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COLLAGEN SYNTHESIS-STIMULATING FACTOR FROM THE CULTURE MEDIUM OF SILICA-TREATED MACROPHAGES.
M. Aalto and E. Kulonen (Turku, Finland)

Rat peritoneal macrophages (non-elicited) were cultured in Dulbecco-Vogt medium (without serum) with and without SiO_2. The media were changed every third day. The effects of the soluble fibrogenic factor in the media were studied on the incorporation of proline into collagen and other proteins in cell-free protein synthesis and also in granulation-tissue slices (Aalto et al.:Exp. Cell Res. 97, 193, 1976; Aho & Kulonen: Exp. Cell Res. 104,31,1977). Attention was also paid to the protein synthesis in the macrophages, the activity of alkaline ribonuclease and to the effect of polyvinylpyridine-N-oxide (PVNO). - The medium of SiO_2-treated macrophages increased the incorporation of proline by 50-100%. RNase activities were 30-40% lower in the media of SiO_2-treated macrophages. PVNO inhibited these effects of SiO_2. Gel filtration chromatography of the media with Sephadex G-100 revealed a large shift of the location of RNase as the effect of SiO_2 and the "SiO_2 factor" could be separated from the bulk of the nitrogenous material.

INVOLVEMENT OF RIBONUCLEASE IN THE INTERACTION OF MACROPHAGES AND FIBROBLASTS, WITH REFERENCE TO SILICOSIS.
Sirpa Aho and E. Kulonen (Turku, Finland)

Macrophage ribonuclease is suggested to regulate the RNA turnover and, hence, the protein synthesis in the adjacent fibroblasts. SiO_2-treated peritoneal macrophages liberate to the 20.000g-supernatant a factor which seems to stabilize the polysomes in granulation-tissue fibroblasts where the synthesis of collagen and other proteins is found to be increased over the respective control level with SiO_2 omitted (Aalto et al.: Exp. Cell Res. 97,193, 1976; Aho & Kulonen: Exp. Cell Res. 104, 31, 1977). The activity of alkaline RNase of the macrophage preparations is actually decreased after a treatment with SiO_2 and correlates inversely with the protein synthesis in the granuloma slices. The characterization of this endonuclease is in progress. Corresponding experiments were made also in vivo with sponge implants imbibed with live macrophages, SiO_2 particles or both. The presence of SiO_2 decreased the free RNase activity in the 27.000g-supernatant and enhanced the amount of polysomes and the collagen synthesis per cell.

HUMAN ARTICULAR CARTILAGE PROTEOGLYCANS IN AGING
R. Amadò, U. Marti and H. Neukom (Zurich, Switzerland)

Cartilage samples of apparently normal human femoral heads of different age were sequentially extracted with water and 4 M guanidinium hydrochloride. Two clearly different groups (A and B) of cartilage could be observed according to the extractability by the two solvents. After ultracentrifugation under dissociative conditions the proteoglycan subunits (PGS) were isolated and their amino acid composition as well as hexosamine, uronic acid and neutral sugar content determined. The results indicate that the protein moiety of the PGS from A and B is not subject to alteration during aging. Chromatography on Sepharose 2B shows that the hydrodynamic size of the PGS of group A is not significantly altered by aging, whereas the PGS of group B behaves like free chondroitinsulfate chains. The yield of extractable PG in normal cartilage (A) decreases significantly during aging, whereas in samples with a high yield of water soluble material (B) nearly all hexosamine containing substances could be extracted, independent of the age of the sample. The results suggest the presence of two distinct PG populations in human cartilage of the femoral head.

THE MECHANISM OF INHIBITION OF JOINT CONTRACTURE BY D-PENICILLAMINE: CHANGES INDUCED IN COLLAGEN CROSS-LINKING PATTERNS USING A LOW DOSAGE SCHEDULE. D. Amiel, W.H. Akeson, & G.L. Mechanic (San Diego, Calif., USA)

Alterations in the [^{3}H] $NaBH_4$ reduced cross-links of collagen from periarticular connective tissue from 9 week immobilized rabbit knee joints have shown an increase in dihydroxylysinonorleucine (DHLNL), hydroxylysinonorleucine (HLNL) and histidinohydroxymerodesmosine (HHMD). A low dosage schedule of D-penicillamine 60 mg/kg body weight 3X weekly is presently under investigation during the period of immobilization to inhibit the process of intermolecular cross-linking. We are reporting direct evidence that D-penicillamine interferes with the formation of intermolecular Schiff base cross-links of collagen during a period of 9 weeks of immobilization in the rabbit knee joint and corroborates the earlier indirect findings of Nimni and his co-workers.

An increase in peptidyl aldehyde, the cross-link precursor, is found, and the [^{3}H] $NaBH_4$ reduced cross-links are significantly lessened in animals treated with D-penicillamine.

THE EFFECT OF BOVINE TENDON GLYCOPROTEIN ON THE FORMATION OF COLLAGEN FIBRILS FROM SOLUTION.

J.C. Anderson, R.I. Labedz and M.A. Kewley. University of Manchester, England.

Glycoprotein (m.wt. approx. 60 000) was extracted from bovine tendon with 3M-$MgCl_2$, and was obtained, together with a low m.wt. proteodermatan sulphate, by affinity chromatography on concanavalin A-Sepharose. The glycoprotein was obtained free of proteoglycan by chromatography on Sephadex G200. The formation of collagen fibrils under physiological conditions of ionic strength, pH and temperature was markedly affected by the presence of small amounts of bovine tendon glycoprotein. The absorbance of the gels at 400 nm was reduced, and they took longer to form. Over the range of concentration tested, the negative specific absorbance $-\Delta A_{sp}$, and the specific retardation R_{sp}, were both proportional to the glycoprotein concentration. When added during the nucleation phase, glycoprotein was still able to exert its effect almost fully, and so must act to inhibit the later stages of fibril formation. Several pieces of evidence showed that glycoprotein acts via a weak binding to the collagen molecule. Electron microscopy established that fibrils formed in the presence of glycoprotein had a normal cross-striation pattern, but were significantly thinner than fibrils formed in control gels. The results suggest that glycoprotein could act in tissues to help regulate the diameter of collagen fibrils.

SCAR TISSUE IN THE CENTRAL NERVOUS SYSTEM.

D.E. Ashhurst and M. Berry (Birmingham, England).

The barrier hypothesis for the failure of axons in the central nervous system (CNS) to regenerate suggests that growing axons may be unable to penetrate the dense scar that develops at the site of the lesion. Since little is known about the nature of the scar tissue, an analysis of this tissue is essential to the elucidation of the problem of regeneration in the CNS.

A knife wound was stereotactically placed in the cerebrum of 40 day-old rats and 10, 30 and 50 days later, the lesioned tissue was processed for electron microscopical and histochemical examinations. The lesion is readily recognised by the presence of macrophages which fill most of the space between the cut surfaces of the nervous tissue. The debris from the damaged tissue has been removed by 30 days and many foot-processes of astrocytes abut on to the lesion. The macrophages and fibroblasts in the lesion are separated from the nervous tissue by a gap of at least 80nm which is filled by electron dense, amorphous material, which is thought to be the fused basement membranes of the opposing cells. In places this gap is wider and typical collagen fibrils appear in the matrix. The presence of glycosaminoglycans in this matrix has been shown histochemically.

PROPERTIES OF CORNEAL KERATAN SULFATE PROTEOGLYCANS

I. Axelsson and D. Heinegård (Lund, Sweden)

Half of the keratan sulfate in bovine corneal stroma can be recovered in a proteoglycan fraction free from other glycosaminoglycans (I. Axelsson and D. Heinegård, Biochem. J. 145 (1975) 491-500). These proteoglycans exhibited a polydisperse but essentially unimodal distribution when they were chromatographed on agarose gels in the presence of 4 M guanidinHCl or 1 % SDS or when they were analysed by sedimentation velocity centrifugation in 6 M guanidinHCl. The weight average molecular weight was 72 000 from sedimentation equilibrium centrifugation in 6 M guanidinHCl. The proteoglycans formed aggregates at physiological ionic strength and pH. The aggregation was enhanced by reduction and alkylation of the proteoglycans, probably because of exposure of groups capable to interact with other proteoglycans. The presence of disulphide bridges in the proteoglycans was established and their role for the conformation of the proteoglycans was studied in the ultracentrifuge. Unspecific interactions between the corneal proteoglycans and other proteoglycans were demonstrated. Oligosaccharides closely associated with the proteoglycans were characterized. A tentative model for corneal keratan sulfate proteoglycans is suggested.

INDICATIONS OF STRUCTURAL HETEROGENEITY FOUND IN BIOSYNTHESIS OF HYALURONIC ACID FROM MINOR SUBSTRATES.

E. Baxter, B.J. Clarris and J.R.E. Fraser (University of Melbourne)

Hyaluronic acid appears to contain small amounts of aminoacid, and of saccharides other than the fundamental constituents of its polysaccharide chain. However, the nature of the association of these substances with the polysaccharide is less certain than in the case of the proteoglycans, and direct analysis of native material is rendered difficult by the minute proportions of the substances of interest. The problem has been examined by biosynthesis from isotopic substrates in synovial cell culture. Labelled secretory products including hyaluronic acid have been purified and separated with repeated gel filtration and density grade ultracentrifugation. Substrates such as acetate, glucosamine and glucose are incorporated in the major polysaccharide component. Other substrates such as fucose and leucine are also incorporated in the purified hyaluronic fractions, but behave differently after specific enzymic degradation of the hyaluronic acid. The degree of incorporation of the latter group of substrates differs from that of the former in response to stimulation of hyaluronic acid synthesis by dibutyryl cyclic adenosine monophosphate. These findings suggest that minor components may have a specific role in the structure and synthesis of mammalian hyaluronic acid.

COLLAGEN OF DUPUYTREN'S DISEASE.

S. Bazin (1), M. Le Lous (1), A.J. Bailey (2), T.J. Sims (2), G. Gabbiani (3), G. D'Audiran (3), A. Bzowski (1) and A. Delaunay (1).
(1) Paris, France; (2) Langford, Bristol, England; (3) Geneva, Switzerland.

Human palm aponeurosis affected by Dupuytren's disease are characterized by an abnormally high proportion of collagen. This collagen has been newly synthesized as revealed by a higher solubility, a larger swelling in acid buffer and a higher proportion of reducible cross links compared to the collagen of age matched normal aponeurosis.

Distinct differences in the cross link pattern could be seen in various parts of the dissected Dupuytren's aponeurosis : the ratio of dehydrodihydroxylysinonorleucine to dehydrohydroxylysinonorleucine was 2,5/1 in "nodules" ; 1,5-1,7/1 in "contractures" and in "apparently unaffected" parts of the aponeurosis. In normal aponeurosis, traces of dehydrohydroxylysinonorleucine were present only.

Substantial amounts of type III collagen were found in the three distinct parts of the diseaded aponeurosis in contrast to collagen from the aponeurosis of normal adult subjects.

These results show that Dupuytren's disease is not a strictly focal but a more systemic affection than usually considered.

EFFECT OF LECTINS AND LECTIN DERIVATIVES ON LYSOSOMAL ENZYME ENDOCYTOSIS BY HUMAN FIBROBLASTS. H. Beeck, K. Ullrich and K. von Figura (Münster, W.-Germany)

Pretreatment of human skin fibroblasts with Concanavalin A (Con A), divalent succinyl-Con A (S-Con A), monovalent S-Con A and wheat germ agglutinin (WGA) reduces lysosomal enzyme endocytosis. Con A prooved to be more effective than S-Con A, which was a stronger inhibitor than monovalent S-Con A. The inhibition was dose dependent and reversible. Complexes between lysosomal enzymes and lectins were formed and assayed for endocytosis. Complexes with S-Con A, monovalent S-Con A and WGA were endocytosed at a lower rate. The Con A-enzyme complexes are heterogeneous. A few complexes are still recognizable and transfer high amounts of enzyme into the cells, when they become internalized. At later phases when the medium is depleted of the recognizable complexes, no further enzyme endocytosis is observed. The nonrecognizable complexes contain more than 90% of the enzyme activity.
These results suggest the involvement of carbohydrates in the recognition site of the enzymes and in the receptor site of the cells and the requirement of free motile cell receptors.

Characterization of Two New Collagen Alpha Chains From Rabbit Articular Cartilage. Paul Benya*, and Marcel E. Nimni, University of Southern California School of Medicine, Los Angeles, Ca. U.S.A.

We have studied the collagen synthesized by rabbit articular chondrocytes in monolayer and cartilage sliced cultures. In both cases, 5-20% of the collagen produced can be accounted for by two new α chains, X and Y. The amount of these chains is dependent on the nature and age of the cultures. Carboxymethylcellulose chromatography, SDS-electrophoresis, differential salt precipitation, and the solubility properties of these chains have been recently described ("Biochemistry" Vol. 16, page 865, 1977). In the present work, fourth passage monolayer cultures were used as a source of unlabeled and 3H-proline labeled X and Y chains. A homogeneous preparation was obtained by CM-cellulose chromatography and SDS-electrophoresis. The amino acid analysis of X and Y chains are different from each other, but both have common features that distinguish them from other interstitial collagens: decreased alanine and increased aspartate, glutamate, and hydroxylysine. Analysis of the cyanogen bromide peptides of these chains, by both size and charge, unambiguously demonstrate that the primary sequence of these chains differ from those of collagen types I, II, and III. Supported by NIH Grants AM10358 & AM16404.

LOW ANGLE X-RAY AND NEUTRON DIFFRACTION STUDIES OF THE INTERVERTEBRAL DISC

C. Berthet, D.J.S. Hulmes, A. Miller & P. Timmins (E.M.B.L. & I.L.L., Grenoble, France)

The 670A periodicity of collagen fibrils gives rise to a series of Bragg reflections, of various intensities, in both low angle X-ray and neutron diffraction patterns of rat tail tendon. Recently these data have been interpreted in detail in terms of a model for the axially projected fibril structure based on the amino acid sequences of the α1 and α2 chains of rat/calf skin (Miller et al.(1975)Brookhaven Symp.,III-86;Hulmes et al.(1977) J.Mol.Biol.110,643).This work provides a basis for interpretation of diffraction data from other connective tissues in almost in vivo conditions.For example, the technique has unequivocally demonstrated the location of hydroxyapatite in the gap region of collagen fibrils in calcified turkey leg tendon(White et al. (1977) Nature 266,421).

We have recently obtained both low angle X-ray and neutron diffraction patterns from the annulus fibrosus of the intervertebral disc. The Bragg reflection intensities differ from the rat tail tendon data.There is thus a difference in axial fibril structure between tendon and the annulus fibrosus. This could be due to either specific collagen-proteoglycan interactions or the different collagen types.Further analysis should reveal the location of these differences along the collagen fibrils.

APPLICATION OF THERMOANALYTICAL METHODS IN THE STUDY OF POLYSACCHARIDES

Magdolna Bihari-Varga /Budapest,Hungary/

Through the use of thermoanalytical techniques /differential thermal analysis, thermogravimetry / investigations were made concerning the behaviour of glycosaminoglycans/GAG-s/ and other polysaccharides, on being heated from 20° to 90°C. In order to correlate observed transitions with particular physical or chemical changes related measurements of other parameters /e.g. study of degredation reactions, chemical analysis of non volatile products etc./ as a function of temperature were made and a method was developed for the quantitative assay of polysaccharides.
The kinetic parameters associated with the thermal decomposition of polysaccharides have been obtained by thermogravimetric techniques and the activation energy and the order of water-, and GAG decomposition has been determined. Correlations between the chemical structure and the thermal stability of the investigated macromolecules could be demonstrated.

COLLAGENOLYTIC ACTIVITY IN SUBCUTANEOUS VS. INTRAMUSCULAR RABBIT V_2 CARCINOMA

C. Biswas, W.P. Moran, K.J. Bloch and J. Gross (Boston, Massachusetts)

Collagenolytic activity has been demonstrated in homogenates of tumors obtained by subcutaneous injection into rabbits of V_2 carcinoma which was carried in muscle and derived from a different cell line than reported by McCroskery, Richards and Harris (1975). This enzyme activity is detected after short preincubation with trypsin whereas tumors obtained by intramuscular injection of V_2 tissue suspension yield activity only after culturing the tissue in vitro. Extracts of intramuscular tumors are inactive with or without trypsin preincubation.

The extract of intramuscular V_2 tumor has been found to inhibit collagenase activity present in the extract of subcutaneous tumor. These results suggest that (1) failure to detect collagenase activity in our intramuscular V_2 tumor extract could be explained by the presence of a high concentration of inhibitors which appeared to be composed of several molecular species, (2) the environment of tumor growth might influence the control of collagenolytic activity.

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The pericellular pool of cultured chondrocytes

S. Björnsson and D. Heinegård (Lund, Sweden)

Chondrocytes were isolated from fetal bovine tracheas by collagenase digestion. Cultures were established on Petri dishes in Ham's medium supplemented with fetal calf serum or in medium without serum. In both cases a pericellular pool of glycosaminoglycans was deposited during culture. The pericellular pool is defined as the proportion of glycosamino glycans which can not be removed from the cells by extensive washing but require mild proteolytic digestion to be solubilized. There was a constant turnover of glycosaminoglycans in the pericellular pool with a half life of three days. Extraction of the cultured cells with guanidine-HCl indicates that the pericellular pool contained a large proportion of aggregating proteoglycans. The proteoglycan monomers in the pericellular pool were larger compared with those in the medium pool.

STUDIES ON GOBLET CELL MUCUS FROM RAT SMALL INTESTINE.
I. Carlstedt and L.-Å. Fransson (Lund, Sweden)

An insoluble glycoprotein complex (RI-DOC) has been isolated from rat small intestinal mucosa. Purification is accomplished by gentle extraction of the tissue with deoxycholate. By this treatment surrounding tissue components are solubilized virtually completely. RI-DOC contains no residual collagen, nucleic acids, phospholipids and deoxycholate.
Antibodies against RI-DOC have beenlocalized to the goblet cells and to the intestinal surface.
RI-DOC is resistent to pancreatic proteases, chaotropic salts and hydrogen bond breaking agents, but can be solubilized by papain or by pronase digestion, by dithiothreitol, by cyanogen bromide and by the shear produced by a Waring Blendor. Different gel chromatographic patterns are obtained when split products from these various degradation techniques are studied further. This provides the basis for structural studies on RI-DOC.
After papain digestion 60% of RI-DOC is recovered as high molecular weight glycopeptides representing some 80% of the total amount of such glycopeptides in the tissue. Thus, the major part of the goblet cell mucus in the rat intestinal mucosa exists in an insoluble form.
RI-DOC contains an "endogenous" proteolytic activity. The nature of this enzyme is not yet known. However, the supression of this enzyme is a prerequisite for the controlled degradation of RI-DOC.

HORMONAL CONTROL OF PRODUCTION OF COLLAGENASE AND ITS INHIBITOR BY CULTURED NON-GRAVID RABBIT UTERUS.
Elizabeth C. Cartwright, Gillian Murphy and John J. Reynolds (Cambridge, England).

Cultured non-gravid rabbit uterus has been shown to produce significant amounts of collagenase, comparable with that produced by post-partum tissue. We have also identified an inhibitor of collagenase (MW 30,000) which is synthesized during the first few days of culture; following this, collagenase appears, but only in a latent form. This species can be activated non-enzymically using 4-amino phenyl mercuric acetate (APMA), implying the presence of an enzyme/inhibitor complex. Furthermore, recombination of the inhibitor with activated enzyme restores the latency. Having found that oestradiol (1μM) and aminophylline (1mM) affect the levels of collagenase production, we have carried out further experiments involving the effects of steroid hormones and cyclic nucleotides on production of the enzyme and its inhibitor by non-gravid uterus. The results obtained are presented.

GEL ELECTROPHORESIS STUDIES ON LINK PROTEINS AND OTHER NON COLLAGENOUS PROTEINS EXTRACTED FROM CARTILAGE

F. Chaminade, V. Stanescu, R. Stanescu and P. Maroteaux (Paris, France)

Articular cartilage of young adult baboons was extracted with 4 M guanidinium chloride or with 3 M $MgCl_2$ with proteolysis inhibitors. The proteins were separated from proteoglycans by ion-exchange chromatography in 8 M urea, concentrated, reduced with β-mercaptoethanol and submitted to SDS-PAGE.

Bands corresponding to collagen and 6 non collagenous proteins were found. The latter had a MW between about 27000 and about 87000 (Weber and Osborn method). Two of the bands had the same migration as the two main bands detected by SDS-PAGE in the AlD4 fraction obtained by density gradient centrifugation followed by ion-chromatography purification. The two bands had a MW of about 46000 and 41000. These bands were absent or very faint in the A4 fraction of an associative gradient and in the 0.15 M NaCl cartilage extract, purified on DEAE-cellulose.

The method allowed the analysis of link and other non collagenous proteins extracted from small cartilage biopsies obtained from chondrodysplastic dwarfs.

SERUM HYDROLASE ISOENZYME PATTERNS IN 3 PATIENTS WITH I-CELL DISEASE (MUCOLIPIDOSIS II) SYMPTOMS. ADDITIONAL α-L-FUCOSIDASE ABNORMALITY IN 1 CASE

M.A. Chester, B. Hultberg and S. Sjöblad (Lund, Sweden)

Seven glycosidases in the sera from 3 patients with I-cell disease have been studied. The isoenzyme pattern from each was normal under the conditions used with one exception (see below). The characteristic increase in enzymic activity was most pronounced in the isozymes with acid pH optima. One patient showed a profound abnormality in α-L-fucosidase isozymes which is, however, probably independent of this disease and may be due to a normal polymorphism.

SPECIFIC RESPONSE OF FIBROBLASTS FROM THE PRE-TIBIAL AREA TO A SERUM FACTOR PRESENT IN PRE-TIBIAL MYXEDEMA.

Herman S. Cheung, Michael B. Kamiel, Leonard Spolter, John T. Nicoloff, and Marcel E. Nimni (Los Angeles, California)

Fibroblasts from skin of patients with pre-tibial myxedema (PTM) were grown in tissue cultures containing DMEM supplemented with 1% penicillin and streptomycin and 20% human serum. Upon reaching the monolayer stage, one set of cells was radioactively labeled with ^{3}H glucosamine and tested for hyaluronic acid synthesis in the presence of serum from PTM patients, while a control set employed normal human serum. Fibroblasts responded to PTM sera by synthesizing approximately three times more hyaluronic acid than the cells incubated in normal human serum. Fibroblasts cultured from skin of the back, prepuce, and El Man 1101 fibroblasts did not respond to PTM sera. Neither crude immunoglobulin nor purified 7S gamma fractions from sera of PTM patients produced these effects. These findings strongly suggest that the serum of PTM patients contains a nonimmunoglobulin fibroblast stimulating factor which increases the production of hyaluronic acid from fibroblasts derived from the skin of the lower extremities from both normal and diseased individuals.

CYTOLOGICAL VARIATIONS IN ESTABLISHED SYNOVIAL CELL LINES

B.J. Clarris and J.R.E. Fraser (University of Melbourne)

In contrast with primary cultures, synovial cell lines maintained by serial passage tend to adopt a fairly uniform fibroblast-like appearance; usually distinguishable from that of cultures from other sources but much the same in different synovial lines in standardized growth conditions. Two distinct variations from this cytological pattern can be induced at this stage. These are best recognized by negative medium phase contrast. One is characterized by increased numbers and extent of small cytoplasmic organelles that take up supravital stain; by clear vacuoles, amorphous juxta-nuclear structures, and dense nuclear membrane. This pattern is typically induced by exposure to indigestible neutral sugars. The other consists of widely spread cytoplasm which develops optically dense ridges in a lace-like or filigree appearance enclosing structureless transparent areas. These changes develop gradually on exposure to dibutyryl cyclic adenosine monophosphate. A predominance of one or the other of these cytological patterns has been found with several experimental treatments, which are also accompanied by changes in cell function.

PROTEOGLYCANS OF BOVINE THORACIC AORTA.
E.G.Cleary and P.Muthiah (Adelaide, S.Australia,)

Proteoglycans (PGs) have been extracted from crushed bovine thoracic aorta at 4°C, in the presence of enzyme inhibitors, by direct extraction with 4M GuHCl and by sequential extraction with 0.4M GuHCl, followed by 4M GuHCl. These yielded 74.2%, 53.8% and 25.7%, respectively, of the uronic acid-containing material.

The aortic PGs have a higher protein : uronic acid ratio than cartilage PGs and thus have a lower flotation density.

Gel chromatography under associative condition (0.4M GuHCl) showed that a significant proportion of the PGs were excluded from 2B Sepharose. On reprocessing the excluded peaks under dissociative conditions, a broad PG peak, still excluded from 2B Sepharose, and a discrete protein peak were obtained. This latter protein had an apparent molecular weight of 135,000 daltons. Its amino acid analysis was similar to that reported for "link-protein" from cartilage PGs.

Remixing the associated PG and protein components under associative conditions led to reversible reaggregation as shown by gel filtration chromatography on 2B and 4B Sepharose. This phenomenon could not be demonstrated, however, in the analytical ultracentrifuge.

ROTATIONAL DIFFUSION STUDIES OF POLYGLUTAMIC ACID IN POLYMERIC NETWORKS
W.D. Comper, M. Ehrenberg, R. Rigler and T.C. Laurent (Uppsala and Stockholm, Sweden)

Studies are presently being performed to ascertain the effects of a polymeric network on the various rotational modes of rod-like molecules. The spectrum of fluorescent lifetimes and rotational movement of dansyl labelled polyglutamic acid (viscosity average M.Wt = 36,000) in the presence of dextran have been measured by a pulsed fluorescence technique with single photon counting. At pH 4.7, polyglutamic acid is known to take on a helical conformation but with an overall compact structure (i.e. a helix with 'kinks'). The addition of dioxane stiffens the helix to yield a more rod-like molecule. This transition is sensitively monitored in our system by a change in the spectrum of fluorescent lifetimes for the dansyl probe. The results obtained to this stage demonstrate at least three measurable rotational relaxation times of dansyl labelled polyglutamic acid in 30% dioxane at pH 4.7. A relatively long, together with two faster relaxation times of 8nS and 1nS have been observed. The 8nS relaxation time corresponds closely to that predicted for rotation of the rod model of the molecule, or a large segment of it, about its long axis. The two fast rotational relaxation times are not significantly affected by the presence of the dextran polymer network. This system should serve as a working model for connective tissues where some insight into the molecular motion of rod-like molecules or stiff random coils, such as collagen and glycosaminoglycans, in network structures of the extracellular matrix may be gained.

ISOLATION OF PROTEOGLYCANS FROM HUMAN EMBRYONIC SKIN FIBROBLAST CULTURES.
L.Cöster,I.Carlstedt and A.Malmström (Lund,Sweden)

Fibroblasts grown in monolayers were allowed to incorporate ^{35}S-sulfate and ^{3}H-leucin for 72 hours.Proteoglycans were prepared from the culture medium (PGM),from a EDTA wash of the cells (PGEDTA) and from a guanidineHCl (GuHCl) extract of the cells (PGC).All fractions were purified by ultrafiltration followed by gel chromatography on Sepharose 4B.PGM represented 77%,PGEDTA 8% and PGC 15% of the total proteoglycan material.
Preliminary studies on PGM indicated that a large proportion of the material existed as a macromolecular aggregate.In order to avoid disassembly of this structure dissociative methods were not used in the preparation procedure. Thus, chromatography on Sepharose 4B in phosphate-buffered saline (PBS) resolved PGM into one exluded (PGM-A) and one included (PGM-B) component. PGM-A was further studied on Sepharose 2B.When chromatographed in PBS the proteoglycans were eluted in the void volume.However,chromatography in 6 M GuHCl resulted in partial inclusion.To exclude the possibility of unspecific aggregation with extraneous proteins,PGM-A was subjected to CsCl density gradient centrifugation in 4 M GuHCl.Rechromatography of the purified proteoglycans on Sepharose 2B in PBS and GuHCl displayed the same difference in chromatographic behaviour as described above.Whether these findings represent an aggregation or a conformational change of the proteoglycans are not yet known.

STRUCTURE OF AGGREGATING DERMATAN SULPHATE.
L.Cöster and L.-Å.Fransson (Lund,Sweden)

The structure of aggregating and non-aggregating dermatan sulphate species (DS-A and DS-N) was investigated.Degradations by periodate oxidation - alkaline elimination,by hyaluronidase and chondroitinase AC showed that DS-A contained regions of the carbohydrate structure -(GlcUA-GalNAc)$_m$ - -(IdUA-GalNAc)$_n$ - where m and n= 1-3. In DS-N block regions of the two repeat periods prevailed.DS-A was subjected to various degradations prior to affinity chromatography on DS-gels.Whereas degradation by chondroitinase AC abolished binding to the DS-gel,hyaluronidase digestion produced fragments which retained affinity to the ligand.These fragments which were of decasaccharide-size (and higher) were composed of the alternating sequences shown above.The IdUA residues of these sequences were generally non-sulphated.
It is proposed that "mixed" regions composed of IdUA- and GlcUA- containing periods constitute the junction zones og aggregating chains.

PERMEABILITY OF CHONDROCYTE CULTURES
G. J. Cumming, C. J. Handley and B. N. Preston (Clayton, Australia).

The integrity of connective tissues is dependent upon the nutrition of chondrocytes, which in turn relies upon transport of material to and from the cells. Factors which may inlfuence this transport include obstruction by extracellular components, the charge carried by the extracellular polyanions and the physical characteristics of the permeant species [1].

We have studied the permeability of an "artificial" cartilage produced by culturing chondrocytes on a "Millipore" base. The extracellular matrix formed is similar in structure to an ultra-thin cartilage slice. The permeability of this structure to solutes of varying size, shape and charge has been measured. The passage of small uncharged solutes is unhindered by the aqueous phase of the matrix whilst that of micro-ions is understood in terms of electrostatic interactions with the anionic proteoglycans.

The factors controlling the flux of macromolecular solutes are numerous and complex. The behaviour of monodisperse macromolecular species is dependent upon shape and configuration of the solute. The transport of compact particles is in marked contrast with that of flexible polymers. The behaviour of polydisperse solutes is complicated by "filtration" effects.

(1) Preston, B.N. & Snowden, J. McK (1973) in "Biology of Fibroblast" (E. Kulonen & J. Pikkarainen eds.) Academic Press, Lond. pp 251-230.

ACIDIC RESIDUES INVOLVED IN COLLAGEN MINERALIZATION
N. R. Davis (Edmonton, Canada)

A considerable body of in vivo and in vitro evidence suggests a central role for collagen fibrils in the mineralization of bone and dentin. Indeed, the majority of mineral appears to be inside collagen fibrils. If the lateral packing of collagen molecules is sufficiently tight that only single ions (but not ion clusters) may enter, nucleation must be catalysed from within the fibrils. Experiments in this laboratory indicate that acidic amino acid residues are essential for the catalysis of nucleation displayed by hard tissue collagens. Lysine, arginine and histidine residues do not appear to be involved in catalysis of nucleation. A maximum of 4 acidic residues per chain appear to be required for catalytic activity. These residues are protected from specific carboxyl group modification reagents when bone, dentin and predentin collagens are lightly calcified prior to exposure to modification reagents. Subsequent decalcification allows these residues to be specifically labelled with radioactive reagents.

Enzymatic conversion of UDP-N-Acetylglucosamine to UDP-N-Acetylgalactosamine in epiphysial-plate cartilage.

G. De Luca, S. Rindi, A. A. Castellani.
Institute of Biological Chemistry, University of Pavia, Italy.

The activity of UDP-N-Acetylglucosamine 4'-epimerase in newborn-pig epiphysial-plate cartilage was investigated. Oxidized NAD is required for enzyme activity, but UDP-N-Acetylgalactosamine formation was also detectable after the addition of NADP to the incubation mixture. The pH optimum, determined by using Tris-HCl buffer, resulted in a range between pH 8.5 and 9.0. A K_m value of 3×10^{-3} M was determined for UDP-N-Acetylglucosamine. The effect on the enzyme activity of some nucleotide-sugars, precursors of ChS and KS chains, was also investigated.
NADH acts as an inhibitor of enzyme activity: the inhibition by NADH is strongly dependent on NAD concentration and is markedly increased as the pH is lowered. It is relevant that this regulation mechanism is also effective on UDP-glucose dehydrogenase and UDP-glucose 4'-epimerase activities, suggesting that the value of NAD/NADH ratio may play a very important regulatory role on the biosynthesis of glycosaminoglycan precursors and could be very effective at physiological pH.

IN VITRO, SULFATE INCORPORATION IN OSTEOGENESIS IMPERFECTA CONGENITA (OIC) AND TARDA (OIT)
E.E. Delvin, E. Lopez and F.H. Glorieux (Montreal, Canada)

OIC and OIT are two heritable disorders of connective tissue whose molecular defects remain as yet unraveled. Reports claiming either defect of in vitro glycoaminoglycan (GAG) biosynthesis or imbalance of GAG content in dentin, prompted us to re-evaluate GAG synthesis in fibroblast culture using ^{35}S uptake as a probe. Confluent monolayers of reference (R,n=6), OIT (n=7) and OIC (n=3) skin fibroblasts were labeled with carrier free $Na_2{}^{35}SO_4$ (10 μCi) for 48 hours. The media were then removed. The cells were washed, removed by trypsinization and disrupted by ultrasonic waves. Protein and $Na_2{}^{35}SO_4$ content were measured on the resulting suspensions and results expressed as DMP/mg Prot.±s.d.. At the time of harvesting $Na_2{}^{35}SO_4$ uptake of OIT cells (4340 ± 883) was not significantly different from that of R(3668 ± 960). One OIC strain displayed normal tracer uptake. However, two of the OIC cell lines studied had, for a total of 9 experiments, a significantly higher incorporation of $Na_2{}^{35}SO_4$ (6929 ± 419,$p<0.01$) than either R or OIT cells. Although methodology difference could not be ruled out, these results are in opposition with a earlier report by Solomons (Ped. Res. 9,318,1975) and cast doubt on the use of this parameter for prenatal diagnosis.

Supported by the Shriners of North America.

COLLAGENASE SECRETION BY CULTURED HUMAN SKIN FIBROBLASTS
E.E. Delvin and M. Trudel(Montreal, Canada)

Mammalian collagenase (E.C. 3.4.24.3) splits native collagen fibrils into two soluble fragments. To study the mechanisms by which human fibroblasts regulate the secretion of this enzyme, skin explants obtained during surgical procedures were diced ($1mm^3$) set in plastic Petri dishes and cultured as described by Dayer et al (PNAS,73,945, 1976) with or without fetal and newborn calf sera. The medium was collected for three 5 day periods. The explants were then removed, the cells trypsinized and set back in culture until they reach confluency. Collagenase was assayed after trypsin activation, by the stabilized gel method using purified rat skin collagen labeled with [2,3,(N)] L- proline. Collagenolytic activity (% Label solubilized ± SEM) was 22.0 ± 5.7, 37.0 ± 5.5 and 25.8 ± 5.2 in the first, second and third passage respectively. Absence of serum in the medium reduced collagenase secretion by 74%. No activity could be elicited in long term fibroblast culture. These results infer that collagenase secretion in culture is dependent upon the culture conditions and that standardization of methods is a prerequisite before results obtained in different laboratoires could be equated.

Supported by the Shriners of North America.

INTERACTION OF THE SERUM-DERIVED AGF AND DENATURED COLLAGEN

W. Dessau and F. Jilek

A high molecular weight fraction (MW > 400 000) with selective affinity to denatured collagen was isolated from the sera of various species. This protein was previously described as AGF and shown to be present on the surface of peritoneal exudate cells. A close relationship with the plasma protein CIG could be established by biochemical and immunological methods. A radio-immuno-assay was developed for AGF with ^{125}I-collagen and goat anti-AGF-serum. In this RIA denatured type I - III collagen reacted with AGF. Binding sites were located on the α1(I)-CB7-, α2-CB5- and α1(II)-CB10-peptide. These data agree with previous observations of Kleinman et al. on the specificity of a serum factor promoting the attachment of fibroblasts onto collagen. The role of AGF in the serum of rheumatic patients as autoantibodies to collagen is being investigated.

INTERACTION OF PLASMA LIPOPROTEINS AND GLYCOSAMINOGLYCANS.

N. Di Ferrante, P.V. Donnelly and R.L. Jackson (Houston, Texas USA)

Formaldehyde-treated sheep erythrocytes, treated with 1:2000 tannic acid, were coated with either low density or high density lipoproteins (LDL or HDL-coated cells) and suspended in 1:200 normal rabbit serum. The presence of the antigens on the cells was demonstrated with specific antisera. High sulfated plasma glycosaminoglycans (GAG), eluted from ECTEOLA cellulose with 2.0 M NaCl, agglutinated LDL and HDL-coated cells at a concentration varying between 0.06 and 14 µg as hexuronate. The agglutination of LDL-coated cells was prevented by 0.25 M NaCl, while that of HDL-coated cells was still present at 0.4 M NaCl. Exhaustive dialysis of the plasma GAG against distilled water gave a retentate (containing all the original hexuronate) which did not agglutinate any longer LDL or HDL-coated cells. The dialysate, which contained no hexuronate but galactose and minimal amounts of protein, had all the agglutinating activity of the original non-dialyzed preparation of GAG. Since infrared analyses of the dialysate suggested the presence of keratan sulfate, this GAG was prepared from bovine cornea, homogenized in 6 M guanidinium Cl and fractionated on ECTEOLA cellulose column. The fractions obtained with increasing concentrations of NaCl, after deproteinization with SP Sephadex C50, agglutinated LDL-coated cells, even after exhaustive digestion with chondroitinase ABC.
(Supported by NIH grants HL-18692-01 and HL-20447-01.)

Bacterial invasion and antigen elimination as a cell surface interaction phenomenon.

L. Edebo, J. Hed, E. Kihlström, K.-E. Magnusson, O. Stendahl and C. Tagesson. Department of Medical Microbiology, University of Linköping, S-581 85 Linköping, Sweden.

Physico-chemical surface properties of *Salmonella typhimurium* 395 MS and R bacteria have been investigated by hydrophobic interaction chromatography and partition in aqueous polymer two-phase systems containing dextran, poly(ethyleneglycol) and palmitoyl-poly(ethyleneglycol). These characteristics have been compared to the biological properties of the bacteria. S bacteria, beeing hydrophilic and uncharged, resist phagocytosis by polymorphonuclear phagocytosis *in vitro*, do not tend to invade HeLa cells *in vitro* and attach poorly to the mouse small intestine *in vitro*. R bacteria, which are more hydrophobic and expose negative surface charge, have greater tendency to interact with the above mammalian cells. Sensitization of S bacteria with IgG or serum IgA antibodies make the bacteria more hydrophobic, negatively charged and liable to interaction with mammalian cells, whereas binding of secretory IgA to R bacteria has an opposite effect. We suggest that the above properties are important *in vivo* with respect to bacterial invasion, the function of antibody in antibacterial immunity, and the elimination and localization of antigen-antibody complexes.

BINDING OF IMMUNOGLOBULINS G TO COLLAGEN
Y. Eeckhout, H. Riccomi, C. Cambiaso, G. Vaes and P. Masson (Brussels, Belgium)

Native salt-soluble collagen purified from guinea pig skin was found to agglutinate polystyrene particles coated with human immunoglobulins G. The agglutination of the particles was measured at room temperature with a Technicon Auto Counter. The percentage of agglutinated particles was proportional to the collagen concentration in a range of 0.1 to 0.7 mg/ml; it was also dependent on the concentration of IgG used to coat the particles. Collagen did not agglutinate particles coated with either human serum albumin or human transferrin. The agglutinating capacity of the collagen preparations was abolished by heat-denaturation or by digesting the collagen with bacterial collagenase but it remained unaffected by treatment with trypsin. Preliminary experiments suggest that collagen binds to the Fc portion of the human immunoglobulins G. Our data indicate that the native triple helical region of collagen exhibits a specific affinity for the IgG-coated particles; they could shed some new light on the mechanism whereby immune complexes are trapped in collagenous tissues and exert their pathological effects.

ZONE CHROMATOGRAPHY: A NEW TECHNIQUE FOR SEPARATING NATIVE COLLAGEN MOLECULES.
H.P. Ehrlich (Boston, MA, USA)

The heterogeneity of collagen is well established with four having been characterized and more recently newer types being described. A column chromatographic technique, which separates native types I, II, III and basement membrane collagens has been developed. The basis of the method is the differences in salt insolubility of the different collagens. Separation of a mixture of native chick collagens has been accomplished by application of the material in solution to molecular sieve columns packed with a desalting type bed material equilibrated in 5M sodium chloride at neutral pH. The column is then eluted with a discontinuous salt gradient from 4.3M to 0.15M sodium chloride. The identity of the separated collagens has been done by polyacrylamide gel electrophoresis of denatured chains and the cyanogen bromide derived peptides and by the ultrastructure of SLS crystallites.

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ELECTROPHORETIC IDENTIFICATION OF GLYCOSAMINOGLYCANS (GAG) IN AVIAN RUDIMENTS.
R. Evangelisti, A. Caruso, G. Stabellini and P. Carinci (Ferrara, Italy)

A current hypothesis suggests that glycosaminoglycans (GAG) may be developmentally involved in mesenchymal regulation of epithelia differentiation. We have therefore investigated the composition in GAG of avian skin and lung in vivo and in vitro at different incubation stages. GAG were extracted according to Breen and coworkers's method (1970),and fractionated by two-dimensional electrophoresis (Hata and Nagai, 1972). Our results indicated significant changes in GAG composition during rudiments development

CHRONOLOGICAL SEQUENCES OF CHANGES IN CONNECTIVE-TISSUE METABOLISM OF RAT SKIN INDUCED BY A HIGHLY POTENT FLUORINATED GLUCOCORTICOID

B. E x e r (CIBA-GEIGY, Basle, Switzerland)

The changes in the content of water, fat and protein of the skin and the incorporation of ^{35}S-sulphate were followed up at daily intervals after a single epicutaneous application of the corticoid.

The first change noticed already after a few hours, was water loss, followed after 1 -2 days by a very heavy decrease in the fat content of the skin. Simultaneously we observed a reduction in the content of "higher" skin proteins, resulting in a increase in the collagen fraction of skin proteins.These processes lead to shrinkage of the skin,with an increase in the collagen content per unit surface area.

The observed inhibition of radioactive sulphate incorporation is due to various processes e.g. transport inhibition, decrease in cell number and in synthesyzing enzyme concentration.

STRUCTURE OF SELF-ASSOCIATING HEPARAN SULPHATE SPECIES

L.-Å Fransson (Lancaster, England)

Heparan sulphate preparations fractionated according to charge density were characterized by gel chromatography, ^{13}C-NMR-spectroscopy and chemical analyses. Preparations with IdUA to GlcUA ratios between 0.4 and 0.7 and N-acetyl to N-sulphate ratios between 0.5 and 0.7 displayed associative behaviour in gel chromatography experiments. Degradation of such species via periodate oxidation-alkaline elimination afforded a series of oligosaccharides containing N-sulphated GlcN, both IdUA and GlcUA and with varying degree of sulphation. Nonsulphated segments [-(GlcUA-GlcNAc)$_n$-] were entirely split into monosaccharide derivatives. Di-tetra- and hexasaccharides with low sulphate (mainly as N-sulphate) and high GlcUA content were completely degraded by repeated periodate oxidation-alkaline elimination. However, treatment of these saccharides with HNO_2 caused N-desulphation but no cleavage of hexosaminidic bonds. Oligosaccharides with these features were also obtained from heparan sulphates which were isolated after tryptic digestion of fibroblasts.

THE GLYCOSAMINOGLYCANS OF PLASMA IN RHEUMATOID ARTHRITIS AND IN RENAL FAILURE

C.Friman,M.Juvani,E.Storgårds and B.Kock(Helsinki,Finland)

Plasma glycosaminoglycans(GAG) were determined in 35 patients with rheumatoid arthritis(RA),in 35 patients with renal failure(RF) and in 50 healthy controls.10 ml of EDTA-plasma was passed through a DE-52 anion-exchange column,which was washed with 0.2M NaCl and eluted with 1.0M NaCl in 0.1M sodium acetate,pH 4.0.Hexuronate was assayed from the eluate with m-hydroxydiphenyl = free GAG of plasma.The run-through plasma and 0.2M NaCl wash were treated with trichloroacetic acid,dialysis papain proteolysis and passed through an AG 1x2 anion-exchange column,which after washing with 0.15M NaCl was eluted with 2.0 M NaCl and hexuronate assayed = bound GAG of plasma.A statistically significant increase in free GAG concentration was found in RA. In RF both free and bound GAG were significantly increased and a positive correlation between plasma creatinine and plasma GAG concentrations was observed. GAG patterns of plasma were studied by electrophoresis on cellulose acetate in various buffers.

CONFORMATION OF HEPARIN IN SOLUTION.
EVIDENCE FOR UNDISTORTED 1C(L) IDURONIC ACID MOIETIES.

G.Gatti, B.Casu (Milan,Italy) and A.S.PERLIN (Montreal, Canada)

Resolution of all the ^{1}H-NMR signals of heparin in D_2O solution was achieved by resolution-enhancement techniques at 270 MHz, and ^{2}signals were unequivocally assigned by homonuclear spin-decoupling.

The value of the interproton vicinal coupling constants, checked by computer-simulation of the spectra, indicate that the conformation of the glucosamine moieties is C1(D), and that of the iduronic acid moieties 1C(L). When allowance is made for the effect of electronegative substituents antiperiplanar to the considered protons, the calculated values are consistent with substantially undistorted chairs.

Variations of temperature (30°-90°C), pH (3-8) and concentration (0.5-20%) affect only the chemical shift of a new protons, but not the coupling constants. This indicates slight changes in the conformation of the polymer chain, the conformation of both moieties remaining substantially unchanged. The conformation of the L-iduronic moiety of the disaccharide obtained by deaminative hydrolysis of heparin is essentially the same as in the polymer.

GLYCOSAMINOGLYCANS OF THE BOVINE PERIODONTAL LIGAMENT: CHANGES ASSOCIATED WITH TOOTH ERUPTION

G. Gibson (Edmonton, Canada)

A major reorganization and reorientation of the collagen fibres of the periodontal ligament occurs as the ligament develops in association with tooth eruption and root growth. We have shown that during this period there is a concomitant change in the relative proportions of the various glycosaminoglycans present within the ligament matrix. The ligament matrix contains hyaluronic acid and the proteoglycans of chondroitin 4 and 6 sulphate and dermatan sulphate. Our results demonstrate that there is a dramatic decrease in the ratio of hyaluronic acid to sulfated glycosaminoglycans during the transition from the non-functional to the completely functional state of the ligament. A similar synthesis and accumulation of hyaluronic acid has been shown by a number of workers to be associated with early stages of growth and reparative processes, involving new synthesis of connective tissue elements and the active migration and proliferation of participating cells. With the development of the ligament there is a loss of hyaluronic acid in parallel with a dramatic increase in a specific chondroitin sulfate fraction (that precipitating in 40% athanol, 5% calcium acetate). This fraction may be involved with the formation and structural stability of the typical obliquely organized fibres of the functional periodontal ligament. The level of the other glycosaminoglycans remains constant during development.

THE ENZYMATIC BASIS OF EXCESSIVE KERATANSULFATURIA. L. Ginsberg, D.T. Di Ferrante, C.T. Caskey and N.M. Di Ferrante (Houston, Texas USA)

Phenotypes with excessive keratansulfaturia (KSuria) are designated as Morquio disease (MPS IV). Data from Dorfman's and our laboratory suggest that Morquio has a defective sulfatase, specific for 6 sulfate. KS has 6 sulfate esters on N-acetylglucosamine and on galactose; it was thought that both could be removed by the same enzyme. In our experiments, classical Morquio fibroblasts release little or no sulfate from chondroitin-6-sulfate tetrasaccharides. We have found two patients with KSuria with different enzyme defects. A 14-year-old girl had mild skeletal involvement, normal intellect, abundant KSuria, normal N-acetylgalactosamine-6-sulfate sulfatase but deficient ß-galactosidase. A 5-year-old boy with retarded growth and intellect, mild skeletal involvement, coarse and excessive hair and clear corneas had KSuria and heparansulfaturia (HS). The only group common to KS and HS is N-acetylglucosamine-6-sulfate; we synthesized this substrate to measure this specific sulfatase. Extracts from Morquio fibroblasts and normal fibroblasts released equal amounts of inorganic sulfate from this substrate, while extracts of the patient failed to do so. Thus, excessive KSuria may be caused by at least three enzyme defects: 1) N-acetylgalactosamine-6-sulfate sulfatase, classical Morquio, MPS IVA; 2) ß-galactosidase, mild Morquio, MPS IVB; 3) N-acetylglucosamine-6-sulfate sulfatase, Morquio-Sanfilippo intermediate, which might occupy the vacant position V of McKusick's classification. (Supported by USPH grants GM-00081-03, HL-18692-01 and the Howard Hughes Medical Institute.)

THE LOCALIZATION OF PROTEOGLYCANS /PG/ AND GLYCOPROTEINS /GP/ IN THE HYALINE CARTILAGE

T. Glant, G. Lévai and Cs. Hadházy /Debrecen, Hungary/

The localization of PG and GP in the hyaline cartilage were studied with FITC and with horseradish peroxidase labelled antibodies. Hyaluronidase digestion increased the intensity of immune reactions, pronase digestion, or extraction with 4.0 M/lit guanidinium hydrochloride, abolished the staining. In electron micrographs immunoperoxidase reactions gave a homogenous staining in the endoplasmic reticulum, in the juxtanuclear region, and in several smooth-walled /exocytotic/ vesicles and elongated areas situating subjacent to the cell membrane. In the intercellular matrix fine filaments beaded with small granules could be shown forming an irregular network which contained collagen fibers in the interstices. Immunohistochemical reactions suggest that filaments and granules may represent the GP and the PG respectively in PG-aggregates. The fact that hyaluronidase digestion enhanced the immune reaction in extracellular spaces but never in intracellular organells, is suggestive of the possibility that the caracteristic onformation of the molecules secreted by chondrocytes, and the aggregation of PGs, take place extracellularly.

THE EFFECT OF DICHLOROMETHANE DIPHOSPHONIC ACID (Cl_2MDP) ON THE BIOSYNTHESIS OF RAT BONE AND CARTILAGE COLLAGEN IN VIVO AND IN VITRO

H.L. Guenther, H.E. Guenther and H. Fleisch (Berne, Switzerland)

Cl_2MDP, a compound related in structure to pyrophosphate but resistant to metabolic destruction inhibits the dissolution and the formation of Ca-phosphate crystals in vitro. In vivo it prevents bone resorption and the calcification of soft tissues. Previously these effects were attributed to physicochemical actions, but there have been growing indications that Cl_2MDP may alter the cellular activity. To test the cellular hypothesis and to assess any changes in the synthesis of bone and cartilage collagen in respect to bone development, new born rats were daily injected with Cl_2MDP 10 mg P/kg for 10 days. Bone explants and epiphyseal cartilage were subsequently cultured in MEM containing ^{3}H-proline but no Cl_2MDP. In addition isolated rat calvaria and rabbit ear cartilage cells were cultured in MEM until confluence with 0.25 mM Cl_2MDP. The results showed that Cl_2MDP drastically promotes the incorporation of ^{3}H-proline in either tissue or cell type. Furthermore it appears that the intermolecular crosslinkage of bone collagen was decreased. The molecular structure of collagen was not changed. It appears that Cl_2MDP influences its target cell by changing the collagen metabolism.

INORGANIC PYROPHOSPHATASE IN BONE

M.Hämäläinen, M.Kaivosoja, L.K.Korhonen (Oulu, Finland)

The existence of a specific inorganic pyrophosphatase (PP_iase), distinct from alkaline phosphatase in bone has been suggested but is often regarded as questionable. In the present investigation, several features of PP_iase activity have been demonstrated, which suggested that it represents an enzyme protein different from those splitting phosphomonoesters and ATP. PP_iase was largerly destroyed during extraction with n-butanol, which facilitated the solubilization of alkaline phosphatase and ATP splitting enzymes and only partially destroyed acid phosphatase. Two major groups of phosphate esters and pyrophosphatases splitting enzymes were separated by gel filtration from homogenates of rat bones. The first pool contained high ATP-ase and phosphomonoesterase activities, but only low activity against inorganic pyrophosphate (PP_i) in the presence of $MgCl_2$. The second pool was most active against PP_i at pH 7.5 in the presence of excess $MgCl_2$ and only slightly hydrolyzed phosphomonoesters or ATP. Immunodiffusion showed that these two pools contained two distinct proteins. It was concluded that there exists a specific inorganic pyrophosphatase distinct from phosphomonoesterases and ATP-ases in bone tissue.

Grants: Sigrid Juselius Foundation, Finland

HETEROGENEITY OF HYALINE CARTILAGE PROTEOGLYCANS

M. Hatt, J.P. Pearson and R.M. Mason (Charing Cross Hospital Medical School, Hammersmith, London, W6 8RF, England).

The major proteoglycan fraction (A_1) extracted from bovine nasal septal cartilage separates into two bands on electrophoresis in large pore composite agarose-acrylamide gels (Roughley & Mason, 1976). These are not artefacts due to degradation of proteoglycans by endogenous cartilage enzymes during extraction and purification (Pearson & Mason, 1977). Proteoglycans extracted from adult human costal cartilage under conditions inhibiting endogenous proteinase activity also show similar heterogeneity on large pore gel electrophoresis.
A partial separation of the two species can be achieved by gel filtration of dissociated proteoglycan (D_1), the component with the slow electrophoretic mobility emerging immediately after the void volume of the column. Both species are found to varying extents in the remainder of the column effluent inferring that each is polydisperse with respect to molecular weight. The ratio of galactosamine to glucosamine decreases for successive fractions eluted from the column suggesting that the slow electrophoreting component may be a predominantly chondroitin sulphate proteoglycan whilst the fast electrophoreting component contains proportionately more keratan sulphate. Analytical data on bovine and human cartilage proteoglycan components separated by electrophoresis will be presented and the heterogeneity of these molecules discussed.

FIBROBLAST SURFACE PROTEIN, FIBRONECTIN, AS AN EXTRACELLULAR MATRIX COMPONENT.

K. Hedman, M. Kurkinen, J. Wartiovaara, and A. Vaheri, (Helsinki, Finland)

Fibronectin (also known as the LETS protein) refers to a glycoprotein detected in plasma and tissues of vertebrates. Immunofluorescence of tissue sections shows fibronectin to be present in primitive mesenchymal and loose connective tissues, in blood vessel walls and characteristically in various basement membranes. Cultured human fibroblasts and astroglial cells synthesize and secrete or shed the glycoprotein into the surrounding medium. In immunofluorescence of cultures of fibroblastic cells, external (i.e. with surface fixation) fibronectin is seen as discrete strands on, below or between the cells.

Immunoelectron microscopy with peroxidase and ferritin showed that fibronectin was in fibroblast cultures present in extracellular "in vitro matrix" material and, to a lesser degree, also associated with the plasma membrane. The matrix material had an amorphous or partly filamentous ultrastructure. It was distributed as patches or strands above, below and between the cells often mediating distant cell-to-cell or cell-to-substrate contacts. The localization of fibronectin in vitro is consistent with its distribution in vivo and suggests that it mediates cell-matrix interactions.

PARTIAL PURIFICATION AND CHARACTERISATION OF A PLATELET ENZYME DEGRADING HEPARAN SULPHATE AND HEPARIN.

C.-H. Heldin, Å. Oldberg, Å. Wasteson, C. Busch, B. Glimelius and M. Höök (Uppsala, Sweden).

Human platelets contain an endoglycosidase degrading heparan sulphate (FEBS Lett. 64, (1976), 218-221). Using radioactive heparan sulphate as substrate, an assay for this enzyme has been developed. The assay is based on the decreased precipitability with cetyl pyridinum chlorid, of depolymerised heparan sulphate as compared to undegraded polymer. The enzyme has been partially purified from a platelet homogenate, using ionexchange chromatography on CM-Sephadex and affinity chromatography on a column of immobilized heparan sulphate, hereby increasing the specific activity about 500 times. After purification the enzyme activity declined rapidly. This could partly be prevented by adding serum albumin to the preparation. The molecular weight of the enzyme is about 60,000, estimated from gel chromatography on Sephadex G-200. Preliminary investigations of the substrate specificity, shows that, besides heparan sulphate, heparin is attacked by the enzyme. The heparin degradation products thus obtained, have lost the affinity for immobilized antithrombin. Furthermore, the enzyme is released from the platelets during the release reaction, induced by e.g. thrombin or collagen. These results suggest a possible role for the enzyme in hemostasis, promoting blood coagulation by heparin consumption.

Aortic glycosaminoglycans and collagen related to biomechanical properties.

P. Helin, H. Oxlund and I. Lorenzen (Copenhagen, Denmark)

In a previous study (Helin et al.: J. Atheroscler. Res. 1969, 10: 359) we observed a decreased susceptibility of aorta of rabbits during shedding period (from September to December) to hemodynamic injury elicited by systemic hypoxia. The hypothesis was advanced, that the phenomenon might be explained by an increased elasticity. In present study the biomechanical properties of aortas from 15 shedding rabbits were compared to those of 15 non-shedding animals. In each aorta the load-strain curv of the thoracic segments were analyzed. The segments of the shedding animals showed a lower inclination of the curve while neither the load nor the strain values exhibited any differences. These results indicate a greater elasticity of the vascular wall during shedding. The aortas of shedding animals contain an excess of hyaluronicacid while the content of sulfated glycosaminoglycans is reduced. The content of dermatansulfate has been found to be correlated to the content of collagen (Helin et al.: Atherosclerosis 1976, 24: 259). The shedding of rabbits may reprecent a key phenomenon for elucidation to the role of glycosaminoglycans and collagen for the mechanical properties of the artery and its vulnerability to hemodynamic injuries.

OXYGEN TENSION AND CONSUMPTION IN THE CANINE INTERVERTEBRAL DISC

S Holm (Göteborg, Sweden), A Maroudas (London, England), G. Selstam (Göteborg, Sweden), A Nachemson (Göteborg, Sweden)

No measurements have been made in the past of the tension or the consumption rate of oxygen (Qo_2) in the intervertebral disc. Work on articular cartilage suggests that although the major metabolic pathway in this tissue appears to be anaerobic, an aerobic pathway also exists, being possibly limited to one part of the cell population (Rosenthal, 1941; Lane et al, 1976). Furthermore, sulphate incorporation into cartilage is dependent upon O_2 tension (Lane et al, 1976). In view of the above findings it appeared of interest to investigate the O_2 consumption in different regions of the intervertebral disc. O_2 tensions were measured with a Clarke electrode and the consumption with a Gilson respirometre. The rate of O_2 consumption in the total disc was found to be dependent on O_2 tension and lay in the range 0.0020-0.015 ul/mg wet tissue x hour. Qo_2 varied in different parts of the disc being higher in the more cellular regions (the anulus fibrosus edge and the vertebral cartilaginous part of the endplate). The Qo_2 was roughly estimated in vivo with a modified oxygen probe and was found to be in the same range as the in vitro determinations. Using the results from Qo_2 and Fick's law of diffusion it has been possible to calculate the concentration profile for O_2 in the disc. This investigation shows that O_2 diffuses into and is consumed by the cells in the intervertebral disc.

α-L-IDURONIDASE ACTIVITY MEASURED USING NATURAL SUBSTRATES DERIVED FROM HEPARIN.

John Hopwood and Vivienne Muller (Adelaide Children's Hospital, Australia)

A deficiency of the lysosomal hydrolase α-L-iduronidase is responsible for the pathogenesis of Hurler and Scheie diseases. The artificial substrate phenyl-α-L- iduronide (I) has enabled this enzyme activity to be assayed in homogenates of a variety of tissues. 'Natural' substrates O-(α-L-idopranosyluronic acid)-(1→4)-2,5-anhydro-D [^{3}H-1] mannitol 6 sulfate (II) and O-(α-L-idopranosyluronic acid)-(1→4)-2,5-anhydro-D [^{3}H-1] mannitol (III) were prepared by deaminative cleavage of heparin, reduction of the disulfated disaccharide fragment with sodium borotritide, followed by acid hydrolysis under controlled conditions and fractionation by ion exchange chromatography. Incubation of whole cell homogenates prepared from human cultured skin fibroblasts, cultured amniotic cells or peripheral blood leucocytes with II and III gave 2,5-anhydro-D [^{3}H-1] mannitol 6 sulfate (IV) and 2,5-anhydro-D [^{3}H-1] mannitol respectively which can be separated from II and III by electrophoresis. Incubation of II with whole cell homogenates prepared from cultured skin fibroblasts and leucocytes isolated from patients with Hurler's disease gave IV at a rate fifty times less than that found for control preparations. Using whole cell homogenates of leucocytes, α-L-iduronidase was observed to have the following properties when incubated with II: a maximum hydrolytic activity at pH 3.4; a reaction rate greater than twenty times that found with III; Michaelis-Menten kinetics with a Km of 11 μM; potent inhibition by sulfate ion and III, and, unlike that reported for I, inhibition by chloride ion.

CARTILAGE PROTEOGLYCANS IN AGEING
SVEN INEROT and DICK HEINEGARD (Univ. of Lund, Lund, Sweden)
Proteoglycan aggregates were prepared from bovine tracheal cartilage of 2 fetuses (20 and 39 weeks gestational age) and of 9 steers (1 - 12.5 years old). The cartilage was extracted with 4 M guanidine-HCl and the aggregate fraction was prepared by associative CsCl density gradient centrifugation in 0.4 M guanidine-HCl. Analytical data indicate that the protein contents show a small increase while the glycosaminoglycan contents show a small decrease with increasing age in the aggregates prepared from the fetuses and the youngest steer. The values obtained for the older cartilages showed no variation with age.The proteoglycan monomers were liberated by reduction and alkylation and chromatographed on Sepharose 2B. The size of the monomers from these youngest cartilages became gradually smaller with increasing age, while the monomers of the older cartilages had the same size. Chromatography on Sepharose 2B of the aggregate fraction indicated that the proportion of aggregates to monomers did not change significantly with age. The data obtained then indicate that the composition of extractable proteoglycans from tracheal cartilage remain relatively constant from birth till old age. Constrasting results have been obtained with canine articular cartilage using similar techniques. In this tissue the size of the proteoglycan monomers decrease continuously with increasing age, primarily due to a decreasing chondroitinsulfate content and a decreasing size of the chondroitin sulfate rich region of the proteoglycan monomer.

THE EFFECT OF A GLUCOSAMINE-DERIVATIVE ON THE $Na_2{}^{35}SO_4$ INCORPORATION OF GRANULOMA TISSUE IN VITRO.
L. Jakab, F. Melicher, J. Fehér /Budapest, Hungary /

It is well known that the synthesis of glycosaminoglycanes /GG/ having important role in the structure and function of the connective tissue ground substance can be inhibited by different pharmacons. These compounds may affect different steps of the biosynthetic route, but their effect is not limited on the metabolism of GG-s. It would be important therefore to study the effect of such materials which are in connection only with the GG metabolism. Previously we showed that derivatives of glucosamine could inhibit the $Na_2{}^{35}SO_4$ incorporation, which might be in correlation with the GG metabolism. This way we studied the effect of N-acetyl-4-6-/p-oxy-benzyliden/-D-glucosamine on the $Na_2{}^{35}SO_4$ incorporation in vitro. The incubation of rat granuloma tissue was followed for 9 and 24 hours. The GG-s were isolated and the radioactivity was measured after that. The glucosamine derivative being in lower concentrations than 1 mM inhibited the $Na_2{}^{35}SO_4$ incorporation, but its inhibitory activity was very pronounced in 2 mM concentration. The mechanism and significance of the observations are discussed.

SOME PROPERTIES OF PHOSPHOPROTEIN FROM RAT INCISOR DENTIN.
M. Jontell, G. Granström, B. Persliden and A. Linde (Gothenburg, Sweden)

Phosphoprotein, rich in serine and aspartic acid, was separated from decalcified dentin matrix by non-hydrolytical extraction procedure. The phosphoprotein was purified using different chromatografic technics. To release the phosphoproteinfrom dentin matrix, decalcification with either 0.5M HAc and subsequent extraction with 1M NaCl or decalcification with neutral EDTA were used. The remaning organic matrix was degraded with periodate. No covalently bound phosphoprotein was found in this. IR-spectra of the two phosphoprotein preparations didnt show any difference. The phosphoprotein shows one class of high affinity Ca^{2+} binding sites with a KD= 2.9×10^{-6}M. If extrapolated, about 446 nmoles of Ca^{2+} are bound per mg protein. By assuming a mol. wt. of 30.000 daltons 1 mole binds 13.4 moles of Ca^{2+} with high affinity. Short time incubation was done with isolated odontoblasts and L-(^{14}C)serine. After homogenisation in 1M NaCl and a subsequent dialysis, the soluble material was applied to a DEAE-cellulose column. One radioactive peak was obtained at an ion strength of 0.25M NaCl. Investigations of characteristics, synthesis and distributions in the tissue of the phosphoprotein are being carried out.

ULTRASTRUCTURAL AND BIOCHEMICAL INVESTIGATIONS ON STRUCTURAL GLYCOPROTEINS AND PROTEOGLYCANS.

Anna Kádár and Magdolna Bihari-Varga /Budapest, Hungary/

Structural glycoproteins and proteoglycans were isolated from various tissues and their composition, structure and organization was studied by transmission and scanning electronmicroscope and by biochemical and thermoanalytical investigations. Similar methods were used to demonstrate structural alterations taking place in the ground substance of the aorta and cartilage of experimental animals treated with BAPN, in copper-deficiency, as well as after cholesterol feeding.
Changes in human arterial matrix in atherogenesis could also be demonstrated. The effect of antilipemic-antiatherosclerotic drugs /among them Chinoin 123, a synthetic pyrido-/1,2a/-pyrimidine derivative/ on the cholesterol induced changes in the composition and structure of the rabbit aorta proteoglycans has also been studied.

SUBUNIT ISOLATION AND STRUCTURE OF CARTILAGE PROTEOGLYCAN.

N.Katsura,H.Takita and N.Kasai (Sapporo,Japan)

Sperm whale cartilage proteoglycan(PG) subunit(PGUS) was prepared as follows;3M $MgCl_2$ extraction,CPC purification,-SS-bond reduction,-SH modification with mercury orange,Str. HAase digestion,ultracentrifugation(precipitate was designated as PGUI), ultrafiltration with diaflo XM 300,Ultrogel AcA 22 chromatography.PGUS has ca. 10^5MW,galactosamine 31.7%,glucosamine 4.6% and ca. 6% of amino acids which consist of Lys 1,His 1,Arg 2,Asp 4, Thr 6,Ser 8,Glu 10,Pro 10,Gly 10,Ala 5,Cys(1),Val 5,Met(1),Ileu 2,Leu 6,Tyr(1),Phe 2.PGUS shows a single spot on the two dimensional electrophoresis(1).PGUI is abundant in Asp,Glu,Pro,Gly, Ala,Cys,low in sugar,and eluted faster than PGUS on gel column. A1-D1 does not give a buoyant band but is distributed in 1.5-1.9g/ml of CsCl density gradient and gives a sharp buoyant band at 1.2-1.3g/ml of Metrizamide.Both never gave a single spot on the electrophoresis.Density gradient preparations do not give a homogeneous PG subunit.
A speculative structure of PG is;ca.300nm stiff rod central core protein(PGUI) is carrying 10-16 PGUSs and PGUS is ca.24nm subunit core protein carrying two doublets of chondroitin sulfate chains. This model explains excellently analytical data and the electronmicroscopic images.
(1)R.Hata & Y.Nagai:Anal.Biochem.45,462,1972

BIOSYNTHESIS OF SKIN COLLAGENS IN NORMAL AND DIABETIC MICE

P. KERN (Paris - France)

In vitro synthesis of collagens was studied on minced mice skin incubated with labelled proline.
After neutro and acido soluble extractions, insoluble dermis was digested by pepsin and collagens type I and III separated by differential solubilization in neutral salt solutions.
Collagens type I and III were characterised by gel electrophoresis and amino acid analysis. "Pulse-chase" experiments demonstrated linear incorporation of radioactivity in hydroxyproline of different collagen fractions.
Qualitative and quantitative alterations occurring in skin collagens of hereditary diabetic KK mice will be discussed.

CELLULAR LOCALIZATION OF GLYCOSAMINOGLYCAN DEGRADATION IN RAT LIVER

Lena Kjellén, Håkan Pertoft and Magnus Höök (Uppsala, Sweden)

After an intravenous injection of ^{3}H-labelled dermatan sulphate to rats, the polysaccharide was eliminated from the circulation in less than 40 min. A large pool of polysaccharide was found in the liver which was further examined.

After collagenase perfusion of the liver and fractionation of the cells by isopycnic centrifugation the major part of ^{3}H-radioactivity was recovered in the non-parenchymal cell fraction. The recovered ^{3}H-polysaccharide had been partly degraded as demonstrated by gel chromatography.

Analysis of fractionated rat liver cells after injection of ^{3}H-chondroitin sulphate gave identical results.

Supported by grants from the Swedish Medical Research Council.

H_2O IN CARTILAGE, H.Kleeberg and W.Luck (Marburg, GFR)

Near-Infrared-Spectroscopy is proving a valuable tool for H_2O-structure studies in biological systems. Starting with freshly dissected bovine nasal cartilage the process of controlled H_2O-desorption is investigated with a specially developed micro-spectrometer. The method permits a characterization of the structure of H_2O present in the tissue at the different desorption steps.

H_2O in fresh cartilage (360g/100g dry weight) can be divided into three fractions: 20g/100g dry cartilage are bound firmly to the matrix; additional 72g probably are located adjacent to the first fraction and/or to macromolecules; the remaining H_2O on an average is H-bonded more frequently than pure water at the same temperature.

The results are compared with those of the H_2O-structure in collagen and glycosaminoglycans,

PROPERTIES OF A HEPARAN SULFATE DEGRADING ENDOGLUCURONIDASE.
U. Klein and K. von Figura (Münster, W.-Germany)

A heparan sulfate degrading endoglucuronidase was isolated and partially purified from human placenta by ion exchange chromatography on CM 52 cellulose and DE 52 cellulose and by affinity chromatography on Concanavalin A-Sepharose 4 B and heparan sulfate-Sepharose 4 B. The endoglucuronidase has a molecular weight of approximately 110 000 estimated by gel filtration. Its catalytic activity is stimulated by glutathione and suppressed by N-ethylmaleinimide. The partially purified endoglucuronidase exhibits no activity towards carboxyl-reduced heparan sulfate though this polysaccharide is recognized by the enzyme as shown by mixing experiments with heparan sulfate. Low molecular heparan sulfate (MG $\sim$ 3000) is not attacked by the endoglucuronidase. N-desulfated heparan sulfate is only a weak substrate for the endoglucuronidase.

The aminosugar adjacent to the glucuronic acid residue present at the reducing terminal of the heparan sulfate fragments liberated by the endoglucuronidase appears to be exclusively N-acetylated glucosamine.

BIOSYNTHESIS ROUTE FOR VARIOUS CHONDROITIN SULFATE SIDE CHAINS IN CALF RIB CARTILAGE.

T.O. Kleine (Marburg/L., W. Germany)

Time course experiments with slices of calf ribs indicate a biosynthesis route for chondroitin sulfate side chains of proteoglycans where very short undersulfated chains of type A (showing a C_4/C_6 ratio of esterification of 2-4 to 1) represent precursors for long higher sulfated type A chains as well as for long type B chains (C_4/C_6 ratio of 5-7 to 1) and slightly oversulfated hybrid type C chains which consist of dermatan-4-sulfate chondroitin-4-,-6-sulfate copolymers. Analysis of the constituents of the side chains and experiments with metabolic inhibitors support this statement: 6-diazo-5-oxo-L-norleucine inhibits the formation of type B and C chains but stimulates that of type A chains. SeO_4^{2-} suppresses more synthesis of type A chains. With puromycin and cycloheximide long type C chains are overlabeled.

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HUMAN MAST CELLS INFLUENCED BY ADRENOCORTICAL STEROIDS

T. Kobayasi and G. Asboe-Hansen (Copenhagen, Denmark)

Urticaria pigmentosa lesions were injected intradermally with hydrocortisone sodium succinate. Twenty-four hours later a skin biopsy was taken. Besides degranulation, the mast cells showed vacuolation, appearing like empty progranules around the Golgi areas. The granules lost their normal ultrastructure. Normal mature granules were reduced in number. The Golgi complex showed abnormal structures. The nuclei showed homogenisation of chromatin granules.

THE NATURE OF URINARY SIALOGLUCIDES IN MUCOLIPIDOSIS AND IN MUCOPOLYSACCHARIDOSIS

M. Koseki, T. Orii* and K. Tsurumi (Fukushima and Gifu*, Japan)

We observed sialogluciduria in mucolipidosis and in mucopolysaccharidosis. Some amount of sialic acid-containing compounds was isolated from the urine of two patients, one with mucopolysaccharidosis type I and the other with type II, and a patient with newly recognized mucolipidosis (Orii et al., 1972) by fractionation on charcoal column followed by gel filtration and ion exchange chromatography. Two sialooligosaccharides, which were designated as monosialo-oroso-N-pentaose and disialo-oroso-N-octaose, were characterized from the major sialoglucide fractions. The structural study revealed that these sialooligosaccharides had a close relation to the carbohydrate side chains of glycoproteins having GlcNAc-Asn linkage. The increased excretion of the oligosaccharides may be regarded as the consequence of the imcomplete catabolism of the oligosaccharide chains contained in this type of glycoproteins. Therefore, the result suggests that the catabolic pathway of sialoglycoproteins is markedly disturbed in mucopolysaccharidosis besides the defect in the mucopolysaccharide catabolism, and the mucolipidosis described here may be considered as an inborn error of metabolism of glycoprotein-heterosaccharide, which involves deficiency of one of the isozymes of sialidase.

Monosialo-oroso-N-pentaose: NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-2)Manα(1-3)Manβ-(1-4)GlcNAc

Disialo-oroso-N-octaose: NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-2)Manα(1-3)[NeuAcα-(2-6)Galβ(1-4)GlcNAcβ(1-2)Manα(1-6)]Manβ(1-4)GlcNAc

THE MARFAN'S SYNDROME: A STUDY OF COLLAGEN SYNTHESIS IN TISSUE FROM THE AORTA

T. Krieg, P.K. Müller and K. Kühn (Martinsried, Germany)

Tissue specimens from the aorta were obtained after heart surgery from a patient with Marfan's syndrome. Media and adventitia were carefully separated and collagen synthesis of both layers was examined after a pulse of 24 hours with radioactively labeled proline. The characterization of the newly synthesized collagen showed that cells in adventitia and media produced only little type I collagen. On the other hand, the experiments showed that the newly synthesized collagen was no more soluble than that of controls. These results may indicate that the low content of type I collagen rather than a defect in crosslink formation may be responsible for the reduced mechanical strength of the aortic tissue often observed in patients with Marfan's syndrome. That would mean that a disturbance in the regulation of genes coding for different collagen chains may represent the prime event which eventually initiates the gradual development of an aortic aneurism.

THE RESISTANCE OF CARTILAGE TO ENDOTHELIAL AND TUMOR CELL INVASION

K.E. Kuettner, B. Pauli and E. Harper (Chicago,IL & San Diego,CA; USA)

Hyaline cartilage is unique for studies on invasion since it lacks its own capillary blood supply and is very rarely invaded by malignant tumors. Extracts of cartilage inhibit endothelial cell growth in vitro. Previous studies (BBRC, 72: 40, 1976) show that normal human skin collagenase is inhibited by a cartilage-derived cationic protein. We examined three types of cells (human osteosarcoma, human metastatic mammary carcinoma and bovine endothelial cells) for collagenase production and observed that preincubation of these enzymes with the cartilage inhibitor resulted in significantly decreased collagenase activity (Science, in press). Human tumor cells were cultured in the presence and absence of human cartilage growth plates in an organ culture system. The cells were found to grow into the bony side of the explant, thereby eroding the bone. On the cartilage side, however, the tumor cells were unable to invade or destroy the tissue. Since cartilage is "non-permissive" to invasion, we suggest that the inability of cells to encroach upon cartilage may be due to the endogenous specific inhibition within its matrix. We further speculate that the naturally occurring resistance to invasion by tumors is due to the same inhibitor. If it is hypothesized that invasiveness of a tumor is related to its degree of collagenase production, then it is also possible that invasiveness can be regulated by the presence or absence of anti-collagenolytic substances such as the specific collagenase inhibitor. Supported by NIH-NCI grant CA21566 and in part by NIH grants AM-09132, CA-18377, NO1-CT-33219, PO1-CA17086 and the Hulbert Fund.

STIMULATORY EFFECT OF EXOGENOUS HYALURONIC ACID DISTINGUISHES CULTURED FIBROBLASTS OF MARFAN'S DISEASE FROM CONTROLS.

S.I. Lamberg (Baltimore, Maryland)

Fibroblasts cultured from patients with Marfan's disease are known to stain metachromatically and accumulate increased amounts of glycosaminoglycan (GAG), predominantly hyaluronic acid (HA). A previous report (JID, Oct. 73) indicated that a greater rate of synthesis rather than a decreased rate of degradation accounted for this increased accumulation. Factors that might influence control of synthesis were investigated. Inhibition of protein synthesis by cycloheximide (> 90%) had only a modest effect on inhibiting GAG accumulation (30%) while serum depletion had a more profound effect (70%) on GAG accumulation than on protein synthesis (20-30%). Exogenous HA restored new accumulation of HA in Marfan-derived fibroblast cultures towards normal (50-80% recovery) while having almost no effect on cultures derived from normal controls. The effect was specific for HA and was not due to stimulation of new protein synthesis.

EFFECT OF SODIUM FLUORIDE ON PROLIFERATION, TOTAL PROTEIN-COLLAGEN- AND GLYCOSAMINOGLYCANPRODUCTION IN CULTURED FIBROBLASTS

U. Langness, R. Schors, E. Prange and R. Lescow,
(Kiel, Germany)

L 929 fibroblasts were cultured as monolayens. Sodium fluoride was added in therapeutical concentrations. Decrease of proliferation was found, but was not significant. In contrary total protein content increased significantly, parallel to increase of collagen containing substances and glycosaminoglycanconcentrations in cells plus medium. The result correspond with the clinical and radiological experience that bone formation can be stimulated by sodium fluoride.

THE DISPERSITY OF BIOLOGICAL, CHEMICAL AND PHYSICAL PROPERTIES OF HEPARINS.

Sigmund E. Lasker (New York, USA)

Heparin used in almost all research studies has originated from many manufacturers with production facilities in various parts of the world. The source materials whether bovine lung or porcine intestinal or other tissues, are collected are prepared by diverse techniques.

For clinical purposes, however, where clotting time, partial thromboplastin time and lipoprotein lipase activity are criteria of activity, commercial preparations may appear to be essentially equivalent. In contrast, numerous studies of different commercial preparations or size-charge fractionated fractions have demonstrated analytical and physical and structural (NMR) variations. The multiplicity of in vitro biological actions of heparin, including specific binding properties, the effect of the ionic strength on inhibition of clot formation, and action on rigidity of surface layers of fibrinogen, appear to be unrelated to USP activity or molecular weights.

Commercial heparins and heparins fractionated by column chromatography remain disperse mixtures of biologically active and inactive components that may inhibit or enhance particular biological test systems.

BIOSYNTHESIS OF LENS CAPSULE COMPONENTS BY EPITHELIAL CELLS OF THE LENS IN LONG TERM CULTURE

M. LAURENT (Paris - France)

The epithelial cells of the lens are maintained in long term culture. In vitro they produce an important complex of collagen and glycoproteins similar to the lens capsule structure.
The collagen molecule has been studied by electron microscopy, immunology and biochemistry.
It has been shown that these fibrils produced in vitro by the cells were collagenase sensitive and reacted positively with an antiserum against collagen type IV. In the oldest cultures the collagen appeared as a fibrous long spacing pattern. The collagen molecule has been purified by immunoprecipitation by the use of antiserum against lens capsule collagen and analysed by polyacrylamide gel electrophoresis.

SUBCELLULAR TARGETS OF TWO FACTORS MODULATING COLLAGEN SYNTHESIS IN FIBROBLASTS.

P. Lehtinen and E. Kulonen (Turku, Finland)

SiO_2-liberated macrophage factor (Aalto et al.: Exp. Cell Res. 97, 193, 1976) was incubated with sliced granulation tissue, which was then homogenized and fractionated to subcellular particles with sucrose-density-gradient centrifugation. The SiO_2-factor increased the incorporation of proline to proteins, including collagen, in the medium, cytosol and especially in those fractions which contained the rough endoplasmic reticulum. Embryonic-chick tendon fibroblasts were incubated with labelled proline in the presence of granulation-tissue extract (Aalto & Kulonen: FEBS Lett. 49, 70, 1974). A decrease was found in the radioactivity of collagen in the medium and in all the subcellular fractions except in those which contained the smooth endoplasmic reticulum and the secretory vesicles indicating a difficulty in the intracellular translocation of collagen.

THERMAL PROPERTIES OF SOME MUCOPOLYSACCHARIDES, CETYLPYRIDINIUM CHLORIDE AND LYSOZYME. J. J. Lim and E. A. Balazs (New York, N. Y., U.S.A.)

Thermal transition temperatures and heat released or absorbed during the transitions of the substances mentioned below have been obtained by using a differential thermal analyzer in the range between a room temperature and about 260°C. The substances considered are: Na-hyaluronate (HA), chondroitin sulfate-6 (CSA-6), heparan sulfate (HS), heparin (H), and their complexes with lysozyme (Lyso) and cetylpridinium chloride (CPC). The HA yielded an endothermic transition at about 130°C and an exothermic at about 253°C; the Lyso, two endothermic at 123°C and 169°C and one exothermic at 232°C; the CPC, two endothermic transitions at 90°C and 220°C; chondroitin sulfate, one endothermic at 171°C and one exothermic at 226°C; heparin, one endothermic at 155°C and one exothermic at 265°C; heparan sulfate, one endothermic at 190°C and one exothermic at 263°C. The complexes yielded different transition temperatures from the individual polyanions. For example, the exothermic transitions observed in HA and Lyso disappeared but instead Lyso-HA yielded an endothermic transition at about 221°C. Since this transition reaction was not observed in the Lyso and the HA alone, it may correspond to some new bond(s) formed between these two compounds. An interpretation of these shifts in the transition temperatures and the changes in the heat associated with these transitions will be presented.

ULTRASTRUCTURE OF NORMAL AND DIABETIC VENOUS ENDOTHELIUM

M.O. LONCHAMPT (Paris - France)

The venous endothelial cells in control and diabetic patients were studied in electron microscopy. The ultrastructure of the cell-coat of diabetic endothelium has been compared with normal one's by ruthenium red staining. The cell-coat was found thinner in diabetic than in control patients of the same age. The thickness of the cell-coat decreased in normal aging.

Moreover, the ultrastructure of the endothelial cells exhibited in diabetic and oldest control patients an increase of Weibel-Palade bodies (specific markers of these cells), probably due to a response to injury.

"Matrix-streaks": A peculiar pattern in the cartilage of the femoral head of aging human subjects

K. Lothe, M.A. Spycher and J.R. Rüttner (Zurich, Switzerland)

Articular cartilage from normal and osteoarthrotic femoral heads in subjects of various ages has been examined histologically and by electron microscopy. In normal to slightly arthrotic specimens from middle-aged and old subjects, peculiar "streaks" may be observed in the matrix of the deep zone of the pressure areas. The "streaks" appear histologically as oblique bands extending from the pericellular halos into the adjacent matrix. Ultrastructurally, the "streaks" seen in thin sections show collagen fibres which form regular undulations. This phenomenon occurs in different planes around the chondrocytes. There is histochemical evidence that the "streaks" result from a focal lack of acid mucopolysaccharides combined with the pressure forces on the cartilage. Their appearance is assumed to be an early sign of cartilage disintegration associated with aging. Different fixation media, directions of cutting and cutting temperatures did not influence their morphological appearance.

CULTURE OF CHONDROCYTES FROM ELASTIC CARTILAGE

K. Madsen and S. Lohmander (Dept. of Histology, Karolinska Institutet, Stockholm, Sweden)

Chondrocytes were isolated from the ear cartilage of young rabbits by collagenase digestion. The cells were cultivated as monolayers and in suspension culture in Ham's F-12 medium supplemented with 10% fetal calf serum. Proteoglycans (PG) were isolated from the culture medium and intercellular matrix and characterized. Freshly isolated chondrocytes synthesized and secreted large amounts of PG, of which a great proportion was aggregated. The size of the constituent PG-monomers did not differ significantly from the size of monomers isolated from hyaline cartilage. When cultivated for several weeks in monolayer the cells eventually ceased producing aggregated PG. The transfer of such cells to a spinner bottle, however, restored the synthesis of PG aggregates. Thus the chondrocytes of rabbit ear cartilage seem to behave similarly to rabbit articular chondrocytes in the response to varying culture conditions.

BIOSYNTHESIS OF HEPARIN: PURIFICATION OF THE C-5-URONOSYL EPIMERASE FROM MOUSE MASTOCYTOMA.

A. Malmström, L. Rodén, D.S. Feingold, I. Jacobsson, G. Bäckström and U. Lindahl (Lund, Sweden; Birmingham, Pittsburgh, USA; and Uppsala, Sweden)

In the course of heparin biosynthesis the L-iduronic acid component is formed by C-5-epimerization of D-glucuronic acid after incorporation of the latter into the growing polysaccharide chain.

The epimerase catalyzing this reaction has been purified 10 000-fold from mouse mastocytoma tissue by a procedure starting with ammonium sulfate fractionation of a 105 000 x g supernatant. The enzyme was subsequently purified by consecutive affinity chromatographies on concanavalin A, heparan sulfate, a desulfated re-N-sulfated heparin derivative and Cibacron blue covalently bound to Sepharose.

Epimerase purified by this procedure yielded two major components on SDS--polyacrylamide gel electrophoresis. The purified enzyme had the same K_{av} as bovine serum albumin on Sepharose 6 B gel chromatography.

In the course of the purification of the epimerase a protein cofactor was removed. Addition of this factor to the purified enzyme resulted in 10-15-fold stimulation of epimerase activity. The factor has been partially purified by ion-exchange chromatography.

PROTEOGLYCANS AND PROTEOGLYCAN AGGREGATES IN EARLY EXPERIMENTAL OSTEOARTHROSIS - C.A. McDevitt, H. Muir and M.E.J. Billingham* (London and Macclesfield*, England)

An animal disease model (J. Bone Jt. Surg. 59B, 24, 1977) was used to investigate the changes in structure and metabolism of proteoglycans and proteoglycan aggregates in early osteoarthrosis (OA). Proteoglycans were extracted with 4M GuHCl containing protease inhibitors and isolated by associative equilibrium density gradient centrifugation. The sedimentation coefficients of the proteoglycan aggregates was reduced in the OA specimens compared with controls. The proportion of proteoglycan aggregates was also reduced in the pathological preparations as assessed by gel chromatography and analytical ultracentrifugation. No difference was evident in the hydrodynamic size and sedimentation coefficient of the monomeric proteoglycans of A1 fractions of OA and control specimens. In vivo administration of 35-SO_4 and gel chromatography of A1-D1 fractions mixed with hyaluronic acid (HA) demonstrated that the newly deposited proteoglycans in OA cartilage were similar in size to controls and could interact with hyaluronic acid. Separation of glycosaminoglycans that were labelled in vivo with 35-SO_4 suggested that the higher GALN/GLCN of OA proteoglycans was due to the synthesis of a new chondroitin sulphate rich proteoglycan in the disease. Thus proteoglycans with altered glycosaminoglycan composition, but with intact HA-binding sites are synthesised in the early phase of the disease. Nevertheless the quality of the proteoglycan aggregates is impaired.

IN SITU INTERACTIONS BETWEEN LOOSE CONNECTIVE TISSUE MACROMOLECULES F.A. Meyer and R. Angelovitz (Rehovot, Israel)

An attempt has been made to investigate the interactions occurring between various structural elements in loose connective tissue using the umbilical cord. The components of interest are the proteoglycans (hyaluronic acid, chondroitin sulphate and dermatan sulphate) and the skeletal elements (collagen and elastin). Tissue slices have been treated with various enzymes including streptomyces hyaluronidase, testicular hyaluronidase, chondroitinase ABC, trypsin, pronase, elastase and collagenase and with chemical reagents. Combinations of the various treatments have also been employed. The swelling behavior of the treated tissues in phosphate-buffered saline, pH 7.3, was determined as well as the release of tissue components. The results obtained will be discussed in relation to our earlier concepts of loose connective tissue structure (1-3).

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A HERITABLE DEFECT IN COLLAGEN FIBRILLOGENESIS RESULTING IN FRAGILE HYPEREXTENSIBLE SKIN
R.R. Minor and D.F. Patterson (Cornell University, Ithaca, N.Y. and The University of Pennsylvania)

A family of cats has been developed with an autosomal dominant defect in the packing of collagen in both the fibrils and fibers in the dermis. The clinical presentation of the propositus was for self inflicted lacerations, and the tensile strength and modulus of stress of his skin were found to be 10 to 20% of that of control cat skin. Electron microscopy showed that the diameter of collagen fibrils in control cat skin had a Gaussian distribution with a mean of 76.8$\pm^2$nm. In contrast, the diameter of these fibrils in affected skin had a bimodal distribution with mean diameters of approximately 60 and 120 nm. In control skin, none of the collagen fibrils had a diameter of >104 nm, whereas in affected skin the diameter of 56% of the fibrils was >104 nm. In addition, there was an extreme disorganization in the orientation of the fibrils within many of the collagen fibers in affected skin. When this propositus was mated with four unrelated clinically normal females, 50% of both male and female kittens were similarly affected. In the heterozygous kittens, there was a consistent correlation of the defects in the packing of dermal collagen and the decrease in the tensile strength and modulus of stress in the skin. To-date, no viable homozygous kittens have been obtained from matings of two affected cats. These findings show that this defect in collagen fibrillogenesis is transmitted as an autosomal dominant trait, and suggest that heterozygotes may synthesize both normal and abnormal collagen molecules. Such a defect may be the consequence of an abnormality in a structural gene which results in a structural defect in a specific collagen polypeptide chain. Thus, this model should prove to be a valuable system for studies of collagen fibrillogenesis, as well as a model for one of the dominant forms of Ehlers Danlos Syndrome in man.
Supported by USPHS Grant GM 20995 and Am 14526.

CHANGE IN A METABOLIC ACTIVITY OF RABBIT ARTICULAR CARTILAGE ORGAN-CULTURED IN AN IN VITRO SYSTEM.
D. Mitrovic, M. Gruson and L. Cohen-Solal (Paris, France)

The metabolic, histochemical and ultrastructural modifications induced in rabbit articular cartilage during timed in vitro incubations at 37° C (10 minutes to 18 hours) using Krebs phosphate glucose nutrient medium were studied. It was found thatafter 10 minutes of incubation the chondrocytes oversynthesized the matrix macromolecules for at least the next 6 hours. This was suggested by :

1 - increased incorporation of ^{35}S-sulfate and ^{3}H-glycine during the first 6 hours of incubation;

2 - an intensification of metachromasia which in addition spread out into the superficial layer that normally is always orthochromatic. Only the most superficial layer corresponding to one or two rows of the cells did not change its staining pattern at any moment during the incubation ;

3 - a rapidly acquired abundant rough endoplasmic reticulum, enlarged Golgi area and numerous newly synthesized proteoglycan molecules.

This study poses fundamental questions about the mechanisms that regulate matrix synthesis by the cells.

INTERACTION OF ELASTIN PEPTIDES WITH AORTIC GLYCOPROTEINS.
M. Moczar and E. Moczar (Créteil, France)

14C acetyl labelled elastin peptides were used to study the interaction between crosslinked elastin and glycoproteins. Such interactions were claimed to be important during the morphogenesis of elastic fibrils. Coacervate forming peptides (I) (MW : 70,000) and non coacervable fractions (II) (MW : 12,000) both containing desmosines were obtained by hydrolysing elastin with 1M KOH in 80 % EtOH for 4 h (I) and 18 h (II) resp. (κ-elastin). The adsorption of I and II as a function of concentration was studied at 20 and 4°C on : A) glycoproteins (MW 36,000, 16,000) from aorta, B) actin from skeletal muscle and C) fibrous elastin. At 20°C, the initial saturation of A, B and C occured at lower concentration with II than with I. At this point A), bound 5-10 times more I than did B), but A, B and C bound the same amount of II. The initial saturation phase is specific for I and not for II. The extent of binding I decreased with the molecular weight of the glycoprotein. This saturation phase was followed by an enhanced binding (aggregation) of I and II above the concentration of 22 µg/ml (I) and 15 µg/ml (II). The aggregation phase showed no specificity for I or II. At 4°C, I and II were not bound to B. The extent of binding of I and II to A and C, decreased about tenfold at 4°C during the saturation phase and there was no enhanced binding (aggregation). These results confirm the existence of conformation-specific interaction between structural glycoproteins of the aorta and coacervable peptides from κ-elastin.

POLARIZATION MICROSCOPICAL STUDIES ON THE SUBMICROSCOPIC STRUCTURE OF GLYCOSAMINOGLYCANS IN EMBRYONIC CONNECTIVE TISSUES
L.Módis/Debrecen, Hungary/

Human and chicken hind limb cartilages and corneae of different embryonic ages were investigated with topo-optical reactions specific for glycosaminoglycans /GAG/ and collagen. These staining reactions result in optical anisotropy and permit conclusions on the submicroscopic orientation of the macromolecules stained. At the early stage of differentiation a small degree of orientation of polycarboxylated GAG could be detected. The molecules are ordered parallel to the cell surface in the cartilage matrix, and parallel to the collagen fibrils in the secundary cornea stroma. An increase in ordering of GAG molecules coincides with the gradual emergence of the collagen structure and with the appearance of sulfated GAG chains. A prevalence of axiparallel orientation is characteristic of the ordering collagen and GAG chains in both connective tissues. This organization comes about gradually during corneal differentiation which starts from the subendothelial stroma and advances towards the epithelium. Despite intensive cell staining, no oriented GAG structures could be detected intracellularly.This finding suggests that extracellular interactions of GAG and collagen are indispensable for the development of their ordering.

HETEROGENEITY OF THE GLYCOSAMINOGLYCANS IN ARTICULAR CARTILAGE PROTEOGLYCANS

KATSUMI MURATA and ANDERS O. BJELLE (Tokyo, Japan; Umeå, Sweden)

The present study reports on a comparative heterogeneous structure of chondroitin sulfate(CS) chains in proteoglycans from articular cartilage under associative and dissociative conditions. The fact that enzymic degradation of CS chains in cartilage proteoglycans with chondroitinases produces uniform disaccharide units made it possible to define a structural heterogeneity of CS chains.
Under associative and dissociative conditions, the distribution of the AGAG components was as follows: the ratio of 4-sulfated disaccharide units to total AGAG increased with decreasing density gradients whereas that of 6-sulfated disaccharide units to total AGAG increased with increasing density gradients. The ratio of disulfated disaccharide units to total AGAG increased somewhat with decreasing density gradients whereas that of non-sulfated disaccharide units tended to decrease. Although the cartilage proteoglycans macromolecules were heterogeneous, a certain regularity was observed with respect to the distribution of sulfate and the degree of sulfation in the chondroitin sulfate chains of the proteoglycans.

SPECIFIC STIMULATORY EFFECT OF PROSTAGLANDINS ON THE BIOSYNTHESIS OF HEXOSAMINE-CONTAINING SUBSTANCES

S.MUROTA, M.ABE, K.OTSUKA and W.C.CHANG (TOKYO, JAPAN)

Effect of various prostaglandins(PG) on the production of hexosamine-containing substances by cultured fibroblasts were studied. $PGF_{2\alpha}$ was found to have the most potent stimulatory effect on the production of the macromolecules by the cells. PGD_2, $PGF_{1\alpha}$ and PGE_2 were the next most potent stimuli. The stimulatory effect of $PGF_{2\alpha}$ was significant even at the low concentration of 100 pg/ml, and it could be seen as early as 2 hours after exposure to $PGF_{2\alpha}$. Further analysis showed that 80 % of the increase of the hexosamine-containing substances was in acidic glycosaminoglycans and the rest was in glycoproteins. Further analysis of the glycosaminoglycan fraction revealed that hyaluronic acid was the most stimulated component among the several acidic glycosaminoglycan components. Prior to the increase of hyaluronic acid, hyaluronic acid synthetase was induced by $PGF_{2\alpha}$, while collagen synthesis was greatly inhibited in the same experimental condition. Thus, the stimulatory effect of $PGF_{2\alpha}$ was specific to the mucus substances, and it did not seem to occur via c-AMP. These findings suggest the possibility of one of the proper derivatives of $PGF_{2\alpha}$ being used as an anti-ulcer drug.

BIOCHEMICAL HETEROGENEITY OF FIBROBLASTS FROM PATIENTS WITH OSTEOGENESIS IMPERFECTA

P.K.Müller and T.Krieg (Martinsried, Germany)

Skin and bone specimens from patients with Osteogenesis imperfecta were used to investigate the synthesis of collagen in tissue explants and also in outgrowing fibroblasts. Using biochemical and immunochemical methods we identified two groups of patients, one of which was characterized by an excessive synthesis of type III collagen, while collagen synthesis in the other group was within the range of healthy controls. In some instances we found enhanced synthesis of type III collagen in fibroblasts from relatives who did not reveal any clinical manifestations. In one case abnormal collagen synthesis was found only in the bone of the affected individual, while fibroblasts derived from a skin biopsy had normal collagen synthesis These data indicate that synthesis of a higher proportion of type III collagen by fibroblasts of the skin is not a sufficient criterion for Osteogenesis imperfecta but may allow the identification of a carrier person. Furthermore, normal collagen synthesis by fibroblasts does not rule out the possibility that a defect in collagen synthesis might be present in osteoblasts only.

THE EFFECT OF GLYCOSAMINOGLYCAN ON THE FIBRIL FORMATION OF COLLAGEN TYPE I AND III.

M.Németh-Csóka /Budapest,Hungary/

Glycosaminoglycans /GAG/ influence differently the in vitro collagen fibril formation. The effect of GAG on the collagen fibril formation of type I and III is studied. Collagen was extracted from pepsin digested insoluble rat skin collagen and fractionated according to Epstein /1974/. The in vitro fibril formation was carried out by heat gelation at pH 7°2 , I=0°16 , T: 25-32 °C. The fibrillary precipitation of collagen type III was more rapid than that of collagen type I under the same conditions. Chondroitin 4-sulphate enhanced whereas heparin delayed fibril formation in both collagen types. Electronmicroscopically the precipitated fibrils showed crosstriation of native like collagen. There was not found any significant difference in the diameter of the fibrils. The biological significance of these results is discussed briefly.

THE BINDING OF CALCIUM TO HEPARIN.

I.A.Nieduszynski, G.Melino & I.Hampson (Lancaster, England)

The binding of calcium to heparin was studied using a flow dialysis technique and the experiments represent the competition for sites between calcium and sodium ions. For dilute solutions of heparin in 0.15M $NaCl$, pH 7.0, two classes of binding site were observed with K_{diss} = 3 x 10^{-3}M and 2.5 x 10^{-2}M. However, at higher heparin concentrations (26 mg/ml) a third class of site was observed with K_{diss} = 5 x 10^{-4}M.

Modified forms of heparin which had been de-N-sulphated and re-N-acetylated, and carboxyl-reduced were also examined as was heparin at pH 2.0. In these cases two classes of binding site were observed.

In heparin the increase in the apparent strength of calcium binding with heparin concentration was interpreted in terms of calcium-promoted chain-chain associations. These results indicate that calcium binding studies at low glycosaminoglycan concentrations may have underestimated the strength of binding which may occur in proteoglycans where the glycosaminoglycan chain concentrations are high.

THE BINDING OF HEPARIN TO ANTITHROMBIN. STUDIES BY ULTRAVIOLET DIFFERENCE SPECTROSCOPY AND CIRCULAR DICHROISM.

B. Nordenman and I. Björk (Uppsala, Sweden)

The interaction between the anticoagulant polysaccharide heparin and the plasma protease inhibitor antithrombin leads to activation of the inhibitor, which results in an accelerated rate of inhibition of various coagulation proteases. Two forms of heparin, one with high anticoagulant activity and the other almost devoid of such activity, may be separated by affinity chromatography on antithrombin-Sepharose. The binding of these two heparin fractions to antithrombin has been characterized by ultraviolet difference spectroscopy and circular dichroism. Each fraction binds to antithrombin in a molar ratio of 1:1, probably to the same site on the protein, but the affinities of the two fractions differ widely. The binding constant of the high-activity fraction at physiological ionic strength is in the order of 10^{7} M^{-1}, while that of the low-activity fraction is about 5 x 10^{-4} M^{-1}. Neither heparin fraction causes a change of the far-UV CD-spectrum of antithrombin, indicating that heparin binding does not lead to significant changes of the secondary structure of the protein. In contrast, the high-activity fraction, but not the low-activity fraction, induces a change of the aromatic CD-spectrum, suggesting a local perturbation of the protein structure in the neighbourhood of some aromatic amino acids. Acid and alkaline UV-difference spectra in the absence and presence of the two heparin fractions also indicate such changes. These conformational changes may be related to the heparin-induced activation of the inhibitor.

BIOCHEMICAL AND ULTRASTRUCTURAL STUDIES OF THE SYNTHESIS OF ELASTIN, PROTEOGLYCANS AND COLLAGEN BY NEONATAL RAT AORTIC SMOOTH MUSCLE CELLS IN TISSUE CULTURE.

B.W. Oakes, A.C. Batty, and C.J. Handley, Monash University, Australia.

High density multilayer cultures of neonatal rat aortic smooth muscle cells ($1x10^6$ cells/$25cm^3$ Falcon flask) were grown for 3-4 weeks using daily medium changes. After a 3-4 day lag period, the cells grew exponentially for 10-12 days followed by a further slow increase in cell numbers. The elastin and proteoglycan content of the cultures was measured by gravimetric analyses after alkali extraction and hexuronic acid analyses respectively, continued to increase with age of the culture after an initial 4-5 day lag period. Using differential digestion of the glycosaminoglycans with the enzymes chondroitin lyase AC and chondroitin lyase ABC, 40% of the uronic acid was associated with a fraction corresponding to chondroitin sulphate and hyaluronic acid and 60% with a fraction corresponding to dermatan sulphate. Collagen content as determined by hydroxyproline analyses tended to parallel cell numbers in that a rapid increase occurred between days 5-10 of incubation followed by a further slow increase with age. Amino acid analyses of alkali-extracted elastin showed an elastin profile with the presence of desmosine and isodesmosine and a high lysine content similar to that described by Looker and Berry, J. Anat. 113 (1972). Transmission electron microscopy revealed large elastin aggregates coalescing between the deeper layers of "embryonic smooth muscle cells" together with collagen fibres and proteoglycan granules when ruthenium red staining was used.

BINDING OF CELL MEMBRANES TO ISOLATED RAT HEPATOCYTES.

B. Öbrink, B. Wärmegård, H. Pertoft (Dept. Medical & Physiological Chemistry, The Biomedical Center, Uppsala, Sweden)

We have earlier shown that plasma membrane preparations from rat liver specifically stimulate aggregation of rat but not chicken liver cells, and that similar membrane preparations from chicken liver stimulate aggregation of chicken but not rat liver cells. Other subcellular membranes from rat liver had no effect. We have now developed a method for the determination of binding of membranes to rat hepatocytes. Radiolabelled membranes were incubated with cells and the rate and extent of binding were determined by separating unbound membranes from cells with bound membranes according to differences in buoyant density in a new density gradient medium consisting of colloidal silica coated with polyvinylpyrrolidone. It was found that rat liver plasma membranes bound to a high extent (60 µg membrane protein/10^6 cells at 60 min) whereas mitochondrial rat or chicken membranes hardly bound at all. However, also membranes from chicken liver, prepared in the same way as plasma membranes from rat liver, bound significantly to the rat hepatocytes, but the extent of binding was only 1/3 of that shown by rat plasma membranes. The binding of radioactive rat plasma membranes could be inhibited by preincubation of the cells with unlabelled rat plasma membranes but not with unlabelled rat mitochondrial membranes.

THE CELL-ASSOCIATED AND SECRETED FORMS OF HYALURONATE PRODUCED BY ROUS SARCOMA VIRUS-TRANSFORMED CHONDROCYTES - M. Okayama, Y. Takagaki, A. Kaji and B.P. Toole (Boston, U.S.A.)

Transformation of chick embryonic chondrocytes with Rous sarcoma virus results in changes in morphology, onset of motility and a 20-fold increase in incorporation of isotopic precursors into hyaluronate by these cells. Approximately one-fifth of newly synthesized hyaluronate is associated with the cell layer. After exhaustive extraction with chloroform:methanol (2:1) and chloroform:methanol:water (10:10:3), the cell-associated and secreted forms of hyaluronate were digested with polysaccharidases and then re-extracted with chloroform:methanol:water. Lipid-like material was recovered from such extracts derived from cell-associated hyaluronate but not from secreted hyaluronate. These results suggest that hyaluronate may exist in two forms, one of which is associated with cellular lipid. Synthesis of hyaluronate was not inhibited by tunicamycin, which blocks formation of polyisoprenol-pyrophosphate-N-acetyl glucosamine derivatives.

ISOLATION AND CHARACTERIZATION OF A HEPARAN SULFATE PROTEOGLYCAN FROM RAT LIVER PLASMA MEMBRANES.

A. Oldberg, L. Kjellén and M. Höök (Uppsala, Sweden)

Heparan sulfate is the only sulfated glycosaminoglycan synthesized by rat hepatocytes. The polysaccharide appears at the cell surface in a proteoglycan form. (Å. Oldberg et al. Biochem. J. 164, 75 ,1977)
Heparan sulfate proteoglycans appear to be associated to the plasmamembrane in two ways as 50-70 % of the proteoglycan molecules can be solubilized by sodium chloride or heparin related polysaccharides, whereas the remaining proteoglycan molecules are not released unless hydrophobic interactions in the membrane are broken with the help of detergents.
In the present investigation heparan sulfate proteoglycans are extracted from a purified rat liver plasmamembrane fraction using a mixture of Triton X-100 and heparin. The heparan sulfate proteoglycan is subsequently purified by ionexchange chromatography on DEAE-cellulose, gel chromatography, and density-gradient centrifugation in CsCl.
The characteristics of the heparan sulfate proteoglycans will be discussed.
Supported by the Swedish Medical Research Council.

INTRAMUSCULAR COLLAGEN AND MUSCLE PROTEIN: COMPARISON OF SYNTHESIS RATES IN VIVO AND IN VITRO AND IN DIFFERENT MUSCLES.

R. M. Palmer, P. J. Reeds, G. E. Lobley and R. H. Smith (Aberdeen, Scotland)

Rabbits infused to constant s.r.a. with ^{3}H proline showed synthesis rates for mixed muscle protein and intramuscular collagen in the ratio 2:1 (m. longissimus or mixed thigh muscle).

In 8 h organ culture, rates were 10-20% of the rate measured in vivo in the same animal; but the ratio of muscle protein to i.m. collagen synthesis rates was similar to that found in vivo.

In vivo red muscle (soleus) protein had approximately twice the synthesis rate of white muscle (psoas). In 8 h culture, although both rates were depressed, the ratio between the two muscle types remained the same.

ARE GASTRIC MUCINS HETEROGENOUS ?

M.Paulsson, I.Carlstedt and S.Gardell (Lund, Sweden)

Porcine stomachs were dissected into the cardiac, fundic and pyloric glandular regions. Mucosal scrapings from each were extracted with 6 M guanidine HCl. Papain digestion, followed by gel chromatography on Sepharose 6B, was used to prepare high molecular weight glycopeptides from both extracted material and residues. A major population of glycopeptides, eluted at or close to the void volume, was studied further. Total solubilization of such glycopeptides derived from the fundic and pyloric regions was accomplished with guanidine, whereas only 80% of the corresponding glycopeptides from the cardiac region were brought into solution. Rechromatography of the high molecular weight glycopeptides on Sepharose 4B resolved three components called glycopeptides (GP) A, B and C respectively. GP-A was found exclusively in the cardiac region and was almost entirely confined to the fraction insoluble in guanidine. GP-B was found in all three glandular regions but not in the guanidine insoluble fraction of cardiac mucins. GP-C was found only in the fundic and pyloric regions. The results indicate the existence of three discrete populations of gastric mucins, possibly derived from the cardiac glands (GP-A), the surface epithelium (GP-B) and the mucous neck cells / pyloric glands (GP-C), respectively.

COLLAGENASE IN SYNOVIAL FLUID
L.Peltonen and L.K.Korhonen (Oulu, Finland)

Collagenase production has been demonstrated from rheumatic synovial cells but if the enzyme is present in synovial fluid is a matter of controversy. We analyzed collagenase activity of synovial fluid samples from 65 patients using ^{14}C labelled collagen. According to the clinical status of patients the samples were divided into four groups: active (RA I) and inactive (RA II) rheumatoid arthritis, degenerative arthrosis (DA) and "normal samples" (N) from meniscectomies. Collagenase activity was discovered in every sample at least after trypsin activation. After centrifugation all activity was found in sediment; however, no correlation between cell count and collagenase activity existed. High activities (over 1500 dpm/ml) were found in 65% of samples from RA I but such values were not obtained from samples of DA and N groups. Analysis of variance and paired tests of significance showed difference at P 0,025 level between RA I and RA II and between RA I and DA; DA and N groups did not differ significantly. Collagenase inhibitors, α_2-macroglobulin and α_1-antitrypsin were studied using immunodiffusion. The quantitative differences between clinical groups falled within normal variation. The collagenase activating effect of trypsin was inversely propotional to the amount of inhibitors thus suggesting the binding of these inhibitors as the main mechanism of collagenase activation by trypsin.

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SERUM INHIBITION OF BONE ARYLSULPHATASE
L.Peltonen and L.K.Korhonen (Oulu, Finland)

Arylsulphatases of bone tissue have not been previously purified and characterized. Because they obviously degradate acid mucopolysaccharides of the intercellular matrix of bone and cartilage, we studied them in the course of investigations concerning arthritis. The soluble arylsulphatases were partially purified and characterized from rat bone. Gel filtration of tissue extracts gave one peak of activity which had a mol. w. of 60-70.000. The enzyme was further fractionated into three components using electrofocusing. These fractions have mol. w. of 32-36.00, one of them had an optimum pH at 5.5, and two others at 5.0. The effects of selected inhibitors and activators, as well as the K_m values were similar in all three components. The arylsulphatases of rat bone are largerly similar to the arylsulphatase B of other tissues. The peak of activity after gel filtration was clearly inhibited by serum. After electrofocusing one of the fractions was inhibited, one activated and no clear effect was observed on the third. The sum effect of serum on the pooled preparations was a linear inhibition.

Grants: Sigrid Juselius Foundation, Finland

NEUROFIBROMATOSIS CELLS SYNTHESIZE TYPE I COLLAGEN
R. Penttinen, T. Marttala and T. Hollmén (Turku, Finland)

Patients suffering from v. Recklinghausen's neurofibromatosis often develop tumors that are considered to be of neural origin. Tumors of a typical v. R. patient contained approx 38% lipids by dry weight and an unexpectedly high amount (31%) of collagen by lipid-free dry weight. Slices of tumors and skin of the same patient were incubated with ^3H-(U)proline and the radioactive proteins analyzed for their content of collagen. 62-66% of total radioactive proteins synthesized by the tumor slices was collagen in comparison with 26-28% synthesized by the skin slices calculated from radioactive hydroxyproline. SDS-polyacrylamide electrophoresis showed a pattern typical of type I collagen after limited pepsin digestion. - Cells were cultivated from skin and tumor samples. The tumor cells differed in their morphology and in their slow growth rate from the skin fibroblasts. They synthesized, however, procollagen as judged from results of DEAE- and CM-cellulose chromatographies, and SDS slab gel-electrophoresis followed by radioautography. The reason for the high capacity of the cells in neurofibroma tumor slices to synthesize collagen thus explaining the appearance of tumors, is not known.

Supported by the Finnish Cultural Foundation.

ENDOCYTOSIS OF ^{35}S -PROTEOGLYCANS BY HUMAN SKIN AND AORTIC FIBROBLASTS.
R. Prinz, J. Schwermann, E. Buddecke and K. von Figura (Münster,W.-Germany)

Fibroblasts grown from human skin and intima of human aorta were used to study the endocytosis of ^{35}S -proteoglycans (^{35}S -Pg) isolated from the secretions of these cells. The uptake follows a saturation kinetic with a half maximal uptake at ^{35}S -Pg-concentrations of 89 nmol disaccharides (Dis)/ml and 11 nmol Dis/ml for skin and aortic fibroblasts, respectively. Maximal uptake per cell and hour was calculated to be $2x10^{-3}$ pmol Dis and $4x10^{-3}$ pmol Dis for skin and aortic fibroblasts, respectively. Up to a concentration of 1000 nmol Dis/ml the association of ^{35}S -Pg with the cell surface follows zero order kinetics. The endocytosis rate depends on the medium pH and is independent from the serum concentration. The endocytosis of ^{35}S -Pg was inhibited by the following substances: chondroitinsulfate proteoglycan, chondroitinsulfate, dermatansulfate, heparansulfate, heparin, hyaluronate, dextransulfate, synthetic polypentosan sulfate (SP54), octa- and tetrasaccharide from chondroitinsulfate. No inhibition was produced by non charged macromolecules such as dextrans of different molecular weights and glycogen. Glycosaminoglycans such as heparin and heparansulfate bind to the cell membrane but in contrast to the proteoglycans they become afterwards not internalized.

Changes in the soluble collagen of the human uterine cervix associated with parturition and menopause.
M. van der Rest, P. Kleissel, F. Naftolin and F.H. Glorieux (Montreal, Canada)

Seven samples of uterine cervix were obtained, two taken during the proliferative phase of the menstrual cycle (group A), one at 16 week pregnancy (B), two intra-partum (C), and two post-menopausally (D). We analyzed their reducible crosslink composition and studied the collagen in acetic acid soluble and insoluble fractions. Type I collagen was found to be dominant in the insoluble fraction with 20% to 38% type III as evaluated by electrophoresis of the CNBr peptides. In all samples, dihydroxylysinonorleucine was the most abundant crosslink. Despite a rather constant hydroxyproline content [19(±6.5)Hyp residues/1000 amino-acid residues], the acetic acid soluble fractions demonstrated striking variations in the electrophoretic patterns of their collagen. There was no difference between groups A and B, where intense β,α1 and α2 bands are seen. They account for 35.5% (±1.5%) of the stained protein. In group D only one intense and complex α1 band is seen. In the two intra-partum samples (C), faint collagen bands represents 4.3% and 16.1% respectively of the stained proteins. Less than 7.5% of the total hydroxyproline is acetic acid soluble in all except the two intra-partum samples where 16% and 30% hydroxyproline is acid soluble. These results are interpreted as an evidence that the cervical dilatation is made possible by a pre-partum degradation of collagen in this tissue.

ADHESION OF RAT HEPATOCYTES TO COLLAGEN. K. Rubin, A. Oldberg, L. Kjellén, M. Höök, B. Öbrink (Dept. Medical & Physiological Chemistry, The Biomedical Center, Uppsala, Sweden)

Adhesion of rat liver cells, isolated by collagenase perfusion, to reconstituted, native fibres or films of denatured collagen (type I) has been studied. Binding occurred at 37° and 22° but not at 4°. The cells bound faster to the native fibres but otherwise no differences in the adhesion to native or denatured collagen were observed. In contrast to adhesion to tissue culture plastic, serum was not required for binding to collagen. There was an absolute requirement for divalent cations, the order of efficiency being $Mn^{++} = Mg^{++} > Ca^{++}$. High concentrations of soluble denatured collagen inhibited the cell-collagen adhesion but D-galactose or UDP-galactose had no effects. Treatment of the collagen substrates with galactose oxidase, glucose oxidase or periodate did not change the cell binding, which is in contrast to rat hepatocyte binding to films of asialoceruloplasmin which was completely abolished by treatment with galactose oxidase or periodate. Cell binding to asialoceruloplasmin showed an absolute dependence on Ca^{++} which could not be substituted by any other divalent cation.

SYNTHESIS OF AN ELASTIN COMPONENT OF ABOUT 70,000 DALTONS BY POLYSOMES FROM CHICK EMBRYO AORTAE.

L. Ryhänen (Oulu, Finland), P. Graves (Piscataway N.J., U.S.A.) G.M. Bressan (Padova, Italy) and D.J. Prockop (Piscataway, N.J., U.S.A.)

Both a molecular weight in the range of 70,000 and 140,000 have been suggested for the newly translated elastin molecule. We studied the problem by characterizing the products synthesized by purified chick embryo aortae polysomes. When purified polysomes were incubated with several different labeled amino acids in conditions which allowed the completion of the polypeptide chains, two main labeled polypeptide peaks were found by polyacrylamide gel electrophoresis in the presence of SDS, one of which comigrated with standard proα chains of procollagen, the other with standard chick tropoelastin. When [^{14}C] -glycine was used, about 20% of the ^{14}C-protein was soluble in propanol-butanol-water mixtures used to extract tropoelastin and consisted mainly of polypeptides of about 70,000 daltons. When chain completion was carried out in the presence of [^{35}S]-methionine, an amino acid absent from tropoelastin, no significant amount of label was incorporated into polypeptides of about 70,000 daltons. When an amino acid very abundant in tropoelastin was used, [^{14}C]-valine, the radioactivity was recovered mainly in the 70,000 dalton peak. The 70,000 daltons component could be precipitated by anti-elastin antibodies. Since the system used to complete the polypeptide chains seems to be devoided of proteolytic activity, the data suggest that the major gene product for elastin is about 70,000 daltons.

CORRELATION OF LYSOZYME ACTIVITY WITH PROTEOGLYCAN BIOSYNTHESIS IN EPIPHYSEAL CARTILAGE

A. Schmidt and E. Buddecke (Münster, West-Germany)

Pig epiphyseal cartilage previously incubated in vitro in the presence of sodium [^{35}S] sulfate or [^{3}H] thymidine was either analyzed by autoradiography or separated into 9 morphologically defined consecutive layers and investigated for ^{35}S-incorporation into the guanidinium chloride extractable proteoglycans and for lysozyme activity. Lowest ^{35}S-incorporation and lysozyme activity were determined in the zone of resting cells, but a successive increase in the rate of proteoglycan synthesis and lysozyme activity occurs towards the diaphyseal cartilage-bone junction with the maximum at the lower columnar cell zone and a sharp reduction of both parameters at the hypertrophic zone. Maximum of ^{35}S-incorporation and [^{3}H] thymidine do not coincide. The guanidinium chloride soluble proteoglycans exhibit macromolecular polydispersity, fractions excluded from as well as retarded by Sepharose 2B gel could be separated and were detected in all zones. The close correlation of proteoglycan biosynthesis and lysozyme activity is discussed with respect to the fact that cartilage lysozyme is copurified with a neutral protease capable of degrading proteoglycan subunits.

THE DIFFERENT ACTION OF Fe(II)- and Fe(III)-IONS ON HYALURONIC ACID

O. SCHMUT and H.HOFMANN (Graz,Austria)

Hyaluronic acid with a protein content of less than 2% was prepared from bovine vitreous humour. The action of $FeSO_4$ and $FeCl_3$ respectively on hyaluronic acid solutions was investigated. Fe(II)-ions lowered the viscosity of the solutions but no precipitation could be observed. Fe(III)-ions caused only a slight decrease of the solutions' viscosity but a water insoluble Fe(III)-hyaluronic acid-precipitate was formed. Different parameters and the biological significance of these reactions are discussed.

^{1}H-NMR of OH and NH groups of GAG polymers, oligomers and monomers

J.E.Scott, F.Heatley, D.Moorcroft (Manchester), A.Olavesen (Cardiff), B.Casu (Milan).

Individual resonance signals from NH and OH protons are clearly resolved and have been assigned in the 300 MHz spectra of Na glucuronate, galacturonate, N-acetyl-chondrosinate, hyalobiuronate (and higher oligomers from HA) in DMSOd^6 solution. Spectra of quaternary ammonium complexes of GAG polymers (CSA, 'C', 'D', and dermatan sulphate) in DMSO showed multiple, differentially deuteratable NH and OH signals, NH protons were observable in H_2O solution, but OH protons were only apparent in monomers at low temperature (< 0°C).

It was necessary to work in very dilute (< 0.1% w/v) DMSOd^6 solutions, to obtain clear signals. Differential concentration and temperature dependences of line widths and chemical shifts of individual hydroxyls suggest aggregation phenomena at higher concentrations. At least one NH signal in HA and CHS oligomers showed a temperature dependence consistently lower than in monomers and N-acetyl-chondrosinate. NH coupling (8-9 hz) was easily measurable for small molecules in DMSOd^6 or H_2O.

Electron dense stains for connective tissue polyanions

J.E.Scott, Carolyn J.P.Jones, T.W.Kyffin (Manchester, England)

Copper, palladium and platinum forms of a cationic 'phthalocyanin' (N tetramethyl, tetrapyridino-tetraazaporphin) have been synthesised, purified and characterised by analyses, electrophoresis, U.V., visible, I.R. and NMR spectra. These intensely blue dyes have been assessed for electron density and staining behaviour in a number of tissues. The Pd and Pt forms give noticeably improved contrast over the Cu derivative, but the best results are obtained by 'staining' the stained E.M. grids with sodium tungstate, when the polyvalent dyes take up tungstate anion in situ, with considerable specificity. (Compare the increased uptake of Cl^- anion by Alcian Blue in similar situations, Scott, Quintarelli & Dellovo, (1964) Histochemie, 4, 73-86).

Used according to critical electrolyte concentration principles, (Scott, 1973, Trans.Biochem.Soc. 1, 787-806) the cinchomeronic tetraazaporphin (ibid) in aqueous 0.3 M $MgCl_2$ containing formalin or glutaraldehyde shows proteoglycan very clearly and specifically. In e.g. rat tail tendon the tungstate treatment faintly stains characteristic bands on the collagen fibrils, thus permitting the relationship between the proteoglycan and collagen to be defined.

SYNTHESIS BY CULTURED FIBROBLASTS OF A GLYCOPROTEIN RELATED TO THE MICROFIBRILLAR PROTEIN OF ELASTIC TISSUES.

C.H.J. Sear, M.A. Kewley, M.E. Grant and D.S. Jackson (Manchester, England).

Cultured human skin fibroblasts synthesise and release into the extracellular space a fucosylated glycoprotein of apparent molecular weight 135000 - 140000 which is not digested by highly purified bacterial collagenase. This glycoprotein accumulates in the cell layer when fibroblasts are cultured for extended periods, and may be extracted using buffered solutions of 5M-guanidinium chloride. The glycoprotein is precipitated by a specific antiserum raised in rabbits against a microfibrillar protein preparation derived from bovine ligamentum nuchae [Kewley et al. (1977) Immunol. 32, 483-489] suggesting that this glycoconjugate may play a structural role in elastic tissues. Further results employing cultured fibroblasts of bovine origin will be presented.

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THE MAIN NEUTRAL PROTEASE OF RAT SKIN: ITS MAST CELL ORIGIN AND PARTICIPATION IN BREAKDOWN OF THE INTERCELLULAR MATRIX

H.Seppä, M.Järvinen, K.Hämäläinen & L.Peltonen (Oulu, Finland)

A neutral protease (NP) was purified from rat skin. The purest preparation was homogenous in SDS gel electrophoresis, the molecular weight was 29 000. NP preferably hydrolyzed substrates containing tyrosine and phenylalanine residues. NP was inhibited by DFP, TPCK, LBTI, SBTI and rat serum. Treatment of rats with compound 48/80 for five days reduced the amount of NP in the skin to 1.7 % of the control. This suggests that NP, which represents the main neutral protease of rat skin, is derived from the mast cells.
A hypothesis of the biological role of NP is presented: in acute inflammation it attacks the proteins of the intercellular matrix and thus promotes the diffusion of plasma to the site of injury. Hyaline cartilage was used as a model of intercellular matrix. The enzyme removes the normal stainability of the cartilage with toluidine blue and Alcian blue, releasing uronic acid. Work on the possible activatory effect of NP on rat procollagenase is in progress. Work on the effects of NP on the permeability of the cartilage is being started.

A DOUBLE HELIX FOR HYALURONIC ACID IN THE PRESENCE OF 'WATER STRUCTURE BREAKING' CATIONS.

J.K.Sheehan (Lancaster, England)

The interaction of hyaluronic acid with cations has been studied using X-Ray fibre diffraction techniques. Films, cast from solutions of hyaluronate prepared in the presence of the cations potassium, ammonium, rubidium and caesium have been crystallised and all show the presence of the same fibre crystalline phase. The symmetry of the diffraction pattern indicates directly the presence of a double helix conformation and detailed model building and structure factor calculations show it to be composed of left handed, antiparallel, form fold helices. The details and implications of the model will be discussed and preliminary infra red and solution data pertaining to this problem will be presented.

METABOLISM OF GLYCOSAMINOGLYCANS (GAG) IN HUMAN EMBRYONIC LUNG FIBROBLASTS I. Sjöberg and Lars-Åke Fransson (Lund, Sweden)

Fibroblasts in monolayer were allowed to incorporate $^{35}SO_4^{2-}$ and (1-^{3}H)-glucosamine for 12 h. The extracellular GAGs were recovered from the medium and extracted sequentially from the cell layer with EDTA, trypsin digestion and dithiothreitol (DDT). By extraction of the residual cells with trichloroacetic acid one cell soluble and one insoluble residue were obtained. As in earlier studies of established cell lines and skin fibroblasts heparan sulphate was enriched in the trypsin digest. The galactosaminoglycans were subdivided into a GlcUA-rich and an IdUA-rich component. The former was found in the medium, EDTA extract and the trypsin digest and to some extent in the insoluble cell fraction, while the latter was found in the DTT extract and in the insoluble cell fraction. The metabolic fate of the different fractions were examined by pulse-chase experiments. A comparison of the $^{35}S/^3H$ ratios for the different fractions indicated that GAGs in the medium are derived both from the cells and from the trypsin released material. While the secreted galactosaminoglycans were GlcUA-rich their precursors were mostly IdUA-rich.

STUDIES ON PROTEOGLYCANS FROM BOVINE CORNEA

P.Speziale,M.Sosso,L.Galligani and C.Balduini(Pavia,Italy)

Proteoglycans were extracted from bovine cornea with 4M guanidinium chloride and were fractionated by CsCl density gradient centrifugation,under associative and dissociative conditions. Under associative conditions two peaks were found(density=1.43 and 1.64 gr/ml).
The heavier fraction was eluted as a single retarded peak on Sepharose 2B,but resulted in two fractions when chromatographed on DEAE-Sephadex:the first(eluted with 0.75M NaCl)seems to contain mainly proteo-ChS chains and the second(eluted with 1.25M NaCl) mainly proteo-KS chains.Each of these two proteoglycans was more retarded on Sepharose 2B with respect to the original sample from density gradient centrifugation.However a complete reaggregation was obtained by recombination of the two fractions.
The lighter fraction(density=1.43 gr/ml) was also retarded by Sepharose 2B and resulted on DEAE-Sephadex in a single peak,eluted with 1.25M NaCl.Chemical analyses of the carbohydrate and protein moieties confirm that corneal proteoglycans are composed of different subunits.

DISTRIBUTION OF FIBRONECTIN - A NEW CONNECTIVE TISSUE PROTEIN - IN NORMAL HUMAN TISSUES

S. Stenman and A. Vaheri (Helsinki, Finland)

Fibronectin is a glycoprotein which occurs in several immunologically cross-reactive forms in plasma and connective tissue of vertebrates. It is a major cell surface-associated protein (also called LETS protein) of cultured fibroblasts. It is lost from the cell surface after malignant transformation although synthesis continues. The name fibronectin is proposed to emphasize the fibrillar distribution of the protein on the surface of cultured cells and its interactions with collagen and fibrin. We stained sections of normal human tissues by immunofluorescence with specific anti-human fibronectin sera. The same sections were later stained for reticulin, collagen and elastin with conventional histologic methods. Fibronectin was ubiquitously present in basement membranes of all studied tissues, including thyroid gland, glands and ducts of the breast, gastric and colonic mucosa, nerves, endothelium of arteries, veins and capillaries and ducts of testis and epididymis. In striated muscle it occurred in the sarcolemma, and in the muscle layer of blood vessels and the gut on the surface of smooth muscle cells. Fibronectin was detected in sinusoid linings of the liver. Spleen and lymph nodes contained fibronectin in the capsule, in the loose connective tissue stroma and in the lining of sinuses. The surface of fat cells contained fibronectin and it was also detected at various sites of loose connective tissue. In all these sites the distribution of fibronectin corresponded to that of histologically demonstrated reticulin.

PHYSICO-CHEMICAL PARAMETERS OF HEPARIN AND HEPARIN-LIKE POLYSACCHARIDES FROM SOLUTION

S. S. Stivala (Hoboken, New Jersey 07030)

Numerous physico-chemical parameters from solution have been determined for commercial heparin and various heparin-like polysaccharides using such methods as viscometry, light and small angle X-ray scattering, sedimentation analysis, electrofocusing, chromatography, etc. The physical parameters relating to biological function will be discussed including an assessment of molecular weight and charge distributions.

POSSIBLE NEUROHUMORAL REGULATION OF THE CONNECTIVE TISSUE DIFFERENTIATION IN THE AVIAN EMBRYO
G. Strudel (Nogent-sur-Marne, France)

Injection of cholinergic agents in young chick embryos determine notochordal, neural and somite malformations which cause in older embryos morphological spinal column defects and chemically abnormal vertebral cartilage. Cholinergic antagonists, cholinergic receptor ligands, adrenergic blocking agents, inhibitors of the biosynthesis of neurotransmitters or depleting agents, inhibit the effects of cholinergic agonists and protect the embryos from spinal column defects.

KERATAN SULFATE EXCRETION IN URINE OF PATIENTS WITH ALPHA-FUCOSIDOSIS
H. W. Stuhlsatz, H. Greiling, J. Gehler and M. Cantz (Aachen, Mainz, GFR)

An alpha-L-fucosidase defect was observed in the skin fibroblasts of two children. Other lysosomal enzymes were found to be normal as ß-D-glucuronidase. ß-D-glucosidase, alpha-D-galactosidase, alpha-N-acetyl-D-glucosaminidase, ß-N-acetyl-D-glucosaminidase, alpha-D-mannosidase, or increased as arylsulfatase A and acid phosphatase. The non-dialysed urines og these fucosidosis patients were separated into two fractions by chromatography on Biogel P-2. The first fraction containing the GAG was further fractionated on Dowex 1 X 2 by stepwise elution with increasing NaCl concentrations. Mainly in the fractions eluted with 1.25, 1.5, 2.0, and 3.0 M NaCl we have assayed and characterised keratan sulfate and chondroitin sulfate both attached to the same peptide core. Compared with the excretion of normal children of the same age we found a 3- to 7-fold increase of keratan sulfate in the last two fractions. As keratan sulfate contains alpha-fucose terminal groups, this increase of keratan sulfate excretion could result from impaired degradation by the defect alpha-fucosidase.

DETECTION OF LUBRICATING GLYCOPROTEIN (LGP-I) IN THE SYNOVIAL MEMBRANE AND ITS SYNTHESIS BY SYNOVIAL CELLS IN CULTURE.
D.A. Swann, K.J. Bloch and R.W. Dubois (Harvard Medical School, Boston, Mass.)

The major component in the articular lubricating fraction from bovine synovial fluid is a high molecular weight glycoprotein (LGP-I, Biochem. J. 161, 473-485, 1977). An antiserum prepared against LGP-I in rabbits did not cross-react with other connective tissue or plasma constituents and appeared to be monospecific. By using this antiserum and fluorescein-conjugated goat anti-rabbit γ-globulin, LGP-I was detected in the synovial membrane, but not in the articular cartilage. LGP-I was also present in the medium obtained from synovial membrane cells maintained in culture, suggesting that this tissue is responsible for the synthesis of LGP-I. These data support the concept that the boundary lubrication of articular cartilage by synovial fluid in vivo is dependent upon the synthesis of a joint-specific glycoprotein by the synovial membrane.

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CONFORMATIONAL FEATURES AND CATIONIC BINDING OF α-ELASTIN.
A.M.Tamburro, V.Guantieri, D.Daga-Gordini, B.Bombardelli and G.Abatangelo (Padova,Italy).

α-Elastin has been studied by CD in different organic solvents/water mixtures in order to establish the effect of the solvents on the structural properties of the protein. The effect of pH and temperature was also investigated.

In addition the binding of calcium and paramagnetic ions to α-elastin was studied by CD, NMR and fluorescence. The results indicate the presence of α-helices and β-bends in water/ethylene glycol and water dioxane mixtures. Binding of Ca^{++} is probably involving both carboxyl groups and acyl oxygens, depending by solvent composition.

ON THE MOLECULAR WEIGHT DEPENDENCE OF THE ANTICOAGULANT ACTIVITY OF HEPARIN
A. Tengblad, L. Thunberg, M. Höök, U. Lindahl and T.C. Laurent (Uppsala, Sweden)

Heparin (HEP) is a heterogeneous polysaccharide which contains several different types of disaccharide residues. Through work by Lam et al. (Biochem.Biophys.Res.Comm. 69, 570 (1976)) and Höök et al. (FEBS Lett. 66, 90 (1976)) it is known that only a fraction of all HEP molecules bind to antithrombin (AT). The binding region involves approx. a dodecasaccharide sequence in HEP (Hopwood et al. FEBS Lett. 69, 56 (1976)). A hypothesis is proposed according to which the disaccharides in HEP are distributed in a random fashion and the amount of HEP that binds to AT is a function of the probability that the molecules contain one or more correct dodecasaccharide sequences per molecule. This probability increases with increasing length of the polysaccharide chain. HEP was fractionated with regard to molecular weight and the binding to AT of each fraction was measured. The data agreed with the hypothesis, and indicated that one out of 40 dodecasaccharide sequences has the structure required for binding to AT. The molecular weight dependence of the anticoagulant activity of HEP may essentially be explained by the hypothesis. The molecules which contain the binding site do, however, also show a molecular weight dependence of their activity. An attempt has been made to explain this by a similar reasoning for the binding of thrombin to HEP.
Supported by the Swedish Medical Research Council.

FORMATION OF ELASTIC FIBERS IN CELL CULTURES AND TRANSPLANTS
J. Thyberg and A. Hinek (Stockholm, Sweden)

Aortic smooth muscle cells and ear cartilage chondrocytes were enzymatically isolated and subsequently maintained in culture or as intramuscular transplants. In *culture* the cells rapidly formed a confluent monolayer and thereafter grew in multiple overlapping layers. At all phases of their growth the cells maintained a characteristic fine structure with bundles of filaments and a prominent endoplasmic reticulum and Golgi complex. Large amounts of extracellular matrix was formed in the cultures, including numerous mature elastic fibers. Likewise, the *transplants* were made up of typical smooth muscle cells and chondrocytes respectively, surrounded by an extracellular matrix consisting of collagen fibrils, proteoglycan granules and elastic fibers in various stages of development. In both systems, three partly overlapping stages could be distinguished in the extracellular elastogenesis. First, small bundles of 10-12 nm microfibrils appeared. These bundles then became associated with small conglomerates of dense, amorphous material. Finally, in the mature elastic fibers, such material had fused into confluent areas within which no microfibrils were detected. By using Spurr low viscosity embedding medium a dense, selective staining of the amorphous component of the elastic fibers was obtained. Otherwise, the elastic fibers have been reported to remain unstained after routine preparation for EM including staining with uranyl and lead salts. Dense, elastin-like material was also noted in Golgi vesicles and occasionally in the ER.

MICROTUBULES AND THE GOLGI COMPLEX

J. Thyberg, S. Moskalewski, A. Hinek, S. Nilsson and U. Friberg
(Stockholm, Sweden)

During recent years much interest has been paid to the role of microtubules (MT) in cellular secretion, e.g. the release of collagen and proteoglycans from connective tissue cells. MT-disruptive drugs have been found to inhibit secretion of many cell products. Based on these findings it has been suggested that MT function in the transport of secretory vesicles from the Golgi complex (GC) to the cell surface. EM studies in our laboratory have however indicated that the role of MT may be more complex. Chondrocytes cultured in monolayer or as aggregates showed a prominent GC consisting of dictyosomes (stacks of cisternae) gathered juxtanuclearly. MT were particularly numerous within this Golgi area. Exposure of the cells to colchicine or vinblastine led to disappearance of MT, dispersion of the dictyosomes throughout the cytoplasm, clustering of lysosomes and increased autophagy. Individual dictyosomes often displayed an altered structure, possibly due to a disturbance of their functional relationship to the endoplasmic reticulum. Similar alterations have been demonstrated in pancreatic islet cells, PHA-stimulated lymphocytes and neuroblasts treated with colchicine or vinblastine *in vitro. These observations indicate that intact MT are of fundamental* importance for normal structure and function of the GC. It is thus possible that the secretory inhibition and other disturbances caused by MT-disruptive drugs could also be due to a defective transfer of material to the dictyosomes and/or a defective packaging and release of material from them.

STRUCTURAL STUDIES ON URINARY GLYCOSAMINOGLYCANS. EVIDENCE OF THE β-D-CONFIGURATION OF THE GLUCURONIC ACID RESIDUES IN HEPARAN SULPHATE.

M.E.Tira, A.Calatroni and C.Balduini (Pavia, Italy)
G.Torri, R.Moretti and B.Casu (Milan, Italy)

Glycosaminoglycans were isolated from the urines of patients affected by different types of mucopolysaccharidoses and were purified by ion-exchange chromathography on Dowex 1x2 Cl^-, gel-filtration on Bio-Gel P-30 and fractional precipitation with ethanol. The samples of heparan sulphate and dermatan sulphate were pure and homogeneous, according to chemical analyses, electrophoresis and gel-filtration. Most fractions gave well defined ^{1}H-NMR spectra (at 270 MHZ in D_2O). The HS fraction eluted with 0.75 and 1.0 N NaCl, accounting for more than 60% of total HS, showed only two prominent signals in the region of anomeric protons. The coupling costants of these signals unequivocally indicate an α-D-configuration for the glucosamino residues, and a β-D-configuration for the glucuronic acid residues.

BASEMENT MEMBRANE COLLAGENS: ISOLATION BY HEAT GELATION FRACTIONATION.

Robert L. Trelstad (Boston, MA USA)

The collagenous components in basement membranes appear to be heterogeneous and consist of several distinct species. The collagen present in the renal glomerulus and lens capsule, referred to as Type IV collagen, represents one species and that isolated from placental membranes and other tissues represents another. The latter molecule is comprised of α chains which provisionally have been termed A and B by Burgeson et al. (PNAS 73:2579-2583) and Chung et al. (BBRC 71:1167-1174). We have found that the basement membrane collagens present in the mixture of collagens from pepsin solubilized human and avian tissues can be selectively isolated by heat gelation. Neutral solutions of pepsin solubilized collagen are subjected to heating at 34-36°C for 16 hours. The preparation is then centrifuged and the gelled pellet separated from the non-gelled supernatant. The basement membrane collagens of both the Type IV and A-B type are present in the non-gelled supernatant and can then be further purified by salt precipitation. Although mixtures of both basement membrane types are seen in most tissues, preferential relative distributions are apparent.

POSSIBLE ALTERATION IN THE PROCESSING OF COLLAGEN IN THE MARFAN SYNDROME

H.H. Turakainen*, R.P.K. Penttinen*, G.R. Martin** and V.A. McKusick*** (*Turku, Finland; **Bethesda, Md. and ***Baltimore, Md., USA)

Amino acid analyses of skin specimens from 8 patients suffering from Marfan syndrome showed a statistically significant increase in the concentrations of serine ($p < 0.02$), phenylalanine ($p < 0.025$) and lysine ($p < 0.005$; t-test) in comparison with 5 control specimens. A suggestive decrease in the concentrations of hydroxyproline, glycine and valine was also noted. - Skin fibroblasts from age- and sex-matched Marfan and control skins were labeled with ^{3}H-glycine and ^{3}H-proline and the cell layer extracted with 1M NaCl, 0.5M acetic acid and 2M $CaCl_2$. No difference was observed in the solubilities of the labeled proteins and their collagenase sensitive fractions between both cultures. However, there was a slight increase in the ratio of collagenous proteins in the culture medium/cell layer in the Marfan cultures compared with the controls. Pulse labeling for 140 minutes followed by a chase for variable lengths of time showed again a high ratio of collagen in medium/cell layer in the Marfan cultures. On the basis of these results we suggest that there is an alteration in the processing of collagen with other proteins to proper tissue matrix in the Marfan syndrome.

Supported by the Finnish Cultural Foundation.

INHIBITION OF LYSOSOMAL ENZYME RECOGNITION BY MONOSACCHARIDES AND GLYCOSIDES: ROLE OF D-MANNOSE AND L-FUCOSE.

+K. Ullrich, ++G. Strecker, +K. von Figura (+Münster, W.-Germany, ++Lille, France)

Recognition of α-N-acetylglucosaminidase by human fibroblasts was inhibited by the following monosaccharides (in the order of their inhibitory effect): L-fucose, D-mannose, D-lyxose and D-arabinose. These sugars have in common the configuration at C_2. Mixing experiments with L-fucose and D-mannose indicate that both monosaccharides compete for recognition with α-N-acetylglucosaminidase at the same receptor site of the cells. On a molar basis the effect of α-methylmannoside was similar to that of D-mannose, whereas the α-anomeric p-nitrophenylglycosides of L-fucose and D-mannose inhibited much stronger than the monosaccharides. Other methyl- and arylglycosides tested had no inhibitory effect.

The trisaccharide Man $\underline{\alpha 1\text{-}3}$ Man $\underline{\beta 1\text{-}3}$ GlcNAc was the strongest inhibitor. The recognition of β-N-acetylglucosaminidase was inhibited by the same monosaccharides as α-N-acetylglucosaminidase, whereas β-glucuronidase recognition was inhibited only by D-mannose. These experiments suggest a crucial role of terminal fucose and/or mannose residues for lysosomal enzyme recognition.

NUTRITION OF THE INTERVERTEBRAL DISC

J. Urban (London, England), S. Holm (Göteborg, Sweden), A. Maroudas (London, England), A. Nachemson (Göteborg, Sweden)

The intervertebral disc is the largest avascular structure in the body. Thus the penetration of nutrients to the centre of the disc and the disposal of the products of cell metabolism depend on solute exchange with the outside. It has been shown both in vitro on human discs and in vivo on dog discs that the major routes for solute transport are through the anulus fibrosus (AF) from the surrounding blood vessels and through the central portion of the endplate. The in vivo experiments made it possible to compare the rate of transport for the sulphate ion as obtained from the experimental results with that predicted from the basic concepts of molecular diffusion and the agreement was found to be very good. The amount of sulphate which penetrated into the nucleus pulposus (NP) via the endplates accounted for approximately 30% of the total, the remaining 60% being supplied from the periphery of the AF. In the case of an uncharged solute, such as methyl glucose, the route via the endplates becomes relatively more important and may account for as much as 50-60% of the total in the adult dog. Once the rates of diffusion of various solutes are known, it becomes possible to assess whether the rate of supply is sufficient to balance the cell requirements provided the latter have also been measured. To date we have measured the rate of metabolism of the sulphate ion in vivo in the dog. The rate of utilisation of glucose and oxygen by the cells in the AF and the NP of the dog has been determined in vitro.

HISTOCHEMISTRY OF EPIPHYSEAL PLATE
K.Väänänen and L.K.Korhonen (Oulu, Finland)

Distribution of the six enzyme activities, namely acid phosphatase (AcP), alkaline phosphatase (AP), β-glucuronidase (Glu), nonspcific esterase (nsE), succinate dehydrogenase (SDH) and β-hydroxybutyric-dehydrogenase (β-HBD), have been studied in the proximal femoral epiphyses of rats, aged from one to eight weeks. Age depending changes in activity were most distinct with AP. This activity became stronger with age but was strictly limited to the lower zones of epiphyseal plate. The AcP activity was most evident in the proliferative cells. Some diffuse staining was also observed in the matrix, especially in hypertophic and calcification zones. The maximal activities of two lysosomal enzymes, β-Glu and AcP were dissimilar. The activity of nsE was strongest in the proliferative zone of epiphyseal plate and changed very little with advancing age. Fairly weak activity of SDH and β-HBD was observed in the epiphyseal cartilage. Some observations concerning the staining of the articular cartilage and bone cells were also made.

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CARTILAGE DEGRADATION BY MACROPHAGES, FIBROBLASTS AND SYNOVIAL CELLS IN CULTURE.
G. Vaes, P. Hauser, G. Huybrechts-Godin and Ch. Peeters-Joris (Brussels, Belgium)

Rabbit bone marrow macrophages and fibroblasts as well as synovial cells from rabbits or humans have been cultivated in contact with ^{35}S-labelled dead cartilage. The degradation of cartilage proteoglycans (CPG) and collagen was monitored by the release of ^{35}S-soluble material and hydroxyproline. Macrophages degraded completely the CPG over 3 days culture. This effect was inhibited by cycloheximide or serum and linked to the synthesis and secretion of a CPG-degrading metal-dependent neutral protease that is inhibited by EDTA, cysteine and serum (but not by Dip-F). This secretion was parallel to that of collagenase but leveled off sooner than that of lysozyme or β-glucuronidase. A limited number of fibroblasts (from carcass, synovium or skin) cultures only resulted in the degradation of the CPG as well as in the accumulation of collagenase in the medium. However, primocultures of synovial cells from arthritic (Dumonde-Glynn) rabbits or rheumatoid patients degraded extensively both the PG and the collagen of the cartilage. These in vitro models are thus suited for the study of the factors controlling tissue degradation in arthritis.

ON THE NATURE OF COLLAGEN AND THE PRESENCE OF OXYTALAN FIBRES IN HUMAN ATHEROSCLEROTIC PLAQUES.
B. Veress, Anna Kádár, and H.Jellinek /Budapest, Hungary/

The typical cells of atherosclerotic plaques /AP/ are the "modified" smooth muscle cells /SMC/ synthetising precursor molecules of connective tissue fibres. In the present study the collagen fibres of human AP were examined to detect if there is any difference between them and those of the media. Transmission electron microscopy /TEM/ and polarizing optical analysis were applied prior to and following sulfation and trypsin digestion which method is specific for collagen /Romhányi et al. 1973/.
Polarization microscopy revealed that the collagen fibres in the AP and the adventitia were more resistant to proteolysis than those of the media. The same was found by TEM. Moreover, TEM showed the presence of fibrous long-spacing collagen as well as oxytalan fibres in AP.
It can be concluded that either the precursors produced by SMC or their extracellular assembly are different from that of the normal media.

INTRACELLULAR LECTIN BINDING SITES IN CULTURED FIBROBLASTS
I.Virtanen, S.Stenman and J.Wartiovaara(Helsinki,Finland)

Intracellular localization of lectin binding sites in cultured human embryonic fibroblasts was studied using FITC-conjugated Concanavalin A (Con A), wheat germ agglutinin (WGA), and Ricin Communis agglutinin II (RCA II) specific for mannosyl and glucosyl, N-acetyl glucosamine, and galactosyl moieties of glycoproteins and -lipids respectively.
To make cells permeable to lectins the cultures were fixed in paraformaldehyde and treated thereafter with 0.05% NP40.
With Con A a large perinuclear area was stained corresponding to the localization of rough endoplasmic reticulum as judged by phase-contrast microscopy and enzyme cytochemistry.
WGA and RCA II gave characteristically a distinct cap-like perinuclear staining which was localized to Golgi region by enzyme cytochemistry.
Our results indicate that in cultured fibroblasts the intracellular distribution of saccharide moieties is strictly segregated to different organelles with the terminal sugars (eg. galactose) being localized only to the Golgi region. This result suggests that terminal maturation of glycoproteins secreted by these cells (eg. collagen and fibronectin protein) occurs contrary to some reports beyond the endoplasmic reticulum at the Golgi apparatus.

RHEUMATOID DISEASE IN CULTURED HUMAN SYNOVIAL FIBROBLASTS.
E. Vuorio (Turku, Finland)

Differences persistent through subsequent generations were detected between cells cultured from normal and rheumatoid synovial tissue. (1) Isolated plasma membranes of rheumatoid cells had decreased activities of marker enzymes. A protein with mw 74000 was present in rheumatoid membranes only. (2) Rheumatoid cells produced increased amounts of underpolymerized hyaluronic acid. Various possibilities are discussed to explain the defect in the macromolecular assembly of hyaluronate as the cause of the lower average molecular weight and greater polydispersity in rheumatoid arthritis. (3) In all cultures the ratio of type I and III collagen synthetized was similar. Seropositive patients differed from seronegative and normal controls: their synovial fibroblasts synthetized *in vitro* an extra protein with collagen-like sequences.

THE ACIDIC GLYCOSAMINOGLYCANS OF HUMAN TERM PLACENTA BLOOD VESSELS
L. Wasserman, A. Ber, J.A. Goldman and D. Allalouf (Petah Tikva, Israel)

1. The composition of acidic glycosaminoglycans in pooled blood vessels, mostly of fetal origin, from a normal human term placenta was investigated by chromatography on a cetyl pyridinium chloride-cellulose column, cellulose acetate electrophoresis, susceptibility to testicular hyaluronidase and thin layer chromatography of the products of digestion with bacterial chondroitinases.
2. The results indicate the presence of hyaluronic acid (36%), chondroitin-6-sulfate (27%), dermatan sulfate (21%), heparan sulfate (8%) and chondroitin-4-sulfate (8%). Several of the fractions obtained from the cetyl pyridinium-cellulose column exhibited anticoagulant activity, which might be of physiological importance for the maintanance of the fluidity of the intensively circulating placental blood.

THE EFFECT OF HYALURONIC ACID AND CHONDROITIN SULPHATE ON THE LOCALISATION OF PROTEOGLYCAN IN GINGIVAL ORGAN CULTURES.
Ole W. Wiebkin and John C. Thonard. (Adelaide, South Australia.)
Some of the proteoglycans synthesised by cultured gingival slices are localised as intercellular ground substance. Macromolecules extracted from either gingival epithelial cultures or from underlying gingival connective tissue were precipitated with cetylpyridinium chloride and proteoglycans were characterised by critical electrolytic elution. Earlier studies using cultures of either gingival or amniotic epithelium indicated that tissue integrity correlates positively with the degree of sulphation of ground substance proteoglycan and that total hyaluronic acid (HA) synthesis is limited in cultures where cell/cell contact is minimal. Radioautographic studies show that HA added to the medium of gingival organ culture (>0.05ug/ml) inhibited intercellular localisation of $^{35}SO_4$ incorporation but in pulse chase experiments did not effect the incorporation of $^{35}SO_4$ into intracellular proteoglycan. The effect of chondroitin sulphate and heparin on the localisation of de novo synthesised proteoglycan in gingival organ cultures was to cause deposition of pockets of intercellular label in the epithelium, particularly when chondroitin sulphate was included with the pulse alone. Since HA does not inhibit proteoglycan synthesis by fibroblasts from skin and synovia, it is postulated that in the epithelium, HA may play a role in controlling secretion of matrix and ground substance proteoglycans in a similar fashion to other avascular tissues e.g. cartilage, thus contributing to the maintenance of tissue integrity.

Immunochemical studies of cartilage proteoglycans.

Jörgen Wieslander & Dick Heinegård (University of Lund, Sweden).

Precipitating antibodies specific for the substructures of bovine nasal cartilage proteoglycans were prepared. Rabbits were immunized with fragments prepared by treatment of proteoglycans with trypsin and chondroitinase as described elsewhere (Heinegård and Axelsson, (1977) J.Biol.Chem., 252, 1971-1980). Rabbits immunized with the link protein fraction, produced antibodies which reacted only with the link protein fraction and not with the proteoglycan monomer. Rabbits immunized with the hyaluronic acid binding region produced antibodies specific for this antigen and not reacting with the link proteins. Immunization with a proteoglycan monomer preparation gave antibodies reacting with the hyaluronic acid binding region, as well as antibodies directed against other regions of the monomer protein core. Antibodies reacting with the polysaccharide containing part of the proteoglycan protein core, could be raised by injection of chondroitin sulfate - and keratan sulfate peptides (the A1-T-A1 fraction).
The antibodies have been used to design methods for sensitive determination of proteoglycan substructures in various samples including cartilage extracts.

DIFFUSION OF HYALURONATE IN CONCENTRATED SOLUTIONS.

K.O. Wik (Uppsala, Sweden)

In a previous article (1) a method for the determination of the intradiffusion (self-diffusion) coefficient of polymers at high concentration was described. Using fluorescin-labelled hyaluronate the same technique has been used to study the diffusion of hyaluronate in concentrations up to 4 mg/ml, which gives a density of polysaccharide chains approximately corresponding to that of the intercellular matrix.

It was found, that while the ordinary translational diffusion coefficient increases with concentration, the intradiffusion coefficient decreases due to increased frictional resistance. At a concentration of 4 mg/ml the ordinary diffusion coefficient is 80 times larger than the intradiffusion coefficient. This factor is a measure of the thermodynamical properties (as expressed by the virial expansion) of the system, and reflects the large non-ideality due to intermolecular interaction in hyaluronate solutions.

(1) T.C. Laurent, L-O. Sundelöf, K.O. Wik and B. Wärmegård
Diffusion of Dextran in Concentrated Solutions.
Eur. J. Biochem. 68(1976), 95-102.