The Significance of Alternative Forms of Xanthine Oxidase in Different Mouse Tissues

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Sackler (1965) has shown that at least two forms of xanthine oxidase exist in the rat. On starch-gel electrophoresis the liver enzyme has a slower mobility (more cathodal) than that of the duodenum. The liver xanthine oxidase activity may be influenced by a number of epigenetic factors. Spandoni & Ticca (1968) report that the injection of xanthine into developing rats brings about a premature appearance of the liver enzyme and that continued administration of xanthine induces a 73% increase in activity over the control animals. Investigations by Stirpe & Della Corte (1969) have suggested that a dehydrogenase is the predominant form of the enzyme in rat and human liver *in vivo*, but that it may be converted *in vitro* into the oxidase type by various treatments. Reported here are preliminary investigations on the mouse xanthine oxidase system and the attempts that have been made to clarify the relationships between different tissue types.

Polyacrylamide-gel electrophoresis and starch-gel electrophoresis have revealed that at least two forms of xanthine oxidase also exist in mouse tissues, with the liver enzyme again being more cathodal than the duodenal form. Most of the liver enzyme was precipitated out between 30 and 50% saturation with ammonium sulphate whereas the duodenal form was precipitated out between 40 and 60% saturation with ammonium sulphate. K_m determinations with the substrates xanthine and 2-amino-4-hydroxypteridine revealed little difference, however, between the different enzyme forms. When the electrophoretic gels were stained for xanthine dehydrogenase activity rather than the oxidase activity, it was noted that the kidney enzyme gave a much more intense reaction. Further, the incorporation of phenazine methosulphate into the assay system caused a 600% increase in activity of the kidney enzyme, 300% for the liver and 200% for the duodenal form. The significance of these results is not clear at present, although the possibility that the kidney enzyme is predominantly a dehydrogenase cannot be excluded.

In order to clarify the possible relationship between these enzyme forms various attempts were made to interconvert them. When the liver enzyme was incubated at 0° or 37°C with high concentrations of a crude commercially obtained (BDH Chemicals Ltd., Poole, Dorset, U.K.) trypsin preparation, its electrophoretic mobility was changed so that it behaved like the duodenal

form. Incubation at 37°C, but not at 0°C, with a duodenal extract also converted the liver into the duodenal form. When duodenal xanthine oxidase is prepared in the presence of soya-bean trypsin inhibitor, it subsequently appears to be very similar, electrophoretically, to the liver enzyme. These results may indicate that the different tissue forms may be due to an artifact of preparation, and the significance to other systems is self-evident.

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Conformation Changes in Chemically Modified Alcohol Dehydrogenase

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Various types of modified alcohol dehydrogenases have been prepared by reaction of enzyme thiol and amino groups and have been found to have interesting properties. Thus iodoacetate was found to inactivate liver alcohol dehydrogenase by formation of a reversible complex, followed by irreversible alkylation of a single thiol in each polypeptide chain. The latter occurred by either an internal or an external mechanism (Reynolds & McKinley-McKee, 1969; Reynolds, Morris & McKinley-McKee, 1970). The inactivation kinetics in the presence of other ligands (e.g. nucleotides, coenzymes etc.) enabled their binding to be studied. The experiments supported the hypothesis that the phosphate of AMP and of the AMP moiety of the oxidized and reduced coenzymes were bound by a positively charged lysine residue. Further evidence for this was proved by enzyme modified with pyridoxal 5'-phosphate. Although iodoacetate seems to bind reversibly to the positive lysine residue, the covalently bound carboxymethyl group may not be near this site, particularly if alkylation is external.

In this connexion the carboxymethyl-enzyme proved interesting in having residual activity (Reynolds & McKinley-McKee, 1970). It also bound the oxidized and reduced coenzymes. Although the dissociation of the binary enzyme-coenzyme complexes may not be the rate-limiting steps as with the native enzyme, the binding properties of the carboxymethyl-enzyme were comparatively similar. Binding is certainly not altered as much as would be expected from introducing an extra anionic group into the active site. This suggests that carboxymethylation does not