

units per molecule, were first perceived and demonstrated by Davies and Stark, who devised a peerless reagent, or class of reagents, for the purpose. These are the dicarboxylic acid imido-esters, which are highly reactive towards primary amino groups, work in aqueous solution under mild conditions and contain a flexible paraffin backbone, which allows the maximum efficiency of cross-linking. Most work to date has been done with the suberic acid derivative (eight carbons), dimethyl suberimidate. There are now signs that protein chemists are taking these reagents to their bosoms, for the realization has dawned that a detailed examination of the distribution of the different oligomers in the reaction product may be capable of revealing more about the structure than just the number of subunits (though even that is nothing to be scoffed at).

An adumbration of the kind of information that one might hope would emerge from carefully designed experiments is to be found in a paper by Carpenter and Harrington (*J. Biol. Chem.*, **247**, 5580; 1972). They have examined on detergent-acrylamide gels the products of cross-linking with dimethyl suberimidate of bovine lens leucine aminopeptidase. There has been some disagreement about the number of subunits in this protein, which has a molecular weight of 320,000. Exposure to the cross-linking reagent leads to the appearance of six bands (including the monomer) in the electrophoresis, which supports the bulk of the earlier evidence, in favour of six identical subunits. They do not appear in anything like the same concentrations, however: dimers and tetramers showed strongly, whereas the trimer, and especially the pentamer, components were very faint. This already permits some inferences about the arrangement of the subunits within the hexamer. With the assumption of symmetry—that is to say structural equivalence of identical subunits—one can envisage four schemes, one of which can be excluded on the basis of the results. This is the planar heterologous hexagonal arrangement, with six-fold symmetry, in which all subunit interfaces are equivalent.

In such a case, although the relative weight of the different oligomers will depend on the extent of cross-linking, there is nothing to militate against trimers and pentamers. In the isologous planar hexamer, on the other hand, where alternate subunits are reversed, so that each makes a different kind of contact with either of its neighbours and the rotational symmetry is three-fold, the preferential formation of dimers, by way of one preferred type of interface, and then of multiples of dimers, can readily be visualized.

Other possibilities are the two forms of a dimer of trimers. The trimer will take the form of an equilateral triangle, and two such arrangements may associate in the eclipsed or in the rotationally staggered form, leading to a trigonal prism and an octahedral geometry respectively. The former would have three, and the latter four, distinct types of inter-subunit contacts. The authors have not attempted to take the analysis further by examining the predictions from these models for possible relative concentrations of oligomers.

Aspartate transcarbamylase is now an old chestnut, but two new papers lead to a more firmly based model for the relationship between the six catalytic and six regulatory subunits. Davies and Stark did in fact show, by the use of dimethyl suberimidate, that the former were trimers and the latter dimers. Now Cohlberg *et al.* (*Biochemistry*, **11**, 3396; 1972) have excluded any possibility that the formation of dimers by the regulatory subunit is adventitious, by making cross-linked dimers, using dimethyl pimelimidate, purifying them, and showing that a hydrodynamically normal enzyme results when these are mixed with the catalytic component. They find, moreover, that the regulatory subunits containing zinc are stable dimers in solution, whereas in the absence of zinc, monomers are in evidence. Taken together with the results of some superior electron microscopy by Richards and Williams (*ibid.*, 3393), a fairly explicit geometry for the whole protein has been deduced.

The electron micrograph of the enzyme dried in thin stain layers reveals three globules, presumably catalytic subunits, in the form of an

equilateral triangle. Outside this is another triangle rotationally displaced by 60° from the first, the apices of which are evidently pairs of regulatory subunits. In thicker stain the molecules appear to lie on their sides, and there is a clear gap between two layers, each presumed to contain three catalytic subunits, in the form of eclipsed triangles. Cohlberg *et al.* note that the two catalytic trimers seem not to be in contact, and in the absence of regulatory dimers have no tendency to associate in solution. Moreover, as the regulatory subunits do not associate beyond the dimer, there should be no important contacts between dimers. The model, which is based on these considerations, and conforms with the crystallographic requirement for both three- and two-fold symmetry, consists of V-shaped arms of regulatory dimers, each linking two catalytic subunits in the upper and lower triangle, which are related by a 120° rotation.

A striking case of functional control by subunit interaction is described by Segal and Stadtman (*Arch. Biochem.*, **152**, 356; 1972), working with the twelve-subunit hexagonal enzyme, glutamine synthetase. The activity is regulated by adenylation of subunits, which apparently occurs in a random manner, and the enzyme is stimulated by divalent metals. The effect of cobalt is of particular interest. It promotes the activity of the unadenylated enzyme, but it turns out that the resulting level of activity is not linearly related to the degree of adenylation (which it is in mixtures of a slightly and an extensively adenylylated preparation). Instead it correlates with the number of unsubstituted chains adjacent to other unsubstituted chains, calculated on the

RNA Virus Genomes lack Poly(U) Tracts

THE widespread belief that the tracts of adenylic acid residues to be found at the 3' terminal ends of some messenger RNA molecules of eukaryotic cells and their viruses are added after the RNA has been synthesized by RNA polymerase and are not, therefore, specified by complementary poly(dT) or poly(U) tracts in DNA or RNA genomes rests on circumstantial evidence, as Marshall and Gillespie stress in next week's issue of *Nature New Biology* (November 8). They also point out that, at least in mammalian cells, there are quite long stretches of A-T base pairs in the nuclear DNA, and the possibility that the poly(A) tails of cell messengers are genetically coded cannot be summarily dismissed.

Because of their size and complexity the genomes of animal cells do not readily lend themselves to detailed analysis, but the genomes of various

animal RNA viruses are sufficiently small for an experimenter to be able to detect and locate tracts of poly(U), which, if they exist, might code for the poly(A) tails of the messenger RNAs of these viruses. Marshall and Gillespie have, therefore, used labelled poly(A) as a probe in hybridization experiments for any poly(U) tracts in the genomic RNAs of some eleven oncogenic and non-oncogenic RNA viruses which replicate in mammalian cells of various species. Apparently none of these RNA virus genomes hybridize significantly with poly(A) in conditions optimal for specific poly(A)/poly(U) hybridization. Of the viruses Marshall and Gillespie have tested, three—Sendai virus, Newcastle disease virus and vesicular stomatitis virus—produce in infected cells messengers with poly(A) tails; these tracts must be added to messengers after transcription.