enzymes (e.g. CPS and OTC) and the sensitivity (called 'elasticity' by Kacser) of their common intermediate [carbamoyl phosphate (CP) in the case of CPS and OTC]. A low sensitivity corresponds to a high control strength of OTC was calculated to be 0.015, showing that the enzyme exerts hardly any control on the flux under these conditions.

Since no well-characterized inhibitors of CPS are known, this procedure could not be used to determine the control strength of CPS.

Kacser & Burns (1974) have shown that there is a direct relationship between the control strengths of two adjacent enzymes (e.g. CPS and OTC) and the sensitivity (called 'elasticity' by Kacser & Burns, 1974) of the enzymes towards their common intermediate [carbamoyl phosphate (CP) in the case of CPS and OTC]. A low sensitivity corresponds to a high control strength and vice versa, sensitivity being defined as the fractional increase in enzyme rate induced by a fractional change in substrate or product concentration.

The sensitivity of CPS towards CP ($k_{sCP}$) can be calculated from the data of Table 1. 4mM-Norvaline decreases citrulline synthesis from 13.17 to 10.95 nmol·min$^{-1}$·mg protein$^{-1}$, whereas intramitochondrial CP increases from 0.062 to 0.848 nmol·(mg of protein)$^{-1}$. It is clear that CPS is very insensitive towards CP, as reflected in the calculated elasticity coefficient:

$$\frac{dS}{dJ} \approx k_{sCP} \approx 0.013$$

The sensitivity of OTC towards CP ($k_{sOTC}$) was determined by using malonate to inhibit citrulline synthesis (Wanders et al., 1981). 4 mM-Malonate decreased citrulline production from 13.2 to 5.1 nmol·min$^{-1}$·mg protein$^{-1}$; concomitantly, intramitochondrial CP decreased from 0.062 to 0.019 nmol·(mg of protein)$^{-1}$, resulting in an elasticity coefficient of 0.88. Thus OTC is far more sensitive (67-fold) than CPS towards CP. The control strength of CP can now be calculated from the ratio of the two elasticities multiplied by the control strength of OTC as determined via norvaline, giving a value of $0.015 \times (0.88/0.013) = 1.02$. This result shows that, in the presence of saturating concentrations of ornithine, all control is exerted by CPS.

Table 1. The effect of norvaline on citrulline synthesis

<table>
<thead>
<tr>
<th>Norvaline added (mM)</th>
<th>Malonate added (mM)</th>
<th>Citrulline production (nmol·min$^{-1}$·mg protein$^{-1}$)</th>
<th>Ornithine$\text{m}$</th>
<th>Intramitochondrial CP (nmol·(mg of protein)$^{-1}$)</th>
<th>Intramitochondrial ATP (nmol·(mg of protein)$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>13.17</td>
<td>0.50</td>
<td>0.062</td>
<td>12.4</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>10.95</td>
<td>0.23</td>
<td>0.848</td>
<td>12.8</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>8.75</td>
<td>0.53</td>
<td>1.902</td>
<td>12.9</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>7.53</td>
<td>0.57</td>
<td>5.885</td>
<td>12.6</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>4.35</td>
<td>0.58</td>
<td>9.943</td>
<td>13.0</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>5.12</td>
<td></td>
<td>0.019</td>
<td>5.0</td>
</tr>
</tbody>
</table>

where $J$ refers to pathway flux, $I$ to the inhibitor concentration and $s$ to the substrate concentration. The control strength of OTC was calculated by using a $K_s$ for norvaline of 70 mM (Lusty et al., 1979), a $K_s$ for ornithine of 0.4 mM (Lusty et al., 1979) and an intramitochondrial ornithine concentration of 1.5 mM (see Table 1); the term

$$\frac{1}{dJ} / \frac{dS}{dI}$$

is derived from the data of Table 1. By using these values, the control strength of OTC was calculated to be 0.015, showing that the enzyme exerts hardly any control on the flux under these conditions.

The thermodynamic basis for the partial control of oxidative phosphorylation by the adenine-nucleotide translocator

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The question of whether the adenine-nucleotide translocator (AT) is rate-limiting in oxidative phosphorylation has led to confusion because of different interpretations of the term 'rate-limiting'. Higgins (1965) defined the extent to which an enzyme controls the flux through a pathway 'flux Control' (H. V. Westerhoff, A. K. Groen & R. J. A. Wanders, unpublished work), 'control strength' (Higgins, 1965; Kacser & Burns, 1973), or 'sensitivity' (Heinrich & Rapoport, 1974) and thus made it possible to study control in exact terms. Moreover, the sum of all controls on a metabolic flux equals 1 (Kacser & Burns, 1973; Heinrich & Rapoport, 1974). We experimentally established the distribution of the control on respiration among the different enzymes involved in oxidative phosphorylation (Groen et al., 1982); the flux control exerted by any one enzyme rarely exceeds 50% and the controls depend on the work load imposed on the mitochondria.

Kacser & Burns (1973) showed that the distribution of flux controls among enzymes is largely determined by the ratio of their 'elasticities', i.e. by the degrees to which their reactions would be accelerated by increases in the concentrations of...
common substrates. However, the control theory (Higgins, 1965; Heinrich & Rapoport, 1974; H. V. Westerhoff, A. K. Groen & R. J. A. Wanders, unpublished work) did not incorporate compartmentation and had been formulated in terms of concentrations, so that $\Delta W_i$, $\Delta P_i$, and ion gradients could not be accounted for. We have adapted this control theory and derived and measured experimentally which enzyme characteristics must be held responsible for the control on respiration by the AT. We have defined (H. V. Westerhoff, A. K. Groen & R. J. A. Wanders, unpublished work) the $\Delta_{m}H_i\eta$, coefficient of an enzyme i, as (cf. Kacser & Burns, 1973; Heinrich & Rapoport, 1974):

$$
\Delta_{m}H_i\eta \text{def} \frac{1}{\Delta_{m}H_i} \left( \frac{\partial \Delta_{m}H_i}{\partial \Delta_{m}H_j} \right) \Delta G_i, \Delta G_j, \Delta G_k
$$

Like elasticities (Kacser & Burns, 1973), $\Delta G$ coefficients are properties of the individual enzymes and not of the system. We have shown that 'connectivity' theorems (Kacser & Burns, 1973) are also valid for coefficients, which makes it possible to relate flux controls to properties of individual enzymes, e.g.:

$$
Z_{e_{\Delta_{m}H_i}} + Z_{e_{\Delta_{m}H_j}} + Z_{e_{\Delta_{m}H_k}} = Z_{e_{\Delta_{m}H_j}} + Z_{e_{\Delta_{m}H_k}} + Z_{e_{\Delta_{m}H_i}}
$$

(o, p, at, i, pt, st refer to the respiratory chain, $H^+-$ATPase, AT, proton leak, phosphate translocator and substrate translocator respectively; the Z's are flux controls and the e's are coefficients). Since it is difficult to clamp $\Delta G_i\eta$, the experimental determination of $\Delta_{m}H_i\eta$, is not wholly feasible. For the case when $\Delta G_i\eta$ relaxes after a $\Delta_{m}H_i\eta$, we have defined the 'overall phosphorlyative' $\Delta_{m}H_i\eta$ coefficient as:

$$
\Delta_{m}H_i\eta \text{def} \frac{1}{\Delta_{m}H_i} \left( \frac{\partial \Delta_{m}H_i}{\partial \Delta_{m}H_j} \right) \Delta G_i, \Delta G_j, \text{but } \Delta G_k \text{ adjusting}
$$

Then, for well-coupled mitochondria, with low flux control by the $H^+-$ATPase (Groen et al., 1982):

$$
1/Z_{e_{\Delta_{m}H_i}} = 1 + 1/\Delta_{m}H_i/\Delta_{m}H_i
$$

Thus the control on respiration by the AT depends on the effects that $\Delta_{m}H_i\eta$ has on respiration and phosphorylation.

This ratio of $\Delta_{m}H_i\eta$ coefficients equals the ratio of the changes in phosphorylation and oxidation rate upon the addition of a little protonophore. Fig. 1 shows an experiment in which we used an oxygen electrode and a pH electrode (ADP phosphorylation leads to alkalinization). The ratio of the relative changes in the two rates upon addition of protonophore was about 0.3, which gives a mechanistic explanation for a 20% control on respiration by the AT, which is in agreement with the value we determined independently (Groen et al., 1982; Tager et al., 1983; H. V. Westerhoff, A. K. Groen, R. J. A. Wanders, J. A. Bode & K. Van Dam, unpublished work).

**Fig. 1. Determination of the ratio of the two 'overall' $\Delta_{m}H_i\eta$ coefficients near State 3 in oxidative phosphorylation**

Alkalization (-----) is used as a measure of ATP synthesis, the rate of disappearance of oxygen (——) as a measure of oxidation rate. Where indicated, 0.20 mM- (Na)ADP and 54 pmol of S13 (5-chloro-3-1-butil-2'-chloro-4'-nitrosalicylanilide)/mg of protein were added to the suspension of essentially 0.43 mg of mitochondrial protein/ml 6.7 mM-(Na)ATP and 10 mM-succinate.


**Stimulated uptake of inosine in working skeletal muscle**

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Exchange of purine material between tissues has been extensively reported (Lajtha & Vane, 1958; Pritchard et al., 1970, 1975; Murray, 1971) and has often been discussed in terms of purine salvage. The role of skeletal muscle, which constitutes 40% of the body mass (Munro, 1969), is less clear, although it is known that exercising or anoxic muscles lose purines (Alertsen et al., 1958; Nasrallah & Al-Khalid, 1964; Bern et al., 1971), and that preformed purines are readily utilized by skeletal muscle (Woo et al., 1977). In the present experiments we have investigated uptake of various purines during exercise, when one would expect the muscle to be in negative purine balance. Inosine was readily taken up, whereas adenosine and hypoxanthine were not.

Male MFI mice (8–9 weeks old), fed on standard Oxoid diet, were anaesthetized intraperitoneally with Nembutal (60 mg/kg; May & Baker, Dagenham, U.K.). The hamstring group of muscles in the left leg was exposed and stimulated via the sciatic nerve (5 Hz with 0.3 ms 20 V pulses), as described previously.