that it is a single chain protein. The sequence analysis shows it has a total of 84 amino-acids with a molecular weight close to 9,100 as calculated from the sequence and from sedimentation equilibrium data. The first thirty amino-acids are identical with those of the *B* chain of insulin and the last twenty are identical to those of the *A* chain. From the amino-acid sequence, Chance and his colleagues propose that the polypeptide chain of proinsulin folds in a spiral so that the first thirty residues are opposed to the last twenty-one, with disulphide bridges between cystines 7 and 70 and cystines 19 and 83. Conversion of proinsulin to insulin would simply involve cleaving the spiralled polypeptide chain between alanine at position 30 and arginine at 31 to yield the *B* chain, and between arginine 63 and glycine 64 to yield the *A* chain. This would produce insulin with *A* and *B* chains linked by two disulphide bridges and a single peptide, or several peptides, consisting of the residues between 31 and 63 of the proinsulin molecule.

In an attempt to simulate this conversion *in vitro*, Chance, Ellis and Bromer have subjected proinsulin to limited digestion with trypsin. The product of this reaction is a molecule identical with insulin except that it lacks the carboxy-terminal alanine residue of the *B* chain and which has 29 residues instead of 30 with lysine 29 as the carboxy-terminus. This was expected because of the specificity of trypsin and it suggests that if trypsin is involved *in vivo*, it can only be involved in the cleavage of the arginine 63—glycine 64 peptide bond of proinsulin. Presumably some other enzyme cleaves the alanine 30—arginine 31 bond.

An Enzyme Observed

from our Molecular Biology Correspondent

THE nuclease from *Staphylococcus* has been selected for a study in great depth by Anfinsen and his group. It is an interesting enzyme, which contains no disulphide bonds or thiol groups, and its sequence was determined in Anfinsen's laboratory. In a recent paper (Cuatrescasas *et al.*, *Biochim. Biophys. Acta*, **159**, 417; 1968) evidence was presented that acetylation of no more than two tyrosine residues caused a diminution in RNase activity, while leaving the DNase activity unimpaired. Thus 1–2 tyrosines are evidently implicated in the active site. It was also noted that 8–10 lysines could be blocked with no observable change in catalytic parameters towards DNA (including the Michaelis constant, V_{max}, and the pH optimum).

A captivating account of recent progress in the study of structure and function of this enzyme was given last week by Anfinsen at the IUPAC Symposium on the Chemistry of Natural Products in London. The concession that proteins and nucleic acids may be viewed as natural products produced both this and the remarkable contribution by Khorana on the synthesis of genes—and with them a strong sensation of organic chemists on all sides turning in their grooves.

Anfinsen began his talk with a description of further modifications of the nuclease, with particular reference to the important tyrosine residues. Tetranitromethane was used as a modifying reagent, and at low concentrations, in the absence of substrates, tyr-85 in the sequence is found to be nitrated. In the presence of inhibitor, on the other hand, tyr-115 only is attacked.

As a step towards the mapping of the active site the following ingenious device was employed. Reduction of the nitration product gives an aminotyrosine residue; the pK of this group is low, so that a pH may be found at which this residue alone is reactive towards a sulphone or dinitrofluorobenzene reagent. A bifunctional reagent is in fact used, and after reaction the pH is raised so as to enable the other functional group to react at whatever suitable side chain is within its radius. The site of attachment turns out to be the other reactive tyrosine residue or a neighbour. Thus, tyr-115 cross-links to tyr-85, and tyr-85 to tyr-115 or lys-116.

The effect of the substrate on the enzyme, the subject of earlier studies by physical methods, has been strikingly demonstrated by the changed reactivity towards proteases: whereas the isolated enzyme is rapidly degraded, the addition of inhibitor renders it indefinitely stable towards a variety of proteases. Limited treatment of the free enzyme with proteases causes specific cleavage near the beginning of the chain, with the loss of the first five residues. There is no loss of activity or change in conformation, and indeed the product is crystallographically isomorphous with the native enzyme. If trypsin is used, however, a second fission occurs at the 48–49 or 49–50 positions, and the resulting fragment adheres non-covalently to the remainder of the chain, in a manner reminiscent of the S-peptide of pancreatic ribonuclease, discovered by F. M. Richards. The product has diminished activity (some 10 per cent of native), but the phenomenon is of great relevance to the heroic task, which Anfinsen has set himself, of total synthesis of the enzyme.

The peptide fragment has indeed already been synthesized, and when it was added to the natural protein core, part of the activity was regained. The synthesis also produces imperfect products which, because of the length of the fragment, preponderate, and a procedure has been explored for the extraction of the genuine article: the core protein is attached covalently to a Sepharose support, and this is used as a column to trap the correct peptide. The method is complicated, however, apparently by the competitive effect of the incorrect peptides. Anfinsen enlarged on the scope and limitations of the Merrifield solid-state peptide synthesis technique, and described a number of improvements including a variant in which the support is a soluble polypeptidyl protein, which is recovered after each step by ethanol precipitation. Success has also been achieved in the complete elimination of sulphur from the protein, so as to diminish difficulties of synthesis. Methionine auxotrophs of the *Staphylococcus* have been found, and will grow when the isosteric unnatural amino-acid, norleucine, is substituted for methionine in the medium. About half the nuclease isolated from such a culture then contains norleucine in place of methionine. The methionine-containing enzyme is destroyed by cleavage with cyanogen bromide, leaving the fully active norleucine-analogue.

One of the chief long-term aims of this formidable programme will be to substitute for individual residues in various parts of the chain, and determine their role in the function of the enzyme, and the maintenance of its conformation. X-ray studies of the enzyme are progressing in another laboratory, and are now at the 4 Å level and, reinforced by these, many more revelations may be anticipated.