by the electron-microscopic findings of Widmann & Fahimi (1975) that foetal Kupffer cells are self-proliferating and are not derived from a precursor cell.

The biochemical role of urea formation by macrophages remains to be elucidated in further experiments. As one of the functions of macrophages is phagocytosis, urea formation might be necessary during degradation of endocytosed proteins.

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Isocitrate Dehydrogenase and Malate Dehydrogenase in Synaptic and Non-Synaptic Rat Brain Mitochondria: A Comparison of their Kinetic Constants

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A good deal of evidence suggests that brain mitochondria are heterogeneous both in their enzyme content and complement (Van den Berg, 1973; Lai & Clark, 1976; Lai et al., 1977). However, the connection between the heterogeneity of brain mitochondria and metabolic compartmentation is, at present, poorly understood (Van den Berg, 1973; Lai & Clark, 1976). We have developed a method (Lai & Clark, 1976) whereby a relatively pure and metabolically active fraction of brain mitochondria may be isolated from the synaptosomal fraction; this population of synaptic mitochondria has been shown to have metabolic and other characteristics distinctly different from those of a population of non-synaptic mitochondria prepared by the Clark & Nicklas (1970) method (Lai & Clark, 1976; Dennis et al., 1977). In view of our finding that synaptic mitochondria metabolized pyruvate and glutamate at rates lower than those by non-synaptic mitochondria and that these differences could be partially accounted for by the differences in the Michaelis parameters of the enzymes associated with pyruvate and glutamate metabolism in these two mitochondrial populations (Lai & Clark, 1976; Dennis et al., 1977), we have further studied the kinetic properties of isocitrate dehydrogenase and malate dehydrogenases in these two mitochondrial fractions with the view of ascertaining whether or not these parameters may shed more light on the relations between the heterogeneity of brain mitochondria and metabolic compartmentation.

Non-synaptic and synaptic mitochondria were prepared from eight adult male rat forebrains as previously described (Lai & Clark, 1976). The activities of NAD⁺-linked and
Synaptic and non-synaptic mitochondria were prepared from eight adult male rat forebrains as described by Lai & Clark (1976). $V_{\text{max}}$ (nmol/min per mg of mitochondrial protein) and $K_m$ values were usually obtained from Lineweaver-Burk plots.

<table>
<thead>
<tr>
<th></th>
<th>Non-synaptic mitochondria</th>
<th>Synaptic mitochondria</th>
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<tbody>
<tr>
<td>NAD$^+$-linked isocitrate dehydrogenase</td>
<td>$V_{\text{max}}$</td>
<td>133.3</td>
</tr>
<tr>
<td></td>
<td>$K_m$</td>
<td>91 $\mu$M-NAD$^+$</td>
</tr>
<tr>
<td>NADP$^+$-linked isocitrate dehydrogenase</td>
<td>$V_{\text{max}}$</td>
<td>36.5</td>
</tr>
<tr>
<td></td>
<td>$K_m$</td>
<td>11 $\mu$M-NADP$^+$</td>
</tr>
<tr>
<td>NAD$^+$-linked malate dehydrogenase</td>
<td>$V_{\text{max}}$</td>
<td>10526</td>
</tr>
<tr>
<td></td>
<td>$K_m$</td>
<td>29 $\mu$M-Oxaloacetate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>69 $\mu$M-NADH</td>
</tr>
</tbody>
</table>

NADP$^+$-linked isocitrate dehydrogenase (EC 1.1.1.41) and NAD$^+$-linked malate dehydrogenase (EC 1.1.1.37) were assayed at 25°C as described by Lai & Clark (1976).

**Isocitrate dehydrogenase**

A Lineweaver-Burk plot of NAD$^+$-linked isocitrate dehydrogenase activities in non-synaptic mitochondria at various isocitrate concentrations was a curve concaving upwards indicating that either this enzyme may exist as a mixture of enzymes having different affinities for isocitrate or that it may be an allosteric protein showing the property of positive co-operativity. A similar curve was also obtained with the Lineweaver-Burk plot of NAD$^+$-linked isocitrate dehydrogenase activities in synaptic mitochondria at different isocitrate concentrations. Thus the NAD$^+$-linked isocitrate dehydrogenase in both synaptic and non-synaptic brain mitochondria may be allosteric proteins with the characteristics of positive co-operativity. This is compatible with the observations of Ogasawara *et al.* (1973), who found that the plot of enzyme rates against isocitrate concentrations for partially purified rat brain NAD$^+$-linked isocitrate dehydrogenase was sigmoidal. For NAD$^+$-linked isocitrate dehydrogenase, the $V_{\text{max}}$ and $K_m$ values for NAD$^+$ and isocitrate in synaptic mitochondria were slightly higher than those in non-synaptic mitochondria (Table 1). However, the $V_{\text{max}}$ and $K_m$ values for NADP$^+$-linked isocitrate dehydrogenase in both synaptic and non-synaptic mitochondria were essentially the same (Table 1).

**Malate dehydrogenase**

NAD$^+$-linked malate dehydrogenase in both synaptic and non-synaptic mitochondria appeared to show substrate inhibition at oxaloacetate concentrations greater than 0.4 mM. The $V_{\text{max}}$ and $K_m$ values for NADH and oxaloacetate in synaptic and non-synaptic mitochondria were not markedly different (Table 1).

**Conclusions**

The NAD$^+$-linked isocitrate dehydrogenase in both synaptic and non-synaptic rat brain mitochondria appeared to show some characteristics of an allosteric protein, namely that of positive co-operativity. For NAD$^+$-linked isocitrate dehydrogenase, the $V_{\text{max}}$ and $K_m$ values for NAD$^+$ and isocitrate in synaptic mitochondria were slightly higher than the corresponding values in non-synaptic mitochondria. For NADP$^+$-linked isocitrate dehydrogenase and NAD$^+$-linked malate dehydrogenase, the Michaelis parameters in these two populations of rat brain mitochondria were virtually the same. Thus it is reasonable to conclude that the control of tricarboxylate-cycle metabolism in these two populations of brain mitochondria does not lie at the level of either of these...
two enzymes: in this regard, we have additional evidence to suggest that the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenases may play important roles (Lai et al., 1977; Dennis et al., 1977).

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Phosphoproteins and Related Enzymes in Isolated Synaptic-Junctional Structures

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Brain synaptosomal plasma membranes contain an intrinsic protein kinase that catalyses the transfer of $^{32}$P from $[\gamma^{32}P]$ATP to endogenous membrane proteins (Weller & Rodnight, 1971; Johnson et al., 1971). Isolated synaptic plasma membranes also contain dense-staining junctional structures (Gray, 1959), particularly those of the postsynaptic density (Matus et al., 1975). More recently it has been shown that most of the protein kinase activity and much of the acceptor protein of synaptic membranes is concentrated in detergent-insoluble subfractions (Dunkley et al., 1976; 1977; Weller & Morgan, 1976) that are known to be enriched in postsynaptic junctional structures (Cotman et al., 1974; Matus & Walters, 1975). These isolated postsynaptic-junctional structures can be obtained in two structural forms. One, derived by treating synaptic membranes with Triton X-100, corresponds in morphology to the postsynaptic density seen in intact tissue. Subsequent treatment of these isolated postsynaptic densities with sodium deoxycholate yields a further subfraction consisting of a fibrous network (the postsynaptic lattice) that apparently lies embedded in the postsynaptic density (Cohen et al., 1977; Matus & Jones, 1978).

We have examined the distribution of protein kinase activity and labelled phosphoproteins in isolated synaptosomal plasma membranes, postsynaptic densities isolated with Triton X-100, and postsynaptic lattices prepared with sodium deoxycholate. The synaptosomal membranes were prepared by the method of Jones & Matus (1974), and from them postsynaptic densities or postsynaptic lattices were prepared by detergent treatment and centrifugation as previously described (Matus, 1978). Membrane samples (0.4mg) were phosphorylated by incubation in 1 ml of 30mm-Tris/HCl buffer, pH 7.4, containing 1 $\mu$mol of ATP and 40 pmol of $[\gamma^{32}P]$ATP (The Radiochemical Centre; 15 Ci/mmol). Protein-bound radioactivity was assayed by the method of Rodnight et al. (1975).

The distribution of $^{32}$P-labelled synaptic-membrane phosphoproteins after detergent treatment is shown in Table 1.

The specific activity of protein-bound $^{32}$P in the Triton X-100-insoluble postsynaptic-densities fraction was approximately twice that in untreated synaptic membranes. The specific activity of protein $^{32}$P in the deoxycholate-insoluble postsynaptic lattice fraction was further enriched, being almost 2-fold greater than that in the postsynaptic densities. This experiment was also performed with the addition of 50 $\mu$M-cyclic AMP to the incubation medium. As expected kinase activity was stimulated under these conditions and modest increases of $^{32}$P incorporation over the basal rate occurred in both the synaptic membrane fraction and the two junctional fractions. The relative