Amino Ketone Formation and Aminopropanol-Dehydrogenase Activity in Rat-Liver Preparations

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1. Rat tissue homogenates convert DL-1-aminopropan-2-ol into aminoacetone. Liver homogenates have relatively high aminopropanol-dehydrogenase activity compared with kidney, heart, spleen and muscle preparations. 2. Maximum activity of liver homogenates is exhibited at pH 9.8. The K_m for aminopropanol is approx. 15 mM, calculated for a single enantiomorph, and the maximum activity is approx. 9 μ moles of aminoacetone formed/mg. wet wt. of liver/hr. at 37°. Aminoacetone is also formed from L-threonine, but less rapidly. An unidentified amino ketone is formed from DL-4-amino-3-hydroxybutyrate, the K_m for which is approx. 200 mM at pH 9.8. 3. Aminopropanol-dehydrogenase activity in homogenates is inhibited non-competitively by DL-3-hydroxybutyrate, the K_i being approx. 200 mM. EDTA and other chelating agents are weakly inhibitory, and whereas potassium chloride activates slightly at low concentrations, inhibition occurs at 50–100 mM. 4. It is concluded that aminopropanol-dehydrogenase is located in mitochondria, and in contrast with L-threonine dehydrogenase can be readily solubilized from mitochondrial preparations by ultrasonic treatment. 5. Soluble extracts of disintegrated mitochondria exhibit maximum aminopropanol-dehydrogenase activity at pH 9.1. At this pH, K_m values for the amino alcohol and NAD⁺ are approx. 200 and 1.3 mM respectively. Under optimum conditions the maximum velocity is approx. 70 μ moles of aminoacetone formed/mg. of protein/hr. at 37°. Chelating agents and thiol reagents appear to have little effect on enzyme activity, but potassium chloride inhibits at all concentrations tested up to 80 mM. DL-3-Hydroxybutyrate is only slightly inhibitory. 6. Dehydrogenase activities for L-threonine and DL-4-amino-3-hydroxybutyrate appear to be distinct from that for aminopropanol. 7. Intraperitoneal injection of aminopropanol into rats leads to excretion of aminoacetone in the urine. Aminoacetone excretion proportional to the amount of the amino alcohol administered, is complete within 24 hr., but represents less than 0.1% of the dose given. 8. The possible metabolic role of amino alcohol dehydrogenases is discussed.

The finding that aminoacetone was produced from DL-1-aminopropan-2-ol by a wide variety of micro-organisms (Turner, 1965) led to the discovery of aminopropanol-dehydrogenase activities in cell-free extracts of *Escherichia coli* (Turner, 1965, 1966; Higgins, Pickard, Turner & Willetts, 1966). The presence of L-threonine dehydrogenase (Neuberger & Tait, 1960, 1962; Green & Elliott, 1964) activity in this micro-organism, due to an apparently distinct and specific enzyme, prompted the suggestion (Turner, 1966) that a coupling of the two dehydrogenases could be involved in the observed conversion of L-threonine into the D-1-aminopropan-2-ol moiety of vitamin B₁₂ by *Streptomyces griseus* (Krasna, Rosenblum & Sprinson, 1957). (See Scheme 1.) The feasibility of such a mechanism was first proposed by Neuberger

& Tait (1960). L-Threonine-decarboxylase activity has never been reported in any biological preparation.

Aminopropanol has also been isolated from rat urine and human urine, and L-threonine demonstrated to serve as a precursor of the amino alcohol in the rat (Smith & Jepson, 1963*a,b*). This suggested that a coupled oxidoreductase system, with aminoacetone as the intermediate, could be involved in this case also. Aminoacetone formation from L-threonine has been reported in liver preparations from rats (Tschudy, Welland, Collins & Rechcigl, 1964; Green & Elliott, 1964), guinea pigs (Urata & Granick, 1963) and bull frogs (Hartshorne & Greenberg, 1964).

The present paper describes the detection of 1-aminopropan-2-ol-dehydrogenase activity in rat

Scheme 1. (1) L-Threonine dehydrogenase. (2) Aminopropanol dehydrogenase. (3) Rapid spontaneous non-enzymic decarboxylation (Laver, Neuberger & Scott, 1959).

tissues, and gives some properties of the enzyme in rat-liver preparations. Dehydrogenase activities for L-threonine and DL-4-amino-3-hydroxybutyrate are also described. Evidence for the activity of aminopropanol dehydrogenase *in vivo* has been obtained from experiments in which rats were injected with aminopropanol.

A preliminary account of some of this work has been published previously (Higgins *et al.* 1966).

EXPERIMENTAL

Materials. DL-1-Aminopropan-2-ol and L-threonine were obtained from British Drug Houses Ltd., Poole, Dorset. DL-4-Amino-3-hydroxybutyrate and ox-heart lactate dehydrogenase (75 units/mg.) were purchased from Koch-Light Laboratories Ltd., Colnbrook, Bucks., and NAD⁺ from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. All other chemicals were of A.R. grade, or the highest quality available commercially.

Black and white hooded male rats, weight approx. 175 g., were obtained from Animal Suppliers (London) Ltd., London, N.12.

Methods. Tissue homogenates were prepared in a Potter-type homogenizer by using 10 ml. of potassium phosphate buffer (0.1 M, pH 7.0) per g. wet wt. of fresh tissue. All operations were carried out in the cold with materials chilled to 0–4° before use. Whole homogenates were used for enzyme assays within 1 hr. of the death of the animal.

Mitochondrial preparations were obtained from fresh rat liver by the differential centrifugation of tissue homogenates in sucrose solutions according to the procedure of Hogeboom (1955). Chilled minced liver was homogenized with 9 ml. of 0.25 M-sucrose/g. of tissue. The homogenate was then layered over an equal volume of 0.34 M-sucrose and centrifuged for 10 min. at 700g. The supernatant was centrifuged again for 10 min. at 5000g. The mitochondrial pellet was suspended in 5 ml. of 0.25 M-sucrose/g. of original tissue, by gentle homogenization, and centrifuged for 10 min.

at 24000g. Sedimented mitochondria were washed by resuspension and recentrifugation in the same manner. All operations were carried out at 0–4°, with chilled sucrose solutions, and an MSE 1700 refrigerated centrifuge was employed.

Disintegration of mitochondria was accomplished by treating ice-cold suspensions with ultrasonic vibrations (20 kcyc./sec.) for 90 sec. with a MSE 60 W Ultrasonic Disintegrator. After disintegration, the preparation was centrifuged at 24000g for 10 min.

Dehydrogenase activities were measured by a modification of the colorimetric method for L-threonine-dehydrogenase activity in rat-liver preparations described by Green & Elliott (1964). Reaction mixtures for the assay of aminopropanol-dehydrogenase activity in homogenates contained 125 μmoles of DL-1-aminopropan-2-ol (each enantiomorph), 5 μmoles of NAD⁺, 15 μmoles of sodium pyruvate, 0.1 mg. of lactate dehydrogenase, 100 μmoles of KCl, 400 μmoles of diethanolamine-HCl buffer, pH 9.8, and approx. 18 mg. wet wt. of tissue as homogenate, in total volume 2.5 ml. With soluble enzyme preparations from disintegrated mitochondria, aminopropanol-dehydrogenase activity was assayed in a similar system except that 1 m-mole of aminopropanol and 4 mg. of protein were present, KCl was omitted, the buffer pH was 9.1, and the total volume was 3.0 ml. Aminopropanol solutions were adjusted to the appropriate pH with HCl before use. L-Threonine- and DL-4-amino-3-hydroxybutyrate-dehydrogenase activities were assayed by substituting these substrates for DL-aminopropanol in the above reaction mixtures. Reactions were started by the addition of enzyme preparation to the other components in thin-walled tubes equilibrated at 37° in a water bath. Incubation was for 60 min. at 37°. Reactions were stopped by the addition of 0.3 ml. of 25% (w/v) trichloroacetic acid solution. Precipitated material was removed by centrifugation.

Aminoacetone was determined in 1.0 ml. samples of stopped reaction mixture supernatants, also in urine samples, by the method of Mauzerall & Granick (1956) as described by Turner (1966). Protein was assayed by a modified biuret procedure as described by Turner (1966).

Activities in soluble extracts are expressed as μ moles of aminoacetone produced/mg. of protein/hr.

Thin-layer chromatography, used to differentiate aminoacetone from 5-aminolaevulate, in urine samples, was carried out on cellulose powder with ethanol-n-acetic acid-pyridine-water (95:10:3:3, by vol.) as the solvent (Elliott, 1960a). Amino ketones were detected by spraying with ethanolic ninhydrin reagent.

RESULTS

Distribution of aminopropanol-dehydrogenase activity in rat tissues

Tissue samples were dissected from freshly killed rats and homogenized as described in the Experimental section. Aminoacetone formation from DL-1-aminopropan-2-ol was measured at both pH 7 and pH 8.7. Liver homogenates were found to have markedly greater activity than the other organs or tissues examined, and in all cases activity was higher at the more alkaline pH. The results are shown in Table 1.

Factors affecting enzyme activity in liver homogenates

(a) Homogenate concentration. Aminoacetone formation, measured under the conditions described in the Experimental section, was proportional to homogenate concentrations up to at least 20mg. wet wt. of tissue/ml. at pH 8.7. (b) pH. Optimum activity was found to occur at approx. pH 9.8, a sharp peak being observed between pH 9.5 and 10.2 (see Fig. 1). (c) 1-Aminopropan-2-ol concentration. At the optimum pH, and in

the presence of an NAD⁺-regenerating system, the rate of aminoacetone production was maximal at aminopropanol concentrations greater than approx. 50mm. The Michaelis constant, obtained graphically by the method of Lineweaver & Burk (1934), was determined to be 14.8 and 15.7mm respectively on different occasions. The maximum velocity values obtained from the graphs gave an activity for liver homogenates of approx. 9 μ moles of aminoacetone formed/mg. wet wt. of tissue/hr. at 37°. (d) Potassium chloride concentration. At concentrations above approx. 50mm, potassium chloride inhibited aminopropanol-dehydrogenase activity; at 100mm, 27% inhibition was observed. Slight activation occurred at lower concentrations, optimally at 40mm. At this concentration, in the presence of 8mm-potassium phosphate buffer in which the homogenate was prepared, 5-10% activation was consistently observed. (e) Amino ketone formation from aminopropanol analogues. Both L-threonine and DL-4-amino-3-hydroxybutyrate gave rise to amino ketones when they replaced aminopropanol in reaction mixtures. Activity with threonine was variable, but always less than one-third of the activity with aminopropanol. Activity with 4-amino-3-hydroxybutyrate was always greater on the basis of the colour yield obtained by the assay procedure for amino ketones. Results are shown in Table 2. Although

Table 1. *Aminopropanol-dehydrogenase activity of rat-tissue homogenates*

Tissue homogenates in K₂HPO₄-KH₂PO₄ buffer were prepared as described in the Experimental section. Reaction mixtures contained: 125 μ moles of 1-aminopropan-2-ol (each enantiomorph), 5 μ moles of NAD⁺, 400 μ moles of KCl, 400 μ moles of potassium phosphate buffer (pH 7) or tris-HCl buffer (pH 8.7), and 10mg. wet wt. of tissue as homogenate, in a total vol. of 2.5 ml. Incubation at 37° was carried out for 60 min. Aminoacetone formed was assayed as described in the Experimental section.

Tissue	Aminopropanol-dehydrogenase activity (μ moles/mg. wet wt. of tissue/hr.)	
	pH 7	pH 8.7
Liver	1.94	5.70
Kidney	0.85	1.94
Heart	1.06	1.56
Spleen	—	0.87
Muscle (sciatic)	—	0.23

Fig. 1. Effect of pH on aminopropanol-dehydrogenase activity. Enzyme activities were measured in rat-liver crude homogenate and the soluble enzyme preparation from disintegrated mitochondria, as described in the Experimental section. Potassium phosphate buffers were used at pH 7.0-7.8, tris-HCl buffers at pH 7.8-9.0, and diethanolamine-HCl buffers at pH 9.0-10.5. Activity of crude-liver homogenate, ●; activity of soluble enzyme preparation, ○.

Table 2. *Amino alcohol-dehydrogenase activities in rat-liver homogenates and the effect of 2-hydroxybutyrate*

Activities with aminopropanol as substrate were assayed as described for homogenates in the Experimental section except that a total volume of 2.9 ml. was used. With L-threonine and DL-4-amino-3-hydroxybutyrate, these compounds replaced aminopropanol at the same concentration with respect to single enantiomorphs of the type indicated. With DL-4-amino-3-hydroxybutyrate, the results are expressed as extinction values, measured at 551 μ , observed during assay of amino ketones as described for aminoacetone (figures in parentheses).

Substrate (43 mm)	Addition (at concn. shown)	Activity (m μ moles/mg. of tissue/hr.)	
		Expt. 1	Expt. 2
DL-1-Aminopropan-2-ol	—	5.2	5.3
L-Threonine	—	1.6	1.9
DL-1-Aminopropan-2-ol	L-Threonine (22 mm)	6.5	6.6
DL-1-Aminopropan-2-ol	L-Threonine (43 mm)	6.8	7.5
DL-1-Aminopropan-2-ol	DL-3-Hydroxybutyrate (21 mm)	3.7	3.8
DL-1-Aminopropan-2-ol	DL-3-Hydroxybutyrate (43 mm)	3.6	3.6
		Expt. 3	Expt. 4
DL-1-Aminopropan-2-ol	—	(0.485)	(0.495)
DL-4-Amino-3-hydroxybutyrate	—	(1.138)	(1.065)

the identity of the amino ketone produced was not determined, and the extinction coefficient of the colour due to 4-amino-3-oxobutyrates was not known, it appeared likely that 4-amino-3-hydroxybutyrate was readily oxidized by liver homogenates, although the K_m determined by the method of Lineweaver & Burk (1934) was approx. 200 mm. When rates of amino ketone formation were measured with various concentrations of aminopropanol, aminohydroxybutyrate and a mixture of these substrates, a double reciprocal plot of the results (Lineweaver & Burk, 1934) gave maximum velocity values in the proportions 6.30, 1.25 and 1.90 respectively. The intermediate velocity observed with mixed substrates suggested that the same active site was involved in the oxidation of both substrates. (f) Chelating agents. A variety of metal-chelating agents were found to weakly inhibit aminopropanol-dehydrogenase activity in rat-liver homogenates. The results are shown in Table 3. (g) 3-Hydroxybutyrate. To investigate the possibility that aminopropanol and 3-hydroxybutyrate were oxidized by the same enzyme, as suggested for the hydroxy acid and L-threonine with rat-liver preparations (Green & Elliott, 1964), the effect of 3-hydroxybutyrate on aminopropanol oxidation was examined. It was found that at aminopropanol concentration 43 mm, and with the DL-3-hydroxy acid at the same or half this concentration, approx. 30% inhibition occurred (see Table 2). A double-reciprocal plot, showing the effect of aminopropanol concentration on reaction rates at DL-3-hydroxybutyrate concentrations 25 and 50 mm (with respect to single enantiomorphs), indicated that inhibition was non-competitive (see

Table 3. *Effect of metal-chelating agents on aminopropanol-dehydrogenase activity of rat-liver homogenates*

Activities were assayed as described in the Experimental section. No preincubation of chelating agents with tissue homogenates was carried out.

Metal-chelating agent		Activity (%)			
	Concn. (mm)	...	4	8	12
None			(100)	(100)	(100)
EDTA			98	96	93
8-Hydroxyquinoline			124	105	90
Thiourea			93	69	52
Oxalic acid			89	73	55
Salicylic acid			92	77	68

Fig. 2). Inhibitor constants were determined graphically from a Lineweaver-Burk-type plot, using the expression $K_i = i/(V/V_p - 1)$ for purely non-competitive inhibition (Dixon & Webb, 1964), and found to be 125 and 200 mm respectively at the corresponding hydroxybutyrate concentrations.

Fractionation of rat-liver preparations and the distribution of amino alcohol dehydrogenases in subcellular fractions

Distribution of aminopropanol-dehydrogenase activity in subcellular fractions. Preliminary studies showed that almost all enzyme activity was located in a fraction forming the pellet material when homogenates were centrifuged at 20000g for 20 min. Slight activity remaining in the supernatant

was recovered in the pellet obtained by centrifuging at 100 000g for 60 min. These results suggested that aminopropanol dehydrogenase was associated with heavy particles, possibly mitochondria.

1-Aminopropan-2-ol-, threonine- and 4-amino-3-

Fig. 2. Effect of DL-3-hydroxybutyrate concentration on aminopropanol-dehydrogenase activity of liver homogenate. Enzyme activity was assayed as described in the Experimental section except that various concentrations of aminopropanol were used, as indicated. Results obtained in the absence of inhibitor (Δ) are compared with those obtained at DL-3-hydroxybutyrate concentrations 25 mM ($\circ$) and 50 mM ($\bullet$) with respect to single enantiomorphs. The calculation of K_i values is described in the text.

hydroxybutyrate-dehydrogenase activities in mitochondrial preparations. Rat-liver mitochondria were prepared as described by Hogeboom (1955). The distribution of aminopropanol-dehydrogenase activities between the various fractions obtained at different stages of the procedure is shown in the first part of Table 4. It was concluded that enzyme activity was associated with mitochondria. The activity of mitochondria with L-threonine and DL-4-amino-3-hydroxybutyrate were approx. 60 and 90% respectively when colours obtained in the standard assay for aminoacetone were compared with that obtained by using aminopropanol as substrate. Additional results are given in the second part of Table 4.

Effect of ultrasonic treatment, and of freezing and thawing, on amino ketone production by mitochondrial preparations. Ultrasonic treatment, freezing and thawing twice, and a combination of these disruptive procedures had very little effect on the aminopropanol-dehydrogenase activity of mitochondria. When such preparations were centrifuged after disruption, at 24 000g for 2 min., however, ultrasonic treatment either alone or followed by freezing and thawing was found to have released approx. 70% of the activity into the supernatant fraction. Freezing and thawing alone solubilized only 34% of the activity. The relative activities of aminopropanol, threonine and amino-hydroxybutyrate were approx. 100, 26 and 60%

Table 4. *Distribution of amino alcohol-dehydrogenase activities in subcellular fractions of rat-liver homogenates*

Subcellular fractions were prepared as described in the Experimental section. At each fractionation step sedimented material was washed and carefully resuspended in the same volume of buffer as the original homogenate. Activities were measured in 0.2 ml. samples of such particulate and supernatant fractions. In Expt. 1 the incubation conditions in all cases were the same as described for homogenates in the Experimental section. In Expt. 2 the conditions were as described for soluble preparations, i.e. amino alcohol concn. 330 mM. Activities are expressed in terms of the extinction values observed during colorimetric assay of amino ketones.

Subcellular fraction	Dehydrogenase activity ($E_{551}^{1\text{cm}}$)		
	Substrate ...	Aminohydroxybutyrate	Threonine
Expt. 1			
Whole homogenate	0.66	—	—
Pellet material (700g)	0.22	—	—
Supernatant (700g)	0.52	—	—
Supernatant (5000g)	0.13	—	—
Pellet material (24 000g)	0.48	—	—
Supernatant (24 000g)	0.06	—	—
Expt. 2			
Whole homogenate	0.81	1.68	0.88
Mitochondrial prep. (washed)	0.46	0.44	0.25
Disrupted mitochondria	0.42	0.39	0.24
Particulate submitochondrial fraction	0.13	0.23	0.18
Soluble submitochondrial fraction	0.27	0.16	0.07

respectively in the soluble fraction (see Table 4, Expt. 2).

Properties of aminopropanol-dehydrogenase and related enzymes in soluble extracts of rat-liver mitochondria. Soluble extracts were prepared from mitochondrial preparations as described in the Experimental section.

(a) Effect of pH. Optimum activity for aminopropanol dehydrogenase was found at approx. pH 9.1. This value contrasts with the value of 9.8 found by using whole homogenates. The results are shown in Fig. 1. The effects of pH on activities with 4-amino-3-hydroxybutyrate and threonine were not determined.

(b) Effects of K^+ and Mg^{2+} ions. Potassium ions, added as KCl, were found to be inhibitory at all concentrations between 10 and 80 mM. At 10 mM, inhibition was 32%, whereas at 50 and 80 mM inhibition was 40 and 50% respectively. In view of the slightly inhibitory effect of some metal-chelating agents on the aminopropanol-dehydrogenase activity of whole-liver homogenates, the effect of Mg^{2+} ions on the activity of soluble mitochondrial extracts at pH 9.1 was tested. Although somewhat variable, the results suggested 10–20% activation at a magnesium sulphate concentration of 1.3 mM. At higher concentrations activation was progressively less marked, and inhibition occurred at concentrations above 3 mM.

(c) Effect of 1-aminopropan-2-ol concentration. In contrast with the value of approx. 15 mM found for the K_m of aminopropanol by using whole-liver homogenates, values of 200 mM were obtained on two separate occasions with soluble extracts of disrupted mitochondria. The graphical method of Lineweaver & Burk (1934) was employed. Maximum velocity values of approx. 66 μ moles of aminoacetone formed/mg. of protein/hr. at 37° were obtained. In view of the high substrate concentration required for half-maximum enzyme activity, an aminopropanol concentration of 330 mM with respect to single enantiomorphs was employed in subsequent determinations.

(d) Effect of NAD^+ concentration. When the NAD^+ -regenerating system, i.e. lactate dehydrogenase plus sodium pyruvate, was omitted from otherwise complete reaction mixtures, half-maximum activity of aminopropanol dehydrogenase was found at NAD^+ concentration approx. 1.3 mM. The method of Lineweaver & Burk (1934) was employed.

(e) Effect of thiol and metal-chelating reagents. Iodoacetic acid, at concentrations up to 50 mM, and *p*-chloromercuribenzoate at 0.017 mM, caused virtually no inhibition. L-Cysteine and 2-mercaptoethanol were both inhibitory at 5–20 mM. At the higher concentration these reagents inhibited 14 and 47% respectively. In contrast GSH caused approx.

30% activation at 1–2 mM. A number of metal-chelating agents caused slight inhibition at high concentration. At 30 mM the percentage effects were: salicylate, 11; EDTA, 17; oxalate, 28; thiourea, 39. No inhibition was caused by 8-hydroxyquinoline. These results were corrected for the effect of some reagents on the stability of aminoacetone and the sensitivity of the assay procedure (see Neuberger & Tait, 1962; Urata & Granick, 1963).

Evidence for activity of aminopropanol dehydrogenase in vivo. The activity of L-threonine dehydrogenase has been demonstrated in rats by detecting aminoacetone excretion in the urine after the intraperitoneal administration of L-threonine (Marver, Tschudy, Perlroth, Collins & Hunter, 1966). A similar method was used to demonstrate aminopropanol-dehydrogenase activity *in vivo*. Doses of 60 mg. to 1 g. of aminopropanol/kg. body wt. were administered, as aqueous solutions adjusted to pH 7 with hydrochloric acid, by intraperitoneal injection. The LD_{50} value for aminopropanol in rats is known to be 4.26 g./kg. body wt. (Smyth, Carpenter & Weil, 1949, 1951). Urine samples were collected over 24 hr., and aminoacetone contents measured (Table 5). Concentrated urine samples were examined by thin-layer chromatography on cellulose powder, as described in the Experimental section. Authentic samples of aminoacetone and the amino ketone 5-amino-laevulic acid were used as reference markers. Amino compounds were detected on chromatograms by spraying with ninhydrin. Urine samples gave a yellow spot having the same R_f as aminoacetone.

Table 5. *Urinary excretion of aminoacetone after injection of aminopropanol*

Rats were maintained in metabolism cages for several days before the start of the experiment. Aminopropanol was administered by intraperitoneal injection of 2 ml. samples of sterile, neutral, aqueous solutions of the appropriate concentration. Sterile water was injected as a control. Urine was collected over the 24 hr. period after injection. Aminoacetone in urine samples was assayed colorimetrically as described in the Experimental section. In each case, a sample of urine was taken through the assay procedure from which the heat treatment with acetylacetone was omitted, and used as a blank. This ensured that results were corrected for the presence in urine of Ehrlich-positive materials.

Rat no.	DL-Aminopropanol administered (mg.)	Aminoacetone excreted (μ g./24 hr.)
1	200	89
2	100	79
3	50	44
4	25	27
5	12.5	11
6	0	7

No aminolaeuvate spot was detected. It was concluded that the amino ketone excreted in the urine after aminopropanol administration was aminoacetone. In contrast with the results obtained by Marver *et al.* (1966) by injecting L-threonine, the amount of aminoacetone excreted after aminopropanol administration was proportional to the dose over the range used (see Table 5).

DISCUSSION

The 1-aminopropan-2-ol-dehydrogenase activity found in rat liver, together with the reported presence of L-threonine dehydrogenase in this tissue (Green & Elliott, 1964; Tschudy *et al.* 1964), strengthens the hypothesis that urinary aminopropanol (Smith & Jepson, 1963a,b; Perry & Schroeder, 1963) is formed from L-threonine via aminoacetone. The finding that aminopropanol dehydrogenase, like threonine dehydrogenase in rat liver, is both NAD⁺-dependent and located in the mitochondrial fraction, makes the linked action of the two enzymes appear likely. Although the formation of aminopropanol from aminoacetone has not been demonstrated in the present study, many alcohol dehydrogenases are readily reversible and have equilibrium constants favouring alcohol formation (see Sund & Theorell, 1963).

The suggested coupling of the two dehydrogenases indicates that previously reported measurements of threonine-dehydrogenase activity in rat tissues, based on aminoacetone formation, may have been underestimates. Tschudy *et al.* (1964) noted that relatively anaerobic incubation conditions markedly decreased the yield of aminoacetone from threonine. This could well have been partly due to the conversion of aminoacetone into aminopropanol. Their failure to detect aminoacetone utilization under more aerobic conditions could similarly be accounted for by a lack of the NADH generated by threonine oxidation. A threonine concentration of 200–500 mM has been reported to be required for half-maximum rates of aminoacetone formation in systems *in vitro* (D. P. Tschudy, F. H. Welland & A. Collins, unpublished work, quoted by Marver *et al.* 1966) and a dose of 4 m-moles per rat has been found necessary to affect urinary aminoacetone concentrations in experiments *in vivo* (Marver *et al.* 1966). The isolation of radioactive aminopropanol and apparently related derivatives from rat urine after injection of [¹⁴C]threonine (Smith & Jepson, 1963a) suggests that the metabolism of aminoacetone to such compounds may be significant in whole-animal experiments.

A comparison of the K_m values reported here for aminopropanol oxidation by liver homogenates and the soluble mitochondrial enzyme preparation, i.e. approx. 15 and 200 mM respectively, suggests that

mitochondria may be capable of concentrating the amino alcohol. The oxidation of parenterally administered aminopropanol to aminoacetone by rats shows that the reaction studied *in vitro* is capable of taking place *in vivo*. Although the aminoacetone excreted in the urine represented only a small fraction of the amino alcohol given, it is possible that the extent of conversion was greater but that some of the aminoacetone was further metabolized to methylglyoxal. Aminoacetone oxidation by blood plasma has been reported for a number of mammalian species (Elliott, 1960b; Buffoni & Blaschko, 1963). Evidence for slow aminoacetone oxidation by guinea-pig-liver mitochondria has been presented by Urata & Granick (1961, 1963), although Green & Elliott (1964) and Tschudy *et al.* (1964) could not detect aminoacetone utilization by rat-liver homogenates. These observations suggest that the measurement of aminoacetone formation from aminopropanol provided a valid assay of aminopropanol-dehydrogenase activity in the present study.

The initially considered possibility that both L-threonine and the structurally related 1-aminopropan-2-ol were oxidized by the same enzyme system appears unlikely from the kinetic studies with mixed substrates, and seems certain from the appreciable separation of activities during fractionation of whole-liver tissue. Whereas both activities were found to be located in the intact mitochondrial fraction, threonine dehydrogenase was located in the pellet material after centrifugation of disrupted mitochondria whereas aminopropanol-dehydrogenase activity was readily solubilized. This confirms the work of Green & Elliott (1964), who reported that ultrasonic disruption, or treatment of the ultrasonic residue with either detergent or butan-1-ol, did not solubilize threonine-dehydrogenase activity from rat-liver mitochondria. Attempts to increase mitochondrial activity by freezing and thawing, or by detergent treatment, were found by Tschudy *et al.* (1964) to produce a marked decrease in threonine-dehydrogenase activity. These workers also reported a pH optimum of 7.3–7.4 for threonine dehydrogenase in rat-liver homogenates, which contrasts with the optimum of approx. 9.8 for aminopropanol oxidation found in the present study. The pH optimum for activity with disrupted mitochondria was not reported. The possibility that the oxidation of aminopropanol was catalysed by a relatively non-specific alcohol dehydrogenase of rat liver can be discounted in view of the location of the latter enzyme almost entirely in the supernatant rather than mitochondrial fraction of tissue homogenates (Nyberg, Schubert & Anggard, 1953). The methods used for the preparation of these rat-liver fractions was almost identical in both cases.

The metabolic role of aminopropanol dehydrogenase in mammalian tissue is not known, although Smith & Jepson (1963a) have reported that the injection of radioactive threonine into a rat gave rise to labelled metabolites other than aminopropanol, which were provisionally identified as *N*-methylaminopropanol, *NN*-dimethylaminopropanol and 2-methylcholine. It is conceivable that the latter compound is a phosphatidyl base representing a minor constituent of phospholipids, although its occurrence as such in mammals does not appear to have been reported. The prevention of 'fatty liver' in threonine-deficient rats by aminopropanol administration (Kielbasinska, Smith & Jepson, 1965) suggests that 2-methylcholine formed from aminopropanol may have a lipotropic function similar to choline. The detection of *N*-trimethylaminoacetone in human urine has recently been reported (Grüner, Aurich & Strack, 1966).

Alternatively, 1-aminopropan-2-ol itself may function as a phosphatidyl base, although once again its natural occurrence as such has not been reported. In the rat it has been established that phospholipid formation readily occurs when CDP esters of 1-aminopropan-2-ol and other unnatural bases are incubated with dispersions of liver tissue in the presence of diglycerides (Chojnacki, 1964). Such activity may be due to a lack of specificity in the ethanolaminephosphotransferase system, and the enzymic formation of CDP 1-aminopropan-2-ol has not been reported. It is known, however, that 1-aminopropan-2-ol supplied in the diet of housefly (*Musca domestica*) larvae is readily incorporated into phospholipids in place of ethanolamine (Bridges & Ricketts, 1966).

The oxidation of DL-4-amino-3-hydroxybutyrate to a compound reacting as an amino ketone, although detected in the present work, has not been investigated in detail. On the basis of the distribution of enzyme activities between particulate and soluble fractions of disrupted mitochondria, aminohydroxybutyrate and aminopropanol dehydrogenases appear to be distinct. The product of aminohydroxybutyrate oxidation is likely to be 4-amino-3-oxobutyrate, a compound synthesized by Nielsands, Neuberger & Scott (1959), although the biological decarboxylation of this amino ketone to aminoacetone is a possibility (Neuberger, 1961). It has been suggested by Neuberger (1961) that 4-amino-3-oxobutyrate may be formed biologically from malonyl-CoA plus glycine, and that reduction to the hydroxy compound could give rise to a likely precursor of the betaine carnitine. Aminohydroxybutyrate can replace carnitine as an essential growth factor for the mealworm *Tenebrio molitor* (Carter, Bhattacharyya, Weidman & Frankel, 1952). Recent work with rats, however, suggests that 4-

butyrobetaine rather than aminohydroxybutyrate is the precursor of carnitine (Linstedt & Linstedt, 1961, 1962; Bremer, 1962). Alternatively, 4-amino-3-hydroxybutyrate dehydrogenase may be involved in the catabolism of carnitine. Pseudomonad species are known to degrade L(-)-carnitine via 3-oxocarnitine (Strack, Aurich & Grüner, 1964).

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