how the parts of enzyme molecules remote from the active sites somehow play a part in enzyme function.

The shapes of protein molecules will not easily be understood in detail, but a few general principles seem to have emerged from a comparison of all the X-ray structures now available. One of the structures most recently to have been determined—that of chymotrypsin—resembles the structure of molecules such as lysozyme in that the long protein chain is curled up in such a way that the amino-acid groups which are chemically hydrophobic tend to be buried within the molecule. By contrast, hydrophilic groups lie on the outer surface. That is reasonable enough, although it does raise the question of how the shape of a protein molecule is influenced by such things as the acidity of the medium. It will also at some stage be important to know why the structure of chymotrypsin differs from that of most of the other proteins so far mapped in detail in that only a very small part of the chain is coiled into the helical form which protein molecules tend naturally to form.

What lies ahead ? To begin with, the structures of more protein molecules will be determined, and it is only seemly to remember that a full-scale X-ray analysis is still a tedious and even an uncertain venture. That said, detailed structures of the protein antibody molecules would clearly be of the greatest interest now that the chemistry of these materials is being understood. And for the rest it is abundantly clear that the X-ray structures of protein molecules which have appeared in the past few months are not an end point in themselves but only incentives for other investigations.

1 Avey, H. P., Boles, M. O., Carlisle, C. H., Evans, S. A., Morris, S. J., Palmer' R. A., Woolhouse, B. A., and Shall, S., *Nature*, **213**, 557 (1967).

2 Kartha, G., Bellow, J., and Harker, D., *Nature*, **213**, 862 (1967).

3 Quoted by Barnard, E. A., *Nature*, **215**, 6 (1967).

4 Moore, S., and Stein, W. H., *Methods in Enzymology*, **6**, 819 (1963).

5 Hirs, C. H. W., *Brookhaven Symp. Quant. Biol.*, **15**, 154 (1962).

6 Matthews, B. W., Sigler, P. B., Henderson, R., and Blow, D. M., *Nature*, **214**, 652 (1967).

CELL BIOLOGY

Synthesis of an Enzyme

Nirenberg and Matthaei provided an invaluable method for the investigation of protein synthesis when they discovered that in cell free systems—essentially the pulp of ground-up cells containing ribosomes, transfer RNA and other components necessary for protein synthesis together with an added source of energy—peptide bond formation occurs and amino-acids are incorporated into polypeptides under the direction of endogenous or added messenger RNA. As a result, cell free systems have been increasingly used for studies of protein synthesis *in vitro* and they have yielded otherwise unobtainable data. But there has always been a nagging doubt that since the conditions in a cell synthesizing protein are so different from those in a test-tube, what occurs *in vitro* in a cell free system may not occur *in vivo* within the cell. Such doubts were not diminished when it was found that in a cell free system programmed with a particular messenger RNA, the amino-acids incorporated into polypeptides vary with the ionic environment and especially with the concentration of Mg^{++} ions.

It was something of a relief, therefore, when it was shown that haemoglobin and some RNA phage proteins synthesized *in vitro* have a similar peptide composition to the natural proteins. Unfortunately, however, these proteins are not enzymes; they cannot be assayed for biological activity and so the fidelity of *in vitro* protein synthesis cannot be properly assayed. It is quite possible that even if a protein synthesized *in vitro* had the same amino-acid sequence as the natural product, it could not, in the artificial environment of a test-tube, fold to assume the secondary and tertiary structures which determine its biological activity. Hence the recent demonstration by Salser, Gesteland and Bolle (*Nature*, **215**, 588; 1967) of *in vitro* synthesis of biologically active T_4 bacteriophage lysozyme assumes a double importance. Apart from providing an experimental system for precisely defining the components and conditions necessary for synthesis of an active enzyme their result raises the general level of confidence that can be placed in *in vitro* observations.

T_4 lysozyme, an enzyme made by the phage fairly late in the infection cycle, causes the dissolution of the bacterial cell wall and the release of the progeny particles. It has several obvious advantages for study *in vitro*. There is a very sensitive assay for it; it consists of a single polypeptide chain and is a fairly small protein (molecular weight 18,000) and so must be coded for by a small messenger RNA molecule. This is important because it increases the chances of isolating the messenger RNA undegraded.

Salser *et al.* isolated the RNA made in phage infected *E. coli* and used this to programme a cell free system. The protein made was assayed for lysozyme and showed activity. Moreover the amount of lysozyme made *in vitro*, about 4 per cent of the total protein synthesized, is similar to the amount made *in vivo*. Several controls showed the enzyme had been made *de novo*. For example, RNA from cells infected with a mutant phage that lacks the gene for lysozyme failed to stimulate lysozyme synthesis *in vitro*, and puromycin and chloramphenicol, drugs that inhibit protein synthesis *in vivo*, abolished the *in vitro* synthesis of lysozyme.

It is clear from these experiments that in the environment of a cell free system, the translation of the information encoded in RNA into the sequence of amino-acids in a protein can be sufficiently accurate to yield an active enzyme.

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that this symmetry has something to do with the geometry of the ribosomes from which the RNA molecule has been derived[4]. Another feature of the shape of the molecule to which Sanger has drawn attention is the occurrence of internal regions in which the molecule is coiled into a spiral form. In this respect the long RNA molecule has something in common with the structure of the smaller molecules of what is called transfer RNA first worked out by Holley and his collaborators, the function of which seems to be to involve individual amino-acid molecules in the construction of a protein.

1 See *Nature*, **214**, 865 (1967).

2 Brownlee, G. G., Sanger, F., and Barrell, B. G., *Nature*, **215**, 735 (1967).

3 Holley, R. W., Apgar, J., Everett, G. A., Marquisee, M., Merrill, S. H., Penswick, J. R., and Zamir, A., *Science*, **147**, 1462 (1965).

4 *Nature*, **215**, 690 (1967).

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