must be taken into account during examination of these reactions, since, besides the general danger of denaturation, the complexity of a protein molecule, where many reacting groups form a unique environment, may bring about considerable alterations in the chemical reactivity of these groups, which often does not closely resemble that shown by the same groups in simpler compounds.

Ox liver glutamate dehydrogenase is one enzyme that has recently been the object of chemical modifications. Although these investigations are still at a very preliminary stage, the studies on the active and regulatory sites of this enzyme that we and other investigators are carrying out indicate that both amino and aromatic groups are involved in the reactivity of the enzyme sites. Fisher & Cross (1965) have suggested, on the basis of solvent-perturbation difference spectra, that tryptophan is present at the glutamate-binding site, and these findings are consistent with those of Brocklehurst *et al.* (1970). Colman & Frieden (1966a,b) have shown that enzymic activity is lost and binding constants of allosteric modifiers are altered by acetylation of several lysyl residues. The use of dinitrophenylation (di Prisco, 1967) has led to the finding that this enzyme possesses two separate or perhaps partially overlapping regulatory sites, one for GTP and the other for ADP. The reactivity of the regulatory sites, as well as that of the catalytic ones, which can be selectively protected by the presence of the specific ligand, is lost when, in each site, a set of six lysyl and three tyrosyl residues per molecule of active oligomer, composed of six, probably identical (Appella & Tomkins, 1966), polypeptide chains of molecular weight 53 000 (Eisenberg & Tomkins, 1968) has been dinitrophenylated (G. di Prisco, unpublished work). In agreement with these findings, Anderson, Anderson & Churchich (1966) have shown inhibition by pyridoxal phosphate, which, after reduction and total hydrolysis, was found combined in a Schiff base form with a lysyl residue involved in the reactivity of the catalytic site. The essential role of lysyl and tyrosyl residues in the catalytic and allosteric properties of the enzyme has been confirmed by different techniques, involving reaction with some other group-specific reagents (Brocklehurst *et al.* 1970).

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The Reactivity of Group-Specific Chromophoric Reagents

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The chemical modification of reactive side chains in enzymes can be used to obtain information about conformation, 'essential' groups and the role of such side chains in subunit interactions. In addition, if the reagent has environmentally sensitive optical properties, it can be used to explore the nature of specific sites.

Two group-specific reagents will be described.

2,4,6-Trinitrobenzenesulphonic acid has been used to characterize the reactivities of amino groups in several enzymes (Freedman & Radda, 1969). When the extent of reaction is compared with changes in activity and allosteric response, particular amino groups can be associated with these enzymic functions. 2,4,6-Trinitrobenzenesulphonic acid can also be employed to detect conformational changes (e.g. between the oxidized and reduced forms of cytochrome c).

7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole (Ghosh & Whitehouse, 1968) reacts rapidly with thiol groups to give a fluorescent product. The uses of this reagent for the study of glyceraldehyde 3-phosphate dehydrogenase and muscle phosphorylase will be discussed.

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X-Ray Analysis of an Unusual Reaction in Lysozyme

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The iodination of lysozyme in acid media results in a product that is enzymically inactive. The reaction has been carried out in the crystalline state. X-ray analysis of the product by high-resolution difference Fourier methods show that glutamate-35 and tryptophan-108 have undergone an unusual reaction caused by environmental factors. The loss