neogenesis from serine, and it was decided to investigate further the effects of other amino acids on the incorporation of radioactivity from alanine and serine into glucose.

Hepatocytes were prepared from trout fed on a high-protein diet and incubations were performed essentially as described by Walton & Cowey (1979a). Incubations were at 15°C with 30-50 mg of hepatocytes in a total volume of 1.5 ml. The hepatocytes were preincubated for 20 min in the presence of the amino acid (2 mM) before the addition of either 10 mM-[U-14C]alanine or [U-14C]serine. Incubations were stopped at 0, 1, 2 and 3 h by addition of 3 M-HClO₄. After removal of the protein by centrifugation, 0.5 ml of supernatant was shaken for 1 h with 9.5 ml of water and 1.5 g of Amberlite MB3 resin to remove charged compounds. Radioactivity in the remaining neutral compounds (predominantly glucose) was determined and compared with results obtained from incubations performed without additional amino acids.

The results in Table 1 show that most amino acids (2 mM) had little or no effect on gluconeogenesis from serine or alanine. Some stimulation was noted by alanine and methionine with [U-14C]serine as substrate. However, the most striking effect was observed with 2 mM-leucine, which almost doubled the incorporation rates from both serine and alanine. This effect on serine has been very briefly reported previously (Cowey & Walton, 1979). Leucine concentrations in rainbow-trout liver 18 h after feeding are approx. 1 mM (Walton & Cowey, 1982), so the stimulating effects could have physiological significance. Leucine (2 mM) has little effect on gluconeogenesis from 10 mM-pyruvate or lactate in trout hepatocytes, implying that it exerts its effect on the transamination stage, but the means is unknown.


Quantification of the control exerted by different steps during citrulline synthesis in isolated rat liver mitochondria

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Kacska & Burns (1973) and Heinrich & Rapport (1974) have introduced the concept of control strength to quantify the amount of control that an enzyme exerts on the steady-state flux through a metabolic pathway. The control strength (C) of step i in a metabolic pathway is defined as the fractional change in pathway flux induced by a fractional change in the amount of enzyme i. As will be clear from this definition, specific inhibitors might be very useful to determine the control strength of a particular reaction. Indeed, Groen et al. (1982) have recently been able to determine the control strengths of several steps involved in mitochondrial oxidative phosphorylation by using specific inhibitors.

We have used the concept of control strength to determine the contribution of different steps to the control of flux through the citrulline synthesis pathway. In order to determine the control strength of the ornithine transcarbamoylase (OTC) reaction, the effect of norvaline, a competitive inhibitor of isolated ornithine transcarbamoylase (Lusty et al., 1979), was measured. As expected, norvaline inhibited citrulline synthesis in isolated mitochondria (Table 1).

Several lines of evidence suggested that, in rat liver mitochondria, norvaline inhibits pathway flux only via inhibition of OTC. Firstly, norvaline did not seem to interfere with ATP production; intramitochondrial ATP was not affected (Table 1). Secondly, norvaline did not inhibit carbamoylphosphate synthetase (CPS) in mitochondria lysed with Triton X-100 or permeabised with toluene. Thirdly, norvaline did not affect the transport of ornithine (see column 4 of Table 1). These data suggest that, in mitochondria, norvaline exerts its inhibitory action by competitive inhibition of OTC.

Thus norvaline can be used to calculate the control strength of OTC by using the following equation derived by Groen et al. (1982):

\[ C_i = -K_i \left(1 + S/K_m\right) \frac{dU}{dI} \]
enzymes (e.g. CPS and OTC) and the sensitivity (called 'relationship between the control strengths of two adjacent control strength and vice versa, sensitivity being defined as the 'elasticity' by Kacser [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

$$
\frac{1}{J} \frac{dJ}{ds}
$$

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.

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 ($c_{\text{CP}}$) can be calculated from the data of Table 1. 4 mM-Norvaline decreases citrulline synthesis from 13.17 to 10.95 nmol·min$^{-1}$·(mg of 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:

$$
c_{\text{CP}} = \frac{13.17 - 10.95}{0.062} = 0.013
$$

The sensitivity of OTC towards CP ($c_{\text{OTC}}$) 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 of 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 CPS 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 × (0.88/0.013) = 1.02. This result shows that, in the presence of saturating concentrations of ornithine, all control is exerted by CPS.

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 by its '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 discussion of metabolic control in exact terms possible. 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 exceeded 50% and the controls depended 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