In a session on biometrical exercises Dr T. T. Elkington (Sheffield) said that in his experience most second year students had little appreciation of biological variation; biometric methods should be used primarily to develop a quantitative approach to this study. He discussed and illustrated the various pictorial methods (such as scatter diagrams) available, and their relationship to statistical treatments. In a discussion on the best way of teaching statistics for experimental taxonomy some thought that a separate course was essential, while others took the view that the necessary techniques and their application could be taught as part of the exercises in variation.

In the session devoted to the relationship between field studies and experimental taxonomy, Dr S. M. Walters (Cambridge) expressed the opinion that certain topics (notably ecotypic differentiation) are too difficult to illustrate beyond the level of demonstration. The most fruitful results could arise from a class exercise on some field phenomenon of variation which has already been partly investigated; he illustrated this by an account of class work on *Primula* hybridization in Cambridgeshire woods.

The members of the symposium decided to prepare specimens for a list of wild British species—hybrids and the relevant published references. Dr C. A. Stace (Manchester) offered to act as coordinator of this project, which, it was decided, should then be referred to the Botanical Society of the British Isles with a recommendation that such a list might be prepared and published by the society. It was also decided that copies of duplicated notes, class-sheets and so on, some of which had been exhibited during the meeting, would be sent to Dr D. Briggs (Glasgow), who would prepare a list of titles and origins of such documents, which could be made available to university teachers.

ENZYMOLOGY

Crosslinking the Crystal

One of the sharper tools of the enzyme chemist's trade is the use of bifunctional reagents to crosslink enzyme molecules. The usual reagent is glutaraldehyde, a five carbon chain flanked at each end by an aldehyde group: it has been used to convert several enzymes into insoluble, stabilized matrices which, experimentally, hold many advantages over the native material. Crosslinked crystals can be exposed to a wider range of solvents and *p*H, the possibility of autodigestion is lessened, and the attachment of heavy atoms for X-ray analysis is made simpler.

In the case of carboxypeptidase, lysozyme, and ribonuclease, crosslinking apparently causes only negligible changes in X-ray diffraction patterns, though there may be exceptions to this tendency: Bishop and Richards (*J. Mol. Biol.*, **33**, 415; 1968) recently showed that crosslinking causes some disordering in β lactoglobulin crystals.

Enzyme chemists may rely quite heavily on the reaction, yet they are totally ignorant of its chemistry. In the latest issue of *J. Mol. Biol.* (**37**, 231; 1968) Richards and Knowles demolish the usual supposition, that glutaraldehyde is forming Schiff bases with enzyme lysines, and they suggest an alternative. The glutaraldehyde reaction is rapid and irreversible, facts which preclude the formation simply of Schiff bases. Yet amino-acid analysis of protein hydrolysates does indicate the participation of lysine residues in the reaction.

Richards and Knowles subjected some commercial glutaraldehyde solution to NMR spectroscopy, and found that glutaraldehyde itself was only a minor constituent of the mixture. The material was largely present in polymeric form, together with α-β unsaturated aldehydes arising by water loss from aldol condensation adducts. Unsaturated aldehydes of this sort would give stable Michael-type adducts with lysine amino groups: such a reaction would account nicely for the observed properties of crosslinked protein crystals.

Various oligomers of glutaraldehyde were present in the commercial material, so presumably crosslinks of various lengths would be available. Further, temporary links might be formed if an oligomer reacted at one end by Michael addition and at the other by reversible Schiff base formation. This variety of feasible linkages may of course account for the remarkable efficiency as a crosslinking agent of the solution that comes in the bottle marked "glutaraldehyde".

MUSCLE CHEMISTRY

New Myochemistry

from our Molecular Biology Correspondent

Slayter and Lowey's electron microscopy last year resolved the long standing doubt about the number of major polypeptide chains in the myosin molecule; it has now prompted a thorough quantitative re-evaluation of the properties of the molecule. Because the myosin molecule has two "heads", each presumed to contain one actin-binding site, one might hope to find that there are two corresponding active sites for the splitting of ATP. Arguments over the number of such sites have in the past been befogged by the controversy about the molecular weight. A re-examination has now been carried out by Schliselfeld and Bárány (*Biochemistry*, **7**, 3206; 1968), who have studied directly the attachment of ^{32}P-labelled ATP to myosin under conditions (high concentration of sodium chloride) at which the rate of turnover is slow. The method selected was gel filtration, in which a column is equilibrated with the ligand, which distributes itself between the inside and outside of the gel beads. When the protein is applied to the column it travels as a zone enriched in the ligand, and is followed by a zone of depletion. The size of this concentration disturbance gives the amount of ligand bound and, from values obtained by varying the ratio of total ligand to protein, standard treatments yield the number of sites per protein molecule.

With tracer experiments, very low protein concentrations can be used, and this and the speed of the procedure circumvent any major difficulties arising from turnover of substrate. Taking the best available values for the molecular weight, it is found that 1·4–1·8 binding sites are present in each molecule of myosin, and 1·4–2·2 in heavy meromyosin (myosin which has been separated from most of its long shaft, but still retains its two "heads"). It appears therefore