

## Somatomedin activity in synovial fluid

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**SUMMARY** Abnormalities of synovial fluid, as a lubricant and nutrient, may have relevance to the causation of certain articular diseases. The somatomedin activity in normal synovial fluid obtained from the knee joint of the ox has been studied and compared with the activity in serum from the same animal. The porcine costal cartilage bioassay of Van den Brande and Du Caju (1974) has been used with the isotopes  $^{35}\text{S}$ -sulphate and  $^3\text{H}$ -thymidine. The mean potency ratio of ox synovial fluid in terms of ox serum for  $^{35}\text{S}$ -sulphate incorporation was 0.28 (range 0.19-0.47) and for  $^3\text{H}$ -thymidine incorporation 0.35 (range 0.21-0.63). A significant correlation was found between the somatomedin activity (as measured by  $^{35}\text{S}$ -sulphate incorporation) and the total protein and albumin concentrations in the ox synovial fluids and the ox sera, but there was no significant relationship between the somatomedin potency ratios and the globulin concentrations. The possible relevance of these findings to injury and disease in synovial joints is discussed.

Synovial fluid probably provides the only source of nutrients for articular cartilage in those mature animals which have a calcified basal layer of cartilage and bone (Hodge and McKibbin, 1969). In the immature animal diffusion of nutrients from the underlying bone epiphysis is likely, however, and so the articular cartilage may be nourished partly from the synovial fluid and partly from the epiphysis (Ekholm, 1951, 1955; Brodin, 1955; Brower *et al.*, 1962; Mankin, 1963; McKibbin and Holdsworth, 1966).

It has become evident recently that somatomedins in the blood are important in stimulating cartilage metabolism (Luft and Hall, 1975). While it has been shown that chondrocytes dissociated from rabbit articular cartilage respond to somatomedin produced by perfusion of growth hormone through liver (Ash and Francis, 1975), there is no information relating to somatomedins in synovial fluid, either normal or pathological. Since serum somatomedins are bound to a protein of molecular weight  $\geq 50\,000$  (Liberti, 1970; Van Wyk *et al.*, 1971, 1974; Hintz *et al.*, 1974; Yalow *et al.*, 1975), the diffusion of somatomedins across synovial membranes may be restricted.

We examined the somatomedin activity in normal synovial fluid obtained from the knee joint of the ox and compared it with the activity in (a) serum obtained from the same animal; (b) a human standard reference serum; and (c) solution containing amino acids at the concentrations in normal human plasma. The porcine costal cartilage bioassay of Van den Brande and Du Caju (1974) was used with the isotopes  $^{35}\text{S}$ -sulphate (proteoglycan synthesis) and  $^3\text{H}$ -thymidine (DNA synthesis).

Van den Brande *et al.* (1974) examining plasma, reported species variation in somatomedin with regard to both the slope and the magnitude of the response. Since heparinized plasma was used for their studies and in our work we used serum, blood plasma and serum from the same ox have been compared.

The results show that firstly normal ox synovial fluid has less capacity to stimulate the synthesis of proteoglycans and DNA in porcine costal cartilage than the animal's own serum; and secondly the somatomedin activity in these fluids is related to their total protein and albumin content. The possible relevance of these findings to injury and disease is discussed.

### Materials and methods

**SAMPLE COLLECTION AND PREPARATION**  
Blood and synovial fluid samples were obtained from

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each of 6 oxen freshly killed with a 'captive bolt'. Blood for the serum and plasma comparison was obtained from 5 additional oxen.

Blood for serum was withdrawn from the opened ox heart into a sterile syringe within an hour of death and kept at ambient temperature until returned to the laboratory, where it was incubated at 37°C for 1 hour and left in the refrigerator overnight. Serum was withdrawn from the clotted blood and centrifuged at 500 *g* for 10 minutes. Synovial fluid samples were obtained from the opened knee joint and only fluids uncontaminated with blood were retained. In all but one experiment samples from the right and left knees were pooled in order to obtain a sufficient volume for analysis. The fluids were kept at ambient temperature during transport to the laboratory, where they were incubated at 37°C for 1 hour and then left in the refrigerator overnight. Centrifugation at 700 *g* for 30 minutes provided a clear supernatant for assay. Serum and synovial fluid samples were stored at -20°C until analysed. Human standard reference serum (SRS) consisted of pooled sera obtained from 21 normal adults. It had been dispensed in aliquots and stored at -20°C. SRS was included in every assay. Amino acid solutions were prepared containing amino acids at the concentrations in normal human plasma (Stein and Moore, 1954). The diluent was Krebs's phosphosaline buffer (Krebs and Eggleston, 1940) supplemented with 1 mg/ml glucose and 0.85 mg/ml sodium bicarbonate. The concentration of inorganic sulphate in the buffer was reduced to that in normal human plasma (Miller *et al.*, 1961) for these solutions.

Blood for plasma was collected into a solution of heparin (25 IU/ml, Weddel Pharmaceuticals Ltd.). Blood was obtained from the opened throat of the oxen within a few minutes of death, and stored on ice for up to 2 hours until the plasma could be separated from the blood cells by centrifugation at 500 *g* for 10 minutes. Plasma samples were stored at -20°C until analysed.

#### ANALYSES

##### *Somatomedin bioassay*

Somatomedin activity was assayed by the porcine costal cartilage technique of Van den Brande and Du Caju (1974). Cartilage discs were preincubated for 24 hours at 37°C in Krebs's phosphosaline enriched with amino acids (at the concentration in normal plasma), glucose (2 mg/ml), Hepes buffer (N-2-hydroxyethylpiperazine-N<sup>1</sup>-2-ethanesulphonic acid (Good *et al.*, 1966) 20 mmol/l adjusted to pH 7.5 with 1N sodium hydroxide), and antibiotics penicillin 100 IU/ml, streptomycin 100 µg/ml, and gentamicin 20 IU/ml. The discs were then transferred to a range of dilutions of the test fluids (40% to 5%). The

diluent was Krebs's phosphosaline buffer with added glucose 1 mg/ml and Hepes buffer (to give a final concentration of 20 mmol/l). Gentamicin 20 IU/ml was also added. The discs were incubated for 24 hours at 37°C; the isotopes <sup>35</sup>S-sulphate (carrier free, Amersham) and (6-<sup>3</sup>H) thymidine (20 000–30 000 mCi/mmol) were then added to give a concentration of 2 µCi/ml and the incubation continued for a further 24 hours.

The cartilage discs were transferred to a boiling water bath for 10 minutes and then washed in six changes of cold water for one hour in order to remove any unincorporated isotope. The discs were then transferred to counting vials and digested with 0.5 ml 23N formic acid at 80°C for 45 minutes, scintillation fluid (10 ml Xylene: Triton, 5:1, containing 0.5% 2, 5-diphenyloxazole) was added, and the isotopic uptake determined using a liquid scintillation counter incorporating an automatic external standard. The results were corrected for quenching and converted to disintegrations per minute (dpm).

##### *Sulphate estimations*

The concentrations of inorganic sulphate in the sera, plasma, and synovial fluids were measured by a benzidine sulphate spectrophotometric method (Antanopoulos, 1962). Serum and plasma samples were deproteinized with an equal volume of 10% trichloroacetic acid (TCA). Synovial fluid samples were treated firstly with an equal volume of 1% acetic acid, to precipitate the hyaluronic acid, and then with 10% TCA to precipitate the protein. Standard solutions of 0–50 µg sulphate/ml provided a standard curve from which the sample sulphate concentrations were obtained.

##### *Total protein estimations*

These were performed by the biuret method using reconstituted lyophilized human serum standard analyzed by the Kjeldahl method.

##### *Albumin estimations*

Ox serum and ox synovial fluid samples were analysed by a radial immunodiffusion technique (Mancini *et al.*, 1965) using antiovine albumin serum (Behringwerke). Standard solutions of 9–42 mg/ml bovine serum albumin, as estimated by the biuret method, provided the standard curve from which the sample albumin concentrations were calculated. The standard reference serum was analysed for its albumin content in an AutoAnalyser by a bromocresol green dye binding method (Northam and Widdowson, 1967). This method could not be used for the synovial fluid samples because of their viscosity.

### Statistical analysis

The results were corrected for the slight variations in specific activity of the  $^{35}\text{S}$ -sulphate. Each assay was then analysed by the method for parallel-line assays (Finney, 1964), relating the logarithm of the response to the logarithm of the concentration. The log-concentration-log-response lines were examined graphically and responses to the lowest concentrations were rejected before analysis if they did not differ significantly from the responses to the adjacent concentration. Assays were rejected as statistically invalid if the slopes of the lines of the standard and the test were significantly different at the 5% level, or if the regression of the log-concentration-log-response lines was not significant at the 5% level. Potency ratios, 95% fiducial limits, and an index of precision ( $\lambda$ ) were estimated for each of the valid assays.

### Results

#### Somatomedin bioassay

The results showed that ox synovial fluid had less capacity to stimulate the synthesis of proteoglycans and DNA in porcine costal cartilage than serum from the same animal (Table 1, Figs. 1 and 2). The mean potency ratio of ox synovial fluid in terms of ox serum (ox synovial fluid/ox serum) for  $^{35}\text{S}$ -sulphate incorporation was 0.28 (range 0.19–0.47) and for  $^3\text{H}$ -thymidine uptake 0.35 (range 0.21–0.63). Using the t-test these ratios were significantly less than 1.0 ( $^{35}\text{S}$ -sulphate  $P < 0.001$ ;  $^3\text{H}$ -thymidine  $0.001 < P < 0.01$ ).

The amino acid solutions showed little or no increased isotopic uptake with increasing concentrations for either  $^{35}\text{S}$ -sulphate or  $^3\text{H}$ -thymidine. Only three of the assays for the amino acid solutions and SRS were valid for  $^{35}\text{S}$ -sulphate incorporation, with potency ratios of 0.001–0.005; in contrast, the assays for the ox synovial fluids and SRS were all valid, with potency ratios of 0.02–0.35. As Table 1 shows, none of the assays were valid when  $^3\text{H}$ -thymidine uptake for the amino acid solutions and SRS were compared, while five out of six were

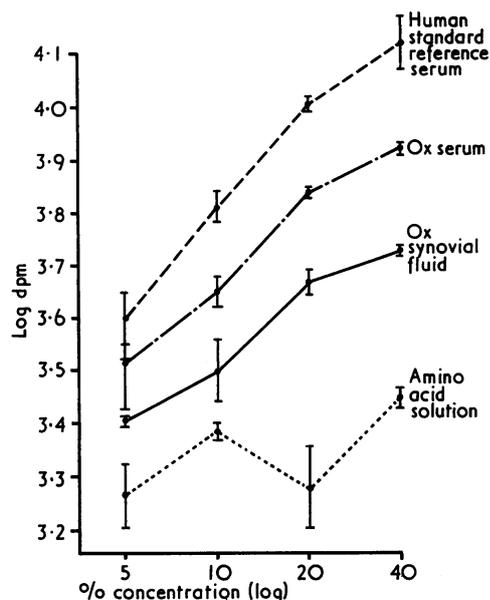


Fig. 1  $^{35}\text{S}$ -sulphate uptake into porcine costal cartilage as a function of the concentration of the different fluids examined. dpm = disintegrations per minute

valid when the ox synovial fluids and SRS were compared. In all the invalid assays the slopes of the amino acid solutions were significantly less than those of the SRS. These results indicate that the synovial fluids had a greater capacity to stimulate the metabolism of porcine costal cartilage than had the amino acid solutions.

The potency ratios of ox serum/SRS were  $< 1$  for both  $^{35}\text{S}$ -sulphate and  $^3\text{H}$ -thymidine incorporation (Table 1). These results were confirmed for  $^{35}\text{S}$ -sulphate incorporation when serum and plasma each obtained from five oxen, were separately compared with human serum and plasma, each pooled from 2 healthy adults. The mean potency ratio for ox serum/human serum was 0.33 (range 0.20–0.42), while that for ox plasma/human plasma was

Table 1 Somatomedin potency ratios for ox synovial fluids, ox sera, and amino acid solutions

Isotope	Ox synovial fluid ox serum		Ox synovial fluid SRS†		Ox serum SRS		Amino acid solution SRS	
	No. of assays (no. valid)	Mean potency ratio and range	No. of assays (no. valid)	Mean potency ratio and range	No. of assays (no. valid)	Mean potency ratio and range	No. of assays (no. valid)	Mean potency ratio and range
$^{35}\text{S}$ -sulphate	6(6)	0.28 (0.19–0.47)	6(6)	0.11 (0.02–0.35)	6(6)	0.36 (0.10–0.70)	6(3)	0.003 (0.001–0.005)
$^3\text{H}$ -thymidine	6(5)	0.35 (0.21–0.63)	6(5)	0.05 (0.02–0.17)	6(4)	0.12 (0.03–0.36)	6(0)	—

\*SRS = Human standard reference serum.

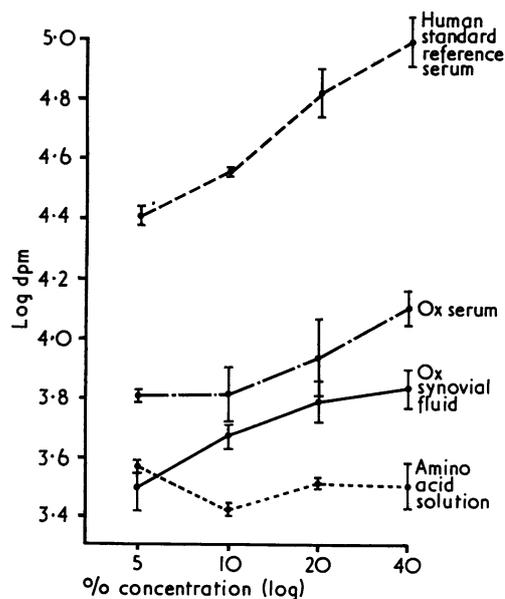


Fig. 2. <sup>3</sup>H-thymidine uptake into porcine costal cartilage as a function of the concentration of the different fluids examined.

0.37 (range 0.23–0.50). These findings are in contrast to those of Van den Brande *et al.* (1974), who found that plasma pooled from 5 cows tested against plasma pooled from 5 humans produced a potency ratio of 1.5 for <sup>35</sup>S-sulphate incorporation.

#### Inorganic sulphate estimations

The concentration of inorganic sulphate was slightly higher in the ox synovial fluids than in the ox sera (Table 2). This is consistent firstly with the Gibbs–Donnan theory of membrane equilibrium, and secondly with sulphate concentrations previously reported (Ropes *et al.*, 1939). The concentration of sulphate in the human SRS was approximately one-quarter that in the ox sera. Thus there is a species difference in the serum concentration of inorganic sulphate between man and ox. Moreover,

this difference shows the need to correct for the specific activity of radioactive sulphate, especially when these fluids from man and ox are being compared.

#### Protein estimations

The total protein concentration in the SRS and the ox sera were similar, but the concentration in the synovial fluids was significantly reduced (Table 2). Although the total protein concentration in the ox sera and the SRS was similar, the ratio of albumin to globulin in each was reversed; for in the SRS there was approximately twice as much albumin as globulin, whereas in the ox sera there was approximately twice as much globulin as albumin. The albumin concentrations in the synovial fluids were approximately 50% of the albumin concentrations in the ox sera, while the globulin concentrations were only about 10% of the globulin concentrations in the ox sera.

#### Relationship between potency ratio and protein content

Correlation coefficients were calculated for the somatomedin potency ratios of the ox fluids/SRS for <sup>35</sup>S-sulphate incorporation compared with the protein concentrations (total protein, albumin, and globulin) in the ox sera and the synovial fluids. There was a significant correlation between the somatomedin potency ratios and both the total protein ( $r=0.63$ ;  $0.01 < P < 0.05$ ) and the albumin concentrations ( $r=0.71$ ;  $0.01 < P < 0.05$ ), but there was no significant relationship between the somatomedin potency ratios and the globulin concentrations ( $r=0.59$ ;  $0.05 < P < 0.10$ ).

#### Discussion

There are several possible explanations for the reduced capacity of the synovial fluids, compared with the sera, to stimulate the synthesis of proteoglycans and DNA in porcine costal cartilage. Firstly, if somatomedins pass freely from the capillaries of the synovial membrane into the synovial fluid then

Table 2. Inorganic sulphate and protein content of ox synovial fluids, ox sera, and human standard reference serum (SRS)

Fluid	Inorganic sulphate ( $\mu\text{g/ml}$ )		Total protein (mg/ml)		Albumin (mg/ml)		Globulin (mg/ml)	
	No. of samples	Mean and range	No. of samples	Mean and range	No. of samples	Mean and range	No. of samples	Mean and range
Ox serum	6	108.0 (83.0–138–.5)	5	69 (64–73)	5	25 (21–32)	5	44 (40–52)
Ox synovial fluid	7	124.5 (109.5–136.0)	6	17 (12–26)	6	14 (11–18)	6	4 (0–8)
SRS	1	25.2	1	76	1	53	1	23

the lower somatomedin activity in the synovial fluid could result from (a) the presence of inhibitors to proteoglycan and DNA synthesis (Salmon, 1972), possibly added by the secretory cells of the synovial membrane; (b) the denaturation of somatomedins by enzymes in the synovial fluid; or (c) the removal of somatomedins by the extensive surface area of the articular cartilage (cf. Daughaday *et al.*, 1968). Secondly, because somatomedins in the blood are bound to a protein of molecular weight  $\geq 50\,000$ , passage across the synovial membrane is probably limited. It is, of course, possible that inhibitors to somatomedins pass more readily into synovial fluid than do somatomedins. Thirdly, as for liver (McConaghey and Sledge, 1970; McConaghey, 1972), kidney (McConaghey and Dehnel, 1972), and possibly muscle (Hall and Božović, 1969; Hall *et al.*, 1970), somatomedins may be produced locally in the synovial membrane in response to, or from, growth hormone.

Recent work examining somatomedins in lymph (from the cisterna chyli) and serum from normal rats has shown that substances inhibitory to  $^3\text{H}$ -thymidine incorporation by chick embryo fibroblasts are present at concentrations above 1% of both the lymph and the serum (Cohen and Nissley, 1975). However, after acidifying and heating the lymph was found to contain approximately 50% of the blood serum somatomedin activity; and activities in both the lymph and the serum were shown to be present in the same fraction after separation on a G50 Sephadex column.

Beaton *et al.* (1975) assayed the somatomedin activity in cerebrospinal fluids (CSF) and sera from 12 adult humans (who were considered to be normal). They found that the CSF from each patient stimulated less uptake of  $^{35}\text{S}$ -sulphate into porcine costal cartilage than the serum. The mean potency ratio for the CSF/serum of the 12 subjects was 0.39 (range 0.21–0.59). Using the same isotope we found the mean potency ratio of ox synovial fluid/ox serum to be 0.28 (range 0.19–0.47), findings which are comparable with those for CSF. Human CSF, obtained at lumbar puncture, contains approximately 0.3 mg/ml total protein (Goldstein *et al.*, 1960), whereas synovial fluids from the knee joint of both ox and man contain approximately 17 mg/ml protein (Table 2; Dittmer, 1961). This suggests that the somatomedin activity in these body fluids is not related to their protein concentration. Our results, however, do show a correlation between somatomedin activity and protein concentration; this aspect was not examined for CSF by Beaton *et al.*

The presence and concentration of serum components in synovial fluid depends on (a) the molecular size of the component and its capacity to pass

through endothelial walls of capillaries, and (b) active transport mechanisms in endothelial cells. Serum proteins are clearly restricted in their passage across the synovial membrane, with albumin entering the synovial cavity more readily than globulins (Table 2). Serum somatomedins are protein bound and we have shown a correlation between the somatomedin activity and the total protein and the albumin concentrations. It is therefore likely that the reduced level of somatomedin activity in ox synovial fluid compared with ox serum is at least partly due to its restricted passage across the synovial membrane, although the possible influence of inhibitors has not yet been excluded.

Synovitis resulting from various disorders and diseases may be associated with increased permeability and lead to a higher level of protein in synovial fluid (Yehia and Duncan, 1975). It is possible then that in such disorders the somatomedins may similarly increase. Somatomedin activity in synovial fluid may also have relevance to the degradation of articular cartilage in osteoarthritis and possibly chondromalacia. A study of somatomedin activity in fluids from diseased synovial joints is therefore needed. This is currently being undertaken together with an evaluation of the effects of human joint fluid upon the metabolism of articular cartilage (Coates *et al.*, 1977).

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