potentiation of release caused by 5mM-arginine was completely abolished. Also, the rate of release in response to a new secretagogue, 20mM-D-xylulose, was significantly diminished.

In studies with mouse islets of Langerhans the rate of release in response to 20mM-glucose was, however, markedly inhibited, as were the effects of 5mM-arginine. The effect of 5mM-leucine was not completely inhibited, and again there was no decrease in the rate of release due to 5mM-theophylline.

Although there may clearly be species differences in the response to azaserine, these results further suggest that the formation of purine nucleotides may be important in the insulin secretory process.

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Howell, S. L. & Taylor, K. W. (1968). *Biochem. J.* **108**, 17.
Mayhew, D. A., Wright, P. H. & Ashmore, J. (1969). *Pharmac. Rev.* **21**, 183.

Moore, E. C. & Le Page, G. A. (1957). *Cancer Res.* **17**, 804.

Separation of Bovine α - and β -Trypsin by Affinity Chromatography

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Affinity chromatography has been used for the selective purification of enzymes (Cuatrecasas, Wilchek & Anfinsen, 1968), and the removal of inert protein from bovine trypsin by affinity chromatography on avian ovomucoids covalently linked to Sepharose has been reported (Feinstein, 1970a,b). Bovine trypsin has been resolved into two major active components, α - and β -trypsin, by ion-exchange chromatography (Schroeder & Shaw, 1968). We report convenient procedures for the isolation of these enzymes by affinity chromatography.

A potent competitive inhibitor of trypsin, *p*-(*p*'-aminophenoxypropoxy)benzamidine (Baker & Erickson, 1968), was coupled at pH 5.0 via the terminal amino group to Sepharose 4B (Pharmacia) and to cellulose powder (Whatman CC31) by using a cyanogen bromide activation procedure (Cuatrecasas *et al.* 1968). The products generally contained 0.6–1.0 μ mol of inhibitor/ml of packed polysaccharide. Trypsin was assayed with the spectrofluorometric titrant 4-methylumbelliferyl *p*-guanidinobenzoate hydrochloride as described by Roberts, Adams, Elmore, Jameson & Kyle (1971). The two forms of trypsin were identified by titration with 4-methylumbelliferyl *p*-guanidinobenzoate

(about 1 μ M) at pH 8.3, since β -trypsin was acylated in a few seconds, but α -trypsin required over 10 min.

A solution of trypsin (lot TRL 81B; Worthington Biochemical Corp., Freehold, N.J., U.S.A.) (20mg) in triethanolamine–50mM-HCl–10mM-CaCl₂ buffer, pH 8.0, was applied to a cellulose-inhibitor column (2.2cm $\times$ 14.3cm) at 4°C, and a peak of inert protein was immediately eluted. Subsequent elution with 50mM-sodium acetate–acetic acid, pH 5.0, yielded a peak of pure β -trypsin (5.1mg). Elution with 50mM-citric acid–50mM-NaCl, pH 2.3, then released all the remaining trypsin (5.2mg), a mixture of the two forms. Gradient elution failed to resolve these. The isolation of β -trypsin was adversely affected by 0.5M-NaCl in the triethanolamine buffer, and by the use of ammonium formate buffers.

When a solution of trypsin (20mg) in 50mM-sodium acetate–acetic acid, pH 4.0, was applied to the cellulose inhibitor column at 4°C, most of it was immediately eluted in a single peak. When, however, the enzyme (50mg) in the same buffer was applied to a Sepharose-inhibitor column (1.5cm $\times$ 25cm) at 4°C, inert protein, α -trypsin and β -trypsin were successively eluted as three incompletely resolved peaks. Selected fractions contained α -trypsin (3.8mg) and β -trypsin (12.4mg). A mixture of the two trypsins (3.4mg) remained bound to the column, and was eluted by 0.1M-citric acid–1M-NaCl, pH 2.0.

Factors such as type of support material, buffer composition, pH and ionic strength strongly influence these separations and will be discussed.

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Baker, B. R. & Erickson, E. H. (1968). *J. mednl Chem.* **11**, 245.

Cuatrecasas, P., Wilchek, M. & Anfinsen, C. B. (1968). *Proc. natn. Acad. Sci. U.S.A.* **61**, 636.

Feinstein, G. (1970a). *FEBS Lett.* **7**, 353.

Feinstein, G. (1970b). *Biochim. biophys. Acta*, **214**, 224.

Roberts, D. V., Adams, R. W., Elmore, D. T., Jameson, G. W. & Kyle, W. S. A. (1971). *Biochem. J.* **123**, 41P.

Schroeder, D. D. & Shaw, E. (1968). *J. biol. Chem.* **243**, 2943.

The Kinetics of Hydrolysis of Synthetic Substrates by Bovine Factor Xa

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Bovine Factor Xa, thrombokinase and autoprothrombin C may be identical (Esnouf & Williams, 1961; Marciniak & Seegers, 1962; Milstone,