from case 2, however, showed a shoulder at pH 4.5 due probably to some residual glucosaminidase A and glucosaminidase B activity. The optimum for both glucosaminidase and galactosaminidase activities of leucocytes from this patient was pH 4.5; here a greater proportion of hexosaminidase A and hexosaminidase B remains, masking the C form. The total hexosaminidase activity of these leucocytes was 4% of control values, whereas activities of the fibroblasts were 1.4 and 2.8% for cases 1 and 2 respectively. The lack of hexosaminidase C activity towards galactosaminide is shown in Fig. 1(b) where the hexosaminidase C peak at pH 5.5–6.0 is now decreased to equal or below that of the residual pH 4.5 forms.

Isoelectric focusing of control fibroblast glucosaminidase showed (Fig. 2c) the characteristic two peaks of A and B forms, the pI values of each being pH 5.5 and 8.2 respectively, whether assayed at pH 4.5 or 5.5. However, focusing of Sandhoff fibroblasts (Figs. 2a and 2b) produced a different major peak, at pH 4.65 (assayed at pH 4.5) or pH 4.8 (assayed at pH 5.5), corresponding to hexosaminidase C activity. This difference in the apparent pI of hexosaminidase C is difficult to reconcile, especially since the results were obtained from common columns. Nevertheless, since the latter pI value (pH 4.8) was obtained when assaying at the pH optimum for hexosaminidase C, this value would seem more likely to be correct. The presence again in case 2 Sandhoff fibroblasts of some hexosaminidase A is evident from the shoulder of activity at pH 5.4 (Fig. 2a) when assayed at pH 4.5 (the pH optimum of this enzyme). Some hexosaminidase B was present as a broad band of decreased activity in both Sandhoff fibroblasts. Further isoelectric focusing studies, in a narrower pH gradient will, it is hoped, provide a better understanding of hexosaminidase C in Sandhoff tissues.

McIlvaine, T. C. (1921) *J. Biol. Chem.* 49, 183–186

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**Turnover of Lysosomal Proteins and Induction and Distribution of Rat Liver Proteinases, after Treatment with Triton WR-1339**

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The turnover rates of proteins can now be assessed by the double-labelling technique of Arias *et al.* (1969): an injection of [14C]leucine is followed after 4 days by one of [3H]-leucine, and the animal is killed 4h later. Proteins which turnover rapidly show a high ratio of 3H/14C radioactivities because of a preferential loss of 14C and incorporation of 3H. Thus the 3H/14C ratio may be used as an index of turnover.

Cytosol, microsomal and ribosomal (see Goldberg & Dice, 1974) proteins from rat liver show a correlation between subunit size and turnover rate, with large subunits turning over fastest, and it has been suggested that free subunits are the main substrates for turnover. The present experiments describe the turnover of lysosomal proteins.

Rats (120–140g) were double-labelled (Arias *et al.*, 1969), and also injected (via the
tail vein) with Triton WR-1339 (85 mg/100 g), 4 days before killing. Lysosomes containing the detergent (‘tritosomes’) were isolated by virtue of their low density. After saline perfusion livers were minced with a Harvard press and homogenized (five passes of a tight Dounce) in 0.25 M sucrose, pH 6.0 (1 g of liver per 3 ml of suspension). The fractionations were by method A given by Beaufay (1972), and the fractions were evaluated by the following assays (in the presence of 0.1% Triton X-100): cathepsin D, Barrett (1972), method 1; cathepsin B1, Barrett (1972), method 1; β-glucuronidase, Dean (1974a). They were also evaluated by determination of protein (Lowry et al., 1951). The ‘tritosomes’ were enriched in lysosomal enzymes (see below). The corresponding gradient fractions obtained with control liver samples were devoid of cathepsins D and B1, and contained little β-glucuronidase.

Rats injected with Triton WR-1339 had greatly elevated total and specific activities of cathepsins D and B1, but only slightly elevated total β-glucuronidase activity, with no change in specific activity (Table 1). Wattiaux et al. (1963) observed no rise in activity of rat liver acid phosphatase under similar circumstances. Thus the induction of lysosomal enzymes by Triton WR-1339 shows some specificity.

Correlated differences in enzyme localization were found (Table 2). The relative specific activities (ratio of fraction specific activity to that in homogenate) of the three enzymes in the ‘tritosomes’ differed considerably from each other and the values varied between preparations. This latter difference may reflect variation in the uptake and distribution of Triton, in addition to experimental variation. Widely differing relative specific activity values for lysosomal enzymes in ‘tritosomes’ have been listed by Tappel (1969), but the present values for cathepsins D and B1 are higher than any of these. Such high values are suggestive of a selective concentration of these enzymes in the ‘tritosome’ portion of the lysosome population. Possibly, newly synthesized lysosomal enzymes, rich in proteinases, are preferentially accumulated in ‘tritosomes’.

The intralysosomal distribution of the enzymes was investigated by treating the ‘tritosomes’ with 0.1% Triton X-100, and separating a membrane (sedimentable) and a soluble fraction by centrifugation at 100000 g for 1 h, at 4°C. Cathepsin B1 and β-glucuronidase were almost entirely restricted to the membrane fraction, whereas cathepsin D was largely soluble. Most lysosomal enzymes are more evenly distributed between mem-

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**Table 1. Activities of lysosomal enzymes in livers of Triton WR-1339-treated and control rats**

| Enzyme activities are expressed as follows: cathepsin D, AΔE280/h; cathepsin B1, nmol of product formed/h; β-glucuronidase, μmol of product formed/h. The values are means ± S.D. from three rats. |
|-----------------|-----------------|-----------------|-----------------|
|                 | Total activity  | Specific activity |
|                 | (units/liver)   | (units/mg of protein) |
| Controls        | Triton-treated  | Controls        | Triton-treated  |
| Cathepsin D     | 41.5 ± 9.0      | 93.1 ± 7.0      | 0.019 ± 0.001   | 0.032 ± 0.001 |
| Cathepsin B1    | 1090 ± 130      | 2380 ± 850      | 0.47 ± 0.03     | 0.82 ± 0.07   |
| β-Glucuronidase | 201 ± 20        | 291 ± 46        | 0.10 ± 0.03     | 0.10 ± 0.02   |

**Table 2. Relative specific activities of lysosomal enzymes in rat liver ‘tritosomes’**

<table>
<thead>
<tr>
<th></th>
<th>Cathepsin D</th>
<th>Cathepsin B1</th>
<th>β-Glucuronidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation 1</td>
<td>133</td>
<td>55</td>
<td>1.7</td>
</tr>
<tr>
<td>Preparation 2</td>
<td>175</td>
<td>76</td>
<td>7.5</td>
</tr>
<tr>
<td>Preparation 3</td>
<td>263</td>
<td>12</td>
<td>5.7</td>
</tr>
<tr>
<td>Average ± S.D.</td>
<td>190 ± 64</td>
<td>48 ± 30</td>
<td>4.9 ± 3.0</td>
</tr>
</tbody>
</table>

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brane and sol (Mahadevan & Tappel, 1967), although a β-glucosidase is membrane-bound (Beck & Tappel, 1968). Microsomal β-glucuronidase is strongly membrane-bound (Dean, 1974b), whereas total lysosomal β-glucuronidase is easily extractable, in contrast with the result with 'tritosomes'. Thus 'tritosomes' are unusual in this respect also; and membrane-binding may affect enzyme specificity and activity.

Membrane and soluble fractions were also concentrated, dissolved in 0.1% sodium dodecyl sulphate-0.05% dithiothreitol, and electrophoresed in sodium dodecyl sulphate-polyacrylamide gels (Neville, 1971). Gel segments (2mm) were cut, and dissolved for scintillation counting.

The 3H/14C ratios varied considerably from segment to segment, reflecting variations in turnover rates of lysosomal proteins. The ratio for the total soluble fraction was higher than for the membrane fraction, suggesting that their average rates of turnover differ in the same sense. No correlation between subunit size and isotope ratio was observed for either 'tritosomal' subfraction. Thus the characteristics of turnover of lysosomal proteins differ from those of other cellular components: one possible explanation is that the lysosomes contained, in addition to functional lysosomal proteins, material from other sources undergoing digestion, which was still preferentially labelled with [14C]leucine. The bias of isotope ratios thus introduced could mask the turnover characteristics of the lysosomal proteins.


The Degradation of Sulphated Insulin by Rat Tissue Preparations

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Bovine insulin reacts with conc. H2SO4 at low temperature with the formation of sulphated insulin in which the aliphatic hydroxyl and phenolic groups have been converted into acid sulphate esters (Reitz et al., 1946). This material is less able to be neutralized by antiserum raised against bovine insulin than is unmodified insulin and it has been used in the treatment of insulin-resistant diabetics (Goldschmied & Laurian, 1968). Electrophoresis of sulphated insulin, however, showed that the reaction product represents a mixture of insulins carrying from one to eight groups/molecule (Thomas, 1971).

A completely sulphated bovine insulin preparation, i.e. one containing eight sulphate groups/molecule, was obtained by ion-exchange chromatography on QAE (quaternary aminoethyl)-Sephadex. When either an isolated rat liver or rat kidney was perfused in vitro with homologous blood containing completely sulphated insulin labelled with 35S an increase in the trichloroacetic acid-soluble 35S was observed, thereby demonstrating that appreciable degradation had taken place (Thomas & Curtis, 1972). The present study