Metabolic homoeostasis of L-threonine in the normally-fed rat

Importance of liver threonine dehydrogenase activity

Michael I. BIRD* and Peter B. NUNN

Department of Biochemistry, King's College London, Strand, London WC2R 2LS, U.K.

(Received 2 March 1983/Accepted 9 June 1983)

Threonine dehydratase, threonine aldolase and threonine dehydrogenase activities were assayed in livers of rats that had been normally-fed, starved for 72 h, fed a high-protein diet or normally-fed and injected with glucagon or cortisone. A modified continuous spectrophotometric assay for threonine aldolase overcame interference resulting from threonine dehydratase activity and revealed that threonine aldolase activity was very low in rat liver, irrespective of the metabolic state of the animal. The concentration of free threonine was determined in livers of animals subjected to the same treatments as described above. Using Michaelis–Menten kinetics to estimate enzyme activities *in vivo* at intracellular threonine concentrations it was calculated that in the normally-fed state, 87% of the threonine degraded was catabolized by threonine dehydrogenase. In other metabolic states (except in glucagon-treated animals) threonine dehydratase was the major enzyme catalysing threonine catabolism. It was concluded that threonine dehydrogenase activity plays a hitherto unrecognized role in the metabolic homoeostasis of threonine in the normally-fed rat and that this enzyme activity, in association with 2-amino-3-oxobutyrate CoA-ligase, accounts for the known rate of glycine formation from threonine in the rat.

Three enzymes catalysing the degradation of L-threonine are known: (i) threonine dehydratase (EC 4.2.1.16), yields 2-oxobutyrate and NH_4^+ ; (ii) threonine aldolase (EC 4.1.2.5), yields glycine and acetaldehyde; and (iii) threonine dehydrogenase (EC 1.1.1.103), yields 2-amino-3-oxobutyrate.

In rat liver threonine and serine are deaminated by a common cytosolic dehydratase (Goldstein *et al.*, 1962), which is highly inducible and responds to a wide range of dietary and hormonal stimuli (see Inoue *et al.*, 1971).

There has been disagreement as to which enzymes are responsible for the cytosolic activities of threonine aldolase, allothreonine aldolase and serine hydroxymethyltransferase (EC 2.1.2.1) [reactions (i)–(iii) respectively] in rat liver (see Malkin & Greenberg, 1964; Riario-Sforza *et al.*, 1969).

However, a highly purified preparation of serine hydroxymethyltransferase from rat liver cytosol (Palekar *et al.*, 1973) catalysed reactions (ii) and (iii), but not reaction (i), indicating that threonine aldolase was a separate enzyme in this tissue. The activity of threonine aldolase is usually evoked to explain the observations of Meltzer & Sprinson (1952) that L-threonine is an effective precursor of glycine in the rat.

The properties of mammalian threonine dehydrogenases are little known (Urata & Granick, 1963; Hartshorne & Greenberg, 1964; Green & Elliott, 1964), although bacterial and avian enzymes have been purified extensively (Boylan & Dekker, 1980; Aoyama & Motokawa, 1981). The product of the threonine dehydrogenase reaction, 2-amino-3-oxobutyrate, either spontaneously decarboxylates to

* Present address: Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, U.K.

form aminoacetone (Laver *et al.*, 1959) or is cleaved in a reaction catalysed by 2-amino-3-oxobutyrate

CoA-ligase (glycine acetyltransferase; EC 2.3.1.29) to form glycine and acetyl-CoA (McGilvray & Morris, 1969; Linstead *et al.*, 1977; Dale, 1978; Bird & Nunn, 1979b).

The aim of the present work was to determine the activities of threonine dehydratase, threonine aldolase and threonine dehydrogenase enzymes in rat liver in order to evaluate the relative importance of the metabolic pathways initiated by these enzymes in rats maintained in different metabolic states. Some of the results have been presented in a preliminary form (Bird & Nunn, 1979a; Bird *et al.*, 1982).

Experimental

Materials

Aminoacetone hydrochloride was synthesized (Hepworth, 1973) and stored at -20°C under N_2 . The melting point [79°C]; see Tschudy *et al.* (1964)], i.r., n.m.r. (^1H and ^{13}C) and mass spectra were consistent with a pure unpolymerized product. The compound was free of ninhydrin-positive contaminants [paper chromatography (Elliott, 1960) and electrophoresis at pH 2.6]. When allowed to react with acetylacetone and Ehrlich's reagent the mean absorption coefficient at 553 nm ($\pm$ S.E.M.) was $73.7 (\pm 0.62) \times 10^3 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ (four batches), in excellent agreement with published values (Urata & Granick, 1963).

L-Threonine (allo-free) and DL-allothreonine were obtained from Calbiochem (Bishops Cleeve, Herts., U.K.). Glucagon, cortisone acetate and pyridoxal 5'-phosphate were obtained from Sigma Chemical Co. (Poole, Dorset, U.K.). NAD^+ , NADH, NADPH, alcohol dehydrogenase (300 units/mg from yeast), lactate dehydrogenase (5 mg/ml, from rabbit muscle) and glutamate dehydrogenase [10 mg/ml, in aq. 50% (v/v) glycerol] were obtained from Boehringer Corp. (London), Lewes, East Sussex, U.K. All other reagents were of analytical reagent grade, or the highest purity commercially available.

Animals and treatments

Male Wistar rats (Charles River, Manston, Kent, U.K.) weighing 200–250 g were housed in a controlled temperature (21°C) and light (07:00–19:00 h) environment and received water and a rat pellet diet (41B; Fox Facilities, Borehamwood, Herts., U.K.) *ad libitum* for at least 1 week before the start of the experiments. Normally-fed animals were maintained on this diet alone. The metabolic state of some animals was altered by using conditions that increase the activity of hepatic threonine dehydratase. The high-protein diet consisted of feeding chopped hard-boiled egg for 4 days. Starved animals were allowed access to water only for 3 days. Cortisone-treated animals were fed normally,

but for 5 days were given daily (at 10:00 h) intramuscular (hind limb) injections of a suspension of cortisone acetate (corresponding to 3.3 mg/100 g body wt.) in sterile 0.9% (w/v) NaCl and were used on day 5 (Peraino, 1967). Glucagon (0.2 mg/100 g body wt.) was injected intraperitoneally to overnight-starved rats, which were used after 4 h (Jost *et al.*, 1970). At the end of each treatment, mean body weights were: normally-fed, 236 g; starved, 178 g; high-protein, 232 g; glucagon-treated, 215 g; cortisone-treated, 204 g.

Preparation of rat liver extracts

Liver (4 g) was homogenized in 2.5 vol. of ice-cold 0.2 M-Tris/HCl buffer, pH 7.6, containing 0.15 M-KCl (Tschudy *et al.*, 1964) using a Potter-Elvehjem-type homogenizer with a Teflon pestle. The homogenate was diluted 10-fold with 0.1 M- KH_2PO_4 (adjusted to pH 7.2 with KOH) containing 10 mM-mercaptoethanol and 0.25 ml (equivalent to approx. 8 mg of liver) was used to assay threonine dehydrogenase activity. This procedure maximized threonine dehydrogenase activity. Dilution of homogenates with Tris/HCl buffers reduced enzyme activity, presumably due to high concentrations of Cl^- (Green & Elliott, 1964). Homogenization in phosphate buffers alone also gave low enzyme activities. Liver (2 g) from the same animal was similarly homogenized in 9 vol. of ice-cold 0.1 M- KH_2PO_4 buffer, containing 10 mM-mercaptoethanol, and adjusted to pH 7.2 with KOH. After centrifugation at 105 000 g for 1 h at 4°C , the supernatant was used to assay threonine aldolase and threonine dehydratase activities. Protein concentration was determined by the method of Lowry *et al.* (1951).

Enzyme assays

Threonine dehydratase. The assay employed NADH and lactate dehydrogenase to continuously reduce 2-oxobutyrates formed from L-threonine [modified from Morris & Peraino (1976)]. The assay mixture contained, in a final volume of 1 ml, 0.1 M- KH_2PO_4 (adjusted to pH 8.4 with KOH), 1 mM-dithiothreitol, 1 mM-EDTA, 0.2 mM-pyridoxal 5'-phosphate, 0.57 mM-NADH, 2 μl of lactate dehydrogenase, and rat liver supernatant containing about 0.5 mg of protein. After a 15 min pre-incubation period at 37°C , the reaction was started by adding L-threonine (final concentration 0.2 M) (test) or deionized water (control). After a lag period of about 10 min the reaction rate (decrease in A_{340}) was linear for at least 10 min. The rate of reaction was proportional to enzyme concentration to a protein concentration of at least 0.5 mg/ml. Control rates of NADH oxidation were zero. Sometimes the assay was modified by omitting NADH and lactate dehydrogenase, and including 5.7 mM-2-oxoglutar-

ate (neutralized), 0.57 mM-NADPH and 40 μ l of glutamate dehydrogenase. The rate of NH_4^+ formation from L-threonine was calculated by subtracting control rates of NADPH oxidation (assay mixture minus 2-oxobutyrate) from the total rate of decrease of A_{340} . 2-Oxoglutarate was not itself reduced by NADPH in the assay.

Threonine aldolase. The assay employed NADH or NADPH and alcohol dehydrogenase to continuously reduce acetaldehyde formed from L-threonine. The method of Palekar *et al.* (1973) was modified using either 0.15 mM-NADH or 0.3 mM-NADPH, as appropriate (Bird & Nunn, 1979a). In the final assays, NADPH was used as the cofactor to eliminate interference (see the Results section). Reactions were started by adding either L-threonine (final concentration 0.1 M) (test) or deionized water (control) to the assay mixture at 37°C. The net change in A_{340} (test rate minus control rate) was linear for at least 20 min.

Threonine dehydrogenase. The basis of the assay was the determination of aminoacetone formed by the rapid, spontaneous decarboxylation of 2-amino-3-oxobutyrate, the product of the dehydrogenase reaction. The method of Hartshorne & Greenberg (1964) was modified using 2.5 mM-NAD⁺ and incubation at 37°C for 30 min. The possibility of aminoacetone catabolism in rat liver homogenates was tested by including 20 mM-, 10 mM- or 2 mM-aminoacetone hydrochloride in the assay mixture in place of L-threonine. After 30 min incubation no loss of aminoacetone was detectable.

Determination of hepatic free threonine concentrations

Livers were rapidly excised (between 12:00 and 15:00 h), freeze-clamped in liquid N₂, weighed and homogenized in an ice-cold saturated solution of picric acid. Excess picric acid and glutathione were removed (Stein & Moore, 1954; Moore *et al.*, 1958). Amino acids were determined (Vega & Nunn, 1969) using Technicon Chromobeads type A resin.

Results

Evidence for interference in the NADH-linked assay for threonine aldolase activity by threonine dehydratase activity

The activity of endogenous alcohol dehydrogenase in 105 000 g supernatant fractions, determined by using 10 mM-acetaldehyde in place of L-threonine in the NADH-linked threonine aldolase assay, was typically 15 nmol of NADH oxidized/min per mg of protein. Similarly, the activity of endogenous lactate dehydrogenase was about 20 nmol of NADH oxidized/min per mg of protein, when determined using 3.3 mM-2-oxobutyrate in

place of L-threonine in the standard threonine dehydratase assay.

Table 1 shows the effects of 3 mM-oxamate and 1 mM-pyrazole, inhibitors of lactate dehydrogenase (Plummer & Wilkinson, 1963) and alcohol dehydrogenase (Krebs & Perkins, 1970) respectively, on the NADH-linked assay for threonine aldolase activity. Most of the apparent aldolase activity was sensitive to oxamate, indicating serious interference from the reduction by endogenous lactate dehydrogenase and NADH of the 2-oxobutyrate produced by the threonine dehydratase reaction. Since threonine dehydratase activity is highly inducible (Table 3), the extent of interference varied in extracts from animals in different metabolic states. Pyrazole (which inhibited exogenous alcohol dehydrogenase activity in the assay medium by >95%) caused only a small decrease in apparent threonine aldolase activity, suggesting that the true hepatic aldolase activity was low (Table 1). Neither 3 mM-oxamate nor 1 mM-pyrazole inhibited dehydratase activity (results not shown) when the NADPH-linked glutamate dehydrogenase assay method was used.

The possibility of interference in the threonine aldolase assay was investigated further by measuring threonine dehydratase activity in the medium normally used for assaying threonine aldolase

Table 1. *Evidence for interference in the standard assay for threonine aldolase*

For comparative purposes, the results displayed were obtained from one experiment using a rat liver supernatant (105 000 g) fraction, although the inhibition studies with oxamate and pyrazole were performed on at least 12 different preparations. For further details, see the Experimental section.

Assay conditions	Enzyme activity (nmol/min per mg of protein)
Standard threonine aldolase assay*	
L-Threonine (0.1 M)	2.91
L-Threonine + oxamate (3 mM)	0.73
L-Threonine + pyrazole (1 mM)	2.44
Threonine dehydratase activity (GDH method)† in threonine aldolase assay mixture omitting NADH and alcohol dehydrogenase	2.24
Standard threonine dehydratase assay†	
GDH method	11.75
LDH method	12.01

* Activity was measured using NADH to reduce acetaldehyde.

† For the GDH method, glutamate dehydrogenase and NADPH were included in the assay. For the LDH method, lactate dehydrogenase and NADH were included in the assay.

activity. For this purpose dehydratase activity was assayed by using the NADPH-linked assay method. Under these conditions dehydratase activity was only 19% of the activity measured under optimal conditions, but was still sufficient to account for most of the apparent threonine aldolase activity (Table 1).

Validity of the NADPH-linked threonine aldolase assay

An assay was developed that did not rely upon the use of inhibitors, but depended on the ability of the commercial yeast alcohol dehydrogenase to continuously reduce acetaldehyde produced from L-threonine, using NADPH as cofactor. This assay was insensitive to 3mM-oxamate and 1mM-pyrazole, and was proportional to acetaldehyde concentration (up to at least 45 μ M) and to protein concentration (up to at least 3 mg of protein/ml).

Activities of the enzymes initiating the catabolism of L-threonine in different metabolic states

Threonine dehydratase. Comparison of the enzyme activities reported here (Table 2) with those found by other workers is difficult, because of the variety of assay conditions used (e.g. Hoshino *et al.*, 1972; Pitot & Peraino, 1964; Nagabhushanam & Greenberg, 1965). The increase in threonine dehydratase activity in treated animals was generally greater than those observed by others (Peraino, 1967; Pestaña, 1969; Jost *et al.*, 1970; Inoue *et al.*, 1971), although these differences may be explained by the variability of published threonine dehydratase activities in fed rats.

The chronic cortisone treatment caused the greatest induction (40-fold) of enzyme activity. Peraino (1967) reported a 7-fold increase in the activity of the enzyme in similarly treated animals, except that the basal diet contained 24% protein compared with the 16% protein contained in the present diet.

Threonine aldolase. To our knowledge there is no published information concerning the activity of rat liver threonine aldolase in different metabolic states. The treatments used in the present work produced only small, probably insignificant, changes in the activity of the enzyme (Table 2).

There is a lack of reliable data for the activity of threonine aldolase in livers from fed rats. From the data of Riario-Sforza *et al.* (1969), the specific activity of threonine aldolase in a rat liver 100 000 g supernatant was calculated as 2.4 μ mol/min per g of tissue, which is 65 times greater than the value determined in the present work (0.037 μ mol/min per g of liver; Table 2). This discrepancy was probably caused by interference from dehydratase activity in the standard spectrophotometric (NADH) assay, a problem that we eliminated. Palekar *et al.* (1973),

Table 2. *Activities in different dietary and hormonal states of the enzymes initiating threonine catabolism in rat liver*
Values from fed rats are means $\pm$ s.e.m. of four separate experiments. Other values are means of two experiments, with individual values in parentheses. The dietary and hormonal treatments are described in the Experimental section. Mean liver weights were: fed, 9.42 g; starved, 4.80 g; high-protein, 9.36 g; glucagon-treated, 8.85 g; cortisone-treated, 9.70 g.

Treatment	Enzyme activity					
	Threonine dehydratase		Threonine aldolase		Threonine dehydrogenase	
	(μ mol/min per g of liver)	(μ mol/min per whole liver)	(μ mol/min per g of liver)	(μ mol/min per whole liver)	(μ mol/min per g of liver)	(μ mol/min per whole liver)
Normal-fed	0.42 $\pm$ 0.046	3.9 $\pm$ 0.45	0.037 $\pm$ 0.003	0.35 $\pm$ 0.05	0.62 $\pm$ 0.04	5.7 $\pm$ 0.13
72 h-starved	8.8 (11.0, 6.6)	41.4 (49.8, 33.1)	0.085 (0.103, 0.068)	0.41 (0.47, 0.34)	0.35 (0.32, 0.38)	1.7 (1.4, 1.9)
High-protein	12.7 (15.1, 10.3)	119.3 (143.8, 94.8)	0.105 (0.114, 0.095)	0.97 (1.08, 0.87)	0.42 (0.42, 0.41)	3.8 (4.0, 3.7)
Normal-fed plus glucagon	2.2 (2.1, 2.2)	18.6 (22.2, 16.0)	0.073 (0.064, 0.082)	0.63 (0.65, 0.62)	0.62 (0.65, 0.60)	5.5 (6.6, 4.5)
Normal-fed plus cortisone	16.5 (16.8, 16.1)	159.4 (155.7, 163.1)	0.074 (0.084, 0.064)	0.72 (0.78, 0.65)	0.38 (0.43, 0.32)	3.6 (4.0, 3.2)

using the NADH assay method, found an activity of 1.22 nmol/min per mg of protein, approximately four times greater than the value shown in Table 2. Again the possibility of interference in the assay by dehydratase activity cannot be eliminated.

Threonine dehydrogenase. In normally-fed rats hepatic threonine dehydrogenase activity exceeded that of threonine dehydratase, whereas in the treated animals, the reverse was true (Table 2). Dietary and hormonal treatments caused only small changes in dehydrogenase activity; we are not aware of any previous attempts to study threonine dehydrogenase in different metabolic states.

The mean threonine dehydrogenase activity in normally-fed rat liver (Table 2) was in good agreement with that of Green & Elliott (1964), but higher than those values reported by Hartshorne & Greenberg (1964), Tschudy *et al.* (1964) or Franchi *et al.* (1973).

Free threonine concentrations in liver

The mean hepatic contents of free threonine (in $\mu\text{mol/g}$ wet wt.) were: normally-fed, 0.26 ± 0.03 (0.35 mm); 72 h-starved, 0.20 (0.27 mm); high-protein, 0.24 (0.32 mm); glucagon-treated, 0.20 (0.27 mm); cortisone-treated, 0.12 (0.17 mm). Concentrations (mm) were calculated assuming that 1 g wet wt. of liver contains 0.75 ml of water. The value for normally-fed rats is the mean ($\pm$ S.E.M.) of six separate experiments; other values are means of two experiments, individual values being within $\pm 12\%$ of the mean.

Contribution of each threonine-degrading enzyme to the overall threonine catabolic activity of liver in vivo

In order to estimate the activity of each enzyme at intracellular concentrations of threonine prevailing *in vivo* the following assumptions were made. (i)

Each reaction obeyed simple Michaelis–Menten kinetics. (ii) The V_{max} for each reaction could thus be determined taking into account the measured activity, the assay concentration of L-threonine and the relevant K_m value. (iii) The K_m values of each enzyme in rat liver for L-threonine were: threonine dehydratase, 87 mm (Nakagawa & Kimura, 1969); threonine aldolase, 20 mm (Malkin & Greenberg, 1964); threonine dehydrogenase, 10.6 mm [determined with partially purified enzyme preparation (Bird, 1980)]. (iv) The cofactors for each enzyme were present at saturating concentrations *in vivo*, allowing the reactions to be regarded as single-substrate reactions. The K_m values of the dehydratase and the aldolase for pyridoxal 5'-phosphate are 0.37 μM (Nakagawa & Kimura, 1969) and 0.11 mm (Karasek & Greenberg, 1957) respectively. The K_m value for NAD^+ was 80 μM [partially purified L-threonine dehydrogenase from rat liver (Bird, 1980)], which is considerably lower than the intramitochondrial concentration of free NAD^+ , estimated as 1.7 mm from the data of Wainio (1970), Williamson *et al.* (1969) and Siess *et al.* (1976). (v) Threonine was distributed evenly throughout the water spaces in liver (extracellular, cytoplasmic and mitochondrial) and was not restricted by mitochondrial permeability barriers. (vi) The reactions *in vivo* were not inhibited or activated by regulatory agents. This statement must be qualified in that the activity of threonine dehydrogenase is probably sensitive to the mitochondrial NAD^+/NADH concentration ratio *in vivo*, since the partially purified enzyme is inhibited 50% by 0.3 mm-NADH in the presence of 2.5 mm-NAD⁺ (Bird, 1980). However, threonine dehydrogenase activity may be greater *in vivo* than we have estimated. Measurement of threonine dehydrogenase activity by determining aminoacetone production underestimates the true enzymic rate, since a proportion of the 2-amino-

Table 3. Calculated activities at intracellular threonine concentrations of the enzymes initiating threonine catabolism in rat liver

The V_{max} value for each enzyme was derived from the activity shown in Table 2, taking into account the threonine concentration in the assay and the relevant K_m value for L-threonine for each enzyme (see the Results section). These values were then used to calculate enzyme activity at intracellular threonine concentrations (see the Results section). The mean activity of each enzyme at intracellular threonine concentrations ($\bar{v}$) was calculated and expressed as a percentage (values in parentheses) of the sum of the activities of each enzyme.

Enzyme	Enzyme activity (nmol/min per whole liver)				
	Normal-fed	72 h-starved	High-protein	Normal-fed plus glucagon	Normal-fed plus cortisone
Threonine dehydratase	22.3 (9.7)	183.8 (77.6)	627.4 (81.6)	82.6 (33.9)	446.1 (86.4)
Threonine aldolase	7.2 (3.1)	6.5 (2.7)	18.3 (2.4)	10.1 (4.1)	7.3 (1.4)
Threonine dehydrogenase	201.4 (87.2)	46.7 (19.7)	123.1 (16.0)	151.0 (62.0)	63.1 (12.2)
Total activity (nmol/min per whole liver)	230.9	237.0	768.8	243.7	516.5
Total activity (nmol/min per g wet wt. of liver)	24.5	49.4	82.1	27.5	53.2

3-oxobutyrate formed does not decarboxylate, but is instead cleaved to glycine and acetyl-CoA (M. I. Bird, P. B. Nunn & L. A. J. Lord, unpublished work).

Values of $\bar{v}$ (intracellular enzyme activity) were calculated and the results were expressed as the percentage contribution of each enzyme to the total rate of hepatic threonine catabolism (estimated by summation of the individual $\bar{v}$ values; see Table 3). These rates were calculated using tissue threonine concentrations in livers freeze-clamped between 12:00h and 15:00h. Such values would vary over a 24h period according to diurnal variations in threonine concentrations.

Discussion

Catabolism of threonine in the rat in different metabolic states

(a) *Normally-fed state.* Hepatic threonine dehydrogenase, threonine dehydratase and threonine aldolase activities were respectively 87%, 10% and 3% of whole-liver threonine catabolic activity (Table 3). These calculations rely on several assumptions (see the Results section).

The validity of these assumptions was evaluated by determining the rate of threonine catabolism in the normally-fed whole animal *in vivo* and comparing this value with the total hepatic catabolic activity for threonine. The rate of threonine catabolism in the normally-fed state *in vivo* was assessed using an approach similar to that of Kang-Lee & Harper (1978), who estimated rates of catabolism by subtracting the amount of threonine required for protein synthesis from the amount of threonine ingested over a specific time period. Normally-fed rats used in the present study each consumed 15 g of a commercial diet (41B) containing 0.51% threonine (Fox Facilities)/day, which was equivalent to a dietary intake of $640\mu\text{mol}$ of threonine/day, if absorption was assumed 100% efficient. Over a 5-day period average body weight gain was 5.8 g/day and, since $60.2\mu\text{mol}$ of threonine is required for a weight gain of 1 g (see Kang-Lee & Harper, 1978), the total requirement of threonine for weight gain (i.e. protein synthesis *de novo*) was $349\mu\text{mol/day}$. Therefore, the quantity of threonine catabolized was estimated as $640 - 349 = 291\mu\text{mol/day}$, which is equivalent to 202nmol/min per whole rat. This value agrees well with the value of 231nmol of threonine catabolized/min per whole liver (Table 3).

The activities of threonine/serine dehydratase and threonine aldolase are located mainly, if not entirely, in rat liver (Karasek & Greenberg, 1957; Rowsell *et al.*, 1979). However, threonine dehydrogenase activity is not located exclusively in rat liver; the activity in kidney and heart was 30% and in brain 10% of the hepatic activity (Green & Elliott, 1964),

but no activity was detected in other tissues. As the relative organ weights in rats (g/100g body wt.) are 3 (liver), 1 (brain), 0.8 (kidneys) and 0.4 (heart) (Waynforth, 1980), it was calculated that 87% of the total body threonine dehydrogenase activity is hepatic in the rat. Thus, the assumption that all threonine dehydrogenase activity is hepatic slightly underestimates the contribution of this enzyme to threonine catabolism in the whole animal. Even so, the concordance of the total threonine catabolic capacity in the liver and the rate of threonine catabolism in the whole animal suggests that the assumptions made (see the Results section) are indeed valid. The conclusion of Franchi *et al.* (1973) that threonine dehydrogenase made only a small contribution to the overall catabolism of threonine in the fed rat may be explained, in part, by the low activity of the dehydrogenase reported by these workers. Threonine dehydrogenase activity is about 8-fold lower when measured in the assay conditions of Franchi *et al.* (1973), as compared with the activity in our assay conditions (P. B. Nunn & M. I. Bird, unpublished work).

(b) *Other metabolic states.* The metabolic regimens adopted in the present study were known to cause a gluconeogenic state in rats associated with the induction of hepatic threonine/serine dehydratase. In the gluconeogenic states (except glucagon-treated animals), threonine dehydratase became the dominant enzyme of threonine catabolism in rat liver, contributing between 77 and 86% of the total hepatic threonine catabolic activity (Table 3). We have no independent means of comparing total hepatic threonine catabolic activity with the rate of threonine catabolism in the whole animal for any of these metabolic states.

Formation of glycine and a two-carbon fragment from threonine

One of the objectives of the present work was to attempt to explain the observation that, in normally-fed rats, 20–33% of dietary threonine was degraded to glycine and 'acetate' units (Meltzer & Sprinson, 1952). We have shown (M. I. Bird, P. B. Nunn & L. A. J. Lord, unpublished work) that preparations of rat liver mitochondria lacking threonine aldolase activity produced glycine from L-threonine in a reaction that probably involved the coupled activities of threonine dehydrogenase and 2-amino-3-oxobutyrate CoA-ligase. At low concentrations of L-threonine the rate of glycine production was several times greater than the rate of aminoacetone formation. Since threonine dehydrogenase activity accounted for nearly 90% of the total threonine catabolized in the normally fed state, it is probable that *in vivo* (with a threonine concentration of 0.35mM) the major proportion of the threonine degraded by this enzyme activity is

converted into glycine and acetyl-CoA, rather than into aminoacetone. Attempts to detect aminoacetone in either freeze-clamped liver or isolated hepatocytes incubated with 0.30 mM L-threonine were unsuccessful (Bird, 1980). Thus, the activity of this mitochondrial system of glycine production is sufficient to account for the original observations of Meltzer & Sprinson (1952).

Physiological roles of the threonine-catabolizing enzymes in rat liver

We conclude that the activity of threonine aldolase in rat liver is uniformly low irrespective of the metabolic state of the animal and that the principal enzymes catalysing the degradation of threonine in this tissue are threonine dehydrogenase and threonine dehydratase. The existence of two enzymes, located in different cellular compartments and each capable of assuming a dominant role in the catabolism of threonine during different metabolic states, raises the question of the physiological functions of the metabolic pathways initiated by these two enzymes and the means by which the flux through the two pathways is regulated.

There is compelling evidence that, in the rat, the major physiological function of threonine/serine dehydratase is to mobilize threonine (or serine) carbon for hepatic gluconeogenesis (see Mak & Pitot, 1981). Hence, the induction of this enzyme activity in gluconeogenic states (in which the enzyme assumes the dominant role in hepatic threonine catabolism) aids the homeostasis of blood glucose concentrations when dietary glucose is lacking (as occurs when feeding a high-protein diet or during starvation). Concomitantly, the dehydratase activity is involved in the homeostatic regulation of free threonine concentrations in the whole animal. This threonine may be derived from either excess dietary sources (high-protein diet) or liver and muscle proteolysis (starvation and other gluconeogenic states; see Ishikawa, 1975).

Conversely, threonine dehydrogenase activity appears to be dominant during the normally-fed state, when the requirement for hepatic gluconeogenesis is low. In this condition the physiological role of the dehydrogenase activity is probably the homeostasis of free somatic threonine concentrations derived ultimately from dietary threonine. If threonine dehydrogenase activity is coupled to that of 2-amino-3-oxobutyrate CoA-ligase in rat liver (Bird & Nunn, 1979b) as occurs in other organisms (McGilvray & Morris, 1969; Linstead *et al.*, 1977; Dale, 1978), the main products of threonine catabolism in the rat during the fed state will be glycine and acetyl-CoA. Glycine may be oxidized by the mitochondrial glycine-cleavage system, or incorporated into a number of important cellular constituents (see Neuberger, 1981). Acetyl-CoA might

be oxidized by the tricarboxylate cycle, or transported from mitochondria via citrate for use in lipid synthesis. Thus, in the normally-fed rat the function of the dehydrogenase might be simply to direct excess threonine carbon for oxidative or biosynthetic purposes. It is noteworthy that the parasite *Trypanosoma brucei* catabolizes L-threonine by a reaction involving threonine dehydrogenase and 2-amino-3-oxobutyrate CoA-ligase and preferentially utilizes the acetyl-CoA produced for lipid synthesis, even in the presence of exogenous acetate (Klein & Linstead, 1976).

M. I. B. was supported by an Instant Award by the S.E.R.C.

References

- Aoyama, Y. & Motokawa, Y. (1981) *J. Biol. Chem.* **256**, 12367–12373
- Bird, M. I. (1980) Ph.D. Thesis, University of London
- Bird, M. I. & Nunn, P. B. (1979a) *Biochem. Soc. Trans.* **7**, 1274–1276
- Bird, M. I. & Nunn, P. B. (1979b) *Biochem. Soc. Trans.* **7**, 1276–1277
- Bird, M. I., Lord, L. A. J. & Nunn, P. B. (1982) *Biochem. Soc. Trans.* **10**, 130
- Boylan, S. A. & Dekker, E. E. (1981) *J. Biol. Chem.* **256**, 1809–1815
- Dale, R. A. (1978) *Biochim. Biophys. Acta* **544**, 496–503
- Elliott, W. H. (1960) *Biochem. J.* **74**, 90–94
- Franchi, G., Lusini, P., Marinello, E. & Pagani, R. (1973) *Ital. J. Biochem.* **21**, 231–238
- Goldstein, L., Knox, W. E. & Behrman, E. J. (1962) *J. Biol. Chem.* **237**, 2855–2860
- Green, M. L. & Elliott, W. H. (1964) *Biochem. J.* **92**, 537–549
- Hartshorne, D. & Greenberg, D. M. (1964) *Arch. Biochem. Biophys.* **105**, 173–178
- Hepworth, J. D. (1973) *Org. Synth.* **5**, 27–29
- Hoshino, J., Simon, D. & Kroger, H. (1972) *Eur. J. Biochem.* **27**, 388–394
- Inoue, H., Kasper, C. B. & Pitot, H. C. (1971) *J. Biol. Chem.* **246**, 2626–2632
- Ishikawa, E. (1975) *Adv. Enzyme Regul.* **14**, 117–136
- Jost, J.-P., Hsie, A., Hughes, S. D. & Ryan, L. (1970) *J. Biol. Chem.* **245**, 351–357
- Kang-Lee, Y. A. & Harper, A. E. (1978) *J. Nutr.* **108**, 163–175
- Karasek, M. A. & Greenberg, D. M. (1957) *J. Biol. Chem.* **227**, 191–205
- Klein, R. A. & Linstead, D. J. (1976) *Biochem. Soc. Trans.* **4**, 48–50
- Krebs, H. A. & Perkins, J. R. (1970) *Biochem. J.* **118**, 635–644
- Laver, W. G., Neuberger, A. & Scott, J. J. (1959) *J. Chem. Soc.* 1483–1491
- Linstead, D. J., Klein, R. A. & Cross, G. A. M. (1977) *J. Gen. Microbiol.* **101**, 243–251
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

- Mak, W. W.-N. & Pitot, H. C. (1981) *Biochem. J.* **198**, 499–504
- Malkin, L. I. & Greenberg, D. M. (1964) *Biochim. Biophys. Acta* **85**, 117–131
- McGilvray, D. & Morris, J. G. (1969) *Biochem. J.* **112**, 657–671
- Meltzer, H. L. & Sprinson, D. B. (1952) *J. Biol. Chem.* **197**, 461–474
- Moore, S., Spackman, D. H. & Stein, W. H. (1958) *Anal. Chem.* **30**, 1185–1190
- Morris, J. E. & Peraino, C. (1976) *J. Biol. Chem.* **251**, 2571–2578
- Nagabhushanam, A. & Greenberg, D. M. (1965) *J. Biol. Chem.* **240**, 3002–3008
- Nakagawa, H. & Kimura, H. (1969) *J. Biochem. (Tokyo)* **66**, 669–683
- Neuberger, A. (1981) *Compr. Biochem.* **19A**, 257–303
- Palekar, A. G., Tate, S. S. & Meister, A. (1973) *J. Biol. Chem.* **248**, 1158–1167
- Peraino, C. (1967) *J. Biol. Chem.* **242**, 3860–3867
- Pestaña, A. (1969) *Eur. J. Biochem.* **11**, 400–404
- Pitot, H. C. & Peraino, C. (1964) *J. Biol. Chem.* **239**, 1783–1788
- Plummer, D. T. & Wilkinson, J. H. (1963) *Biochem. J.* **87**, 423–429
- Riario-Sforza, G., Pagani, R. & Marinello, E. (1969) *Eur. J. Biochem.* **8**, 88–92
- Rowell, E. V., Carnie, J. A., Wahbi, S. D., Al-Tai, A. H. & Rowell, K. V. (1979) *Comp. Biochem. Biophys.* **63B**, 543–555
- Siess, E. A., Brocks, D. G. & Wieland, O. H. (1976) *FEBS Lett.* **69**, 265–271
- Stein, W. H. & Moore, S. (1954) *J. Biol. Chem.* **211**, 915–926
- Tschudy, D. P., Welland, F. H., Collins, A. R. & Rechcigl, M. (1964) *Cancer Res.* **24**, 2033–2037
- Urata, G. & Granick, S. (1963) *J. Biol. Chem.* **238**, 811–820
- Vega, A. & Nunn, P. B. (1969) *Anal. Biochem.* **32**, 446–453
- Wainio, W. W. (1970) *The Mammalian Mitochondrial Respiratory Chain*, Academic Press, New York
- Waynforth, M. B. (1980) *Experimental and Surgical Technique in the Rat*, p. 244, Academic Press, New York
- Williamson, J. R., Scholz, R. & Browning, E. T. (1969) *J. Biol. Chem.* **244**, 4617–4627