The chemical economy of coral reefs, this time in the West Indies, was the theme of Drs D. C. Smith (University of Oxford) and D. H. Lewis (Sheffield). They have developed a technique for studying the movement of photosynthetic products from symbiotic zooxanthellae to their coelenterate hosts. Movements of the various compounds identified seemed to be stimulated by ammonia, and results suggested that the coral reef is almost a closed circuit system with regard to carbon and nitrogen, and, as in tropical rain forest, all nutrients may be locked up in the standing crop.

Three contributors described results obtained during exchange visits to India. Mr P. D. V. Savage (Marine Biological Laboratory, Fawley), who spent three months studying phytoplankton production in the Vellar Estuary, South India, found that the nannoplankton contributed a surprisingly large amount of the total production (phytoplankton less than 20 μm across being responsible for up to 87 per cent of total uptake of carbon-14). Drs A. Trevallion and R. R. C. Edwards (Department of Agriculture and Fisheries, Aberdeen) found that the rate of respiration in some Indian molluscs at 28° C was two to ten times greater than in British species at 12°–16° C, possibly because of differences in activity—the tropical species burrow and grow faster and have shorter life cycles. On the other hand, at 30° C the standard respiration of *Cynoglossus* fishes (tropical soles of six species which lived together in the area studied) was only 1·2 times that of *Pleuronectes platessa* at 10° C.

ENZYMES

Active Sites by Computer

from our Molecular Biology Correspondent

THE creeping computerization that is spreading through biochemistry is now beginning to permeate the activities of enzymologists. As practised by Gass and Meister (*Biochemistry*, **9**, 1380; 1970) it seems in fact a natural application of computer-assisted stereochemistry, and a rational culmination of the widely applied strategy of defining active centres by systematic variation of the structure of substrates or inhibitors.

The enzyme that has been studied for many years in Meister's laboratory, and has been used in these studies, is brain glutamine synthetase. Not only L but also D-glutamate is a substrate, and so also are three of the possible monomethyl derivatives, namely, α-methyl-L-glutamate, *threo*-β-methyl-D-glutamate and *threo*-γ-methyl-L-glutamate. Other substrates are β-glutamate and a cyclic glutamate analogue. Inspection of the steric relations between these various derivatives led to the conclusion that the glutamate skeleton is in a fully extended state when it is bound to the active site of the enzyme, the α-hydrogen atom projecting outwards from a presumed cleft. This in turn led to various other conclusions concerning the disposition of binding groups in the active centre.

What Gass and Meister have now done is to define the geometrical characteristics of the active site quantitatively in terms of the natural substrate, L-glutamate, and then to apply essentially a simple computer model-building routine, with systematic translational and rotational displacement of the molecule, to give the configuration of each substrate best compatible with the active site geometry. The results were delivered directly on a plotter as stereographs, and are published in this form.

In the extended state of the substrate, the hydrogen atoms that may be substituted by methyl groups without loss of substrate activity all point in one direction, presumably away from an active site cleft. Specific binding occurs at the γ-carboxyl group, one of whose oxygen atoms becomes phosphorylated to give the intermediate acyl phosphate, which is formed by reaction with ATP. This step is followed by reaction of the acyl phosphate with ammonia, leading to a putative tetrahedral intermediate, the ammonia being presumed to be bound at a defined site on the protein, so placed as to facilitate the reaction, in accordance with well established principles. The phosphorylation site is determined by the coincidence of one of the carboxyl oxygen positions when L and D-glutamate are compared. The active site is thus operationally defined in terms of the disposition of a few contacts, and attempts can then be made to fit other substrates and inhibitors into it. With D-glutamate, a gross difference in the direction of the γ-carboxyl group, compared with that of the antipode, is at once apparent, and is surmised to be responsible for the difference in reaction rates of the isomers. A comparison of the tetrahedral intermediates involving L-glutamate, on the one hand, and its several analogues on the other, shows differences of geometry which provide a rationalization for rate differences.

The inhibitors, L-methionine sulphone and sulphoximine, when examined in terms of their geometry, appear as analogues of the tetrahedral complexes. The sulphur oxygen atoms of the sulphone seat nicely at the sites occupied by the γ-carboxyl oxygens of the substrate, when the S-methyl group is placed at the presumed ammonia binding site. These are thus unusual inhibitors, which compete with an intermediate, not the substrate. The binding of L-glutamine, a substrate for the reverse reaction, and β-glutamine are also rationalized, and Gass and Meister consider also other features of substrate and inhibitor specificity, all of which fit with greater or lesser conviction into their scheme. The reconciliation of results of this approach with X-ray structures of enzymes, where these are available, should also be of considerable interest.

ENZYME SYNTHESIS

Not Pure Enough

from our Biological Chemistry Correspondent

QUESTIONS have been raised about Merrifield's solid phase preparation of polypeptides, apparently well established by his synthesis of an enzyme, although his product has only 13 per cent of the activity of native ribonuclease (Guttee and Merrifield, *J. Amer. Chem. Soc.*, **91**, 501; 1969). The technique uses an insoluble resin to support the carboxyl end of a peptide which is then extended by stepwise, quantitative addition of further amino-acids to the amino-terminus. The peptide is detached from the polymer support when its chain reaches the desired length.

Research teams in Tübingen and Houston have examined critically some of the potential limitations of the method by considering two questions. Is there any racemization during peptide synthesis? And is