nicotine is not a direct effect on enzyme activity, but is only observed after pretreatment of cells for at least 6 h.

The measurement of protein-bound L-fucose revealed that aortic tissue and cultured arterial wall cells contain a high level of this deoxysugar. There was no significant difference in the sugar content between endothelial cells (23.9 ± 8.9 nmol/mg of protein) and smooth muscle cells (23.9 ± 3.2 nmol/mg of protein). Total aortic tissue exhibits a level of 12.1 ± 2.1 nmol/mg of protein-bound L-fucose.

The results obtained indicate that the arterial wall is a highly active tissue of the body in respect to L-fucose metabolism.

### Serum keratan sulphate levels rise in rheumatoid arthritis patients, but fall in ankylosing spondylitis patients compared with normal controls

**ROB WILL, JUDITH ELSWOOD, LAUREL EDMUNDS and ANDREI CALIN**

Royal National Hospital for Rheumatic Diseases, Bath BA1 1RL, U.K.

**Summary**

Serum levels of keratan sulphate (KS) were found to be significantly elevated in patients with destructive and predominantly seronegative rheumatoid arthritis (RA) compared with a control population. Levels in RA did not correlate with clinical or laboratory indices of joint activity or damage. Conversely levels were depressed in ankylosing spondylitis (AS) compared with controls.

**Introduction**

KS is a glycosaminoglycan which quantitatively is almost entirely confined to hyaline cartilage. Serum levels rise in cartilage breakdown and may be a useful marker of cartilage turnover in rheumatic conditions [1].

**Methods**

1. **Patient selection.** Out-patients with RA and AS who were being assessed in clinical and radiological studies had serum stored at -70°C for measurement of the KS level. Serum was also collected from adult controls who denied a history of joint disease.

2. **Clinical assessment.** In the RA study, data were collected on the level of joint pain and stiffness, the number of swollen and tender joints (Ritchie index), the hand functional index (HFI), a component of the Keitel function test (KFT) [2], the range of joint movement and the Stanford health assessment questionnaire (HAQ). A score for the degree of joint damage (DS) was derived depending upon the loss of joint movement (score 0–20). In the AS group, data were obtained for the degree of spinal pain, stiffness and loss of movement and the level of peripheral joint inflammation (swelling and increased warmth) and damage (loss of joint movement).

3. **Laboratory assessment.** The C-reactive protein (CRP) level was measured in all patients and the rheumatoid factor titre in PA patients.

4. **Serum KS level.** This was measured using an inhibition enzyme-linked immunosorbsent assay (E.L.I.S.A.) technique [3]. Proteoglycan was prepared from the hyaline cartilage of the knee joint of a 73-year-old male cadaver and the 5D4 monoclonal antibody was obtained from ICN Biomedicals Ltd.

**Results**

1. **Rheumatoid arthritis.** The patients are predominantly seronegative (35/42); 34 female, 8 male; mean age 59.7 ± 11.9 years, mean disease duration 15.3 ± 7 years. Serial determinations of KS gave reproducible results and freezing/thawing of the sample four to five times did not influence the KS level. The mean KS level in the RA patients was significantly increased compared with the controls (P < 0.001) (Fig. 1).

2. **Ankylosing spondylitis.** The AS patients (24 male, 6 female) had a mean age of 43.8 ± 10.3 years and a mean disease duration of 23.8 ± 9.8 years. The serum KS level was significantly depressed in the AS patients compared with the controls (P < 0.001) (Fig. 1).

3. **Correlation of clinical parameters with serum KS levels.** The KS level in RA patients did not correlate with the Ritchie index, Keitel, HAQ, peripheral joint damage or CRP. There was a trend for RA patients with more severe joint damage to have higher KS levels. Similarly in AS, clinical

**Fig. 1. Serum KS levels (ng/ml) in RA, AS and controls**

RA (n = 42) versus controls (n = 19): 361 ± 177 versus 227 ± 72 (P < 0.001*). AS (n = 30) versus controls: 154 ± 51 versus 227 ± 72 (P < 0.01)*. Statistics by an unpaired t-test.
measures of spinal pain, peripheral joint inflammation, damage or CRP did not correlate with KS levels.

Conclusions
Serum levels of KS were significantly elevated in this cross-sectional study of predominantly seronegative RA compared with young healthy controls, but did not correlate with measures of current joint activity or indices of joint damage. Destructive or erosive disease of the hands or wrists was an entry criteria for the RA study, but in some of these patients destructive joint disease may have developed many years previously and the serum KS level at the time of the clinical assessment is unlikely to correlate with the degree of joint damage. Conversely, other patients with markedly elevated serum KS levels were likely to be actively resorbing cartilage at the time of the study. Serum levels of KS do not only reflect increased cartilage breakdown, but may also reflect increased proteoglycan synthesis.

Clinical instruments for identifying patients who are actively destroying their cartilage are not currently available. The relationship between serum KS levels and measures of progressive joint damage can probably only be adequately assessed in a prospective study where the rate of progressive joint damage can be measured radiographically.

The low serum KS levels in AS patients are surprising and may reflect depressed cartilage turnover in these patients or cleavage of KS peptide fragments in the joint or in the circulation. Recent work has demonstrated that the 5D4 antibody has a greater avidity for larger KS fragments than smaller ones [4]. An unlikely possibility to explain the findings is a primary modification of the KS epitope recognized by 5D4 in AS.

References

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Heparin induces synthesis of highly charged heparan sulphate by vascular smooth muscle cells

ROGER M. MASON and SUSAN P. WILLIAMS
Department of Biochemistry, Charing Cross and Westminster Medical School, Fulham Palace Road, London W6 8RF, U.K.

Heparin has an antiproliferative effect on vascular smooth muscle cells both in vivo [1] and in vitro [2]. Post-confluent smooth muscle cells synthesize a heparan sulphate with antiproliferative properties which may be involved in maintaining them in a quiescent state [3]. We have investigated the effect of heparin and other polysulfated polysaccharides on the [%s]proteoglycans synthesized by bovine aortic smooth muscle cell cultures during proliferation and when confluent.

Experiments were carried out on passage 2 cells plated at 8000/cm2 on to gelatin-coated dishes in Dulbecco's modified Eagle's medium (DMEM) + 10% (v/v) fetal calf serum (FCS). After 7 h when the cells had attached, the medium was changed to DMEM + 0.4% (v/v) FCS for 72 h, to growth arrest and synchronize the cells in G0 of the cycle. Heparin (Pabyn, Paine & Byrnes Ltd, Greenford, U.K.) was added at a concentration of 100 μg/ml to test cultures and was present throughout the rest of the experiment. Cultures were released from growth arrest by changing the medium back to DMEM + 10% (v/v) FCS with or without heparin and were labelled with [%s]sulphate (50 μCi/ml) for 12-48 h at various times during proliferation and after reaching confluence.

The cell doubling time is about 18 h and control cultures reached confluence at day 5 after release from growth arrest. Under the conditions described, there was a 24 h delay in proliferation in heparin-treated cultures, compared with controls, after which they underwent cell division at a similar rate. Proliferating cultures synthesized several proteoglycans of which about 75% were secreted to the medium. These included a large chondroitin sulphate proteoglycan (CSPG) (47%), a smaller dermatan sulphate proteoglycan (DSPG) (21%) and a small heparan sulphate proteoglycan (HSPG) (6%). A second and different DSPG (14%) was retained in the cell layer, together with a small HSPG proteoglycan (7%), which may be a precursor of the medium variety [4]. Confluent cultures synthesized a similar array of proteoglycans, but the medium CSPG and DSPG were of smaller hydrodynamic size.

Heparin treatment had two effects on proteoglycan synthesis. In control cultures, [%s]proteoglycan synthesis increased 2-fold in the 12 h after release after growth arrest, then reduced by 50% in the next 12 h, during which cell division occurred. The burst in proteoglycan synthesis in heparin-treated cultures was delayed 12 h, but followed the same pattern with a reduction during the subsequent 12 h accompanied by cell division. This effect was therefore clearly related to proliferation, whereas the second effect on heparin occurred whether the cultures were proliferating or confluent. This latter effect involved an increase in the charge of the newly synthesized HSPG so that it eluted from a Mono Q column with 0.83 mM NaCl compared with 0.73 mM NaCl for HSPG from control cultures. Other highly sulphated polysaccharides (100 μg/ml), e.g. pig mucosal heparan sulphate, semi-synthetic heparin analogue (Lutripold-Werk, Munich, F.R.G.) and pentosan polysulphate (Benechemie, Munich, F.R.G.), also induced synthesis of a more highly charged HSPG.

A recent report shows that heparan sulphate synthesized by porcine aortic endothelial cells treated with heparin contained a higher proportion of disulphated disaccharide repeat units than that from control cultures [5], so a variety of cell types may respond in this way.

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