0.2m-NaCl (Antonopoulos et al., 1961), accounted for 1% of the total uronic acid. It contained negligible sulphate but equimolar amounts of hexosamine and uronic acid, and its glucosamine/galactosamine molar ratio was 15.8:1. This fraction, thus identified as hyaluronic acid, interacted with proteoglycan in the gel-chromatographic system. Moreover hyaluronic acid from sources other than cartilage likewise interacted with cartilage proteoglycans. The nature of the interaction is being examined by various methods including viscosity (Hardingham & Muir, 1972), optical rotatory dispersion, circular dichroism and n.m.r. relaxation (see Dea et al., 1973).

We are grateful to T. Wall and Son Ltd., London N.W.10, U.K., for supplying fresh tissue for this study. We thank Miss P. Hobin for technical assistance and the Arthritis and Rheumatism Council for general support.


The Effects of Infusions of Normal Plasma in Three Patients with Sanfilippo’s Syndrome

MICHAEL F. DEAN, PHILLIP F. BENSON and HELEN MUIR
Kennedy Institute of Rheumatology, Bute Gardens, London W6 7DW, U.K., and Paediatric Research Unit, Guy’s Hospital Medical School, London SE1 9RT, U.K.

The mucopolysaccharidoses are a group of inherited metabolic disorders characterized by systemic accumulation and greatly increased urinary output of one or more glycosaminoglycans. Six major types of these disorders have hitherto been recognized and classified by McKusick (1966) on the basis of their clinical features, genetic inheritance and pattern of glycosaminoglycan excretion. The first attempts at treatment by the infusion of normal human plasma were reported by Di Ferrante et al. (1971). They reported changes in urinary glycosaminoglycans, namely a decrease in the excretion of polymeric glycosaminoglycans and an increased excretion of their degradation products, in one case of Hurler’s syndrome (mucopolysaccharidosis type I) and two cases of Hunter’s syndrome (mucopolysaccharidosis type II). The authors also noted a definite clinical improvement after the infusions.

We have applied this treatment to three cases of Sanfilippo’s syndrome (mucopolysaccharidosis type III), two of whom were siblings (D. M. and L. M.). Plasma infusions equivalent to 3.5 litres of blood were given over a period of 3 successive days and 24h urine collections were taken on several days preceding treatment, during infusion and on a number of days afterwards. The urine was collected in sterile bottles that contained a little toluene (approx. 10ml) as a preservative and then frozen at −20°C until required.

After thawing and measurement of total volume, samples were dialysed in dialysis tubing that had been heated to decrease its porosity (Callanan et al., 1957) for 48h at 4°C against distilled water. The glycosaminoglycans were isolated by precipitation with 9-aminoacridine hydrochloride as described by Dean et al. (1971). Material containing uronic acid left in the supernatant solution was isolated on a column of Dowex-1 X2 (Cl- form) (Di Ferrante et al., 1971), after concentration of the solution and removal of residual 9-aminoacridine hydrochloride (Muir & Jacobs, 1967). Uronic acid contents were determined by the method of Bitter & Muir (1962) or by an automated modification
when column effluents were analysed. Glucosamine/galactosamine molar ratios were
determined as described by Tsiganos & Muir (1969), and sulphate contents were
measured by the procedure of Dodgson (1961). Gel chromatography was carried out on
columns (9mm x 500mm) packed with Sephadex G-100 or G-25 suspended in 0.2M-
sodium acetate buffer, pH6.8, which was also used for elution.

Immediately after plasma infusion the output of uronic acid in the urine increased by
50% in two patients (K. F. and D. M.) and by tenfold in a third patient (L. M.). The out-
puts then fell to a point at or below pretreatment values, followed by a rise and fall in a
cyclical pattern. Most of this increase was attributable to polymeric glycosaminoglycans,
which were precipitated by 9-aminoacridine hydrochloride. In all three cases the ratio
of precipitable uronic acid to that isolated from the supernatant solutions rose sharply
after infusion and thereafter fell and rose again in a cyclical manner (Fig. 1). There was,
however, little change in the glucosamine/galactosamine molar ratios of the material in the urine (precipitates plus supernatants) throughout the investigation, apart from slight daily fluctuations. The predominance of heparan sulphate in the urine was thus essentially unchanged by the treatment, with the glucosamine content of the precipitable material being somewhat higher than that isolated from the supernatant solutions.

Most of the precipitable glycosaminoglycans were retarded on Sephadex G-100. The range of molecular weights remained unchanged after infusion, although the elution profiles became less irregular in shape, resembling the profile of glycosaminoglycans isolated from normal urine (M. F. Dean, unpublished work).

In contrast with the precipitable material, that isolated from the supernatant solutions changed considerably in size with treatment, as assessed by gel chromatography on Sephadex G-25. Two types of response were observed. In one patient (K. F.) a second component of lower molecular weight than the first appeared, the proportion of which increased with time during infusion and decreased slowly afterwards. In the other two patients, who were siblings, three components were separated before infusion, whereas after infusion the two smaller ones disappeared, leaving only the largest. The smallest components reappeared gradually after treatment during the course of a few days.

Another difference between the siblings (D. M. and L. M.) and the third patient (K. F.) was that the sulphate content of the glycosaminoglycans changed in opposite ways after treatment. In the siblings the sulphate/uronic acid ratios before treatment were 0.4:1 and 0.6:1 respectively, and these increased to 1.2:1 with infusion, whereas in the third patient (K. F.) the ratio decreased from 1.6:1 to 1.2:1 with treatment (Fig. 2). In all three cases the ratios quickly reverted to pretreatment values within a few days after infusion.

There may be two forms of Sanfilippo's syndrome (Kresse et al., 1971), in one of which there appears to be a deficiency of a heparan sulphate N-sulphatase (Kresse & Neufeld, 1972). The patient K. F. may have this form, since the sulphate/uronic acid ratio of the glycosaminoglycans decreased with treatment, coupled with the appearance of a fragment of lower molecular weight in the supernatant solution. The two siblings, however, appear to represent a different form of the disease, since the sulphate/uronic acid ratio of the glycosaminoglycans increased with treatment, and the largest component isolated from the supernatant solution increased in amount.

![Graph](Image)

Fig. 2. Sulphate/uronic acid molar ratios of precipitable glycosaminoglycans in the urine of three patients with Sanfilippo's syndrome before, during and after infusion of normal human plasma

○, Patient L. M.; ■, patient D. M.; ▲, patient K. F.
We acknowledge the excellent technical assistance of Miss Diane Orton and Mr. S. Brown, and thank the Medical Research Council for a grant to M. F. D., the Arthritis and Rheumatism Council, the Wellcome Trust and the Spastics Society for financial support. The clinical work described, for which the informed consent of the patients was obtained, was carried out with the full approval of the Ethical Committee of Guy's Hospital Medical School.


Canine Articular Cartilage in Natural and Experimentally Induced Osteoarthrosis

C. A. McDEVITT, HELEN MUIR and M. J. POND
Kennedy Institute of Rheumatology, Bute Gardens, London W6 7DW, U.K. and Department of Surgery, University of Glasgow School of Veterinary Medicine, Glasgow G61 1QH, U.K.

Proteoglycans of articular cartilage consist of a heterogeneous population of molecules that differ in chemical composition and hydrodynamic size as assessed by gel chromatography (Brandt & Muir, 1969, 1971a,b; Šimůnek & Muir, 1972a) and gel electrophoresis (McDevitt & Muir, 1971). The influence of natural and experimentally induced osteoarthrosis on the composition of canine articular cartilage and on the extractability of proteoglycans and their heterogeneity was investigated.

The dogs used were: (1) a 13-month-old alsatian with natural osteoarthrosis of the hip and a control dog of the same age; (2) a 2-year-old dog with experimentally induced osteoarthrosis in one knee, the other knee serving as a control. This dog was killed 5 months after induction of osteoarthrosis. In all instances the joints were frozen immediately after death and stored frozen until required.

After thawing, the cartilage of the hip of each dog from (1) and each knee from the dog with induced osteoarthrosis (2) was carefully dissected from the bone at 4°C, cut into small pieces and placed in four separate containers and weighed. A sample weighing 70mg was taken from each for chemical analyses as described by Šimůnek & Muir (1972a). The remainder of the cartilage was extracted with 5ml of 2~CaCl2, pH 6.8, for 48h at 4°C. The extracts from each cartilage specimen were compared on gel electrophoresis in large-pore polyacrylamide–agarose gels (McDevitt & Muir, 1971) that had been shown to separate proteoglycans according to their hydrodynamic size on gel chromatography. The proteoglycan bands in the gels were located by staining with Toluidine Blue (McDevitt & Muir, 1971).

The total weight of cartilage of each pathological joint was higher than in controls (Tables 1 and 2), although the degree of hydration was similar. Compared with the corresponding controls, the uronic acid content of osteoarthritic cartilage was higher in the 13-month-old dog with natural osteoarthrosis (Table 1), but was lower in the 2-year-old dog with induced osteoarthrosis.

In both types of osteoarthrosis the galactosamine/glucosamine molar ratios were higher than controls both in the cartilage itself and in the extracted proteoglycans (Tables 1 and 2). The proteoglycans were more readily extracted from osteoarthritic