderspital Zürich, Switzerland.

Genomic subclones covering the human proα1(II)collagen gene (COL2A1) have been used as probes to study the methylation patterns of the gene in various human cell types exhibiting varying rates of collagen synthesis. For this purpose DNA was isolated from blood cells and cultured fibroblasts and from human fetal liver, skin and cartilage. DNA samples digested with restriction enzyme SmaI were fractionated on agarose gels and transfered to nitrocellulose for Southern hybridization. In all the DNA samples the region around the 5'-end of the gene was found to be undermethylated. In blood DNA the rest of the gene was methylated and no other SmaI bands were detected. In tissues exhibiting type I collagen production (liver, skin and cultured fibroblasts) another region in the central part of the gene was also undermethylated. Only in cartilage DNA the whole gene was undermethylated. The results are in agreement with the hypothesis that certain CpG rich regions of the genome (such as the 5'-end of type II collagen gene) remain always undermethylated. The results also support the observations that actively expressed genes are undermethylated. Why undermethylation was observed in the central region of type II collagen gene in fibroblasts and liver cells, which do not produce type II collagen, remains unexplained.