nine production from homocysteine and serine by extracts of *E. coli* 3/62, a methionine auxotroph specifically lacking the reductase (Guest, Foster & Woods, 1964). Either $H_4PteGlu$ or $H_4PteGlu_3$ serves as cofactor for this process.

Similarly *Coprinus* extracts also stimulate methionine synthesis by extracts of *E. coli* 121/176, another methionine auxotroph, specifically lacking N^5 -methyltetrahydropteroyltriglutamate-homocysteine methyltransferase (Guest, Friedman, Foster, Tejerina & Woods, 1964). Here $H_4PteGlu_3$, but not $H_4PteGlu$, is an effective coenzyme. Like *E. coli*, therefore, *Coprinus* has a methyltransferase specific for conjugated N^5 -methyltetrahydrofolates. This was confirmed by direct assay with $N^5[^{14}C]$ -methyl- $H_4PteGlu_3$ and $N^5[^{14}C]$ -methyl- $H_4PteGlu$. Only the former converts homocysteine into methionine by transmethylation.

However, extracts of *E. coli* grown with added vitamin B_{12} can use N^5 -methyl- $H_4PteGlu$ as a methyl donor in a cobalamin-dependent transmethylation to homocysteine; the same enzyme will also use methylcobalamin as a methyl donor (Woods *et al.* 1964). Even when *Coprinus* is grown with added vitamin B_{12} neither of these activities can be detected in extracts. Further, *Coprinus* contains only traces of endogenous vitamin B_{12} .

Coprinus extracts do not use *S*-adenosylmethionine as a methyl donor to homocysteine, nor does *S*-adenosylmethionine dilute the transfer of radioactivity from $N^5[^{14}C]$ -methyl- $H_4PteGlu_3$ to homocysteine.

We conclude that in *Coprinus* the conversion of homocysteine and serine into methionine is similar to the cobalamin-independent pathway in *E. coli*.

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A Reactive Histidine Residue at the Active Site of Pig Heart Mitochondrial Malate Dehydrogenase

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Pig heart mitochondrial malate dehydrogenase (EC 1.1.1.37) was inactivated by iodoacetamide (3mM). By using radioactive iodoacetamide it was found that 2mol of the reagent were incorporated/mol of enzyme (mol.wt. 70000). The reaction occurred with N-3 of a histidine residue, and from the pH-rate profile pK_a 7.1 was determined for this residue. The enzyme lost no activity on incubation with 50mM-iodoacetate at pH 7.80.

NADH gave essentially complete protection against inactivation by iodoacetamide; NAD^+ , ADP-ribose and *N*-methylnicotinamide in contrast gave only partial protection. The substrates, oxaloacetate and malate, did not protect the enzyme, but a mixture of NAD^+ and oxaloacetate protected completely, demonstrating the formation of the abortive ternary complex enzyme- NAD^+ -oxaloacetate. Oxaloacetate also enhanced protection by ADP-ribose and complete protection could be obtained with the mixture, again demonstrating formation of a ternary complex. Thus the ADP-ribose moiety of the coenzyme is important in promoting substrate binding, perhaps by a conformational change in the enzyme.

From the protection data and use of different alkylating agents, some tentative conclusions have been drawn about the nature of the active site of mitochondrial malate dehydrogenase.

Comparative Effects of Chemical Modification on Human Alkaline Phosphatases

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Human alkaline phosphatases (EC 3.1.3.1) can be divided into at least three classes (see Moss, 1969), but, with the exception of placental phosphatase for which amino acid analyses have been published (Ghosh & Fishman, 1968; Harkness 1968), little is known about the underlying structures of these enzymes. Acetylation of rat intestinal phosphatase (Fishman & Ghosh, 1967) and bovine brain phosphatase (Brunel, Cathala & Saintot, 1969), and treatment of kidney phosphatase with cyanate (Carey & Butterworth, 1969), increases the electrophoretic mobility of the enzymes while decreasing their activity. These treatments have been applied to human hepatic, intestinal and

placental phosphatases so that their effects on enzymes of the three categories could be compared. Enzyme samples were treated with 0.6M-cyanate at pH 10 and 37°C (Carey & Butterworth, 1969) or with 0.1M-acetic anhydride in half-saturated sodium acetate at pH 8 and 0°C (Brunel *et al.* 1969), followed by dialysis against tris-HCl buffer, pH 8.

Compared with control samples all three enzymes were decreased in activity by both reagents by up to 80%, the enzymes from the three tissues differing in the degree of inactivation produced. The inorganic pyrophosphatase activities of the phosphatases were depressed approximately in parallel with their orthophosphatase action. Loss of activity was not accompanied by a change in catalytic characteristics: e.g. K_m values for the hydrolysis of *p*-nitrophenyl phosphate were unaltered and the treated samples responded similarly to activation by Mg^{2+} ions.

Both acetylation and cyanate treatment increased the mobility of the phosphatases on starch-gel electrophoresis, but the three enzymes were affected differently. The mobilities of placental and intestinal phosphatases increased by approx. 20–30%, but that of the liver enzyme by only about 5% in parallel electrophoretic runs. When enzyme extracts in which minor active zones were present were treated these zones were also accelerated.

Cyanate and acetic anhydride probably act similarly, by decreasing the number of positive charges due to ionizable amino groups on the enzyme molecules, and the smaller effect on the mobility of liver phosphatase presumably indicates the existence of fewer of these groups in the molecule of this enzyme than in those from the other two tissues. The similar effects on the mobilities of intestinal and placental phosphatases show a further resemblance between these enzymes. The loss of activity on treatment without a change in catalytic characteristics suggests a general modification of the enzymes, rather than a specific action on groups in the active centres. An ϵ -amino group of lysine has been implicated in the activity of rat intestinal phosphatase (Fishman & Ghosh, 1967), but may not be reactive under these conditions.

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Hydrolysis of Indoxyl Acetate by Bovine Testicular Hyaluronidase Preparations

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Assay procedures generally employed for the assay of mammalian hyaluronidases have many disadvantages. It has been shown by Guilbault, Kramer & Hackley (1967) that crude bovine testicular hyaluronidase preparations can deacetylate indoxyl acetate (deacetylase activity) below pH 7.0 to yield the fluorescent Indigo White. These authors suggest that this property provides a basis for a convenient assay for hyaluronidase and have detailed the procedure to be followed.

In the present study deacetylase activity was examined for different crude commercial bovine testicular hyaluronidase preparations [Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K., 300 i.u./mg; Sigma (London) Chemical Co. Ltd., London S.W.6, U.K., 350 i.u./mg; BDH Chemicals Ltd., Poole, Dorset, U.K., 350 i.u./mg] and for a highly purified preparation kindly supplied by Dr B. Högberg of A. B. Leo, Helsingborg, Sweden (38000 i.u./mg). In spite of the fact that the Leo hyaluronidase is about 100 times as pure as the other preparations its deacetylase activity was of the same order as those preparations, suggesting that the two activities are not related. The Koch-Light and Leo preparations were then selected for more detailed studies. In each case concentrations of polyphlorethin phosphate that were able to inhibit hyaluronidase activity completely (cf. Diczfalussy *et al.* 1953) were without effect on the deacetylase activity. Hyaluronidase was stable at pH 2.2 at 0°C, whereas deacetylase activity was lost under these conditions. At pH 6.0 hyaluronidase activity was lost between 60°C and 65°C, conditions under which deacetylase activity was retained, whereas diluted enzyme preparation (100 µg/ml) lost hyaluronidase activity but retained deacetylase activity when shaken mechanically at 37°C.

Gel chromatography on Sephadex gave different elution diagrams for the two activities. Similar elution diagrams on Sephadex G-100 and G-200 were obtained for the hyaluronidase activity of either the Koch-Light or Leo preparation. Elution diagrams for the deacetylase activity showed differences for each preparation; the Koch-Light preparation had one peak of activity, which was eluted behind the hyaluronidase peak, the Leo preparation showed two peaks of activity, which were eluted in advance of and after the hyaluronidase activity, although complete separation of the activities was not achieved.

These and other results suggest that hyaluroni-