

# Synovial fluid copper and related variables in rheumatoid and degenerative arthritis

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**SUMMARY** Copper, caeruloplasmin, transferrin, albumin, and total protein were measured in the serum and synovial fluid of 40 patients with rheumatoid arthritis and 40 patients with osteoarthritis. A raised synovial fluid copper and caeruloplasmin have been found to be characteristic of rheumatoid effusions. The relation between copper and caeruloplasmin in synovial fluid differs from that in serum. Synovial fluid caeruloplasmin was increased disproportionately in relation to other plasma proteins present in rheumatoid effusions.

In the light of accumulating evidence pointing to an abnormal copper and caeruloplasmin metabolism in rheumatoid arthritis (RA) (Scudder *et al.*, 1978) we decided to study copper levels and related variables in the synovial fluid of RA patients and a control group of patients with degenerative arthritis. Synovial fluid findings were also related to serum levels and the serum/synovial fluid partition of different proteins was calculated.

## Material

Synovial fluid and blood were collected from 40 patients with RA (33 females, 7 males) and from a control group of 40 patients with osteoarthritis (24 females, 16 males). The RA patients were being treated with conventional analgesic and anti-inflammatory drugs (salicylates, ibuprofen, and indomethacin). None was receiving D-penicillamine or sodium aurothiomalate.

## Methods

### SERUM AND SYNOVIAL FLUID COPPER

Copper was measured with a Perkin Elmer model 306 atomic absorption spectrophotometer. Protein-free extracts were prepared by dilution of the serum or synovial fluid with 1 volume of 200 g/l trichloroacetic acid followed by mixing and centrifugation. The supernatants were aspirated directly into an air/acetylene flame.

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### TOTAL PROTEIN AND ALBUMIN

Total protein and albumin were measured by standard autoanalyser methods using the biuret and bromocresol green reactions respectively.

### TRANSFERRIN AND CAERULOPLASMIN

Transferrin and caeruloplasmin were estimated by single radial immunodiffusion (Mancini *et al.*, 1965). Commercially prepared M-Partigen immunodiffusion plates and lyophilised protein standards were obtained from Hoechst Pharmaceuticals, Hounslow, UK. Non-caeruloplasmin-bound copper was calculated as the difference between total and caeruloplasmin-bound copper. Caeruloplasmin-bound copper was calculated assuming that the copper content w/w of caeruloplasmin is 0.32% (Kasper and Deutsch, 1963).

## Results

Copper and caeruloplasmin were significantly increased ( $P < 0.01$ ) in the RA group compared with the osteoarthrotic group. There was a strong positive correlation between synovial fluid copper.

Table 1 Synovial fluid copper and caeruloplasmin (mean  $\pm$  SD) in patients with osteoarthritis (control group) and with rheumatoid arthritis

	n	Copper ( $\mu\text{mol/l}$ )	Caeruloplasmin (mg/l)
Control group	40	7.7 $\pm$ 3.0	141 $\pm$ 62
Rheumatoid group	40	13.8 $\pm$ 4.8	310 $\pm$ 16

Conversion: SI to traditional units—  
Copper: 1  $\mu\text{mol/l} \approx 6.37 \mu\text{g}/100 \text{ ml}$ .

Table 2 Partition of total protein, albumin, transferrin, and caeruloplasmin (mean  $\pm$  SD) between serum (S) and synovial fluid (SF) in patients with osteoarthritis and rheumatoid arthritis

	Total protein (g/l)	SF/S	Albumin (g/l)	SF/S	Transferrin (g/l)	SF/S	Caeruloplasmin (mg/l)	SF/S
<i>Osteoarthritis (n = 40)</i>								
Serum	70.0 $\pm$ 1.8	0.46	39.5 $\pm$ 5.2	0.64	2.51 $\pm$ 0.57	0.63	334 $\pm$ 76	0.42
Synovial fluid	32.0 $\pm$ 7.0		25.4 $\pm$ 6.9		1.58 $\pm$ 0.55		141 $\pm$ 62	
<i>Rheumatoid arthritis (n = 40)</i>								
Serum	69.0 $\pm$ 4.8	0.55	43.4 $\pm$ 3.7	0.61	2.29 $\pm$ 0.67	0.72	451 $\pm$ 87	0.69
Synovial fluid	38.0 $\pm$ 9.0		26.6 $\pm$ 4.8		1.66 $\pm$ 0.65		310 $\pm$ 165	

and caeruloplasmin in both the RA group ( $r = 0.596$ ;  $P < 0.001$ ) and the osteoarthrotic group ( $r = 0.362$ ;  $P < 0.01$ ). The results are summarised in Table 1.

The mean non-caeruloplasmin-bound copper in the osteoarthrotic group was calculated as  $3.53 \pm 0.1 \mu\text{mol/l}$  ( $22.48 \pm 0.6 \mu\text{g}/100 \text{ ml}$ ). In the rheumatoid group there was a calculated copper 'deficit' in terms of the caeruloplasmin detected immunologically. In other words, in rheumatoid synovial fluids copper was insufficient to account for all the caeruloplasmin measured.

**TOTAL PROTEIN ALBUMIN AND TRANSFERRIN**  
The mean total protein content of rheumatoid synovial fluid was significantly higher ( $P < 0.02$ ) than that of osteoarthrotic fluid. Mean levels of synovial fluid albumin and transferrin were not significantly different. The partition ratio for caeruloplasmin in the osteoarthrotic group was 0.42 while in the RA group it was 0.69. These results are summarised in Table 2.

## Discussion

The permeability of synovial membrane to plasma proteins is poorly understood. It is a highly selective process which probably depends on molecular size, molecular weight, and metabolic changes in the joint as well as on the plasma concentration of different proteins. Damage to or destruction of the membrane will result in loss of its ability to selectively limit or exclude certain plasma proteins. The changes induced by acute or subacute inflammation may be superimposed on this pattern. Our results show that a raised synovial fluid caeruloplasmin is characteristic of rheumatoid, as distinct from osteoarthrotic, effusions. This difference, moreover, is considerably greater than the difference between total protein concentrations; and caeruloplasmin concentration in relation to total protein concentration is higher in rheumatoid synovial fluid

than in rheumatoid serum. Whether similar changes occur in other inflammatory effusions remains to be established. The rise in synovial fluid caeruloplasmin (as in serum caeruloplasmin) may be related to the protein's powerful antioxidant action (Al-Timimi and Dormandy, 1977). This may be part of the acute inflammatory response of the body and may have a protective significance.

The relation between copper and caeruloplasmin in synovial fluid differs from that in serum and it differs in osteoarthrotic and rheumatoid effusions. In osteoarthrotic fluid the copper/caeruloplasmin ratio is marginally reduced compared to serum; but the relation still suggests that virtually all the copper is caeruloplasmin-bound. In rheumatoid fluid there is a copper 'deficit', i.e. insufficient copper to account for all the caeruloplasmin as measured by immunodiffusion. It is possible that some of the synovial fluid caeruloplasmin is chemically but not immunologically partially degraded and holds less than the 8 g atoms copper per molecule characteristic of the serum protein fraction.

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