sensitivities to Ca^{2+} inhibition; the physiological importance of the latter is therefore questionable.

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Studies on Equine Trypsinogen and Trypsin

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Equine trypsinogen was prepared by extraction of minced pancreas with 0.25 M- H_2SO_4 , fractionation with $(\text{NH}_4)_2\text{SO}_4$ and chromatography on cellulose citrate (Whatman CT-60) at pH 6.0. Inactive components were also removed from commercial bovine trypsin by this chromatographic system. Equine trypsin was prepared from trypsinogen by autocatalytic activation in the presence of 0.05 M- CaCl_2 .

Purified trypsinogen and trypsin appeared homogeneous on ultracentrifugation and by disc electrophoresis (Reisfeld, Lewis & Williams, 1962). A molecular weight of 25600 ± 1000 for equine trypsin was determined by the high-speed equilibrium method of Yphantis (1964).

The *N*-terminal residues of trypsinogen and trypsin were found to be serine and isoleucine respectively after carbamoylation (Stark & Smyth, 1963; Gertler & Hofmann, 1967) and these were confirmed by dansylation (Gray, 1967). Identical *C*-terminal sequences (Gln, Thr, Ile, Ala)-Ala-Asn were found in carboxypeptidase A digests of *S*-sulpho-trypsinogen and *S*-sulpho-trypsin.

The structure of the activation peptide was found to be Ser-Ser-Thr-Asp₄-Lys. This is similar to the activation octapeptides from pig and sheep trypsinogens but differs markedly in the *N*-terminal two residues of the sequence. The amino acid composition of equine trypsin showed general similarities to the trypsins from cow, pig and sheep (see Schyns, Bricteux-Gregoire & Florkin, 1969) but differed in detail in the content of methionine (1 residue/mol.) and lysine and arginine (both 7 residues/mol.).

The common features of *N*-terminal isoleucine and *C*-terminal asparagine in the trypsins from cow and pig have thus been extended to equine trypsin. Similarly, the common sequence of Asp₄-Lys reported to be in the activation peptides from cow,

pig and sheep trypsinogens has also been found in this study. The functional significance of this sequence has been reported by Abita, Delaage, Lazdunski & Savrda (1969).

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Zinc-Dependent Conformational Stability in the Alkaline Phosphatase of *Escherichia coli*

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Alkaline phosphatase of *Escherichia coli* has been found variously to contain 4, 3 or 2 g.atoms of Zn/mole (Simpson & Vallee, 1968; Reynolds & Schlesinger, 1967; Plocke, Levinthal & Vallee, 1962); at least part of the zinc content is essential to activity, and its absence through chelation or genetic alteration (Schlesinger, 1966) renders the enzyme inactive. Zinc has also been shown (Reynolds & Schlesinger, 1967) to be essential for subunit re-association but not necessary for refolding of the subunit from an extended-coil conformation.

In the present work ultraviolet circular dichroism (CD) has been employed to detect major conformational phenomena related to the presence of zinc, with wild-type enzyme from *E. coli* K 12 and a zinc deficient enzyme from *E. coli* C₁₀F₁₈.

The CD extrema near 221 and 207 nm. remained essentially unchanged when *E. coli* wild-type enzyme was suspended in high urea concentrations; the 192 nm. peak became inaccessible. *E. coli* wild-type enzyme depleted of zinc by dialysis against EDTA behaved quite differently; substantial deviation from natural conformation occurred at above 2 M-urea, characterized by loss of the 221 nm. and 207 nm. peaks and the appearance of a larger peak near 202 nm. Sedimentation-velocity analysis of EDTA-treated enzyme in 6 M-urea revealed that dissociation into subunits had occurred; the low sedimentation coefficient, $S_{20,w}$ 1.7s, was consistent with an extremely extended conformation.

E. coli C₁₀F₁₈ inactive enzyme behaved similarly to EDTA-treated *E. coli* wild-type enzyme when treated with urea solutions. *E. coli* C₁₀F₁₈ enzyme incubated with Zn^{2+} and phosphate with partial

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reactivation (Schlesinger, 1966) gained conformational stability in urea approaching closely that of *E. coli* wild-type enzyme.

This information suggests that the position of zinc may restrain the initiation of helix-coil transition even on transfer to a less polar, strongly hydrogen-bonding, solvent. Zinc deficiency permits randomization to a high degree, leaving less than 3% residual α -helix content. The ability of zinc atoms to constrain the natural conformation under conditions of stress suggests that zinc atoms are bonded to residues widely separated in the primary sequence.

In the aromatic side-chain absorption region at least four weak ellipticity bands were discernible. These bands were less pronounced in the mutant, but approached those of the wild-type on partial activation with Zn^{2+} and phosphate.

E. coli C₁₀F₁₈ was kindly given by Dr A. Torriani. C.N.A.T. is grateful for a Science Research Council Studentship.

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Insulin Conformations

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It is generally recognized that the A chain and the B chain of insulin (a) each contain helical conformations and (b) are joined by two disulphide bonds, namely CyS (A,7)–CyS (B,7) and CyS (A,20)–CyS (B,19) (for extensive references see, e.g., Scheraga, 1961; Rieser, 1967).

An additional intrachain disulphide linkage, namely CyS (A,6)–CyS (A,11), also exists. Further, it has been suggested that some tyrosine hydroxyl groups may be hydrogen-bonded, and in particular that this could involve tyrosine (B,16) with glutamic acid (B,13).

In attempting to investigate the stereochemical and thermodynamic permissibilities, we have constructed numerous correctly proportioned molecular models ['ball and spoke', Courtauld & Corey–Pauling–Koltun (Koltun, 1965) atomic models (Lewin, 1967, 1969a,b)]. From several of these it became clear that the guanidinium group of arginine (B,22) can form a strong resonating ionic linkage with asparagine (A,21) in insulin. This conformation appears to cover one side of the inter-

chain disulphide bridges, namely CyS (A,20)–CyS (B,19), and therefore could afford it partial protection against rupture.

It can be computed that the above guanidinium (arginine)–carboxylate linkage is likely to be ruptured on addition of other guanidinium-group-containing compounds such as polyguanides and protamines, or doubly positively charged cations such as Zn^{2+} , or doubly negatively charged multi-oxyanions such as sulphate. As the rupture of this ionic bond involves exposure of these groups, the possibility arises that these may result in modification of the antigenic properties of insulin. It is therefore relevant to note that Moloney, Aprile & Wilson (1964) have concluded that the antigenicity of insulin was considerably decreased on sulphation. It is also relevant to consider whether commercial preparations of insulin containing zinc and protamine differ in their biological effects from those of pure insulin, not only with respect to prolonged 'activity' insofar as the treatment of diabetes is concerned, but also with respect to intrinsic antigenic properties.

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Order of Strength of Ionic Linkages in Protein Interactions

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Proteins are known to be involved in various ionic linkages, and evaluation of the relative order of strength of these linkages is desirable. The order can be assessed by using stereochemical and physico-chemical considerations of the probability of (a) the existence of the charge and (b) the direction of the electric field in appropriate cases: the latter can influence enforcement of particular conformations. Two factors assist in this evaluation: (1) resonance can play an important part in restricting the direction of the electric field, e.g. in carboxylate, diesterified phosphate and guanidinium (arginine), where the most probable direction is at right-angles to the line joining the centres of the two atoms mainly involved in the charge distribution; (2) co-operative