- Gonzalez, G. & Offord, R. E. (1971) *Biochem. J.* **125**, 309–317
- Guy, O., Bartelt, D. C., Amic, J., Colomb, E. & Figarella, C. (1976) *FEBS Lett.* **62**, 150–153
- Louvard, M. N. & Puigserver, A. (1974) *Biochim. Biophys. Acta* **371**, 177–185
- Maroux, S. & Desnuelle, P. (1969) *Biochim. Biophys. Acta* **181**, 59–72
- Offord, R. E. (1966) *Nature (London)* **211**, 591–593
- Webster, D. & Offord, R. E. (1972) *Biochem. J.* **130**, 315–317

APPENDIX

The Production of the Second Activation Peptide from Trypsinogen Isolated from Individual Pancreases

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The need for a check on the bovine origin of the second peptide [see main paper, Webster & Offord (1977)] was met by isolating trypsinogen from ox pancreases taken at the abattoir (under the supervision of P. C. M.) from freshly killed animals. Trypsinogen was isolated from these pancreases by the method of Northrop *et al.* (1948), scaled down by the appropriate factor. For a single pancreas this factor was 12 and our preparation produced approximately the expected quantity of material. This unfortunately proved insufficient in our hands, when the method of Balls (1965) was used, to give enough crystalline material. However, two pancreases repeatedly gave sufficient material (usually about 30mg) for further study. The crystalline zymogen from such a preparation was activated as described by Maroux & Desnuelle (1969), and the peptide fraction was isolated by using a scaled-down version of the method described in the main paper (Webster & Offord, 1977).

In a typical experiment, 15 mg of activated trypsinogen (in 1.5 ml of activation buffer) was applied to a column (40 cm × 1.2 cm) of Sephadex G-10 packed in 1 mM-HCl and eluted with the same solvent. The effluent was monitored at 240 nm. On electrophoresis of the peptide fraction (0.6–0.75 column volumes) at pH 6.5, and after staining with ninhydrin, both peptides were visible in much the same relative amounts as seen on the purchased material mentioned in the main paper (Webster & Offord, 1977). The

quantity of material obtained after elution from the electrophoresis paper was insufficient to give a satisfactory estimate of the amino acid composition. However, it was at least possible to show that the minor peptide here probably contained the same *N*-terminal amino acid residue as that obtained from purchased material. On dansylation of the peptide fraction from the column by the method of Gray (1972), the only visible spots corresponded to *N*^ε-dansyl-L-lysine, dansylvaline and dansyl-phenylalanine.

* Abbreviation: dansyl, 5-dimethylaminonaphthalene-1-sulphonyl.

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References

- Balls, A. K. (1965) *Proc. Natl. Acad. Sci. U.S.A.* **55**, 392–396
- Gray, W. R. (1972) *Methods Enzymol.* **25**, 121–138
- Maroux, S. & Desnuelle, P. (1969) *Biochim. Biophys. Acta* **181**, 59–72
- Northrop, J. H., Kunitz, M. & Herriot, R. M. (1948) in *Crystalline Enzymes* (Dunn, L. C., ed.), 2nd edn., pp. 262–265, Columbia University Press, New York
- Webster, D. & Offord, R. E. (1977) *Biochem. J.* **167**, 285–287