Agarose isoelectric focusing showed that both AMF and DAP, in common with many other glycoproteins, separated as distinct, closely aligned bands. DAP had a mean pI of 5.12 with a range of 5.05-5.20 and AMF, in agreement with previous reports, a mean pI of 4.75 and a range 4.7-4.8.

After trauma all the purified proteins from serum and liver contained only AMF-like protein (Table 1). The presence of AMF in liver confirms this organ as a source of the protein, although blood lymphocytes may be implicated in its synthesis (Panrucker & Lorscheider 1984).

On day 12 of pregnancy decidual tissue contained only the DAP-like protein. Neither protein was found in the maternal liver, and despite the presence of high concentrations in the decidua, only traces of DAP were found in maternal serum, most being in the AMF form (Table 1). This serum protein may be synthesized by other maternal tissues (Panrucker & Lorscheider, 1984) or possibly be derived from decidual DAP. Later in pregnancy (day 20) the foetal liver contains only AMF-like protein (Table 1). The foetal liver is known to synthesize macrofoetoprotein (Weimer et al., 1967; Sarcione & Bohne, 1969) and on day 20 of pregnancy this is found to be entirely of the AMF form. In the maternal serum, at this time, only traces of DAP-like protein are found but with substantial amounts of the AMF form. This protein persists in the maternal serum on day 1 post partum and its absence from maternal liver (Table 1) indicates that it may originate from previous secretion by the foetal liver.

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Tightly bound metals in cyclic nucleotide phosphodiesterases

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Factors involved in the function of cyclic nucleotide phosphodiesterases are interesting because their malfunction will disturb cyclic AMP levels, which affect hormonal and neural transmission. The enzymes nearly all require free bivalent metal ions, probably Mg$^{2+}$ in vitro, though Mn$^{2+}$, Co$^{2+}$ and some others can often substitute in vitro. The Mg$^{2+}$-independent high-$K_{m}$ (Londesborough & Suoranta, 1983) and Mg$^{2+}$-dependent low-$K_{m}$ (Suoranta & Londesborough, 1984) enzymes from yeast contain zinc and can be inactivated by metal chelators and reactivated by specific bivalent metal ions such as Zn$^{2+}$. Are the mammalian enzymes also metalloproteins, whose levels and properties might depend on the bivalent metal ion status of the animals and on losses and substitutions of metals during purification? The effects of metal chelators on two partially purified mammalian phosphodiesterases are described here.

Salt extracts of rat liver plasma membranes containing the peripheral phosphodiesterase were prepared as described by Marchmont et al. (1981). The enzyme was progressively inactivated by the metal chelators 8-hydroxyquinoline and o-phenanthroline (but not by the non-chelating m-phenanthroline), and re-activated by Zn$^{2+}$ or Mn$^{2+}$, but not by Mg$^{2+}$, Ca$^{2+}$, Cd$^{2+}$, Co$^{2+}$, Cu$^{2+}$ or Fe$^{2+}$ (Londesborough, 1984).

Bovine heart calmodulin-dependent phosphodiesterase from Sigma Chemical Co. (sp. activity, 0.16 units/mg; calmodulin activation, 6.1-fold) was assayed (Suoranta & Londesborough, 1984) at 30°C and pH7.4 in 25 mM-Tris/HCl buffer containing 25 mM-imidazole/HCl, 2.5 mM-MGCl$_2$, 2 mg of albumin/ml, 10 units of calmodulin/ml, 40 mM-CaCl$_2$, and 50 mM-cyclic [1H]AMP. o-Phenanthroline and 8-hydroxyquinoline caused progressive inactivations in buffer A [2.5 mg of albumin/ml of 35 mM-Pipes (1,4-piperazinediethanesulfonic acid)/KOH/25 mM-EDTA/1 mM-2-mercaptoethanol, pH 7.0] that were not reversed by dialysis against buffer B (20 mM-Tris/HCl/1 mM-imidazole/1 mM-MGCl$_2$/50 mM-NCaCl/7 mM-2-mercaptoethanol/50 mM-EDTA, pH 7.4) unless zinc acetate was included (Fig. 1). Addition of Zn$^{2+}$ after the buffer exchange caused reactivation, but Cd$^{2+}$ and Mn$^{2+}$ were much less effective. Untreated enzyme was unaffected by up to 1000 mM-Zn$^{2+}$ in assays. With o-phenanthroline, activities at 50 mM and 500 mM-cyclic AMP were lost at the same rate. The basal and
Alterations of dihydropteridine reductase activity by lead

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5,6,7,8-Tetrahydrobiopterin (BH₄) functions as the essential cofactor for the phenylalanine hydroxylase-catalysed conversion of phenylalanine to tyrosine, the conversion of tryptophan to 5-hydroxytryptophan, catalysed by tryptophan hydroxylase, and for the rate-controlling enzyme of catecholamine synthesis, tyrosine hydroxylase, which converts tyrosine to dihydroxyphenylalanine. The cellular concentration of tetrahydrobiopterin is believed to determine the level of tyrosine hydroxylase activity (Kettler et al., 1974) and thus the rate of catecholamine synthesis.

Abbreviation used: DHPR, dihydropteridine reductase.

DHPR (EC 1.6.99.7) is responsible for the reconversion of the labile species quinonoid dihydrobiopterin, formed during the hydroxylation reactions, back into the active cofactor tetrahydrobiopterin. In conjunction with the pathway of tetrahydrobiopterin synthesis de novo, this salvage path catalysed by DHPR is important in maintaining the cellular concentrations of the enzyme cofactor. Tissues usually contain sufficient DHPR to maintain the cofactor in the fully reduced state (Craine et al., 1972). Lead, a known neurotoxin, has in previous studies in vitro been implicated as an agent capable of altering the activity of DHPR in both rat brain and liver (Purdy et al., 1981). In addition, studies in man have suggested an inhibitory effect of lead on the salvage pathway (McIntosh et al., 1980; Blair et al., 1982). This study investigates the possible effect of lead on DHPR activity by using a rat model.

Male Sprague-Dawley rats were exposed to two concentrations of lead acetate (120 and 480μM) in their drinking water from weaning; a control group of rats received distilled water. Food and water were supplied ad libitum.

After a period of 4 or 12 weeks, the animals were killed by decapitation and the brains removed along with a sample of blood and a femur into lead free tubes. Freehand dissection

Fig. 1. Effect of phenanthrolines and hydroxyquinolines on calmodulin-dependent phosphodiesterase

Enzyme was incubated at 30°C in buffer A containing 2.9 mM-reagent [a: (■) none, (△) m-, (▽) p- and (○) 8-hydroxyquinoline, (▲) p- and (○) none, (△) 8- and 4-hydroxyquinolines and then dialysed (broken lines) against buffer B with or without 80μM-zinc acetate. Where shown, 940μM-metal salts were added after dialysis. For (■) and (○) assays were at 50μM-cyclic AMP. Other assays were at 50μM-cyclic AMP, and (△, ▲, ▽ and ○) contained also 280μM-zinc acetate.

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