p-guanidinobenzoate and with the *p*-nitrophenyl ester agreed closely.

We have also synthesized 4-methylumbelliferyl *p*-trimethylammonium cinnamate chloride, an analogue of the *p*-nitrophenyl ester used for the spectrophotometric determination of α -chymotrypsin (Knowles & Preston, 1968). The technique for the spectrophotometric titration of α -chymotrypsin with this titrant is similar to that described above for titrations with 4-methylumbelliferyl *p*-guanidinobenzoate. Results obtained with 4-methylumbelliferyl *p*-trimethylammonium cinnamate agreed well with those obtained with *p*-nitrophenyl N^2 -acetyl- N^1 -benzylcarbazate (Elmore & Smyth, 1968).

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Observations on Rat Mast-Cell-Granule Protease

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Mammalian mast cells contain proteases that, according to their ability to hydrolyse ester substrates, appear to have active centres analogous to either trypsin or chymotrypsin (see Selye, 1965). Interrelationships between the chymotrypsin-like enzyme of rat peritoneal-mast-cell granules (Lagunoff & Benditt, 1963) and the granule heparin proteoglycan fraction (cf. Lloyd, Bloom, Balazs & Haegermark, 1967a; Lloyd, Bloom & Balazs, 1967b) have now been investigated. The studies were facilitated by the development of spectrofluorometric procedures based on the use of *N*-methoxycarbonyl-L-phenylalanine 4-methylumbelliferyl ester and 4-methylumbelliferyl *p*-trimethylammonium cinnamate chloride. These compounds respectively have been shown to be suitable as steady-state

assay substrate and titrant for α -chymotrypsin (T. Morrison, unpublished work; Roberts, Adams, Elmore, Jameson & Kyle, 1971).

Granule preparations were obtained from fractionated populations of mast cells (purity 97–99% by haemocytometer count) by using methods described by Lloyd *et al.* (1967a). The granule ultrastructure was dissociated by extraction with 2M-sodium chloride followed by sonication for 30s at 4°C before centrifuging at 35000g for 45min. At pH 7.09 and 25°C in 20mM-phosphate buffer containing 0.1M-sodium chloride the clarified extracts accomplished the turnover of *N*-methoxycarbonyl-L-phenylalanine 4-methylumbelliferyl ester and reacted with 4-methylumbelliferyl *p*-trimethylammonium cinnamate in a manner analogous to chymotrypsin.

The unique sensitivity of 4-methylumbelliferyl *p*-trimethylammonium cinnamate as a titrant for chymotrypsin-like active centres was exploited in investigations of the binding of the enzyme to the granule matrix. Granule preparations were suspended at 4°C for 30min in sodium chloride solutions in the concentration range 0.1–2.0M and residual material was then removed by centrifuging at 35000g for 45min. The operational molarity of the enzyme in each of the supernatants was titrated with the titrant and expressed as a percentage of the operational molarity in the supernatant after extraction with 2.0M-sodium chloride, which also liberated all the heparin proteoglycan into solution (cf. Lloyd *et al.* 1967a). Complete recovery of enzyme was achieved after extraction with 1.2M-sodium chloride accompanied by 22% of granule heparin. Declining amounts of enzyme were recovered on extraction with 0.9M-sodium chloride (46.4%) and 0.7M-sodium chloride (7.3%). These findings are in agreement with the view that the heparin proteoglycan is among the most stable of the components of the granule to electrolyte extraction and is likely to contribute materially to the reticular network of the granule matrix (cf. Lloyd *et al.* 1967a,b), to which other granule components are attached by weak electrostatic forces.

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Comparative Studies on Rat Mast-Cell-Granule Protease and a Bovine Liver-Capsule Protease

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When a portion of the 1.2M-sodium chloride extract of rat peritoneal-mast-cell granules (see Lloyd, Morrison & Elmore, 1971) was analysed on a column of Sephadex G-100 previously suspended in 20mM-phosphate buffer, pH 7.0, containing 0.1M-sodium chloride and eluted with this electrolyte solution, a protein zone hydrolysing *N*-methoxycarbonyl-L-phenylalanine *N*-4-methylumbelliferyl ester was excluded from the gel in association with heparin. However, when the extract was analysed on a Sephadex G-100 column suspended in 20mM-phosphate buffer, pH 7.0, containing 1.2M-sodium chloride and eluted with the same solution the enzyme degrading this substrate was retarded by the gel. Fractions containing heparin and protein continued to appear early in the elution (cf. Lloyd, Bloom & Balazs, 1967).

Fractions hydrolysing *N*-methoxycarbonyl-L-phenylalanine 4-methylumbelliferyl ester that were freed from heparin in this way were pooled, concentrated by ultrafiltration by using a Diaflo UM-2 membrane (molecular-weight retention limit 1000; Amicon N.V.) and then desalted by successive addition of water to the concentrate in the ultrafiltration cell. Salt-free concentrates retained the capacity to hydrolyse *N*-methoxycarbonyl-L-phenylalanine 4-methylumbelliferyl ester, reacted with 4-methylumbelliferyl *p*-trimethylammonium cinnamate in a manner analogous to α -chymotrypsin but with a slower acylation rate and catalysed the turnover of the trypsin titrant 4-methylumbelliferyl *p*-guanidinobenzoate (see Roberts, Adams, Elmore, Jameson & Kyle, 1971).

The suitability of bovine liver as an alternative source of mast-cell proteases was investigated. Freshly stripped capsule was disrupted by maceration in liquid N₂ at 45000 rev./min in a VirTis 45 homogenizer and the finely divided material was then suspended in 0.34M-sucrose at 4°C by glass-glass homogenization. The suspension was layered over 0.88M-sucrose and centrifuged at 1500g for 6 min at 4°C. A granule-rich layer that collected at the interface was removed and recentrifuged, and the resulting pellet was then extracted with

1.2M-sodium chloride with glass-glass homogenization before being centrifuged at 35000g for 45 min at 4°C.

A significant purification of an enzyme hydrolysing *N*-methoxycarbonyl-L-phenylalanine 4-methylumbelliferyl ester was obtained when a portion (20 ml) of the 1.2M-sodium chloride extract of bovine capsule material was fractionated on a column (20 cm × 3 cm) of the dipolar ion-adsorbent β -alanine-Sephadex G-75 (Porath & Fryklund, 1970), previously equilibrated with 20 mM-phosphate buffer, pH 5.8, and then eluted with the same buffer. Fractions containing the enzyme, which appeared early in the elution, were pooled and concentrated by ultrafiltration by using a Diaflo UM-2 membrane. A further purification of the enzyme was achieved by gel filtration of the resultant concentrate on Sephadex G-100 in 20 mM-phosphate buffer, pH 7.0, containing 1.2M-sodium chloride. Comparisons of the properties of the enzymes from rat mast-cell granules and bovine liver capsule will be described.

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Effect of a High-Ethanol and Choline-Deficient Diet on the Metabolism of [³H]Oestradiol *in vitro* by Subcellular Fractions of Male Rat Livers

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Male rats weighing approx. 220 g were divided into five groups. They were fed on a basic hypolipotropic diet, i.e. choline-deficient, low-protein and high-fat (French, 1966), with the following additions: group A received 30% (w/v) ethanol in place