Quantification of the importance of individual steps in the control of aromatic amino acid metabolism

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1. The quantitative importance of the individual steps of aromatic amino acid metabolism in rat liver was determined by calculation of the respective Control Coefficients (Strengths). 2. The Control Coefficient of tryptophan 2,3-dioxygenase for tryptophan degradation was determined in a variety of physiological conditions and with a range of activities of tryptophan 2,3-dioxygenase. The Control Coefficient varied from 0.75 with basal enzyme activity to 0.25 after maximal induction of the enzyme by dexamethasone. The remainder of the control for tryptophan degradation was associated with the transport of the amino acid across the plasma membrane, with only very small contributions from kynureninase and kynurenine hydroxylase. 3. The Control Coefficients of tyrosine aminotransferase for tyrosine degradation were approx. 0.70 and 0.20 with basal and dexamethasone-induced tyrosine aminotransferase activities respectively; the Control Coefficients of the transport of the amino acid into the cell were 0.22 and 0.58 respectively. 4. Phenylalanine hydroxylase was found to have a Control Coefficient for the degradation of phenylalanine of approx. 0.50 under conditions of basal enzyme activity; after maximal activation by glucagon, the Control Coefficient decreased to 0.12. The transport of phenylalanine was responsible for the remaining control in the pathway. 5. These results have important implications, directly for the regulation of aromatic amino acid metabolism in the liver, and indirectly for the regulation of neuroamine synthesis in the brain.

INTRODUCTION

The concentrations in the blood of the aromatic amino acids, phenylalanine, tyrosine and tryptophan, result from a balance between dietary intake, protein synthesis and irreversible disposal through metabolism in the liver.

Enzymes of aromatic amino acid catabolism exhibit changes in activity in a number of physiological and pharmacological conditions [1–4], and these changes have been interpreted in terms of the ‘rate-limiting’ roles of particular enzymes, namely tryptophan 2,3-dioxygenase (EC 1.13.11.11), phenylalanine hydroxylase (EC 1.14.16.1) and tyrosine aminotransferase (EC 2.6.1.5) [2, 3, 5–10]. Such interpretations are, however, poorly based, and require more direct evidence. Such evidence may be obtained from measurements, for each reaction, of the forward velocity (as opposed to net flux) and the displacement from equilibrium, and the comparison of these with measurements for other steps in the pathway.

The importance of any one step in the regulation of flux through a pathway under specified conditions is expressed quantitatively by its Control Coefficient (or Strength). The theory supporting this concept was developed by Higgins [11], Kacser & Burns [12] and Heinrich & Rapoport [13] and has been recently reviewed [14, 15]. Practical applications have been discussed and exemplified in several recent papers (e.g. [15–18]).

The transport of the aromatic amino acids by facilitated diffusion has been regarded as being very rapid, and considerably faster than the rates of the subsequent intracellular metabolism [2, 3, 19, 20]. It has, therefore, seemed unlikely that transport was important in controlling metabolic flux. Recent studies in this

laboratory have, however, demonstrated that transport is less fast than assumed hitherto, and that catabolic flux in the cell is sensitive to the addition of inhibitors of the transport system [21, 22].

The concentrations of the three aromatic amino acids in the blood and their ratios are important factors for neurotransmitter synthesis in the brain [23–25]. The activities of tryptophan 2,3-dioxygenase, phenylalanine hydroxylase and tyrosine aminotransferase change as the physiological and nutritional status changes, but not always in the same way and in parallel. The concentrations of the three amino acids in the blood are not regulated simply by the changes in enzymic activities, so that other factors must play a part. Transport into the liver is a plausible factor; this supposition stimulated the present study. We have performed a systematic analysis of the Control Coefficients of components of the systems for the transport and disposal of the aromatic amino acids in liver. The results demonstrate the importance of transport across the plasma membrane for the control of both amino acid catabolism and synthesis of biogenic amines in the central nervous system.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (180–220 g) were used throughout, and were given a standard laboratory diet (CRM diet; Labsure, Poole, Dorset, U.K.) *ad libitum*. Bilateral adrenalectomy was performed as described in ref. [26]; adrenalectomized rats were given 1% (w/v) NaCl instead of drinking water. Rats were given

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dexamethasone phosphate intraperitoneally at 07:30 h (3 mg in 0.9% NaCl/animal) 5 h before liver cell preparation. Diabetes was induced by injection, into the caudal vein, of streptozotocin (80 mg in 0.9% pyrogen-free NaCl/kg body wt.). Diabetes was confirmed by the appearance of glucose in the urine (Clinistix; Ames Co., Slough, Berks., U.K.). Insulin (Neusulin; Wellcome, Beckenham, Kent, U.K.) was administered intraperitoneally twice daily (3 units/animal). Isolated liver cells were prepared from rats 5 days after adrenalectomy and from rats 3 days or 10 days after streptozotocin injection.

Chemicals

All radiolabelled compounds were from Amersham International, Amersham, Bucks., U.K. D- and L-Amino acids and model substrates were from Sigma Chemical Co., Poole, Dorset, U.K. The 'silicone oil' used was a 2:1 (v/v) mixture of Dow Corning 550 and dinonyl phthalate supplied by BDH Chemicals, Poole, Dorset, U.K. The sources of other reagents were as given previously [9, 19].

Purification of radiolabelled compounds

Radiolabelled L-[G-³H]- and L-[benzene-ring-U-¹⁴C]-tryptophan were purified by affinity chromatography [21, 27]. The L-[4-³H]phenylalanine and L-[side-chain-2,3-³H]tyrosine were purified as described in ref. [21].

Preparation and incubation of cells

The procedures were as described previously [21] with the following amendments: (1) perfusions were started at 12:00–13:00 h each day; (2) perfusion media contained 20 mM-D-glucose; (3) cells were washed without albumin in the medium described below; (4) the incubation medium consisted of Krebs buffer [28] containing 2.5 mM-Ca²⁺ and supplemented with 5 mM-D-glucose, 5 mM-L-glutamine (except for experiments with tyrosine, unless stated), 4.5 mM-L-lactate, 0.5 mM-pyruvate and, where relevant, transport inhibitors. The final cell concentration was approx. 3 mg/ml for flux measurements and 6 mg/ml for measurements of amino acid uptake. Fluxes through the pathways of aromatic amino acid metabolism were measured as described previously ([21], and references cited therein), with 50 μ M-L-tryptophan, 50 μ M-L-phenylalanine and 100 μ M-L-tyrosine.

The pathways of aromatic amino metabolism in the liver are shown in Scheme 1.

Briefly, flux from tryptophan through tryptophan 2,3-dioxygenase, kynureninase and picolinate carboxylase was determined by measuring the release of ¹⁴CO₂ and ¹⁴C-labelled non-aromatic products from L-[ring-2-¹⁴C]tryptophan, L-[carboxy-¹⁴C]tryptophan and L-[benzene-ring-U-¹⁴C]tryptophan respectively. The flux from tyrosine through tyrosine aminotransferase was determined by measuring the release of ³H₂O from L-[side-chain-2,3-³H]tyrosine. The flux from phenylalanine through phenylalanine hydroxylase was determined by measuring the release of ³H₂O from L-[4-³H]phenylalanine.

Measurement of amino acid transport

Suspensions of liver cells (1.5 ml; 9 mg dry wt.) were preincubated for 25 min at 37 °C, under an atmosphere of O₂/CO₂ (19:1) and with shaking (100 oscillations/min), before addition of radiolabelled amino acid substrate. At

Scheme 1. Pathways of aromatic amino acid catabolism in liver

Key: (1) transport across plasma membrane (L and T systems); (2) tryptophan dioxygenase (and formamidase); (3) kynurenine hydroxylase; (4) kynureninase; (5) 3-hydroxyanthranilate oxidase; (6) picolinate carboxylase; (7) phenylalanine hydroxylase; (8) tyrosine aminotransferase; (9) 4-hydroxyphenylpyruvate oxidase.

appropriate times, 1 ml portions of the cell suspension were layered on to 250 μ l of the 'silicone oil' and centrifuged (15 s, 12000 g), such that the cells passed through the 'silicone oil' into 100 μ l of 6% (v/v) HClO₄. Rates of transport were calculated from the differences in cell-associated radioactivity between 5 and 12 s (for L-phenylalanine) or between 5 and 15 s (for L-tyrosine and L-tryptophan), over which times transport was linear. The intracellular and contaminating extracellular spaces were determined simultaneously by measurement of the ³H₂O- and [U-¹⁴C]sucrose-permeable spaces as determined by incorporation of label into cell pellets. The intra- and extra-cellular spaces were 2.05 $\pm$ 0.10 μ l/mg dry wt. (mean $\pm$ S.E.M. for three independent observations) and 1.51 $\pm$ 0.07 μ l/mg dry wt. (mean $\pm$ S.E.M. for three independent observations) respectively; these values agree with those obtained previously [3].

The intracellular concentrations of radiolabelled L-tryptophan, L-tyrosine and L-phenylalanine were determined from the time courses of uptake into liver cells. The total cell-associated amino acid was assessed from the L-[4-³H]phenylalanine associated with the cells at steady state (90 s) or by extrapolation of the linear accumulation of radioactive products derived from L-[G-³H]tryptophan or L-[side-chain-2,3-³H]tyrosine to zero time in order to determine the L-tryptophan or L-tyrosine associated with the cells at steady state. There is a rapid binding of aromatic amino acids to liver cells, which is complete within 5 s [29]; this binding was determined by extrapolation of the initial phase of transport to zero time and was subtracted from the total cell-associated material to allow calculation of the intracellular concentration.

The external concentrations of aromatic amino acids used in transport experiments were the same as those used in the determinations of metabolic fluxes.

Fig. 1. Relationship between tryptophan 2,3-dioxygenase activity and flux through the reaction catalysed by the enzyme in intact cells

Details of the procedures for the preparation and incubation of cells and for the measurements of metabolic flux and enzyme activity are given in the Materials and methods section. Flux was measured with 50 μ M-tryptophan and activity in extracts with 2.5 mM-tryptophan. All cell preparations were started at 12:30 h unless otherwise stated. Cells were prepared from rats pretreated in various ways: A, adrenalectomized; B, control; C, starved for 24 h; D, control (cells made at 16:30 h); E, held overnight in laboratory; F, chronic diabetic plus insulin; G, chronic diabetic; H, pretreated with dexamethasone phosphate; I, acute diabetic. All results are means $\pm$ s.e.m. for three separate cell preparations. The inset is a double-reciprocal plot of the data; $J_{\max.} = 10.5$ nmol/h per mg dry wt. and $k = 43$ nmol/h per mg dry wt.

Assay procedures

Tryptophan 2,3-dioxygenase was assayed [30] in cell extracts prepared as described previously [21]. Tyrosine aminotransferase activity was measured radiometrically [31] with 100 μ M-L-[2,3- 3 H]tyrosine; the K_i of this enzyme for amino-oxacetate was determined in liver cell extracts containing 6 mg dry wt./ml.

The viability of cell preparations was assessed on each occasion by measurement of ATP content [32]; all preparations contained more than 8 nmol of ATP/mg dry wt.

RESULTS AND DISCUSSION

Tryptophan metabolism

The relationship between changes in the activity of tryptophan 2,3-dioxygenase measured *in vitro* and corresponding changes in metabolic flux (J) through the enzyme in cell suspensions is demonstrated in Fig. 1. It is apparent that increases in assayable activity, presumably reflecting parallel increases in enzyme protein [33], are not accompanied by proportional changes in the activity expressed in the cell cytosolic compartment.

Empirically the curve in Fig. 1 fits the equation:

$$J = \frac{J_{\max.} \cdot V}{V + k} \quad (1)$$

(where J is the metabolic flux at any point, $J_{\max.}$ is equal to J at infinite V , V is the assayable maximum activity under various conditions, and k is a constant defined as the value of V when $J = J_{\max.}/2$) and the double-reciprocal plot of the data is linear (see inset to Fig. 1). This equation bears an obvious similarity to that which describes the relationship between reaction velocity and substrate concentration for an enzyme obeying Michaelis-Menten kinetics, and has been obtained from theoretical considerations [34]; $J_{\max.}$ and k are complex functions of the kinetic parameters of the enzymes of the pathway.

This non-linear relationship shows that the effect on the flux of changes in the activity of the enzyme depends on the absolute value of that activity. The slope of the

Fig. 2. Relationship between the Control Coefficients of tryptophan transport and tryptophan 2,3-dioxygenase for tryptophan metabolic flux

The curve of Control Coefficients of tryptophan 2,3-dioxygenase (—) was obtained over the range of values of J and V shown in Fig. 1 by using the differentiated form of eqn. (1):

$$\frac{\partial J}{\partial V} = \frac{J_{\max.} \cdot k}{(V + k)^2}$$

The two estimates of control coefficients of tryptophan transport were calculated from eqn. (6) (see the text); the curve shown by ---- was determined assuming that the sum of the Control Coefficients of tryptophan 2,3-dioxygenase and transport is unity (see the text).

tangent drawn at a given point, $\partial J/\partial V$, can be used to evaluate the ratio of the normalized changes:

$$\frac{\partial J/\partial V}{J/V} = C_E^J \quad (2)$$

This expression is designated as the Control Coefficient, where E is any given enzyme [12, 14, 35], and is a measure of the control exercised by that enzyme in the pathway for any given concentration of the enzyme. It is therefore easy to measure the changes in the C_E^J value of tryptophan dioxygenase for tryptophan metabolism, as the amount of the enzyme varies from one condition to another. Fig. 2 expresses this relationship graphically. Thus at low activities of the dioxygenase, such as are found under basal conditions, the enzyme exhibits a high Control Coefficient, and is clearly the major determinant in the regulation of tryptophan disposal; loosely, and in non-quantitative terms, the dioxygenase is sometimes said to be 'rate-limiting'. When the activity becomes greater, however, as after induction by glucocorticoid, the Control Coefficient becomes smaller, reaching, in our experiments, values of only 0.25. Under these conditions, any description of the enzyme as 'rate-limiting' is obviously inappropriate, and a quantitative description of control, namely the value of the Control Coefficient, should replace such terms.

The question now arises: which step, or steps, is, or are, responsible for the residual control in the pathway?

We have previously reported that transport into the cell may be of importance in controlling the further metabolism of tryptophan [22]. We now attempt to quantify the importance of this step.

The effect of a change in the concentration of an external effector (X) on the pathway flux J is defined [12] in terms of the Response Coefficient, R :

$$R_X^J = \frac{\partial J/\partial X}{J/X} \quad (3)$$

The effect of a change in the concentration (X) of any effector on the rate (v_i) of an 'isolated' enzyme is defined in terms of the Elasticity Coefficient ([13]; 'Controllability Coefficient' [12]):

$$\epsilon_X^E = \frac{\partial v_i/\partial X}{v_i/X} \quad (4)$$

The Control Coefficient of an enzyme can be calculated from these coefficients [12], and is given by:

$$C_E^J = R_X^J/\epsilon_X^E \quad (5)$$

The Control Coefficient (C_T^J) of the transport step (T) for tryptophan metabolic flux (J) can be calculated by using a specialized form of eqn. (5), namely eqn. (6):

$$C_T^J = R_{S(out)}^J/\epsilon_{S(out)}^T \quad (6)$$

where $R_{S(out)}^J$ is the Response Coefficient of extracellular tryptophan (S) for tryptophan metabolic flux (J), and $\epsilon_{S(out)}^T$ is the Elasticity Coefficient of extracellular tryptophan (S) for tryptophan transport (T).

$R_{S(out)}^J$ may be readily obtained [36] from eqn. (7):

$$R_{S(out)}^J = \frac{\partial J/\partial S}{J/S} \quad (7)$$

and from a plot of the rate of tryptophan metabolism (J) against various tryptophan concentrations (S) (Fig. 3a).

Groen *et al.* [15] have shown that the Elasticity Coefficient can be expressed as a function of the mass-action ratio (Γ), the equilibrium constant (K) and the maximal rate of the enzyme (V_t). This expression can be used to determine the Elasticity Coefficient of tryptophan transport for extracellular tryptophan (eqn. 8):

$$\epsilon_{S(out)}^T = \frac{1}{1-\Gamma/K} \cdot \left(1 - \frac{J}{V_t}\right) \quad (8)$$

Values for $R_{S(out)}^J$ and $\epsilon_{S(out)}^T$ are shown in Table 1. K was taken as being equal to 1 because transport of the aromatic amino acids into liver cells is non-concentrative

Fig. 3. Concentration-dependence of tryptophan and tyrosine metabolism by isolated liver cells

Details of the procedures for the preparation and incubation of cells and for the measurements of metabolic fluxes are given in the Materials and methods section. The results shown are (a) the tryptophan-concentration-dependence of tryptophan 2,3-dioxygenase flux (● and ○), (b) the tryptophan-concentration-dependence of kynureninase flux (● and ○) and picolinate carboxylase flux (■ and □), and (c) the tyrosine concentration-dependence of tyrosine aminotransferase flux (● and ○). Black symbols refer to basal tryptophan 2,3-dioxygenase activity (a and b) or tyrosine aminotransferase activity (c), open symbols to induced tryptophan 2,3-dioxygenase activity or tyrosine aminotransferase activity. All results are means $\pm$ s.e.m. for three separate cell preparations.

Table 1. Response Coefficients and Elasticity Coefficients of aromatic amino acids

The procedures for the preparation and incubation of cells and measurements of concentrations and rates of reactions were as described in the Materials and methods section. Elasticity Coefficients were calculated by using equations given in the text. The Response Coefficient of extracellular phenylalanine for phenylalanine metabolic flux was calculated from data in ref. [45]. Response Coefficients of tryptophan and tyrosine were calculated from Figs. 3(a) and 3(c). The cytosolic concentrations of glutamate and 2-oxoglutarate were taken from ref. [62], and those of 4-hydroxyphenylpyruvate were calculated from the kinetics of 4-hydroxyphenylpyruvate oxidase [63]. V' values for tyrosine aminotransferase (the rates of reaction at saturating tyrosine concentrations and with other reactants at 'pathway' concentrations) were 100 and 200 nmol/h per mg dry wt. for basal and dexamethasone-stimulated conditions respectively. The equilibrium constants for tyrosine aminotransferase and transport were taken as 0.73 [64] and 1.0 respectively. Abbreviations: TRP, tryptophan; TYR, tyrosine; PHE, phenylalanine; T, transport; TAT, tyrosine aminotransferase; o , extracellular; i , intracellular.

Enzyme	[Substrate] (μM)	[Product] (μM)	Transport maximal activity, V (nmol/h per mg dry wt.)	Metabolic flux, J (nmol/h per mg dry wt.)	Response Coefficient of amino acid for flux	Elasticity Coefficient	Eqn. no(s.)
Tryptophan dioxxygenase							
Basal	TRP $_{o}$, 50	TRP $_i$, 37.5	198	2.52	$R_{\text{TRP}_o}^J$, 1.00	$e_{\text{TRP}_o}^T$, 3.95	(7), (8)
Induced	TRP $_{o}$, 50	TRP $_i$, 12.5	198	7.52	$R_{\text{TRP}_o}^J$, 1.00	$e_{\text{TRP}_o}^T$, 1.28	(7), (8)
Tyrosine aminotransferase							
Basal	TYR $_{o}$, 100	TYR $_i$, 78	171	3.51	$R_{\text{TYR}_o}^J$, 1.00	$e_{\text{TYR}_o}^T$, 4.45	(7), (8)
Induced	TYR $_{o}$, 100	TYR $_i$, 45	171	8.53	$R_{\text{TYR}_o}^J$, 1.00	$e_{\text{TYR}_o}^T$, 1.73	(7), (8)
Basal	TYR $_i$, 78	TYR $_{o}$, 100	171	3.51	—	$e_{\text{TYR}_i}^T$, 4.64	(13)
Basal	TYR $_i$, 78; 2-oxoglutarate $_i$, 310	4-Hydroxyphenyl pyruvate $_i$, 0.75 glutamate $_i$, 4580	—	3.51	—	e_{TAT}^T , 1.20	(8)
Induced	TYR $_i$, 45	TYR $_{o}$, 100	171	8.53	—	$e_{\text{TYR}_i}^T$, 1.90	(13)
Induced	TYR $_i$, 45; 2-oxoglutarate $_i$, 310	4-Hydroxyphenyl pyruvate $_i$, 1.86; glutamate $_i$, 4580	—	8.53	—	$e_{\text{TYR}_i}^T$, 5.85	(8)
Phenylalanine hydroxylase							
Basal	PHE $_{o}$, 50	PHE $_i$, 25	222	5.06	$R_{\text{PHE}_o}^J$, 1.20	$e_{\text{PHE}_o}^T$, 1.96	(7), (8)
+ Glucagon	PHE $_{o}$, 50	PHE $_i$, 5	222	8.90	$R_{\text{PHE}_o}^J$, 1.00	$e_{\text{PHE}_o}^T$, 1.07	(7), (8)

Table 2. Control Coefficients for metabolism of the aromatic amino acids through tryptophan dioxygenase, tyrosine aminotransferase and phenylalanine hydroxylase

Control Coefficients were calculated as indicated in the text. All values are means $\pm$ S.E.M. for three separate liver cell preparations. Abbreviations: J, metabolic flux; POH, phenylalanine hydroxylase; T, transport; TAT, tyrosine aminotransferase; TDO, tryptophan 2,3-dioxygenase.

Conditions	Control Coefficients						Method of determination
	Tryptophan metabolism		Tyrosine metabolism		Phenylalanine metabolism		
	Transport	Tryptophan dioxygenase	Transport	Tyrosine amino-transferase	Transport	Phenylalanine hydroxylase	
Basal		0.75 ± 0.03					Hormonal manipulation of enzymic activity; eqn. (2)
Induced		0.25 ± 0.01*					
Basal	0.22 ± 0.01			0.71 ± 0.02			Inhibitor titration; eqn. (10)
Induced	0.70 ± 0.03*			0.29 ± 0.01*			
Basal				0.71 ± 0.02			Inhibitor titration; eqn. (15)
Induced				0.29 ± 0.01*			
Basal	0.25 ± 0.01		0.22 ± 0.01		0.61 ± 0.01		Determination of Response Coefficients and Elasticity Coefficients; eqns. (6), (7) and (8)
Induced/ phosphorylated	0.78 ± 0.02*		0.58 ± 0.02*		0.93 ± 0.03*		
Basal				0.85 ± 0.10			Ratios of Elasticity Coefficients; eqns. (6), (8), (13) and (14)
Induced				0.19 ± 0.03*			
Basal				0.63 ± 0.09			Measurement of intermediates and kinetic parameters; eqns. (6) and (16)
Induced				0.08 ± 0.02*			
Basal	0.25 ± 0.01	0.75 ± 0.01			0.49 ± 0.01	0.51 ± 0.01	Theoretical calculation; eqn. (2) and Table 3
Induced/ phosphorylated	0.74 ± 0.01*	0.26 ± 0.01*			0.88 ± 0.01*	0.12 ± 0.01*	

* *P* < 0.005 versus corresponding basal values, (Student's *t* test).

* $P < 0.005$ versus corresponding basal values (Student's t test).

[29]. The calculated Control Coefficients of tryptophan transport obtained by this method are shown in Table 2.

When the activity of tryptophan 2,3-dioxygenase is low, the Control Coefficient of transport is 0.25 (Table 2). When the dioxygenase activity is maximally induced, this value is increased to 0.78. When the two Control Coefficients, for transport and dioxygenase, are added together, both at basal and induced activities of the enzyme, their sum is approximately unity. The summation theorem [12, 13] had established that the sum of all Control Coefficients in a pathway must add up to unity. This means that the Control Coefficients of the remaining steps (see Scheme 1) must be very small. This is discussed further below. It therefore appears that, apart from the dioxygenase, the only other important factor controlling the flux is the transport system (Fig. 2). At high activities of the induced enzyme, the transport system is the most important. In fact the apparent maximal flux ($J_{\max}$) at a tryptophan concentration of 50 μ M, obtained from a reciprocal plot of the data of Fig. 1 (see Fig. 1 inset), of 10.5 nmol/h per mg dry wt. appears to be largely determined by the unidirectional rate of inward transport

(10.1 $\pm$ 0.7 nmol/h per mg dry wt.; mean $\pm$ S.E.M. for three independent observations).

An alternative way of estimating the value of C_T^J is with the use of an external inhibitor of transport, such as phenylalanine. An analogous relationship to eqn. (6) can be used where the external effector is an inhibitor (I):

$$R_I^J = C_T^J \cdot e_I^T \quad (9)$$

By determining the effect of an inhibitor on both the pathway flux (J) and the reaction rate of the enzyme under the original pathway conditions (v_i ; see below) when $I \rightarrow 0$, we can obtain the Response Coefficient and Elasticity Coefficient, and therefore the Control Coefficient, of the enzyme. The appropriate expression given by Groen *et al.* [15, 16] is shown in eqn. (10):

$$\left(\frac{\partial J / \partial v_i}{J \cdot \partial I / v_i \cdot \partial I} \right)_{I \rightarrow 0} = C_E^J \quad (10)$$

Fig. 4 shows the fractional effect of phenylalanine concentration (I) on metabolic flux (J) and the rate of unidirectional transport of tryptophan into the cell (v_t).

Fig. 4. Effect of L-phenylalanine on tryptophan transport and metabolic flux through tryptophan 2,3-dioxygenase

Details of the procedures for the preparation and incubation of cells and for the measurements of metabolic flux and rates of transport are given in the Materials and methods section. The fluxes through the dioxygenase in the absence of L-phenylalanine were 2.55 ± 0.20 and 7.50 ± 0.50 nmol/h per mg dry wt. for cells from control (○) and dexamethasone-treated (●) animals respectively. The rate of transport of tryptophan (△) into the cell in the absence of L-phenylalanine was 10.10 ± 0.70 nmol/h per mg dry wt. The tryptophan concentration was $50 \mu\text{M}$ throughout. All results are means $\pm$ S.E.M. for three separate cell preparations.

From the initial slopes of these curves, and the relationships shown in eqn. (10) (given that $\partial v_t/v_t = \partial v_i/v_i$), we can again calculate the Control Coefficient of the transport step for tryptophan metabolism (Table 2). The values agree well with those obtained as described above.

At this point it is worth emphasizing that, although eqn. (2) is the most frequently quoted expression for the flux Control Coefficient, it is merely one specific expression of this coefficient. The general expression [11, 13] is shown in eqn. (11):

$$C_E^J = \frac{\partial J / \partial v_i}{v_i} \quad (11)$$

where v_i is the activity of the isolated enzyme under the original pathway conditions, i.e. with substrates, products and effectors at the specific concentrations found experimentally. This definition, which is used implicitly, but not explicitly, in ref. [15], underlies many of the equations used in the present paper.

From the kinetic properties of the dioxygenase and of the transport system and measurements of intracellular amino acid concentrations, we have calculated the effect of a minimal (1%) change in the activity of the latter on metabolic flux (Table 3). Using eqn. (11) and assuming that the 1% change has a negligible effect on the Control Coefficients, and that the dioxygenase reaction is essentially irreversible, we obtained values for Control Coefficients very similar to those obtained by the other methods (Table 2). This calculation is essentially naive and is dependent on several assumptions as noted above. Although this method cannot be regarded as a general

one, it is interesting that the simple-minded assumptions hold in this case.

It has been suggested [37–39] that both kynureninase (EC 3.7.1.3) and kynurenine hydroxylase (EC 1.14.13.9) may play roles as regulators of tryptophan metabolism. It therefore seemed worthwhile to examine the possibility that these enzymes may contribute to the control of catabolic flux. Experiments with the non-competitive inhibitor amino-oxyacetate have shown, however, that the Control Coefficient of kynureninase for the flux through the kynureninase-catalysed reaction is < 0.004 [17]. Similar calculations based on data showing inhibition of kynurenine hydroxylase by 4-methyl-2-oxopentanoate [39] show that the Control Coefficient of the hydroxylase is < 0.037 , even when dioxygenase activity is greatly increased after administration of dexamethasone.

The enzyme next to kynureninase in the pathway is 3-hydroxyanthranilate oxidase (EC 1.10.3.5). In extracts, this enzyme is inhibited irreversibly by very low concentrations of 4-chloro-3-hydroxyanthranilate [40]. In cell incubations, however, the inhibitor (added directly or as its precursor 6-chlorotryptophan) is considerably less effective [41]. From the data in ref. [41] and taking a maximum value for the K_i of $0.25 \mu\text{M}$ [40], we have calculated a maximum Control Coefficient of the oxidase for flux under basal conditions of 0.0005, an insignificantly small value.

Exceptionally, in pyridoxine deficiency [17], the Control Coefficient of kynureninase (which is pyridoxal 5'-phosphate-dependent) for flux through the kynureninase reaction rises from 0.004 to 0.41. Under the same conditions the Control Coefficient of kynureninase for flux through the dioxygenase is only 0.28; this is consistent with the observation that, in pyridoxine deficiency, a proportion of the flux through the dioxygenase does not pass through kynureninase [17], suggesting that it is diverted into other products, whose concentrations increase in the urine.

The Control Coefficients of tryptophan transport for the flux through the reaction catalysed by kynureninase (shown in Table 4) were separately calculated by using eqns. (5) and (8) and the data in Fig. 3(b). The Control Coefficients of tryptophan 2,3-dioxygenase for the flux through kynureninase (shown in Table 4) were calculated by using eqn. (2) and the data in Fig. 5. The sum of these Control Coefficients, at any activity of the dioxygenase, is again close to 1.0; this confirms that kynureninase, kynurenine hydroxylase and 3-hydroxyanthranilate oxidase cannot play significant regulatory roles under normal nutritional conditions.

Because of the physiological irreversibility of tryptophan 2,3-dioxygenase (see above), picolinate carboxylase (EC 4.1.1.45) cannot exert a significant Control Coefficient for tryptophan degradation. The Control Coefficients of tryptophan transport and tryptophan 2,3-dioxygenase, for flux from tryptophan through picolinate carboxylase (shown in Table 4), were calculated as described above for flux through kynureninase. The sum of these Control Coefficients, at any activity of tryptophan 2,3-dioxygenase, is once more close to 1.0. However, picolinate carboxylase lies at a branch point, and evidence suggests [42] that this enzyme plays an important role in the distribution of tryptophan-derived carbon between the nicotinamide nucleotides and the citric acid cycle. Calculations based on data in [42, 43]

Table 3. Theoretical calculation of Control Coefficients of transport for tryptophan and phenylalanine metabolic fluxes

Experimental procedures were as described in the Materials and methods section. The effect of a small (1%) inhibition of transport on the separate metabolic fluxes (J) was evaluated as follows. The experimental system is expressed in the general form:

$$50 \frac{A}{B} C \xrightarrow{J}$$

where 50 is the concentration of extracellular amino acid (in μM), A and B are the rates of transport into and out of the cell respectively, C is the concentration of intracellular amino acid (in μM) and J is the flux through tryptophan 2,3-dioxygenase or phenylalanine hydroxylase. The units of A , B and J are nmol/h per mg dry wt., and the values of A , B and J are based on results obtained in experiments with three separate liver cell preparations; in each case S.E.M. was $< 10\%$ of mean value. The new values for A , B , C and J after the theoretical 1% inhibition of A are denoted by A' , B' , C' and J' ; these were calculated from the relationships below:

$$C' = \frac{AC}{B + J/0.99}$$

$$B' = 0.99A - (C' \cdot J/C)$$

$$J' = 0.99A - B'$$

Values in the last column show the percentage change in flux as J becomes J' (which is equivalent to the Control Coefficient C_J^J). It is assumed that the reactions catalysed by tryptophan 2,3-dioxygenase and phenylalanine hydroxylase are essentially irreversible and product-insensitive (i.e. that subsequent reactions have negligible Control Coefficients) and that they respond linearly to changes in substrate concentrations in this range.

Conditions	A	B	C	J	A'	B'	C'	J'	Percentage inhibition of flux $\left(\frac{J-J'}{J} \times 100\right)$
Tryptophan metabolism									
Basal tryptophan 2,3-dioxygenase	10.10	7.58	37.50	2.52	9.999	7.4851	37.406	2.5138	0.25
Induced tryptophan 2,3-dioxygenase	10.10	2.58	12.50	7.52	9.999	2.5350	12.407	7.4640	0.74
Phenylalanine metabolism									
Basal phenylalanine hydroxylase	10.15	5.09	25.00	5.06	10.049	5.0140	24.876	5.0350	0.49
Maximal phosphorylation of phenylalanine hydroxylase (glucagon)	10.15	1.25	5.00	8.90	10.049	1.2265	4.9560	8.8220	0.88

Table 4. Control Coefficients of tryptophan transport and tryptophan 2,3-dioxygenase for metabolism of tryptophan through kynureninase and picolinate carboxylase

The procedures for the preparation and incubation of cells and measurements of concentrations and rates of reactions were as described in the Materials and methods section. The Elasticity Coefficients used were those shown in Table 1. The Response Coefficients were determined from the data shown in Fig. 3(c). All values are means $\pm$ S.E.M. for three separate liver cell preparations. Abbreviations: TRP, tryptophan; TDO, tryptophan 2,3-dioxygenase; T, transport.

Flux	Elasticity Coefficient	Response Coefficient	Control Coefficient	Eqn. no(s).
Kynureninase flux				
Transport				
TDO basal	$\epsilon_{\text{TRP}_0}^T$, 3.95	$R_{\text{TRP}_0}^J$, 1.00	C_T^J , 0.25	(6), (7), (8)
TDO induced	$\epsilon_{\text{TRP}_0}^T$, 1.28	$R_{\text{TRP}_0}^J$, 1.00	C_T^J , 0.78*	
Dioxygenase				
TDO basal	—	—	C_{TDO}^J , 0.75	(2)
TDO induced	—	—	C_{TDO}^J , 0.25*	
Picolinate carboxylase flux				
Transport				
TDO basal	$\epsilon_{\text{TRP}_0}^T$, 3.99	$R_{\text{TRP}_0}^J$, 1.00	C_T^J , 0.25	(6), (7), (8)
TDO induced	$\epsilon_{\text{TRP}_0}^T$, 1.32	$R_{\text{TRP}_0}^J$, 1.00	C_T^J , 0.76*	
Dioxygenase				
TDO basal	—	—	C_{TDO}^J , 0.75	(2)
TDO induced	—	—	C_{TDO}^J , 0.25*	

* $P < 0.005$ versus corresponding basal values (Student's t test).

Fig. 5. Relationships between tryptophan 2,3-dioxygenase activity and flux through kynureninase and picolinate carboxylase in intact cells

Details of the procedures for the preparation and incubation of cells and for the measurements of metabolic fluxes and tryptophan 2,3-dioxygenase activity are given in the Materials and methods section. Other conditions and pretreatments are as described in Fig. 1. ●, Kynureninase flux; ○, picolinate carboxylase flux. All results are means $\pm$ S.E.M. for three separate cell preparations.

show that the Control Coefficient of picolinate carboxylase for flux from tryptophan through picolinate carboxylase is at least 0.5, confirming this view of the enzyme's importance. Since the sum of the Control Coefficients in a pathway must always be 1.0, an equal, negative, Control Coefficient, for flux from tryptophan through picolinate carboxylase, must reside in the other branch of the pathway, that leading to the nicotinamide nucleotides [12, 15].

Tyrosine metabolism

Tyrosine metabolism can be analysed quantitatively by methods similar to those applicable to tryptophan metabolism.

Using eqns. (5) and (8) and the data in Fig. 3(c), we have shown that C_T^J (where J and T refer to tyrosine catabolic flux and tyrosine transport respectively) increased from 0.22 to 0.58 as tyrosine aminotransferase activity increased from a basal value of 170 nmol/h per mg to a value of 340 nmol/h per mg after induction by dexamethasone (Table 2). These values for C_T^J are not dissimilar from those calculated for tryptophan transport and indicate once more that transport into the cell is an important site of regulation.

Kacser & Burns [12] have established that the ratio of the Control Coefficients of adjacent steps in a pathway is a function of the ratio of Elasticity Coefficients for their common intermediate metabolite (M), as shown in eqn. (12):

$$\frac{C_1^J}{C_2^J} = -\frac{e_M^2}{e_M^1} \quad (12)$$

We here use this connectivity theorem to calculate Control Coefficients of tyrosine aminotransferase for flux through this enzyme.

The Elasticity Coefficient of intracellular tyrosine for transport, $e_{Tyr(in)}^T$, was obtained from eqn. (13):

$$e_{Tyr(in)}^T = -\frac{\Gamma/K}{1-\Gamma/K} \cdot \left(1 + \frac{J}{V_r}\right) \quad (13)$$

where J is the tyrosine metabolic flux and V_r the maximum rate of the reverse (or back) reaction i.e. of transport of tyrosine from cytosol to extracellular medium {see also eqn. (27) of ref. [15]}. It was assumed that transport was symmetrical and therefore $V_r = V_f$. The Elasticity Coefficient of intracellular tyrosine for tyrosine aminotransferase, $e_{Tyr(in)}^{TAT}$, was calculated by using eqn. (8). Values for both coefficients under conditions of basal and induced aminotransferase activities are given in Table 1.

Using eqn. (14):

$$\frac{C_T^J}{C_{TAT}^J} = -\frac{e_{Tyr(in)}^{TAT}}{e_{Tyr(in)}^T} \quad (14)$$

where C_T^J and C_{TAT}^J are the Control Coefficients of tyrosine transport (T) and tyrosine aminotransferase (TAT) respectively for metabolic flux (J) and the Elasticity Coefficients are as defined above {see eqn. (12) in ref. [14]}, we calculated the values for C_{TAT}^J . As enzyme activity rose from basal to induced values, C_{TAT}^J fell from 0.85 to 0.19 (Table 2).

C_{TAT}^J values were also calculated in two further ways. First, we measured the effect of various concentrations of amino-oxycacetate [2], a non-competitive inhibitor (I), on aminotransferase activity in cell-free extracts and on metabolic flux in cell incubations (Fig. 6). Groen *et al.* [15] have shown that such data may be used to determine Control Coefficients (eqn. 15):

$$C_{TAT}^J = -\frac{K_i}{J} \left(\frac{\partial J}{\partial I} \right)_{I \rightarrow 0} \quad (15)$$

Fig. 6. Effect of amino-oxycacetate on metabolic flux through tyrosine aminotransferase

Details of the procedures for the preparation and incubation of cells and for the measurement of metabolic flux are given in the Materials and methods section. Amino-oxycacetate was added at the start of the pre-incubation period to cells from control (●) or dexamethasone-treated (○) rats. All results are means $\pm$ S.E.M. for three separate cell preparations.

The K_i values for the inhibitor were $30 \pm 2 \mu\text{M}$ and $35 \pm 3 \mu\text{M}$ (means $\pm$ S.E.M. for three separate cell preparations) for extracts from cells from control and dexamethasone-treated animals respectively. The values for C_{TAT}^J derived from eqn. (15) were 0.71 and 0.29 respectively for the basal and induced conditions (Table 2).

Secondly, if it can be assumed that, under the experimental conditions, $1 - J/V$ is close to unity, then C_{TAT}^J can again be determined from C_T^J , measurements of cellular metabolite concentrations and the kinetic properties of the transporter and aminotransferase {eqn. (16); see eqn. (8) in ref. [15]}.

$$C_{\text{TAT}}^J = C_T^J \left([\text{TYR}_{(\text{in})}] - \frac{[\text{HPP}]}{K_{\text{TAT}}[\text{OG}]/[\text{GLU}]} \right) \times \left(\frac{1}{K_T[\text{TYR}_{(\text{out})}] - [\text{TYR}_{(\text{in})}]} \right) \quad (16)$$

(where GLU represents glutamate, HPP 4-hydroxyphenylpyruvate, OG 2-oxoglutarate and TYR tyrosine, 'out' and 'in' refer to the medium and the intracellular compartment respectively, and K_T and K_{TAT} are the equilibrium constants of transport and the aminotransferase respectively). The values for C_{TAT}^J so derived were 0.63 and 0.08 for the basal and induced tyrosine aminotransferase activities respectively (Table 2).

Inspection of Table 2 shows that there is some variation in the values found for C_{TAT}^J by the three methods. The two lower values for the experiments in which activity was increased by previous administration of dexamethasone are less dependable, and may be underestimates because we have, in the absence of appropriate data, ignored any effect of the treatment on the cytosolic [2-oxoglutarate]/[glutamate] ratio. What is clear, however, is that the aminotransferase is a more important determinant of flux under basal conditions than after dexamethasone administration.

The sum of the values for C_T^J and C_{TAT}^J is close to 1.0 at basal activity of the aminotransferase. After dexamethasone administration, however, the sum is less than this, suggesting that a contribution to the control of the pathway is made by a reaction (or reactions) distal to the aminotransferase. Because it is unlikely *a priori* that the reaction catalysed by 4-hydroxyphenylpyruvate oxidase (EC 1.13.11.27), the enzyme adjacent to the aminotransferase, is physiologically reversible, and because of the insensitivity to its products (CO_2 and homogentisate), it is probable that this enzyme is responsible for the residual Control Coefficient [12, 15]. In separate experiments (results not shown), it was found that flux was decreased by the addition of 5 mM-L-glutamine. Glutamine is readily converted into glutamate, which increases the 'backward' rate through tyrosine aminotransferase. This results in an increase in the Control Coefficient of 4-hydroxyphenylpyruvate oxidase and a decrease in the Control Coefficient of tyrosine transport, a consequence of the coincidence of decreased flux and maintained unidirectional rate of transport into the cytosol.

Phenylalanine metabolism

The Elasticity Coefficient of extracellular phenylalanine for phenylalanine transport, $\epsilon_{\text{Phe(out)}}^T$, was calculated, by using eqn. (8), from data derived from experiments in which the rate of transport and the intracellular

concentration of the amino acid were determined (Table 1). The Response Coefficient of extracellular phenylalanine for phenylalanine metabolic flux, $R_{\text{Phe(out)}}^J$, was obtained from data in ref. [43]. From the relationship shown in eqn. (6), the Control Coefficient of phenylalanine transport for metabolic flux, C_T^J , was found to be 0.61 (Table 2).

The reaction catalysed by the hydroxylase exhibits a very large negative free energy change. *In vivo* the reaction is certainly essentially irreversible, because the requirement for phenylalanine cannot be met by formation from dietary tyrosine. In addition, the activity of the hydroxylase in cells is insensitive to the concentration of intracellular tyrosine (A. R. Nicholas, L.-D. Luo, R. G. Knowles & C. I. Pogson, unpublished work). It is reasonable, therefore, to conclude that the residual Control Coefficient (0.39) is the property exclusively of the hydroxylase [12, 15]. It follows that previous descriptions of phenylalanine hydroxylase as 'rate-limiting' [3,6] are misconceived.

The physiological irreversibility of the hydroxylase permits, with the reservations noted above, the analysis of Control Coefficients by consideration of the effects of small perturbations [as, above, for tryptophan metabolism, eqn. (11); Table 3]. On exposure of cells to glucagon the hydroxylase becomes phosphorylated; the extent of this phosphorylation parallels an increase in flux [3, 4]. With a maximally effective concentration of glucagon, the value of C_T^J for phenylalanine metabolism is increased to 0.88, a near-doubling that occurs within 5 min (Table 3; [3]).

Glucagon approximately doubles the rate of hydroxylation of phenylalanine in liver cells. Because the Control Coefficient of the hydroxylase is, at most, approx. 0.5, it can be predicted that the change in the activity of the hydroxylase (assayed under pathway conditions) must be at least 4-fold. This is consistent with data obtained with the isolated enzyme [44].

Of the two values for the Control Coefficient of the transport step under basal conditions (Table 2), the lower may be the less reliable because phenylalanine hydroxylase responds sigmoidally and not linearly to changes in substrate concentration, as was tacitly assumed in our calculations. The sigmoidicity is less in evidence when glucagon is present [45], and the two values found for these conditions are correspondingly similar.

Effect of physiological amino acid concentrations on aromatic amino acid metabolism

Work in this laboratory has shown that aromatic amino acids enter the liver cell through two systems, one the L-system [46], the other a low-capacity higher-affinity more-specific T-system [22, 29] with properties similar to those of the T-system of human erythrocytes [47]. Under physiological conditions, competition for transport should occur with the wide range of L-system and T-system substrates [46]. In liver cell incubations, a mixture of amino acids, at concentrations found in the portal vein (A. J. Dickson, personal communication), inhibits basal metabolism of the three aromatic amino acids equally and by approx. 15%. The degree of inhibition increased markedly when the activities of the dioxygenase, transaminase and hydroxylase were increased (Table 5), because raised activities of the enzymes result in an increase in the Control Coefficients for transport.

Table 5. Effects of a physiological mixture of amino acids on the transport and metabolism of the aromatic amino acids

The procedures for the preparation and incubation of cells and for the measurement of transport rates and metabolic fluxes were as described in the Materials and methods section. The final composition of the amino acid mixture, determined from hepatic portal blood from fed male rats at 10:00 h (A. J. Dickson, personal communication), was: Ala, 0.498; Arg, 0.256; Asn, 0.635; Asp, 0.058; Gln, 0.445; Glu, 0.415; Gly, 0.448; His, 0.082; Ile, 0.082; Leu, 0.145; Lys, 0.568; Met, 0.027; Pro, 0.282; Val, 0.163 (all concentrations mM). The final concentrations of phenylalanine, tryptophan and tyrosine were 50, 10 and 100 μ M respectively in all cases; where appropriate due allowance was made for the addition of radiolabelled substrate at the end of the preincubation period. Where relevant, tryptophan 2,3-dioxygenase and tyrosine aminotransferase activities were induced by previous injection of the rat with dexamethasone phosphate; phenylalanine hydroxylase activity was increased by addition of glucagon to cell suspensions. All results are means $\pm$ S.E.M. for three separate cell preparations. Numbers in parentheses indicate percentages of control values. Differences between incubations with the amino-acid mixture and controls were all significant by Student's *t* test ($P < 0.05$). Differences between the percentage effects of the amino acid mixture on fluxes at basal and elevated activities of the enzymes (II versus III) were all significant by Student's *t* test ($P < 0.05$).

	Transport rate or metabolic flux (nmol/h per mg dry wt.)	
	Control	+ Amino acid mixture
I. Transport into the cell		
Tryptophan	2.10 $\pm$ 0.15	1.41 $\pm$ 0.11 (67%)
Tyrosine	16.30 $\pm$ 0.98	11.25 $\pm$ 0.82 (69%)
Phenylalanine	10.40 $\pm$ 0.62	6.75 $\pm$ 0.52 (65%)
II. Metabolic flux with basal activities of:		
Tryptophan dioxygenase	0.51 $\pm$ 0.02	0.44 $\pm$ 0.02 (86%)
Tyrosine aminotransferase	3.59 $\pm$ 0.14	3.05 $\pm$ 0.14 (85%)
Phenylalanine hydroxylase	4.90 $\pm$ 0.20	4.07 $\pm$ 0.15 (83%)
III. Metabolic flux with elevated activities of:		
Tryptophan dioxygenase	1.47 $\pm$ 0.04	1.04 $\pm$ 0.03 (71%)
Tyrosine aminotransferase	8.15 $\pm$ 0.16	5.87 $\pm$ 0.24 (72%)
Phenylalanine hydroxylase	8.75 $\pm$ 0.26	6.04 $\pm$ 0.18 (69%)

General discussion

A major conclusion of the experiments and calculations reported here is that the assumption that tryptophan dioxygenase, tyrosine aminotransferase and phenylalanine hydroxylase are 'rate-limiting' for their respective pathways [2, 3, 5–10] is ill-founded. Theoretical considerations of the control of metabolic pathways suggest that the expression 'rate-limiting' is frequently misleading, because control is an activity shared between a number of components of any given pathway [12–15]. Our studies of the pathways of aromatic amino acid metabolism support this latter view.

Transport across the plasma membrane is often overlooked as a step in metabolic pathways, probably because no change in chemical composition is involved and because of a general assumption that such transport processes, with equilibrium constants of unity, are unlikely candidates as regulators. Earlier studies on aromatic amino acid metabolism in liver suggested that transport into the cell was a relatively fast event [2, 3, 19, 20]. It is now clear that the control of the metabolism of all three amino acids is largely (for tyrosine) or exclusively (tryptophan and phenylalanine) shared between transport and the first enzyme of each pathway, and that the relative significance of these two steps varies as enzyme activities change. The significant values of the Control Coefficients of transport of the three amino acids under basal conditions imply that the metabolism of the three amino acids is 'co-ordinated' at this step. Thus variations in the time courses of, and sensitivity to, induction of the

three enzymes [48–50] are not necessarily reflected in changes in the ratios of the amino acids in the blood. These ratios, and the absolute concentrations of the amino acids, are important determinants in the synthesis of biogenic amines in the brain [23–25], and it has been suggested that changes in the activities of the three liver enzymes may indirectly affect neurotransmitter synthesis through effects on removal of the amino acids from the blood [51, 52]. Although changes in enzyme activity may indeed be of some significance, it now seems not improbable that competition for transport into the liver cell as well as into the brain [53, 54] may be of equal importance.

The role of the other amino acids as regulatory factors may be of particular significance in pathological or dietary conditions in which concentrations of competing amino acids are increased [55, 56].

Tryptophan 2,3-dioxygenase [57] and tyrosine aminotransferase [58] exhibit diurnal fluctuations in activity. The time of maximal activities, and presumably maximal Control Coefficients of transport for flux, coincides with that of the maximal concentrations of the competing L-system and T-system amino acids in the blood [59, 60]. The consequence must be that the rate of metabolism of tryptophan and tyrosine will be more inhibited at these maxima, so that any diurnal rhythm in such metabolism is damped relative to the enzymic rhythmicity.

The substrate affinities and kinetics of the transport, tryptophan dioxygenase and tyrosine aminotransferase reactions are similar; changes in the concentration of tryptophan and tyrosine in the physiological range

therefore have little effect on the Control Coefficients. In contrast, the relationship between phenylalanine concentration and phenylalanine hydroxylase activity (in the basal state) is sigmoidal [45, 61], so that increases in the former may lead to substantial increases in reaction rate. The rate of uptake into the cell increases approximately linearly with phenylalanine concentrations in the physiological range; this means that increases in phenylalanine concentration will be associated with an increased Control Coefficient of the transport step, and a proportionately decreased Control Coefficient of the hydroxylase. Thus in hyperphenylalaninaemia catabolism of phenylalanine may be more sensitive to competitive inhibition by other amino acids.

From measurement of the Control Coefficient of an enzyme, it is possible to obtain a quantitative assessment of the importance of that enzyme in the control of a pathway under one given set of conditions. A more general statement about the role of the same enzyme in varying the rate of flux through the pathway (under a wide range of conditions) requires understanding of the degree to which the enzyme is sensitive to putative regulators (i.e. its regulability).

In the present paper we have calculated Control Coefficients by several methods and have found good agreement. It is clear, however, that such calculations can be very sensitive to small errors in experimental data; we would therefore urge that Control Coefficients should be ascertained by at least two independent methods.

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