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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 N_2 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 $\times$ 3 cm) of the dipolar ion-adsorbent β -alanine-Sephadex G-75 (Porath & Fryklund, 1970), previously equilibrated with 20mM-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 20mM-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