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The Regulation of Glucose and Pyruvate Formation from Glutamine and Citric-Acid-Cycle Intermediates in the Kidney Cortex of Rats, Dogs, Rabbits and Guinea Pigs

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The suppression by 3-mercaptopicolinate of gluconeogenesis from glutamine or 2-oxoglutarate in rat or dog kidney tubules did not affect the amount of these substrates undergoing complete oxidation. Furthermore, 3-mercaptopicolinate caused an accumulation of lactate in dog tubules. 3-Mercaptopicolinate abolished both gluconeogenesis and substrate oxidation in tubules from rabbit and guinea-pig kidney. These results imply the presence of an alternative pathway to phosphoenolpyruvate carboxykinase/pyruvate kinase for the production of pyruvate from citric-acid-cycle intermediates in the kidney cortex of rats and dogs but not in that of rabbits or guinea pigs. Oxaloacetate decarboxylase (present in the kidney cortex of all four species) or 'malic' enzyme (present in rat and dog but absent in rabbit and guinea-pig kidney cortex) could function in this role. Our observations indicate that 'malic' enzyme is probably implicated in this phenomenon. The lactate production observed in dog tubules in the presence of 3-mercaptopicolinate can be suppressed when aspartate formation is inhibited by 2-amino-4-methoxy-*trans*-but-3-enoic acid. This suggests that the provision of cytosolic NADH from citric-acid-cycle intermediates is facilitated by accumulation of aspartate acting as a 'sink' for cytosolic oxaloacetate.

Plasma glutamine is metabolized by the kidney cortex for ammonia production. The carbon skeleton of glutamine (2-oxoglutarate) is then converted to neutral compounds. Glucose and CO_2 are believed to be the major end products, but the relative importance of these two pathways *in vivo* is still uncertain.

According to classically accepted pathways, both the production of glucose from, and the complete oxidation of, glutamine require flux through the phosphoenolpyruvate carboxykinase [GTP: oxaloacetate carboxy-lyase (transphosphorylating) (EC 4.1.1.32)] reaction. 3-Mercaptopicolinic acid has been shown to be a reasonably specific inhibitor of this reaction (Kostos *et al.*, 1975; Robinson & Oei, 1975; Jomain-Baum *et al.*, 1976). Therefore 3-mercaptopicolinate would be expected to suppress both the gluconeogenic and the oxidative pathways of glutamine metabolism. However, in experiments with isolated tubules from rat kidney cortex, we found that gluconeogenesis could be completely inhibited by 3-mercaptopicolinate with no effect on glutamine oxidation (Vinay *et al.*, 1978*a*). This implies the formation of pyruvate from citric-acid-cycle intermediates in rat kidney by a pathway not involving phosphoenolpyruvate carboxykinase. Two such pathways could involve 'malic' enzyme [L-malate: $NADP^+$ oxidoreductase (oxaloacetate-decarboxylating) EC 1.1.1.40] or oxaloacetate decarboxylase (EC 4.1.1.3). This paper extends our earlier findings in rat and includes studies in species where the intracellular distribution of phosphoenolpyruvate carboxykinase is known to be bimodal (cytosolic and mitochondrial) as opposed to the totally cytosolic distribution in the rat (Soling & Kleineke, 1976). The effect of 3-mercaptopicolinate in kidney tubules from rats, dogs, rabbits and guinea pigs is reported. The maximal activities of both 'malic' enzyme and oxaloacetate decarboxylase have been determined in each species. Oxidation of glutamine is affected by 3-mercaptopicolinate only in species lacking renal 'malic' enzyme, irrespective of the cellular distribution of phosphoenolpyruvate

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carboxykinase. The results support the thesis that 'malic' enzyme may function to provide pyruvate from citric-acid-cycle intermediates when the phosphoenolpyruvate carboxykinase reaction is inhibited by 3-mercaptopicolinate. Some of these results have been presented in preliminary form (Vinay *et al.*, 1978*a*; Watford *et al.*, 1979*b*).

Materials and Methods

Animals

Wistar rats, New Zealand White rabbits, guinea pigs and mongrel dogs were maintained on species-specific commercial diets (Purina) and water *ad libitum*. Animals were anaesthetized with pentobarbital, intraperitoneally for rats and guinea pigs and intravenously for rabbits and dogs. All animals were well-fed unless otherwise stated.

Chemical and enzymes

L-[U-^{14}C]Glutamine and L-[U-^{14}C]glutamate were obtained from Amersham/Searle (Arlington, IL, U.S.A.). Aquasol scintillation fluid was obtained from New England Nuclear (Boston, MA, U.S.A.). Glutamine, 2-oxoglutarate, glutaminase (grade V) and amino-oxyacetate were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other enzymes and co-enzymes were from the Boehringer Corp. (Montreal, Quebec, Canada). Bovine serum albumin (fraction V) was from Miles Laboratories (Elkhart, IN, U.S.A.).

3-Mercaptopicolinate and 2-amino-4-methoxy-*trans*-but-3-enoic acid were gifts from Dr. N. W. DiTullio of Smith, Kline and French, Philadelphia, PA, U.S.A. and Dr. W. E. Scott of Hoffman La Roche, Nutley, NJ, U.S.A., respectively.

Isolated kidney tubules

Kidney tubules were isolated by a modification of the method of Guder & Wieland (1971) in that slices of cortex were cut prior to incubation with collagenase and hyaluronidase. The modification gives both increased yields and a preparation of tubules of more uniform length than the original method which involved the use of a tissue press (P. Vinay, unpublished work). Tubules were incubated in bicarbonate-buffered saline (Krebs & Henseleit, 1932) containing 2.5% (w/v) dialysed bovine serum albumin, as previously described for rat tubules (Vinay *et al.*, 1978*a*). Oxygen consumption and $^{14}CO_2$ production were measured as described by Vinay *et al.* (1978*b*). Substrate oxidation was estimated by drawing carbon and ammonia balances as described by Vinay *et al.* (1980*a*), as well as from measurements of $^{14}CO_2$ production from uniformly labelled substrate after correction for isotopic dilution (Vinay *et al.*, 1978*b*).

Determination of metabolites

Metabolites were assayed enzymically in the neutralized perchloric-acid extracts. Glucose was determined by the method of Slein (1963), lactate by that of Hohorst (1963*a*) and ammonia by that of Kun & Kearny (1974). Malate, citrate, and 2-oxoglutarate were measured as described by Hohorst (1963*b*), Dagley (1974) and Bergmeyer & Bernt (1974), respectively. Alanine and glutamate were measured by the methods of Williamson (1974) and Bernt & Bergmeyer (1974) respectively. Aspartate was determined according to the method of Bergmeyer *et al.* (1974) as modified by Crow *et al.* (1978). Glutamine was routinely determined as glutamate after hydrolysis with glutaminase (Lund, 1974).

Measurement of enzyme activities

Kidney cortices were homogenized in 10vol. of ice-cold 0.25M-sucrose containing 5mM-Tris/HCl, pH 7.4, and 1mM-dithiothreitol, by four passes of a motor-driven Teflon pestle. The homogenates were fractionated according to the procedure of Johnson & Lardy (1967). The nuclear fractions were retained and used for enzyme measurements. Mitochondrial activities in the fractions were 'solubilized' by freezing and thawing three times (Elliott & Pogson, 1977). Samples were stored in ice and assayed the same day.

The cross-contamination of the fractions was estimated from measurements of lactate dehydrogenase (Wroblewski & LaDue, 1955) and glutamate dehydrogenase (Schmidt, 1974) which were used as markers for the cytosolic and mitochondrial compartments respectively. All assays of enzymic activity were carried out at 37°C using at least two different concentrations of enzyme and the values shown have been corrected for cross-contamination of the fractions.

'Malic' enzyme was measured according to the method of Ochoa (1955) and oxaloacetate decarboxylase by that of Wojtczak & Walajtys (1974).

Results and Discussion

Metabolism of glutamine by kidney tubules from fed rats and dogs

We have reported that the inhibition of gluconeogenesis from glutamine by 3-mercaptopicolinate in isolated rat-kidney tubules did not suppress glutamine oxidation (Vinay *et al.*, 1978*a*). In those studies we did not measure malate or lactate, which were assumed not to accumulate. However in subsequent experiments using tubules from dog-kidney cortex we found a considerable accumulation of both malate and lactate. Therefore, it was necessary to assay for these metabolites in the rat.

The results are presented in Table 1. Under control conditions with glutamine (5mM) as substrate, no significant production of either malate or lactate was found. The presence of 3-mercaptopicolinate (0.5mM) caused a small accumulation of malate with no effect on lactate. Net oxidation of glutamine was unaffected. Therefore these results slightly change the quantitative aspects of our earlier results, but not the overall conclusion that inhibition of the phosphoenolpyruvate carboxykinase reaction in rat tubules does not inhibit glutamine oxidation.

The intracellular distribution of renal phosphoenolpyruvate carboxykinase is totally cytosolic in the rat, but it is present in both the cytosolic and mitochondrial compartments in most other species (Soling & Kleineke, 1976). As the dog has been extensively used for investigations of renal glutamine metabolism and as this species exhibits predominantly (75%) mitochondrial phosphoenolpyruvate carboxykinase activity (Vinay *et al.*, 1980*b*), the effect of 3-mercaptopicolinate in dog-kidney tubules was compared with that found in the rat. The utilization of glutamine (5mM) by dog tubules (Table 1), was accounted for by glucose (29%), glutamate (32%), alanine (11%) and a small production of lactate (<5%). The remainder (20–25%) was calculated to have undergone complete oxidation to CO_2. Complete inhibition of gluconeogenesis in isolated kidney tubules from dogs and other species possessing mitochondrial phosphoenolpyruvate carboxykinase activity requires higher concentrations of 3-mercaptopicolinate than in the rat (Watford *et al.*, 1980). The presence of 2.5mM-3-mercaptopicolinate completely suppressed gluconeogenesis from glutamine in dog tubules (Table 1), increased glutamine utilization and caused accumulation of aspartate, malate and lactate, without affecting glutamate or alanine production. Again the amount of glutamine undergoing complete oxidation was not affected. The lack of inhibition of glutamine oxidation by 3-mercaptopicolinate, and the accumulation of lactate in the dog, suggest that there is an alternative pathway for pyruvate formation from citric-acid-cycle intermediates, not involving phosphoenolpyruvate carboxykinase, as in the rat. Alternatively, glutamine oxidation could be entirely supported by intact intramitochondrial phosphoenolpyruvate carboxykinase activity in this species. To clarify this point, studies were performed using rabbit and guinea-pig kidney tissue (see below).

Table 1. *Effect of 3-mercaptopicolinate on glutamine or glutamate metabolism in isolated kidney tubules from fed rats, dogs and guinea pigs*

Tubules were prepared as described in the Materials and Methods section, and incubated with L-[U-^{14}C]glutamine or [U-^{14}C]glutamate with or without 3-mercaptopicolinate. Results are expressed as μmol of metabolite produced (+) or removed (−)/30min per g wet wt. The values are means ± S.E.M.; $n = 6$, 8 and 4 for rat, dog and guinea pig respectively.

		Rat		Dog		Guinea pig	
Metabolite	Incubation with ...	5mM-Glutamine	5mM-Glutamine + 0.5mM-mercaptopicolinate	5mM-Glutamine	5mM-Glutamine + 2.5mM-mercaptopicolinate	5mM-Glutamate	5mM-Glutamate + 2.5mM-mercaptopicolinate
Glutamine		−95.4 ± 5.8	−76.7 ± 5.2	−47.2 ± 2.8	−60.1 ± 4.6	+46.8 ± 9.3	+36.3 ± 0.9
Glutamate		+34.7 ± 2.8	+40.5 ± 4.0	+15.1 ± 1.5	+12.9 ± 1.1	−99.4 ± 6.2	−73.9 ± 2.5
Aspartate		−0.2 ± 0.4	+12.5 ± 1.3	−1.3 ± 0.5	+18.7 ± 3.0	+3.7 ± 0.9	+18.7 ± 2.6
Alanine		—	—	+5.2 ± 0.8	+3.5 ± 1.7	—	—
Glucose (as C_3 moieties)		+34.1 ± 3.1	+0.8 ± 0.02	+13.6 ± 0.9	+1.1 ± 0.7	+14.7 ± 2.4	+0.1 ± 0.1
Lactate		+0.3 ± 0.2	+0.3 ± 0.2	+2.2 ± 0.5	+6.7 ± 1.0	+8.1 ± 0.9	+3.5 ± 0.8
Malate		<0.01	+3.1 ± 0.1	<0.01	+6.2 ± 1.1	<0.01	+6.1 ± 1.2
NH_3		+172.5 ± 13.2	+123.5 ± 12.5	+69.7 ± 4.9	+76.8 ± 8.1	<5	<5
Glutamine oxidized to CO_2:							
from $^{14}CO_2$		29.8	34.7	11.0	7.0	23.5	10.0
from NH_3 balances		34.9	34.5	9.7	7.0	—	—
from C_3 balances		26.8	22.9	12.5	10.9	25.4	8.2

Effect of 3-mercaptopicolinate in kidney tubules isolated from fed guinea-pigs and rabbits

Two possible pathways to phosphoenolpyruvate carboxykinase/pyruvate kinase are via 'malic' enzyme and via oxaloacetate decarboxylase. Saggerson & Evans (1975) have reported that 'malic' enzyme activity is present in the kidney cortex of rats, but

not in that of guinea pigs or rabbits. On the other hand, phosphoenolpyruvate carboxykinase is present in both the cytosolic and mitochondrial compartments in both species (Soling & Kleineke, 1976; Watford *et al.*, 1980). Therefore to test the hypothesis that 'malic' enzyme could provide pyruvate from citric-acid-cycle intermediates when phosphoenolpyruvate carboxykinase is inhibited in the kidney cortex of dogs and rats, we have studied the effects of 3-mercaptopicolinate in isolated kidney tubules from guinea pigs and rabbits.

As glutamine is not readily utilized by tubules isolated from fed guinea-pig kidney (results not shown) glutamate was used as substrate in this species. The metabolism of L-[U-^{14}C]glutamate (5mM) by guinea pig tubules is presented in Table 1. Glutamate was metabolized to glucose (15%), lactate (8%), glutamine (47%), aspartate (<5%) and CO_2 (25%). When gluconeogenesis was inhibited by 2.5mM-3-mercaptopicolinate, malate, 2-oxoglutarate and aspartate accumulated. Lactate production and both glutamate removal and oxidation were markedly depressed. There was also a decrease in glutamine production, presumably because of diminished ammonia availability from decreased glutamate metabolism. Net oxidation of glutamate to CO_2 decreased by 50% (Table 1).

Kidney tubules isolated from fed rabbits do not readily use glutamine or glutamate as gluconeogenic substrates, possibly because of inhibition of glutamate dehydrogenase by ammonia in this species (Klahr, 1971). Therefore 2-oxoglutarate (5mM) was used as substrate for rabbit-kidney tubules. In control incubations (Table 2), 2-oxoglutarate uptake could be accounted for by glucose (36%), glutamate (21%), glutamine (11%), malate (7%) and lactate (10%), with less than 15% undergoing complete oxidation. The addition of 3-mercaptopicolinate (5mM) increased the production of malate and aspartate, with complete inhibition of both glucose formation and substrate oxidation, and suppression of lactate production.

These results indicate that a difference in intracellular distribution of phosphoenolpyruvate carboxykinase is not responsible for the maintenance of glutamine oxidation in the dog, and that an alternative pathway to phosphoenolpyruvate carboxykinase/pyruvate kinase is present in rat and dog kidney cortex but is absent in that of rabbits and guinea pigs.

Metabolism of 2-oxoglutarate by isolated kidney tubules from rats and dogs

To rule out the possibility that the differences observed between isolated tubules from rats and dogs and those from rabbits and guinea pigs (Tables 1 and 2), were due to the use of different substrates,

Table 2. *Effect of 3-mercaptopicolinate on the metabolism of 2-oxoglutarate in isolated kidney tubules from rats, dogs and rabits*

Tubules were prepared as described in the Materials and Methods section, and incubated with 5mM-2-oxoglutarate with or without 3-mercaptopicolinate (0.5–5mM). Results are expressed as μmol of metabolite produced (+) or removed (−)/30min per g wet wt. The values are means ± S.E.M.; $n = 3$, 5, and 5 for rat, dog and rabbit respectively.

	Rat			Dog		Rabbit	
Incubation with ..	5mM-2-Oxoglutarate	5mM-2-Oxoglutarate +0.5mM-mercaptopicolinate	5mM-2-Oxoglutarate	5mM-2-Oxoglutarate +2.5mM-mercaptopicolinate	5mM-2-Oxoglutarate	5mM-2-Oxoglutarate +5mM-mercaptopicolinate
Metabolite						
2-Oxoglutarate	−71.2 ± 7.1	−79.2 ± 2.5	−59.1 ± 4.7	−66.1 ± 5.1	−53.7 ± 6.3	−55.5 ± 1.4
Lactate	+2.4 ± 1.0	+0.3 ± 0.3	+4.0 ± 0.7	+6.5 ± 0.6	+5.5 ± 0.6	+3.1 ± 0.5
Malate	+4.4 ± 0.6	+16.2 ± 0.2	+2.5 ± 0.7	+22.7 ± 6.4	+4.0 ± 0.8	+32.2 ± 1.4
Glucose (as C_3 moieties)	+32.6 ± 0.3	+3.0 ± 1.1	+19.0 ± 1.7	+2.5 ± 0.7	+19.3 ± 2.4	+1.0 ± 0.5
Aspartate	<0.01	<0.01	−2.0 ± 0.2	+6.6 ± 0.7	+0.7 ± 0.5	+5.9 ± 0.3
Alanine	—	—	+4.7 ± 0.3	−3.1 ± 0.12	—	—
Glutamate	+0.1 ± 0.1	+0.6 ± 0.6	+12.5 ± 1.2	8.4 ± 2.6	+11.3 ± 1.0	+7.7 ± 0.3
Glutamine	+11.9 ± 1.1	+28.8 ± 3.9	—	—	+6.1 ± 1.4	+7.6 ± 4.0
Recovery of C_3 moieties (%)	72	62	69	66	87	104
2-Oxoglutarate oxidized to CO_2 (from C_3 balance)	19.9	30.3	18.3	22.9	6.93	0

studies were performed with rat and dog tubules using 2-oxoglutarate as substrate. Table 2 shows that, in the rat, 2-oxoglutarate (5mM) was metabolized to glucose (46%) and glutamine (17%), with small accumulations of lactate and malate, and approximately 28% underwent complete oxidation to CO_2. 3-Mercaptopicolinate (0.5mM) decreased glucose and lactate production and increased malate and glutamine accumulation, with no significant effect on the amount of substrate undergoing complete oxidation. Aspartate did not accumulate in these experiments. In the dog, the 2-oxoglutarate (5mM) metabolized could be accounted for by glucose (32%), glutamate (24%), alanine (9%), lactate (8%) and malate (5%). The addition of 3-mercaptopicolinate (2.5mM) increased the concentrations of aspartate, lactate and malate and decreased those of glucose, glutamate and alanine. Again there was no effect on complete oxidation of substrate. The large accumulation of malate in the experiments shown in Table 2 is obviously a 'substrate effect' (compare with Table 1). However, complete oxidation of 2-oxoglutarate, in the presence of 3-mercaptopicolinate, in both rat and dog tubules confirms that the species differences reported in this paper are independent of the substrate used.

Alternative pathways for the formation of pyruvate not involving phosphoenolpyruvate carboxykinase

Oxaloacetate decarboxylase. While the spontaneous decarboxylation of oxaloacetate readily occurs *in vitro*, this is not thought to happen in the more stable environment of the cell. Oxaloacetate decarboxylase activity has been demonstrated in rat liver (Dean & Bartley, 1973; Lopes-Cardozo & Van den Bergh, 1974; Wojtczak & Walajtys, 1974), kidney cortex (Wojtczak & Walajtys, 1974; Alleyne *et al.*, 1978), brain (Wojtczak & Walajtys, 1974) and intestinal epithelial cells (Watford *et al.*, 1979*a*). Oxaloacetate decarboxylase could therefore explain the pyruvate formation seen in our experiments. It would also indirectly provide the cytosolic NADH (via the cytosolic malate dehydrogenase reaction) needed for the formation of lactate in dog tubules. Oxaloacetate decarboxylase activity could be measured in the kidney cortex of all species studied (Table 3) when 1mM-dithiothreitol was present in the homogenization medium. The enzyme activity was not affected by 3-mercaptopicolinate at concentrations up to 5mM. The total activity could account for the pyruvate formation seen with 3-mercaptopicolinate. However, if oxaloacetate decarboxylase was involved in pyruvate formation from citric-acid-cycle intermediates during phosphoenolpyruvate carboxykinase inhibition in our experiments with rat and dog tubules, this pathway should also have been operative in the rabbit and guinea pig, in contrast to our observations. We therefore felt that, in the rat and dog, oxaloacetate decarboxylase activity is not responsible for the maintenance of renal oxidation of glutamine, glutamate and 2-oxoglutarate in the presence of 3-mercaptopicolinate. Furthermore, it is not certain that oxaloacetate decarboxylase activity is a distinct enzyme. Other enzymes do exhibit such activity, including pyruvate kinase (Creighton & Rose, 1976*a*,*b*; Jursinic & Robinson, 1978), 'malic' enzyme (Ochoa, 1955; Schimarlik & Cleland, 1977) and phosphoenolpyruvate carboxykinase (Jomain-Baum & Schramm, 1978; Columbo *et al.*, 1978). In the last case, it could be argued that the decarboxylase function of phosphoenolpyruvate carboxykinase was not inhibited by 3-mercaptopicolinate, thus explaining the formation of pyruvate observed in our experiments. Rognstad (1979) has suggested that the oxaloacetate decarboxylase activity of rat hepatic phosphoenolpyruvate carboxykinase can operate when the normal activity is inhibited by 3-mercaptopicolinate. However, it is difficult to imagine why this would not operate in the rabbit and guinea pig. Dean & Bartley (1973) reported that the mitochondrial oxaloacetate decarboxylase of rat liver is inhibited by low concentrations of KCl, citrate and CoA derivatives, and exhibits a K_m for oxaloacetate of about 0.23mM. As the intracellular content of oxaloacetate is of the

Table 3. *'Malic' enzyme and oxaloacetate decarboxylase activities in kidney cortex of rats, dogs, rabbits and guinea pigs*
Activities were determined as described in the Materials and Methods section and have been corrected for cross-contamination of the fractions; they are expressed as μmol of pyruvate formed/min per g wet wt. at 37°C and are means ± S.E.M., where *n* is the number of experiments. The nuclear fractions contained none of these activities when corrected for cross-contamination and are therefore omitted.

	Oxaloacetate decarboxylase				'Malic' enzyme		
Species	*n*	Homogenate	Cytosol	Mitochondria	*n*	Homogenate	Cytosol	Mitochondria
Rat	4	3.10 ± 0.25	1.43 ± 0.12	1.98 ± 0.28	5	2.22 ± 0.16	1.66 ± 0.16	0.54 ± 0.05
Dog	4	0.80 ± 0.10	0.29 ± 0.07	0.56 ± 0.10	8	1.02 ± 0.13	0.28 ± 0.05	0.74 ± 18
Rabbit	4	2.20 ± 0.10	0.58 ± 0.16	1.57 ± 0.26	4	<0.10	—	—
Guinea pig	3	1.50 ± 0.12	0.74 ± 0.01	0.70 ± 0.11	3	0.22 ± 0.05	0.22 ± 0.05	<0.01

order of 5–15 nmol/g in the rat (Hems & Brosnan, 1971) and in the dog (Vinay *et al.*, 1980*b*,*c*) it seems unlikely that this enzyme could function as a decarboxylase *in vivo*. Wojtczak & Walajtys (1974) reported an apparent K_m for oxaloacetate of 0.55 mM but suggest that the real K_m could be much lower since the keto form of oxaloacetate was likely to be the true substrate. However, under their conditions of assay, more than 70% of the oxaloacetate would be present as the keto form (see Pogson & Wolfe, 1972) and therefore the K_m would become 0.39 mM, still much higher than the oxaloacetate concentration *in vivo* in any intracellular compartment.

'Malic' enzyme. 'Malic' enzyme has been reported to be present in rat-kidney cortex (Brdiczka & Pette, 1971; Richards & Knox, 1972; Tsoncheva, 1974; Saggerson & Evans, 1975; Alleyne *et al.*, 1978) but absent from that of rabbits and guinea pigs (Brdiczka & Pette, 1971; Saggerson & Evans, 1975). We are not aware of any previous measurements of renal 'malic' enzyme activity in the dog. We found 'malic' enzyme activity in both rat and dog kidney cortex (Table 3). It was barely detectable in that of guinea pig and absent in that of rabbit. The enzymes were not affected by 3-mercaptopicolinate (up to 5 mM). The activity in rats and dogs is sufficient to account for the maximum rates of pyruvate formation observed in the presence of 3-mercaptopicolinate in those species and the K_m for malate is 0.2–0.6 mM (Tsoncheva, 1974), a value close to the tissue concentration (Hems & Brosnan, 1971).

As pyruvate formation from citric-acid-cycle intermediates does not occur in the presence of 3-mercaptopicolinate in species lacking renal 'malic' enzyme, we propose that 'malic' enzyme can function in this rôle in rat and dog kidney cortex. 'Malic' enzyme activity, however, implies the production of NADPH, which may be used for fatty acid synthesis or serve some other, as yet undetermined, function. Attempts to increase renal 'malic' enzyme activity in the rat by sucrose feeding (see Young *et al.*, 1964) produced no change in activity, but the hepatic enzyme increased 10-fold (from 0.86 to 8.71 μmol/min per g wet wt). This suggests that this enzyme may not play a role in fatty acid synthesis in the kidney. Similarly, no adaptation to sucrose feeding was found for the 'malic' enzyme of rat enterocytes (Watford *et al.*, 1979*a*). Furthermore, Stark & Frenkel (1974) have shown that induction of 'malic' enzyme does not always correlate with changes in fatty acid synthesis, and have reported (Stark *et al.*, 1975) that induction of rat-liver 'malic' enzyme correlates with the induction of hepatic glutathione reductase. They suggest that the NADPH formed by 'malic' enzyme could be used to reduce the glutathione involved in the γ-glutamyl cycle. Despite the fact that the NADPH-producing dehydrogenases of the hexose monophosphate shunt are reported to increase in acidosis in rat kidney cortex (Dies & Lopspeich, 1967), acidosis caused no change in renal 'malic' enzyme activity in the rat (results not shown). Similar findings were reported by Alleyne *et al.* (1978). The possibility of an NAD^+-linked 'malic' enzyme, as seen in adrenal cortex of rat and calf (Mandella & Sauer, 1975) and rabbit heart (Lin & Davis, 1974), was not considered in the present work since such activity has not been reported in kidney cortex.

Formation of lactate by dog-kidney tubules

When gluconeogenesis from glutamine (Table 1) or 2-oxoglutarate (Table 2) is inhibited in dog kidney tubules by 3-mercaptopicolinate, an accumulation of lactate is observed. Such lactate formation requires not only the production and cytosolic accumulation of pyruvate from citric-acid-cycle intermediates, but also requires a supply of cytosolic reducing equivalents, as NADH. Of the possible pathways for the provision of pyruvate when phosphoenolpyruvate carboxykinase is inhibited, only transfer of malate from the mitochondria and the cytosolic activity of oxaloacetate decarboxylase would provide cytosolic NADH (via the malate dehydrogenase reaction). However, our observations indicate that oxaloacetate decarboxylase is probably not involved. Therefore the question of the provision of the NADH required for lactate synthesis arises.

It is possible that one molecule of the cytosolic malate could be converted to pyruvate via 'malic' enzyme, while another molecule of malate is oxidized to oxaloacetate by the cytosolic malate dehydrogenase reaction, producing the required NADH. However, for this pathway to proceed there must be continued removal of the cytosolic oxaloacetate. A cytosolic removal mechanism must be proposed, since the inner mitochondrial membrane is believed to be impermeable to oxaloacetate (at physiological concentrations). Aspartate could act as a 'sink' for cytosolic oxaloacetate. This would give rise to an accumulation of aspartate in the cytosol as the mitochondrial aspartate translocase is unidirectional in the direction of aspartate efflux (Williamson, 1976). Indeed, we always see concomitant accumulation of lactate and aspartate in these experiments (Tables 1 and 2).

In order to test this theory, we attempted to block aspartate accumulation by inhibition of aspartate aminotransferase with amino-oxyacetate (Wallach, 1960). Under our experimental conditions amino-oxyacetate (up to 2 mM) did not suppress transaminase activity and aspartate accumulation (results not shown), even when using the pre-incubation procedures recommended by Meijer and co-workers (Meijer & Van Dam, 1974; Meijer *et al.*, 1978). At

Table 4. *Effect of 2-amino-3-methoxy-trans-but-3-enoic acid on lactate production in dog-kidney tubules*
Tubules were prepared as described in the Materials and Methods section and were pre-incubated with or without 2-amino-4-methoxy-*trans*-but-3-enoic acid (2.5 mM) for 15 min before the addition of glutamine (5 mM) and 3-mercaptopicolinate (2.5 mM). Results are expressed as μmol of metabolite produced (+) or removed (−)/30 min per g wet wt. and are the means ± S.E.M. of four experiments.

Metabolite	Addition to incubation	Glutamine ... (control)	Glutamine + 3-mercapto-picolinate	Glutamine + 2-amino-4-methoxy-*trans*-but-3-enoate	Glutamine + 3-mercapto-picolinate + 2-amino-4-methoxy-*trans*-but-3-enoate
Glucose (as C_3 moieties)		+13.2 ± 2.0	+2.0 ± 0.9	+11.3 ± 1.8	+0.5 ± 0.3
Aspartate		−1.1 ± 0.7	+25.0 ± 2.0	<0.1	+5.4 ± 1.7
Lactate		+3.2 ± 1.2	+6.7 ± 0.6	+1.5 ± 0.3	+1.5 ± 0.7
Malate		<0.1	+6.2 ± 1.0	<0.1	+5.4 ± 1.4

the present time the effectiveness of amino-oxyacetate and its interaction with carbonyl compounds such as pyruvate is a matter of controversy. The pre-incubation techniques claimed by Meijer *et al.* (1978) to be effective in maintaining transaminase inhibition are disputed by Smith *et al.* (1977). The latter workers (Smith *et al.*, 1977) have recommended the use of an alternative inhibitor of aspartate aminotransferase, L-2-amino-4-methoxy-*trans*-but-3-enoic acid (Rando *et al.*, 1976). After a 15-min preincubation with 2.5 mM-inhibitor, aspartate accumulation was suppressed in dog tubules incubated in the presence of glutamine (5 mM) and 3-mercaptopicolinate (2.5 mM) (Table 4). Furthermore, lactate accumulation was simultaneously decreased. We were unable to measure glutamate in these experiments, as the inhibitor reacts with the glutamate dehydrogenase used in the assay. These results support our hypothesis that, when phosphoenolpyruvate carboxykinase is inhibited in dog kidney tubules, aspartate acts as a 'sink' for the cytosolic oxaloacetate generated when cytosolic NADH is required for lactate production.

Saggerson (1978) also reported that the provision of cytosolic reducing equivalents from pyruvate was not completely suppressed when phosphoenolpyruvate carboxykinase was inhibited by 3-mercaptopicolinate in isolated rat-kidney tubules. However, Saggerson argued that aspartate was unlikely to act as a 'sink' for oxaloacetate in his experiments, as amino-oxyacetate (0.1 mM) was without effect in the formation of lactate from pyruvate in the presence of 3-mercaptopicolinate. As he used a pyruvate/amino-oxyacetate ratio of 50:1 and did not use pre-incubation procedures, it is almost certain that inhibition of the transaminase was nullified in his experiments.

Relevance to metabolism in vivo

The finding that pyruvate can be formed from citric-acid-cycle intermediates when phosphoenolpyruvate carboxykinase is inhibited in the kidney cortex of rats and dogs could be of relevance to some of the published work concerning the relationship of renal gluconeogenesis to ammonia production. Ross (1976) reported that complete suppression of gluconeogenesis by 3-mercaptopicolinate in the perfused kidney of normal rat did not affect ammonia production. However, our results show that suppression of gluconeogenesis does not necessarily mean suppression of disposal of the glutamine carbon skeleton.

Our data do not allow speculation as to the role of the alternative pathway ('malic' enzyme) in the provision of pyruvate *in vivo*. However, it should be pointed out that, in the dog, renal glutamine oxidation is increased during metabolic acidosis while no adaptation of phosphoenolpyruvate carboxykinase activity is seen (Vinay *et al.*, 1980*b*). Simultaneously, the tissue concentration of malate rises above the K_m of 'malic' enzyme for malate, a finding comparable with observations *in vivo* in the dog following renal intra-arterial infusion of 3-mercaptopicolinate (Vinay *et al.*, 1980*c*). Therefore, it is possible for 'malic' enzyme to play a physiological role in adaptation to acidosis in this species.

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