(Sieler, 1970) in the eluted glucagon (60 μ g), indicating that the hormone was essentially pure. After affinity chromatography of ileum extracts, more than 90% of the glucagon-like immunoreactivity had a molecular weight of approx. 12000 on gel filtration at pH 8.8. Thus the antibody-Sephacrose complex, though not discriminating between pancreatic glucagon and glucagon-like immunoreactivity of gut, can be used in association with gel filtration for the purification of the gut glucagon-like immunoreactivity in amounts that will facilitate determination of structure and physiological properties.

We acknowledge financial support from the Medical Research Council.

- Akanuma, Y., Kuzuya, T., Hayashi, M., Ide, T. & Kuzuya, N. (1970). *Biochem. biophys. Res. Commun.* **38**, 947.
- Buchanan, K. D. & McCarroll, A. M. (1971). In *Radioimmunoassay Methods*, p. 266. Ed. by Kirkham, K. E. & Hunter, W. M. Edinburgh: E. and S. Livingstone.
- Cuatrecasas, P. (1970). *J. biol. chem.* **245**, 3059.
- Kenny, A. J. & Say, R. R. (1962). *J. Endocr.* **25**, 1.
- Moody, A. J., Markussen, J., Schaich-Fries, A., Steenstrup C., Sundby, F., Malaisse, W. & Malaisse-Lagae, F. (1970). *Diabetologia*, **6**, 135.
- Sieler, N. (1970). In *Methods of Biochemical Analysis*, vol. 18, p. 259. Ed. by Glick, D. New York: Interscience Publishers Inc.
- Valverde, I., Rigopoulou, D., Marco, J., Faloona, G. R. & Unger, R. H. (1970). *Diabetes*, **19**, 614.
- Weintraub, B. D. (1970). *Biochem. biophys. Res. Commun.* **39**, 83.

Conformational Studies of some Hormonal Peptides in Solution by using Nuclear-Magnetic-Resonance Spectroscopy

By J. FEENEY, G. C. K. ROBERTS and A. S. V. BURGEN. (*Medical Research Council Molecular Pharmacology Unit, The Medical School, Hills Road, Cambridge CB2 2QD, U.K.*)

N.m.r. can provide detailed information about the conformation of small peptides in solution. The basis for such studies is that the coupling constant between the α -proton and its adjacent NH proton (J_{NC}) is related to the ϕ dihedral angle. Side-chain conformational information can be similarly obtained by studying the vicinal coupling constants between the α -CH and β -CH₂ protons. It is also possible to detect the presence of intramolecular hydrogen bonds in peptides by considering the temperature-dependence of the NH chemical shifts. We have used these methods to study oxytocin, [8-lysine]-vasopressin and component peptides of gastrin in aqueous solution.

Oxytocin and vasopressin are found to have similar conformations and it is possible to indicate the range of ϕ , ψ values consistent with the n.m.r. data and the requirement of ring closure. There is no evidence for intramolecular hydrogen-bonding, nor for assuming that the Pro-Leu(Lys)-Gly-NH₂ fragment is folded over the rings.

For the component peptides of gastrin, Asp-Phe-NH₂, Met-Asp-Phe-NH₂, Trp-Met-Asp-Phe-NH₂ and β -Ala-Trp-Met-Asp-Phe-NH₂, the aspartic acid and phenylalanine side-chain conformations were determined: significant but small differences in rotamer population between the inactive tripeptide and active pentapeptide were found. From the observed J_{NC} values and from consideration of peptide potential-energy maps, the most populated backbone conformations have ϕ angles centred around -95° .

Differences in the Binding of Zinc Ions by Insulin and Proinsulin

By T. L. COOMBS and P. T. GRANT (*Natural Environment Research Council Institute of Marine Biochemistry, Aberdeen AB1 3RA, U.K.*) and B. H. FRANK. (*Lilly Research Laboratories, Indianapolis, Ind. 46206, U.S.A.*)

In agreement with previous observations on ox, pig and cod proinsulins (Grant & Coombs, 1970), it has been shown by equilibrium dialysis (ultra-filtration) and by sedimentation-equilibrium and velocity studies that pig proinsulin bound more than 5 mol of Zn²⁺/mol and aggregated to form a mixture of soluble polymers (mainly $s_{20,w}$ 5.1S) in the presence of excess of Zn²⁺. In contrast, pig insulin bound only 1.0 mol of Zn²⁺/mol and was almost completely precipitated from solution. These differences were only manifested when more than 0.3 mol of Zn²⁺ was bound/mol of protein.

Binding data from equilibrium mixtures containing insulin or proinsulin (0.1 mM), free ⁶⁵Zn²⁺ (0.001–1.0 mM), tris-HCl buffer (50 mM) and sodium chloride (0.1 M) at pH 7 at 20°C indicate that each protein contained two main orders of Zn²⁺ binding.

In equilibria containing 0.001–0.01 mM free Zn²⁺ both insulin and proinsulin aggregated to form soluble hexameric polymers ($s_{20,w}$ 3.2S and 3.4S respectively; see also Frank & Veros, 1970) and bound essentially the same amount of Zn²⁺. The bound Zn²⁺ was only slowly removed by ultra-filtration with Zn²⁺-free buffer. The capacity of this site was calculated from a Scatchard plot (Hughes & Klotz, 1963) to be about 0.33 mol of Zn²⁺/mol and the apparent intrinsic association constant was $1.9 \times 10^6 \text{ M}^{-1}$. These results imply