The Metabolic Fate of Chondroitin [35S]Sulphate Proteoglycan in the Rat
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Revell & Muir (1972) have studied the metabolism of 35S-labelled proteoglycan from porcine cartilage after injection into guinea pigs. They concluded that the polymeric material excreted in the urine was the result of proteolytic degradation of the injected proteoglycan to single chains of chondroitin sulphate. These results are particularly interesting with respect to the site(s) and mechanism(s) of proteoglycan degradation, since it has been shown that single chains of chondroitin 4-[35S]sulphate are taken up by rat liver and degraded with the elimination of inorganic [35S]sulphate into the blood (Wood et al., 1973). No other 35S-labelled products could be detected. The present investigation was undertaken to determine the nature of labelled polymeric material excreted in the urine of the rat after the administration of 35S-labelled proteoglycan from cartilage of animals of the same strain. The results show that, in this species, it is not necessary to invoke a proteolytic degradation step to account for the polymeric products in the urine.

Rat xiphoid cartilage was labelled with [35S]sulphate as described by Hardingham & Muir (1972) and then extracted with 4 M-guanidinium chloride, yielding a solution from which a preparation corresponding to proteoglycan 'subunit' was isolated by preparative ultracentrifugation in a CsCl gradient as described by Hascall & Sajdera (1969). After removal of CsCl and guanidinium chloride by dialysis, the proteoglycan preparation [76% chondroitin 4-sulphate, estimated as a fraction of total chondroitin sulphate by method III of Saito et al. (1968)] was found to have a specific radioactivity of 2.2 μCi/

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![Fig. 1. Gel chromatography on Sephadex G-200 of urine from rats with cannulated ureters after injection of 35S-labelled proteoglycan](image-url)

The column was eluted with 0.2 M-NaCl. Distribution of radioactivity in 35S-labelled proteoglycan (○) and in 3 h urine sample (●). Void volume of the column (580 mm × 9 mm) is indicated by $V_0$. 

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Fig. 2. Gel chromatography on Sephadex G-200 of 1h urine sample from rats with cannulated ureters after injection of $^{35}$S-labelled proteoglycan

The column was eluted with 0.2 M-NaCl. Distribution of radioactivity in 1h urine sample. Void volume of column (580 mm x 9 mm) is indicated by $V_0$.

µmol of uronic acid. Per mol of hexosamine it contained 0.08 mol of glucosamine, 0.05 mol of amino acid residues and 1.06 mol of ester sulphate.

Adult male rats (250-350 g) were each injected via the jugular vein with $^{35}$S-labelled proteoglycan (0.9 mg/kg body wt.; 0.9 µCi dissolved in 0.5 ml of 0.95 % NaCl). Urine was collected from cannulated ureters into tubes (suspended in ice) at hourly intervals over 6h. Samples were assayed for radioactivity by scintillation counting before and after fractionation by gel chromatography on Sephadex G-200 (Wood et al., 1973).

Within 6h, 49 % of the injected radioactivity was recovered in the urine, approximately half (23 %) in the first hour. In all but the first 1h urine sample, only one, retarded, peak of radioactivity was detected on gel chromatography (Fig. 1). This material co-chromatographed with authentic inorganic $^{35}$S-sulphate on paper electrophoresis as well as on gel chromatography and was precipitated by addition of Ba$^{2+}$ ions.

In the 1h urine sample only a second peak of radioactivity was observed which was eluted between the main peak of injected material (dissolved in normal urine) and that of inorganic $^{35}$S-sulphate (Fig. 2).

However, the proteoglycan preparation described above contained significant amounts of material of lower molecular weight, as indicated by tailing of its elution profile (Fig. 1). To test whether these could account for the polymeric material in the 1h urine sample, the proteoglycan was freed of them by preparative chromatography on a column of Sephadex G-200. The fractionated proteoglycan (which was now eluted exclusively in a sharp peak between 13 and 20 ml elution volume; Fig. 1) was injected intravenously and the excretion of radioactivity was studied by the methods described above. Several differences were noted. In contrast with the unfractionated proteoglycan, total urinary excretion after 6h was decreased to 38 %, with the maximum occurring only after 3h. The 1h urine contained only 4 % of the injected radioactivity and, on gel chromatography, no significant amounts of any component other than inorganic $^{35}$S-sulphate could be detected in this, or any other, urine sample.

Thus the appearance of $^{35}$S-labelled polymeric material in the urine, of comparable size to single chains of chondroitin sulphate, was not necessarily due to proteolytic
The Methylation of Mercury by the Gastro-Intestinal Contents of the Rat

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Bacteria in the sediments of lakes and rivers, and in soil, have been shown to convert inorganic mercury salts into the more toxic methylmercury and dimethylmercury, which are absorbed from the human gastrointestinal tract much more rapidly than their inorganic counterparts (Jensen & Jernelöv, 1969; Matsumura et al., 1972; Beckert et al., 1974; Clarkson, 1971). Abdulla et al. (1973) demonstrated the methylation of HgCl₂ in rats with jejunal blind-loops, which suggests that small amounts of methylmercury may be formed by the microbial flora of the gut. In the present communication, the ability of suspensions of rat caecal and small intestinal contents to synthesize methylmercury is demonstrated.

Table 1. Synthesis of methylmercuric chloride by gut contents in vitro

Suspensions of caecal or intestinal contents (0.2 g/ml) were incubated for 20h at 37°C in the presence of ²⁰³HgCl₂ (2 μg/ml). The methylmercuric chloride formed was extracted with redistilled benzene and identified by t.l.c. The results of two experiments are shown.

<table>
<thead>
<tr>
<th>Region of gut</th>
<th>Atmosphere</th>
<th>Treatment</th>
<th>Methylmercuric chloride formed (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small intestine</td>
<td>Air</td>
<td>None</td>
<td>1.2, 0.8</td>
</tr>
<tr>
<td></td>
<td>N₂</td>
<td>None</td>
<td>0.4, 0.2</td>
</tr>
<tr>
<td>Caecum</td>
<td>Air</td>
<td>None</td>
<td>4.8, 4.2</td>
</tr>
<tr>
<td></td>
<td>N₂</td>
<td>None</td>
<td>3.2, 2.8</td>
</tr>
<tr>
<td></td>
<td>Air</td>
<td>+vitamin B₁₂ (5 μg/ml)</td>
<td>2.8, 3.6</td>
</tr>
<tr>
<td></td>
<td>Air</td>
<td>+antibiotics*</td>
<td>0.4, 1.2</td>
</tr>
<tr>
<td></td>
<td>Air</td>
<td>Autoclaved†</td>
<td>0.2, 0.0</td>
</tr>
<tr>
<td></td>
<td>Air</td>
<td>Filtered‡</td>
<td>0.2, 0.8</td>
</tr>
</tbody>
</table>

* Chloramphenicol, neomycin sulphate and tetracycline hydrochloride, each at 1 mg/ml.
† The suspension of caecal contents was autoclaved (100 kPa (15 lb/in²) for 15 min) before incubation.
‡ The suspension of caecal contents was centrifuged (10000 g for 15 min) and the supernatant passed through a Millipore membrane filter, pore size 0.45 μm.