take place at the active centre, but that alkylation is external by free iodoacetate in solution. Thus many ligands bound at the active site, including iodoacetate, can protect against alkylation presumably by an induced change in enzyme conformation.

What is the function of the reactive thiol group? The fact that carboxymethylation drastically decreases catalytic activity does not necessarily mean that the thiol group has any positive function at all. Previously inhibition by *p*-chloromercuribenzoate was considered evidence that thiol groups were involved in binding and/or catalysis at the active centre. Criticizing this, it was then suggested that the thiol groups maintained native structure and that reaction with *p*-chloromercuribenzoate disrupted this (Theorell & McKinley-McKee, 1961). However, a reactive thiol group that enables the formation of active carboxymethyl-enzyme need not necessarily be involved in binding, catalysis or maintenance of tertiary structure. It may only be that, when it is modified, a change in the interactions of the polypeptide chain occurs and a much less active enzyme conformation results. Thus for enzyme action so-called 'essential amino acids' may only be essential and have a function when modified.

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Purification of the High-Molecular-Weight Form of Adenosine Deaminase from Rabbit Intestine

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Purified adenosine deaminases from most sources hitherto investigated have been shown to have molecular weights in the region of 35 000. However, recent work has shown that enzymes of higher molecular weight exist. Ma & Fisher (1968) found three adenosine deaminases in the frog having molecular weights of 200 000, 110 000 and 35 000, and these workers have also reported that the enzyme of molecular weight 200 000 is present in the livers of a wide variety of amphibians and mammals (Ma & Fisher, 1969). As yet these high-molecular-weight enzymes have not been characterized.

In this communication a method is presented

for the purification of the high-molecular-weight adenosine deaminase found in rabbit intestine (mol.wt. 260 000). The purification of a low-molecular-weight form of the enzyme (mol.wt. 35 000), which is also present in this tissue, has already been described (Piggott & Brady, 1970).

The initial stages of the purification are essentially those used in the purification of the low-molecular-weight enzyme: extraction with acetate buffer, pH 5.5, precipitation and fractional re-extraction with ammonium sulphate, and chromatography on DEAE-cellulose at pH 5.8. At the DEAE-cellulose step it was observed that the high-molecular-weight enzyme was eluted over a large number of fractions. This necessitated the duplication of all subsequent steps. The enzyme was further purified on Sephadex G-200. The final preparation, which exhibited two protein bands corresponding to enzymic activity on polyacrylamide-gel electrophoresis, had a specific activity of 30 units/mg. This is about ten times less than the value of 300 units/mg obtained with the purified low-molecular-weight form (Piggott & Brady, 1970).

The relationship between the two forms is being investigated. Attempts to convert the high-molecular-weight form into the low-molecular-weight form with urea, detergents and high salt concentrations have not been successful.

Differences have been found in the K_m values and the activation energies of the two forms.

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Inhibition Kinetics of D-Glyceraldehyde 3-Phosphate Dehydrogenase

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The kinetics of ternary-reactant enzymes have been treated theoretically by a number of authors (Cleland, 1963; Dalziel, 1969). In particular Fromm (1967) has suggested the use of inhibitors for determining the kinetic mechanism of such enzymes. This communication describes the inhibition kinetics of rabbit muscle D-glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12).