that the 70% rise in the serine/leucine incorporation ratio in secreted protein from oestrogen-treated liver reflects the synthesis of the yolk protein phosphatidylserine, which has a high serine content. The serine/leucine incorporation ratio of non-secreted protein increased by only 10–20% in oestrogen-treated liver.

In the presence of inhibitors of RNA synthesis (actinomycin D or α -amanitin) inhibition of secretory protein production by oestrogen-treated explants was apparent only after 2–3 days, suggesting that the RNA species necessary for yolk protein synthesis and secretion are comparatively stable.

Liver from control male animals did not respond to oestradiol *in vitro*.

M. J. C. is a Beit Memorial Research Fellow.

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Temperature-Jump Studies on Glutamate Dehydrogenase

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Relaxation kinetics is capable of providing considerable information about the rate constants for the various steps in enzyme-catalysed reactions (Hammes, 1968). Most studies so far have used light-absorption to follow relaxation processes, but fluorescence detection, as used here, has many advantages, particularly for the dehydrogenases, where the coenzyme NADH is fluorescent.

Glutamate dehydrogenase from ox liver was studied as a model for allosteric enzymes. It has been suggested that the rate-limiting step in glutamate oxidation may be the release of NADH and that the inhibitor GTP and the activator ADP act by decreasing and increasing (Iwatsubo & Pantaloni, 1967) respectively the rate of this step. Direct measurement of these rate constants by the temperature-jump method by using fluorescence detection showed that NADH release is two orders of magnitude faster than the overall turnover of the enzyme and hence cannot be rate-limiting. It was found that GTP and ADP do affect the rate of NADH release in the manner predicted, but not sufficiently for this to account for their effects on the enzyme's activity.

From the values of the rate constants the equilibrium constant for enzyme-coenzyme binding was

calculated to be $200\mu\text{M}$, in good agreement with a previously published value (Yielding & Holt, 1967).

It is fairly well established that NADH binds to two sites on the enzyme (Jallon & Iwatsubo, 1971), and hence there is the possibility that binding studies are not relevant to the catalytic mechanism. NADPH binds only to the active site, and a comparison of the two coenzymes shows that the same site is involved in the reaction being followed here, confirming the validity of the above conclusions. These data provide a basis for a model of the spatial relationships of the various substrate-binding sites and the site involved in aggregation.

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Haem-Haem Interactions Associated with Ligand Binding by Cytochrome Oxidase

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The classical CO-ferrocycytochrome a_3 photochemical action spectrum shows no contribution from cytochrome a , nor does the latter's redox state have any effect on the spectrum of the former (Nicholls, 1963). But ligand binding to ferricytochrome a_3 does affect cytochrome a . All terminal respiratory inhibitors can form at least two complexes with cytochrome aa_3 : a cytochrome $a^{2+}a_3^{3+}$ -inhibitor complex and a cytochrome $a^{3+}a_3^{3+}$ -inhibitor complex. The cytochrome $a^{3+}a_3^{3+}$ -azide complex (Muijsers *et al.*, 1968) has similar e.p.r. (electron-paramagnetic-resonance) and visible spectra to cytochrome $a^{3+}a_3^{3+}$ (van Gelder & Beinert, 1969) and $K_d = 25\mu\text{M}$ (pH 7). The cytochrome $a^{2+}a_3^{3+}$ -azide complex shows a shift of the cytochrome a^{2+} α -peak (Gilmour *et al.*, 1967), a unique low-spin e.p.r. signal and $K_d \geq 25\mu\text{M}$. Formation of the cytochrome $a^{3+}a_3^{3+}$ -cyanide complex involves a high-spin $\rightarrow$ low-spin spectroscopic transition in the cytochrome a_3 haem, occurs very slowly ($2\text{M}^{-1}\cdot\text{s}^{-1}$) and has $K_d = 2\mu\text{M}$. The cytochrome $a^{2+}a_3^{3+}$ -cyanide complex has a normal cytochrome a^{2+} spectrum but is formed more rapidly ($4000\text{M}^{-1}\cdot\text{s}^{-1}$) with $K_d \leq 0.1\mu\text{M}$ (van Buuren *et al.*, 1972a,b).

Similarly, reduction of ferricytochrome a_3 appears to decrease the redox potential of cytochrome a , giving rise to biphasic reduction curves with $n < 0.5$ (Minnaert, 1965). The intermediate half-reduced species (Wilson *et al.*, 1972) may be a mixture of