mobilization of tissue calcium. In the present work we have measured the dose-response characteristic for vasopressin-stimulated cyclic AMP in the isolated toad (*Bufo marinus*) bladder, and have investigated, with rat kidney, the possibility that hormonal effects on tissue calcium may lead to an increased uptake of Ca^{2+} into mitochondria.

All toads were rapidly pitthed and the half bladders were excised and weighed. They were preincubated for 1 h in aerated frog Ringer solution and then for a further 20 min, as matched pairs, in the presence of a given dose of [8-arginine]-vasopressin or under control conditions. The tissue was homogenized and the cyclic AMP concentration determined using the technique described by Brooker, Thomas & Appelman (1968). The concentration of cyclic AMP was found to be about 2 pmol/mg wet wt. in control tissue and to be maximally stimulated by 260% at 100 munits of vasopressin/ml.

The Ca^{2+} concentration in rat kidney mitochondria, isolated from a 10% (w/v) homogenate (Rossi & Lehninger, 1963), was measured by atomic absorption spectroscopy in an EDTA-water system after washing of the mitochondria in iso-osmotic sucrose and extraction with 5% (w/v) trichloroacetic acid. The minced tissue was incubated for 20 min in the absence and in the presence of vasopressin (1–500 munits/ml). A dose response characteristic was obtained showing the Ca^{2+} concentration of rat kidney mitochondria to reach a minimum after vasopressin (2 munits/ml) treatment. At higher doses of hormone Ca^{2+} concentration gradually increases, reaching a value close to that of the control at 100 munits of vasopressin/ml.

It is concluded that the concentration of cyclic AMP in toad bladder may not be directly related to the hormonal stimulation of Na^{+} transport across the tissue. Our view of the possible role of cyclic AMP and Ca^{2+} in the mechanism of vasopressin action will be presented.

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Isolation of Glucagon-Like Immunoreactivity of Gut by Affinity Chromatography

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The gut of animals contains glucagon-like immunoreactivity (Moody *et al.* 1970; Valverde, Rigopoulou, Marco, Faloona & Unger, 1970). Antibodies coupled to Sepharose have been used for affinity chromatography of polypeptide hormones (Akanuma, Kuzuya, Hayashi, Ide & Kuzuya, 1970; Weintraub, 1970). We have used antibodies to pancreatic glucagon, that cross-react with glucagon-like immunoreactivity of gut, for affinity chromatography of the latter substance from pig ileum.

An antibody-Sepharose complex was prepared by coupling rabbit immunoglobulin G (260 mg) containing antibodies to crystalline pig glucagon (Novo Terapeutisk Laboratorium, Copenhagen, Denmark) to 100 g of Sepharose 4B (Cuatrecasas, 1970). ^{125}I -labelled glucagon was bound by the complex in 40 mM-phosphate buffer, pH 7.4, containing human serum albumin (5 g/l), thiomersalate (0.25 g/l) and Trasylol (aprotinin; 5000 units/ml) (FBA Pharmaceuticals, Haywards Heath, Sussex, U.K.). The most suitable solvent for the elution of ^{125}I -labelled glucagon from the antibody-Sepharose complex was 4.25 M-ethanol in 4 mM-hydrochloric acid, which can be removed subsequently by freeze-drying. When ^{125}I -labelled glucagon (15 ng) was applied to columns (4.5 cm $\times$ 0.6 cm) at pH 7.4, 8–10% of the radioactivity flowed through the columns. This is attributed to chemical changes in the hormone during iodination. After a wash with water, 65% of the ^{125}I -labelled glucagon was eluted as a single peak by 4.25 M-ethanol in 4 mM-hydrochloric acid. A further 3% was eluted by 6 M-guanidine, pH 2.5, so that more than 20% of the hormone remained tightly bound to the columns. This loss was prevented by pre-washing the columns with 4.25 M-ethanol in 4 mM-hydrochloric acid. Columns bound 30 μg of crystalline glucagon/g of antibody-Sepharose complex and retained their chromatographic properties after at least ten experiments.

When crystalline glucagon, glucagon (2000 ng/g of tissue) extracted (Kenny & Say, 1962) from pig pancreas or glucagon-like immunoreactivity (200 ng/g of tissue) from pig ileum were chromatographed, the elution profiles as determined by glucagon radioimmunoassay (Buchanan & McCarroll, 1971) were identical. Immunoreactivity was eluted in 5 ml as two close peaks. After affinity chromatography of pancreas extracts on larger columns (28 cm $\times$ 1.5 cm), *N*-terminal histidine was detected

(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-Sephadex 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.

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

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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

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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