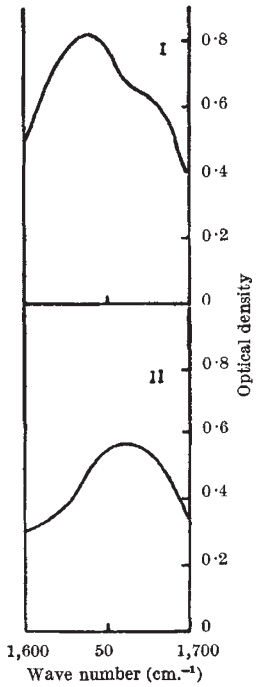
Chain Configurations in Natured and Denatured Insulin: Evidence from Infra-red Spectra

THE range of techniques available for studying the denaturing and renaturing of proteins has recently been increased by the observation[1] that a change from the extended (β) chain configuration of a synthetic polypeptide to the folded (α) form is accompanied by an increase in the frequency of the infra-red absorption band characteristic of the C = O peptide link. There is already evidence[2] that some denaturing processes in proteins are accompanied by an unfolding of polypeptide chains, producing the extended β-form, and it seemed of interest to see if evidence on this point could be obtained from infra-red spectra.

Insulin can be denatured and renatured (in the sense that the biological activity can be removed and later restored) by simple processes. The subject has been reviewed by Sanger[3]. We have measured the C=O absorption band of these two forms of insulin; these are shown in the accompanying figure, whence it may be seen that the frequency does indeed diminish as a result of denaturation. Crystalline natured insulin gives a curve similar to that of insulin precipitated from aqueous phenol.

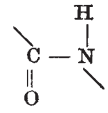
Dr. Lens's observation[4] that insulin can be renatured by aqueous phenol (which he has described to us) directed our attention to the possibility of producing similar results with *m*-cresol. Bamford, Hanby and Happey[5] have shown that synthetic polypeptides when cast from solution in *m*-cresol are predominantly in the α-chain configuration, whereas when cast from solution in formic acid they are in the β-form. It is therefore possible to pass a given synthetic polypeptide through an α–β transformation and back again simply by successive solution and reprecipitations from these two solvents. In addition, it is possible to follow the various stages by means of the C=O frequency-shift described above.



I. Insulin denatured by boiling in dilute HCl solution. II. Insulin precipitated from aqueous phenol (natured)

A sample of crystalline insulin was passed through the same cycle. It showed exactly the same kind of frequency shifts of the C=O band as had been observed in synthetic polypeptides. The film cast from formic acid at a high temperature gave a curve similar to that of the denatured protein (I in figure). It was confirmed by chemical analysis that there was no formic acid in the specimen. This material was redissolved in *m*-cresol and recast as a film. A curve similar to the natured form (II in figure) was obtained.

These results are consistent with the view (though not perhaps excluding other explanations) that natured insulin consists of polypeptide chains in the same α-fold as α-synthetic polypeptides, and that denaturation is accompanied by an extension of the chains into the β-configuration. The type of α-fold, which has been suggested in earlier papers[6,7,1], enables the α–β transformation to occur without any marked change in the packing of side-chains but merely by rotation of the



groups about bonds attached to the asymmetric carbon atom. No rotation of side-chains about the chain axis is required. With this kind of fold in natured insulin, the α–β transformation could occur without breaking the disulphide linkages between chains. It is known that these disulphide linkages must be preserved[3] if the denaturation is to be reversible.

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A Subsidiary Shell Pigment of *Haliotis cracherodii* Leach

IN previous communications[1], I have described investigations of the chief blue pigment extracted by acids from the shell of the gastropod *Haliotis cracherodii* Leach. Important evidence has been produced by Tixier and Lederer[2] against our view, based largely on spectroscopic findings, that this pigment is an indigoid. A joint study of the blue pigment, in collaboration with these workers, is in progress. In the meantime, we have obtained one of the accompanying yellow pigments in sufficient yield to perform spectrographic studies upon it, and upon its zinc complex.

The yellow material forms one of the discrete fractions obtained by chromatography in chloroform upon talc of the mixture formed when shell extracts in 3*N* hydrochloric acid are shaken with amyl alcohol and the epiphase evaporated to dryness. It is soluble in ether and in chloroform, is precipitated as an amorphous powder by adding petroleum ether to the chloroform solution, and shows no visual absorption bands in neutral solvents. Spectrophotometry shows well-defined maxima at about 4400 and 4900 A.

Upon addition of methanolic zinc acetate, a crimson zinc complex, reversibly decomposed by acids, is formed. This material has a strong four-banded spectrum and gives an orange fluorescence in ultraviolet light. It, too, is precipitated by petroleum ether from chloroform, but attempts to crystallize it have so far failed. The plot obtained with the Holiday continuous-recording spectrophotometer is shown in the accompanying graph. The sample examined was homogeneous, both in the free state and as the zinc complex, upon repeated chromatography.