The compartmentation of substantial amounts of transketolase and transaldolase in the plastid is in keeping with the view that all the enzymes of the pentose phosphate pathway are at least partially located in this organelle. In addition this provides further support for the view that a major function of the pentose phosphate pathway in non-photosynthetic cells is to provide NADPH for nitrite assimilation (Sarkissian & Fowler, 1974), the enzymes of which are also located in the plastid (Fowler & Barker, 1977; M. J. Emes & M. W. Fowler, unpublished work).

M. J. E. thanks the Science Research Council for a postgraduate studentship.

Schnarrenberger, C., Oester, A. & Tolbert, N. E. (1972) Plant Physiol. 50, 55–59

Influence of Magnesium Sulphate on the Inhibition of Human Placental Oestriadiol-17β Dehydrogenase by Adenosine Triphosphate

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In Mg²⁺-free buffers (e.g. Tris/sulphate buffer, pH 8.0), NaATP markedly inhibited the reduction of oestrone by human placental oestriadiol-17β dehydrogenase (EC 1.1.1.62) when NADH was coenzyme; changes in reaction rate were much smaller when NADPH was coenzyme (Shaw & Jeffery, 1976). These effects occurred at concentrations of free ATP comparable with the concentrations of total ATP common in vivo. It is generally held that ATP occurs in vivo mainly as the Mg²⁺ complex (e.g. Lehninger, 1975). We have therefore investigated the effect of ATP in the presence of MgSO₄. We chose MgSO₄ because this enzyme is relatively insensitive to sulphate concentration, though it is inhibited by nitrate, chloride and acetate (Warren & Cheatum, 1966).

The enzyme was prepared by homogenizing fresh placenta in suitable buffer, precipitating a broad fraction with (NH₄)₂SO₄ and chromatographing the crude material twice on DEAE-cellulose to obtain a solution of highly purified (but not homogeneous) enzyme. This enzyme preparation was relatively stable in solutions containing 50% (v/v) glycerol, in which form it was stored and used.

Reaction was carried out at 37°C and pH 7.4 in a solution containing, in addition to substrate, coenzyme, and enzyme, potassium phosphate (20mm), glycerol (5% v/v), ethanol (3.33%, v/v) and bovine serum albumin (1mg/ml). The amount of enzyme solution was chosen to give a reaction rate of between 1 and 3nmol of coenzyme oxidized/min in the absence of both ATP and MgSO₄. The reaction was followed spectrophotometrically at 340nm with an Acta model M-VI spectrophotometer (Beckman Instruments, Glenrothes, U.K.). This technique was superior to a sensitive fluorimetric technique previously used by us (Shaw & Jeffery, 1976) because it avoided com-
### Table 1. Reaction rates for the human placental oestradiol-17β dehydrogenase preparation with different substrates and coenzymes

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reaction rate (nmol of coenzyme oxidized/min per ml of enzyme solution)</th>
<th>Rate ratio for NADH/NADPH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reaction rate NADH (50 μM) NADPH (50 μM)</td>
<td></td>
</tr>
<tr>
<td>Oestrone (30 μM)</td>
<td>67.6 15.0</td>
<td>4.51</td>
</tr>
<tr>
<td>16α-Hydroxyoestrone (75 μM)</td>
<td>9.54 28.1</td>
<td>0.34</td>
</tr>
<tr>
<td>Rate ratio for oestrone/16α-hydroxyoestrone</td>
<td>7.09 0.53</td>
<td>—</td>
</tr>
</tbody>
</table>

### Table 2. Percentage changes caused by ATP, MgSO₄, and ATP plus MgSO₄ in the reaction rate for oestradiol-17β dehydrogenase from human placenta

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Change in reaction rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NADH (50 μM) NADPH (50 μM)</td>
</tr>
<tr>
<td>Oestrone (30 μM)</td>
<td></td>
</tr>
<tr>
<td>ATP (500 μM)</td>
<td>-83</td>
</tr>
<tr>
<td>MgSO₄ (1 mM)</td>
<td>+4</td>
</tr>
<tr>
<td>ATP (500 μM) plus MgSO₄ (1 mM)</td>
<td>+34</td>
</tr>
<tr>
<td>16α-Hydroxyoestrone (75 μM)</td>
<td></td>
</tr>
<tr>
<td>ATP (500 μM)</td>
<td>-95</td>
</tr>
<tr>
<td>MgSO₄ (1 mM)</td>
<td>-14</td>
</tr>
<tr>
<td>ATP (500 μM) plus MgSO₄ (1 mM)</td>
<td>-84</td>
</tr>
</tbody>
</table>

Applications arising from quite large changes in fluorescence yield associated with small changes in experimental conditions.

The two substrates used (oestrone and 16α-hydroxyoestrone) behaved somewhat differently with the two coenzymes (NADH and NADPH) (Table 1). With NADH as coenzyme, the very marked inhibition caused by NaATP (500 μM) was lower in the presence of MgSO₄ (1 mM), particularly with oestrone as substrate (Table 2). Small stimulations, found when NADPH was coenzyme (Table 2), were probably non-specific effects.

An important function of this enzyme in vivo (i.e. in human placenta) may be to facilitate the transfer of hydrogen from NADPH to 16α-hydroxyoestrone to give oestriol (the most abundant oestrogen of normal human late pregnancy). Our findings (Tables 1 and 2) considered together with what is known of the concentration in human placenta of NADPH (10 μM), the Michaelis constant for NADPH (<1 μM), and the concentration of ATP (probably within the concentration range 1–6 mM, as MgATP), indicate that changes in the concentration of ATP have little direct effect on this conversion.

The situation is less clear for the conversion of oestrone into oestradiol-17β, for which NADH or NADPH could serve as coenzyme, and the reverse of this reaction, for which NAD⁺ or NADP⁺ could serve. However, small changes in the concentration of MgATP within the concentration range 1–6 mM, such as might occur in vivo, would have relatively small effects, and would seem unlikely to provide the basis for any important regulatory mechanism. The presence in vivo of a separate transhydrogenase that is activated by oestradiol-17β but not by oestrone should be borne in mind (Karavolas et al., 1969).
In the absence of Mg\(^{2+}\), the inhibition by NaATP (200 µM) of the reduction of oestrone (30 µM) by the enzyme, with NADH (50, 100, 150 and 200 µM) as coenzyme, was characterized by an increase in the apparent Michaelis constant (from about 10 µM to about 150 µM) without appreciable change in the apparent maximum velocity. This gave a competitive inhibition constant for ATP of about 14.4 µM. It is consistent with the view that ATP binds reversibly to the NADH-binding site of the enzyme.

Regarding the small effects when NADPH was coenzyme, we suggest that, because the Michaelis constant for NADPH is lower than that for NADH, Na-ATP would be less effective as a competitive inhibitor with NADPH, and other effects (including non-specific activations) might have a greater influence on the reaction rate under the conditions used.

We thank Professor H. M. Keir for his interest and encouragement, Mrs. Laura Selway for skilled technical assistance, and the Medical Research Council for a project grant.


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**Co-operativity in Chlorpromazine–Human Serum Albumin Interactions**

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Association constants (K) and numbers of sites (n) for the binding of chlorpromazine to serum albumin (s) have been reported by a number of workers using various techniques [bovine (Jahnchen et al., 1969; Nambu & Nagai, 1972); human (Novel & Champion, 1973; Huang & Gabay, 1974; Sharples, 1974)]. There are considerable discrepancies between the results, many of which were obtained with chlorpromazine concentrations beyond the theoretical solubility limits of the drug at the pH values used. Because of this it was felt necessary to re-investigate the interaction of chlorpromazine with human serum albumin by using lower drug concentrations and lower drug/protein ratios than reported previously.

Experimental methods using ultrafiltration devices were found to give non-reproducible results because of the large and erratically reversible binding of chlorpromazine to the membranes used (Amicon PM-10 membranes, CF 50A or CF 25 membrane cones and Pellicon PTGC01310 membrane discs).

Equilibrium dialysis with Visking tubing in standard glass tubes was found to give acceptable results, with negligible binding of the substrate to the apparatus. Chlorpromazine solutions at concentrations ranging from 7 to 112.4 µM were placed inside and outside a dialysis bag containing 12.8 µM-human serum albumin. Tubes were left shaking in the dark overnight at 20°C. Tube contents outside the bag were analysed for chlorpromazine spectrophotometrically at 254 nm. In experiments using [³H]chlorpromazine, the drug concentrations both inside and outside the bag were determined by liquid-scintillation counting. Solutions outside the bag were checked for protein by the method of Lowry et al. (1951) and any showing signs of protein leakage were discarded. The purity of the chlorpromazine was checked before and after equilibrium was reached. Under the conditions used there was no detectable degradation of the chlorpromazine during the experiment. For comparison, the binding isotherm of phenylbutazone was determined by the same method as for chlorpromazine.

Fig. 1 shows the binding isotherm for chlorpromazine and that for phenylbutazone. The chlorpromazine isotherm is clearly sigmoidal in shape at the drug/protein ratios used.