Glycine metabolism in rat kidney cortex slices*

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When rat kidney cortex slices were incubated with glycine or [1-¹⁴C]glycine, after correcting for metabolite changes with control slices, product formation and glycine utilization fitted the requirements of the equation:

Evidence is presented that degradation via glyoxylate, by oxidation or transamination, is unlikely to have any significant role in kidney glycine catabolism. It is concluded that glycine metabolism in rat kidney is largely via glycine cleavage closely coupled with serine formation. 1-C decarboxylation and urea formation with glycine in rat hepatocyte suspensions were somewhat greater than decarboxylation or ammonia formation in kidney slices, showing that in the rat, potentially, the liver is quantitatively the more important organ in glycine catabolism. There was no evidence of ammonia formation from glycine with rat brain cortex, heart, spleen or diaphragm and 1-C decarboxylation was very weak.

About 60% of the urinary ammonia in the dog (Van Slyke *et al.*, 1943) and in man (Owen & Robinson, 1963) appears to be derived from the amide group of the glutamine removed from arterial blood by the kidneys. Shalhoub *et al.* (1963) and Pitts *et al.* (1963) showed that glutamine is extracted from the blood by dog kidney to a far greater extent than any other amino acid but found also a significant extraction of glycine, which may be a minor source of urinary ammonia. They also reported that alanine and serine are the kidney's major amino acid additions to the circulation. Similar findings on amino acid exchanges *in vivo* across the kidney have been obtained with the rat (Aikawa *et al.*, 1973) though Squires *et al.* (1976) did not find any efflux of alanine. Some related observations have been made in experiments with rat kidney cortex slices: a notable increase in ammonia production when glutamine is added and to a lesser extent with glycine (Davies & Yudkin, 1952). The rate of glycine deamination with slices is similar to that observed with isolated perfused rat kidneys (Pitts, 1971).

It seems reasonable to attribute the relatively high

rate of ammonia liberation from glutamine to the action of glutaminase (EC 3.5.1.2), a very active enzyme in mammalian kidney extracts (see e.g. Krebs, 1935). Glycine oxidase activity, which would produce ammonia and glyoxylate (Ratner *et al.*, 1944), was invoked as the mechanism of glycine deamination by Davies & Yudkin (1952), and by Canessa-Fischer *et al.* (1963) in relation to dog kidney, but this is not so readily acceptable. There was little evidence then of any other mechanism of glycine degradation and the enzyme was believed to be specific for glycine; this mechanism has continued to be given serious consideration (e.g. see Bender, 1978). Yet it seems most unlikely that glyoxylate is an intermediate in glycine catabolism. Weinhouse & Friedmann (1951) recovered [¹⁴C]-oxalate from the urine of rats after the administration of [1,2-¹⁴C]glyoxylate, but not when the animals were given relatively large doses of [1-¹⁴C]- or [2-¹⁴C]glycine. And it has since become well established that there is another process initiating glycine catabolism which does not involve glyoxylate as an intermediate, the 'glycine cleavage' reaction (EC 2.1.2.10):

* Dedicated to Professor A. E. Braunstein for his 80th birthday (26 May 1982).

The necessary enzyme complex is present in many microbial species, in plants, in bird liver and, of most relevance to the present paper, in mammalian liver and kidney (see the review by Kikuchi, 1973). In many instances this process is linked with the serine hydroxymethyltransferase reaction (EC 2.1.2.1) that converts another molecule of glycine into serine with the regeneration of free tetrahydrofolate:

(in bird liver, Richert *et al.*, 1962; in plants, see e.g. Cossins & Sinha, 1966; in some microbes, see e.g. Sagers & Gunsalus, 1961; Jones & Bridgeland, 1966). The present investigation was undertaken primarily to discover to what extent glycine deamination in intact kidney cells may be accounted for by the glycine cleavage reaction and to what extent there is coupling with serine formation.

Experimental

Male Sprague-Dawley rats, body wt. about 250 g and starved for 24 h, were used.

Tissue slice incubations

Rats were killed by bleeding. Tissue slices were obtained with the aid of a hand microtome (Stadie & Riggs, 1944) using a skin-graft blade moistened with the medium to be employed in the incubations, phosphate saline (Krebs & de Gasquet, 1964) or bicarbonate saline (Krebs & Henseleit, 1932). After removing surface fluid against a perspex sheet, slices were weighed on a torsion balance. Usually kidney cortex slices were about 50 mg. All slice incubations were at 37°C. Except as indicated below each slice was suspended in 3.0 ml of phosphate saline, which included any test substrate, under oxygen with shaking at about 100 cycles/min. Incubations were terminated by the addition of 3.0 ml of 4% (w/v) HClO₄. Slices were disintegrated with a glass rod, the denatured fragments were removed by centrifugation and the supernatants were retained for analysis.

Metabolite determinations

Ammonia was determined with the use of glutamate dehydrogenase (Buttery & Rowsell, 1971) after the neutralization of HClO₄ extracts with KOH. Glucose was determined by the glucose oxidase method of Krebs *et al.* (1964) but with a modified buffer: 0.5 M-Tris/0.25 M-sodium phosphate, pH 7.4. At this Tris concentration reaction with other sugars is insignificant.

Serine was determined colorimetrically in some experiments. Periodate was used to liberate formaldehyde, which was then reacted with chromotropic acid (4,5-dihydroxynaphthalene-2,7-disulphonic

acid) as described by Frisell *et al.* (1954). With this procedure glucose also yields formaldehyde, one mol per mol as with serine. It was checked that the absorbance eventually measured (at 570 nm) is linear with serine or glucose at least to 3 µmol, the same for the same amount of either compound and additive for serine/glucose mixtures. For serine determinations it was possible, therefore, to correct for the small glucose contribution to the absorbance by making glucose determinations on the same kidney extracts. In other experiments serine (and glycine) was determined for us on a JEOL 6AH amino acid analyser by Mrs. Joan Ward (Department of Medical Biochemistry).

Urea was determined colorimetrically with 1-phenyl-1,2-propanedione-2-oxime as described by Archibald (1945). With the HClO₄ extracts from the liver incubations there was a depression (5–10%) in the absorbance obtained for added urea compared with that given by standards. However, reproducibly the percentage depression was the same for a range of internal standards in tests with the same amount of the same liver extract. Accordingly with each extract assayed an additional determination was set up with the same amount of extract and including an internal urea standard (0.25 µmol) so that correction could be made for the interference.

The oxo acid produced by D-phenylalanine metabolism was determined colorimetrically by the non-specific 2,4-dinitrophenylhydrazine method (Greenberg, 1962).

Assay of ¹⁴CO₂ release from [1-¹⁴C]- or [2-¹⁴C]-glycine

Reactions were in Warburg manometer flasks. Every assay required a set of flasks each with the same contents so that ¹⁴CO₂ generated could be measured for different incubation times and a progress curve obtained.

For studies with tissue slices main compartments contained 2.4 ml of phosphate saline including 10 mM-glycine and approx. 1.2 × 10⁶ d.p.m. of [1-¹⁴C]- or [2-¹⁴C]glycine. Centre wells contained 0.3 ml of 1 M-KOH and side arms 0.3 ml of 50% (w/v) HClO₄. After preincubating the flasks a tissue slice was added to each main compartment. Flask entrances were sealed with glass stoppers coated with rubber grease, and flasks were incubated at 37°C normally under oxygen with shaking at approx. 80 cycles/min. The HClO₄ was mixed into the main compartment at 0, 15 or 30 min and incubation was continued for a further 30 min shaking at 120 cycles/min to allow ¹⁴CO₂ displaced into the gas space to be trapped in the centre well. Duplicate samples (100 µl) of the KOH were then taken for radioactivity determination. The scintillation fluid contained 5 g of 2,5-diphenyloxazole and 0.3 g of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene dissolved

in 1 litre of scintillation grade toluene; for each determination with a 0.1 ml aqueous sample a mixture of 14 ml of scintillation fluid with 4 ml of methanol was used.

The efficiency of CO_2 collection for the procedure was investigated with $\text{NaH}^{14}\text{CO}_3$, approx. 3000 d.p.m. in 2.4 ml of 0.05 M-Tris/HCl buffer, pH 8.4. A radioactivity recovery of 90–95% was obtained, maximal from about 20 min after addition of acid.

For assays with cell-free homogenates of kidney the procedure adopted, [derived from methods described by Richert *et al.* (1962) and Sato *et al.* (1969)], had been found to be optimal for the liberation of $^{14}\text{CO}_2$ from $[1\text{-}^{14}\text{C}]\text{glycine}$ in rat liver homogenates. A mixture (1.2 ml) containing 0.02 M-glycine, 0.06 M-potassium phosphate buffer/0.12 M-KCl, pH 7.8, 6.6 mM- MgCl_2 , 0.5 mg of NAD/ml and approx. 1.2×10^6 d.p.m. of $[1\text{-}^{14}\text{C}]\text{glycine}$ was added to the main compartments. Centre wells contained 0.3 ml of 1 M-KOH and side arms 0.3 ml of 50% (w/v) HClO_4 . A sample (1.2 ml) of a preincubated 10% (w/v) kidney cortex homogenate, prepared in 0.1 M-potassium phosphate buffer/0.2 M-KCl, pH 7.8, containing 10 mM-dithiothreitol, was added and the flasks were sealed. After 0, 10 or 20 min incubation under air in the shaker bath the acid was added to the main compartment. In other respects the procedure was as described for tissue slice experiments. Linear progress curves for the radioactivity liberated were obtained at least up to 20 min incubation and the rate of radioactivity liberated was proportional to homogenate concentration.

With hepatocytes the procedure was modified, principally to allow incubation under O_2/CO_2 . Flask side arms contained 0.3 ml of 0.5 M- H_2SO_4 , but initially centre wells were empty. A pre-incubated hepatocyte suspension (2.2 ml, approx. 0.2 g of cells) was added to each main compartment, which contained 0.2 ml of 0.12 M-glycine with approx. 10^6 d.p.m. of $[1\text{-}^{14}\text{C}]\text{glycine}$. Flasks were then gassed with O_2/CO_2 (19:1), the side arm entrances were sealed with greased glass stoppers and the main compartment entrances with Subaseal self-sealing rubber caps (William Freeman & Co. Ltd., Barnsley, Yorks, U.K.). After 0, 10, 20 or 30 min in the incubator-shaker at 37°C the acid was added to the main compartment and 0.3 ml of 1 M-KOH was injected through the rubber cap into the centre well. The procedure for collecting and measuring the released $^{14}\text{CO}_2$ was then applied.

D-Amino acid oxidase assay

Peroxidase was used to couple the H_2O_2 generated in the oxidase reaction with the oxidation of *o*-dianisidine and so obtain a continuous spectrophotometric assay, i.e. an increase in A_{440} . Assay mixtures, 2.9 ml, contained 0.1 M-Tris/HCl buffer, pH 8.3,

3.3 mM-D-phenylalanine or 0.17 M-glycine, 33 μg of peroxidase/ml (Boehringer) and 40 μg of *o*-dianisidine/ml; reactions at room temperature were initiated by adding 0.1 ml of D-amino acid oxidase (Boehringer), 2.5 μg or 50 μg respectively with D-phenylalanine or glycine as substrate.

Radiochemicals

$[1\text{-}^{14}\text{C}]\text{Glycine}$ (56 mCi/mmol) and $[2\text{-}^{14}\text{C}]\text{glycine}$ (54 mCi/mmol), obtained from The Radiochemical Centre (Amersham, Bucks., U.K.), were described as 97–98% pure. After fractionation by descending paper chromatography for 40 h on Whatman No. 1 paper with phenol saturated with water, with both forms of labelled glycine radioautography showed radioactivity largely in the glycine position but radioactive impurity spots were also revealed. The labelled glycines were purified as described by Rowsell *et al.* (1975) and radiochemical purity was confirmed by radioautography after separate paper chromatographic runs in three different development solvents.

Incubations with hepatocytes

Hepatocytes were prepared essentially by the method of Berry & Friend (1969) as modified by Krebs *et al.* (1974) but with collagenase as the only enzyme added to the liver perfusion medium. Cell suspensions in bicarbonate saline (Krebs & Henseleit, 1932) containing 2.5% (w/v) bovine serum albumin were obtained, about 1 g of cells/10 ml for experiments with $[1\text{-}^{14}\text{C}]\text{glycine}$ (see above) and about 1 g of cells/18 ml for urea synthesis. For the latter studies approx. 0.5 g of hepatocytes were incubated in bicarbonate saline containing 5 mM-glycine and 3 mM-ornithine in a final volume of 20 ml under O_2/CO_2 (19:1) at 37°C. Control incubations were made without glycine. Samples (4.5 ml) were inactivated with 0.5 ml of 20% (w/v) HClO_4 at 0, 40, 80 and 120 min. After centrifugation urea was determined on the unneutralized supernatants.

Expression of results

In each experiment duplicate incubations were made for each test. The discrepancy for each pair of values was most commonly <10% and each determination was calculated as the average. These averages were combined for several experiments to obtain the mean $\pm$ s.d.

Results

The release of $^{14}\text{CO}_2$ from $[1\text{-}^{14}\text{C}]\text{glycine}$ was measured for incubations with slices of various rat tissues and with isolated hepatocytes (Table 1). Only with kidney and liver was there a notable generation of $^{14}\text{CO}_2$. Consistently, with tissue slices, this was

Table 1. Liberation of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ glycine in slices of various rat tissues and hepatocytes

Tissue slices were incubated in phosphate saline with 10 mM- $[1-^{14}\text{C}]$ glycine. Approximate fresh slice weights (mg) were: kidney cortex, 100; liver, 175; brain cortex, 100; heart, 150; spleen, 130; diaphragm (pieces), 120. Hepatocyte suspensions were in bicarbonate saline under O_2/CO_2 (19:1). Mean values $\pm$ S.D. are given for three rats.

Tissue slice	$^{14}\text{CO}_2$ liberation ($\mu\text{mol/h}$ per g of tissue)
Kidney	11.3 ± 0.8
Liver	4.8 ± 0.6
Heart	0.35 ± 0.06
Brain	0.18 ± 0.04
Spleen	0.14 ± 0.03
Diaphragm	0.11 ± 0.02
Hepatocyte suspension	14.4 ± 4.0

greater for kidney than liver but for isolated hepatocytes it was above the kidney slice value.

Ammonia formation from glycine was investigated with the same tissues, as was also urea formation with liver. Results from a representative experiment are shown in Fig. 1. Regularly, with kidney slices there was a clear increase in ammonia production for glycine incubations. With liver slices, in control incubations there was some decrease in ammonia from the zero time value of about $2 \mu\text{mol}$ of ammonia/g of tissue, presumably through urea formation, and in the presence of glycine the only evidence of ammonia formation was the maintenance of its concentration close to the zero value (results not shown). However, with liver slices an increase in urea formation was observed for glycine incubations. This was larger with hepatocyte suspensions, i.e. 6.5 ± 2.9 and $17.3 \pm 6.7 \mu\text{mol}$ of urea formed/h per g of cells for control and glycine incubations respectively in experiments with five rats. For the other tissues tested, (diaphragm, heart, spleen and brain), there was ammonia formation in control incubations, especially with brain, but in no case was there any evidence of extra production through glycine deamination.

On the basis of these observations we conclude that liver and kidney are probably the only significant sites of glycine catabolism in the rat.

Glycine was compared with several L-amino acids for the ability to increase ammonia formation in aerobic incubations with rat kidney cortex slices. Again, reproducibly, an increase was observed with glycine though less than with aspartate or glutamate and much less than with glutamine (Table 2). The value for glutamate is almost certainly an under-estimation; it takes no account of the ammonia liberated and then incorporated into glutamine (see

Fig. 1. Ammonia or urea formation with glycine in slices of various rat tissues

Slices were incubated in bicarbonate saline in the presence or absence of 10 mM-glycine under O_2/CO_2 (19:1); with liver 1.5 mM-ornithine was also included. Approximate slice weights were as for Table 1. Zero-time values have been subtracted to show the formation of ammonia or urea for 60 min incubation with added glycine (▨) or in its absence (□).

Table 2. Ammonia formation with different amino acids in rat kidney slices

Slices were incubated for 60 min with 10 mM substrate. Results show the ammonia increase as mean $\pm$ S.D. (number of rats).

Substrate	Ammonia formation ($\mu\text{mol/h}$ per g of kidney)
None	8.8 ± 0.9 (4)
Glycine	21.0 ± 3.0 (4)
Serine	8.8 ± 1.6 (4)
Glutamine	94.6 ± 6.0 (4)
Aspartate	33.1 ± 4.5 (3)
Glutamate	27.4 ± 4.2 (3)

Krebs, 1935). A slight increase ($<2.5 \mu\text{mol}$ of ammonia/h per g kidney) was observed with alanine, leucine, hydroxyproline, methionine and phenylalanine; none with serine, valine, isoleucine or histidine; and with threonine and ethanolamine somewhat less than the control rate was found.

For incubations under nitrogen, ammonia formation in control and glycine incubations was depressed and there was no extra formation for added glycine (Fig. 2). Under nitrogen there was some decrease in ammonia liberation with glutamine, which may be in part explained by a suppression of the deamination of the glutamate released on glutamine hydrolysis,

Fig. 2. Ammonia formation with glycine and glutamine in rat kidney slices under aerobic and anaerobic conditions Ammonia formation under oxygen is shown for control slices (▲) and for incubations with 10mM-glycine (■) or 10mM-glutamine (●). The corresponding open symbols show the formation under nitrogen.

but about 80% of the increment for aerobic conditions was still observed.

If ammonia liberated from glycine in kidney is due principally to glycine cleavage [see eqn. (1), Introduction], and once formed is not consumed in other reactions, the amounts of CO₂ released from C-1 of glycine and ammonia produced should be equal. Experiments were made with kidney slices incubated aerobically in the presence of [1-¹⁴C]glycine or with no added substrate. Taking into account the incomplete recovery of ¹⁴CO₂ under these conditions (see the Experimental section) there was good agreement between the ¹⁴CO₂ released and the extra ammonia formation with glycine (Table 3). Cell-free kidney homogenates were considerably less effective in promoting the 1-C decarboxylation of glycine: values of 4.12 ± 0.47 and 10.9 ± 0.85 μmol of CO₂/h per g kidney were found for homogenates and slices respectively in incubations with [1-¹⁴C]glycine under air with five rats.

Fig. 3 shows the results of an experiment in which analyses were made for ammonia and serine (periodate/chromotropic acid method) in control and glycine-incubated rat kidney slices. A progressive accumulation of serine was observed for each set of incubations and, with added glycine, serine production in excess of controls fairly closely

Table 3. Ammonia and ¹⁴CO₂ formation with [1-¹⁴C]-glycine in rat kidney slices

Slices were incubated with 10mM-[1-¹⁴C]glycine. After ¹⁴CO₂ collection, media were analysed for ammonia. Control (no substrate) rates of ammonia formation were also obtained. Results, (ammonia formation as glycine—control), are given as mean ± s.d. for eight rats.

Formation (μmol/h per g of kidney) of:	
Ammonia	¹⁴ CO ₂
11.6 ± 1.2	10.4 ± 0.8

Fig. 3. Ammonia and serine formation with control and glycine-incubated rat kidney slices

Serine (●), determined by the periodate/chromotropic acid method, and ammonia (▲) are shown for incubations with 10mM-glycine, and for control incubations by the corresponding open symbols.

matched the extra ammonia production. This finding was confirmed by the results of a series of such experiments (Table 4a). A separate series was designed to measure also the accompanying disappearance of glycine, with the use of relatively large

Table 4. *Ammonia and serine formation and glycine utilization in rat kidney slices*

(a) Slices were incubated in the presence or absence of 10 mM-glycine for 60 min. Extracts were then analysed for serine by the periodate/chromotropic acid method and for ammonia. (b) After 120 min incubations slice extracts were analysed for serine and glycine on the amino acid analyser and for ammonia. Results (glycine—control) are given as mean $\pm$ s.d. (number of rats).

	Product formation ($\mu\text{mol/h per g of kidney}$)		Glycine utilization ($\mu\text{mol/h per g of kidney}$)
	Ammonia	Serine	
(a)	12.8 ± 1.7 (6)	12.5 ± 2.3 (6)	—
(b)	12.5 ± 1.3 (4)	13.7 ± 3.1 (4)	27.9 ± 2.6 (4)

amounts of kidney cortex slices, 100–200 mg, in 2 h incubations, to increase the metabolic loss of glycine and so improve accuracy in measuring the decrease. Glycine and serine were both determined on the amino acid analyser. After correcting for the ammonia and serine formation and the slight glycine increase (about $0.5 \mu\text{mol/h per g tissue}$) in control incubations, the rate of glycine decrease was close to twice the ammonia and serine formation (Table 4b). These observations are again consistent with the operation of the glycine cleavage process and, further, with the subsequent incorporation of a large proportion of the methylene radical generated, [reaction (1), Introduction], into serine in the hydroxymethyltransferase reaction (reaction 2). The relatively poor generation of $^{14}\text{CO}_2$ from $[2-^{14}\text{C}]$ -glycine is in keeping with that interpretation. When kidney slices from the same rat were separately incubated with $[1-^{14}\text{C}]$ -glycine or $[2-^{14}\text{C}]$ -glycine the rates of $^{14}\text{CO}_2$ liberation were 11.6 ± 2.3 and $0.48 \pm 0.18 \mu\text{mol/h per g tissue}$ respectively for experiments with four rats.

No glucose formation above the low control value (about $1.3 \mu\text{mol}$ of glucose/h per g tissue) was measurable in rat kidney slice incubations with either serine or glycine. In the same series of experiments positive results were recorded for established gluconeogenic substrates, e.g. with pyruvate and malate about 30 and $35 \mu\text{mol}$ of glucose/h per g tissue respectively.

Experiments were made to explore the possibility of a glycine oxidase involvement in glycine catabolism. According to the findings of Neims & Hellerman (1962), glycine oxidase, originally believed to be a separate specific enzyme (Ratner *et al.*, 1944), is simply one of the catalytic activities of D-amino acid oxidase (EC 1.4.3.3), at least in pig or lamb kidney. If this oxidase has a physiological role in glycine deamination then ammonia formation

Fig. 4. *Effect of benzoate on the catabolism of D-phenylalanine and glycine in rat kidney slices*

Zero time values have been subtracted to show (□) the formation of ammonia in rat kidney slices for 60 min control incubations and in the presence of 10 mM-D-phenylalanine or 10 mM-glycine, and the formation of phenylpyruvate with D-phenylalanine. ▨, the effect of 0.5 mM-benzoate on the incubations.

from glycine in kidney slices should be diminished in the presence of a D-amino acid oxidase inhibitor, e.g. benzoate (Frisell *et al.*, 1956). First it was confirmed that pig kidney D-amino acid oxidase will operate with D-phenylalanine or glycine as substrate (cf. Neims & Hellerman, 1962; Dixon & Kleppe, 1965) and it was shown that reactions with either substrate were inhibited (>95%) by 1 mM-benzoate. It was checked that this inhibitory effect was not on the peroxidase used to couple H_2O_2 generation in the oxidase reaction with chromogen oxidation, i.e. by making assays in the absence of any oxidase substrate but with added H_2O_2 . The effects of benzoate on glycine and D-phenylalanine catabolism in rat kidney slices were now investigated. Reproducibly, with 0.5 mM-benzoate, there was a >90% decrease in oxoacid liberation from D-phenylalanine and a suppression of ammonia liberation close to the control slice rate, but no evidence of any effect on glycine deamination (e.g. see Fig. 4).

Discussion

Glycine was found to be one of a restricted set of amino acids that gave an undoubted increase in ammonia production on incubation with rat kidney slices. Glutamine was far more effective than any other amino acid tested; aspartate and glutamate also gave a substantially greater rate of ammonia liberation than glycine but there was little or no formation in excess of control slices with any other

natural amino acid investigated. Where comparison is possible, the present results agree with Pitts' (1971) findings: a rate of extra ammonia liberation for aspartate about 2.5-fold as much as for glycine for the normal rat kidney and only minor increases for alanine and leucine, with actual increases close to our own values. Deamination rates in tissue slices with different amino acids at relatively high concentrations do not match the differences in extraction *in vivo* by the kidney. In that respect also, glutamine is predominant but the extraction of glycine far exceeds that of aspartate and there is a small glutamate output (in the rat, Aikawa *et al.*, 1973; in the dog, Shalhoub *et al.*, 1963).

Virtual molar equivalence was observed for the extra ammonia and extra serine produced when glycine was added to rat kidney cortex slices incubated aerobically, and when $^{14}\text{CO}_2$ formation was compared with the extra ammonia formation for incubations with $[1-^{14}\text{C}]$ glycine. Glycine disappearance was twice the ammonia and serine formation. These observations are consistent with the operation of glycine cleavage (eqn. 1) and the subsequent transfer of the methylene radical thus generated from glycine C-2 to a second molecule to form serine (eqn. 2); net change:

For a continuing reaction the oxidative regeneration of NAD^+ would be required and we note that ammonia liberation from glycine was entirely dependent on the presence of oxygen.

It was confirmed that glycine is a substrate for D-amino acid oxidase (pig kidney) (Neims & Hellerman, 1962; Dixon & Kleppe, 1965) and benzoate was shown to be an effective inhibitor with either D-phenylalanine or glycine as substrate. Benzoate also powerfully inhibited the release of ammonia and oxo acid from D-phenylalanine by rat kidney slices, but now it had no apparent effect on glycine deamination. If, as the findings of Neims & Hellerman (1962) suggest, glycine oxidase activity is not associated with a specific enzyme but is a property of D-amino acid oxidase, we must conclude that a conversion into glyoxylate and ammonia has no significant role in rat kidney glycine catabolism.

Glycine transamination with pyruvate or 2-oxoglutarate, as a step towards ammonia liberation, also seems improbable. Alanine-glyoxylate aminotransferase activity (550–650 μmol of pyruvate formed/h per g tissue) and glutamate-glyoxylate aminotransferase activity (45–55 μmol of 2-oxoglutarate formed/h per g) can be assayed in crude homogenates of rat kidney but there is no real evidence of reversibility; e.g. with glycine and pyruvate as substrates, alanine formation in excess of the control value is measured as 3–4 μmol /h per g, and

this could be accounted for by other indirect pathways assuming an initial ammonia liberation (E. V. Rowsell & M. M. Al-Naama, unpublished work). Where purified enzymes have been tested, [glutamate-glyoxylate aminotransferase from rat liver (Nakada, 1964); human liver glutamate-glyoxylate aminotransferase and alanine-glyoxylate aminotransferase (Thompson & Richardson, 1966, 1967)], entirely negative results have been obtained for incubations with glycine and 2-oxoglutarate or glycine and pyruvate. The glyoxylate aminotransferases, it would appear, function to form glycine, not to promote its deamination, (see Rowsell *et al.*, 1969). Moreover there is evidence that glyoxylate is not an intermediate in glycine catabolism *in vivo* (see the Introduction), i.e. that neither glycine oxidation nor glycine transamination are involved.

From these considerations and from the positive evidence obtained in the present study we conclude that glycine cleavage is the major process initiating glycine catabolism in rat kidney. In fact, our data match the stoichiometric requirements of glycine cleavage coupled with serine formation (eqn. 3 above) sufficiently well to allow the conclusion that this is the only quantitatively significant process for the metabolic removal of glycine in rat kidney slices.

We find that rat kidney slices are poorly equipped to remove the C-2 of glycine with oxidation to CO_2 . The $^{14}\text{CO}_2$ formed from $[2-^{14}\text{C}]$ glycine was about 4% of that liberated from $[1-^{14}\text{C}]$ glycine. This may reflect, in part, the weak activity in kidney of formyl-tetrahydrofolate dehydrogenase (EC 1.5.1.6) (Kutzbach & Stockstad, 1971) the enzyme responsible for the eventual release as CO_2 of carbon attached to tetrahydrofolate. The close agreement between the ammonia and serine formed from glycine suggests further that the reaction of methylene-tetrahydrofolate with glycine, at least when the glycine concentration is relatively high, is able to compete effectively with any other process by which the kidney cell may dispose of the methylene group.

Since the predominant amino acid additions to the kidney circulation in the normal dog had been found to be alanine and serine, accompanied by a significant extraction of glycine (Shalhoub *et al.*, 1963; Pitts *et al.*, 1963), it was suggested that glycine may be a serine precursor in the kidney; the incorporation of ^{14}C into serine, after infusing $[\text{U-}^{14}\text{C}]$ -glycine into the renal artery, has been described, for the rat (Pitts *et al.*, 1970) and the dog (Pitts & MacLeod, 1972). These particular results, considered in isolation, provide evidence for kidney serine hydroxymethyltransferase activity *in vivo* but do not signify whether the normal conversion is from glycine into serine or *vice versa*; in fact, Pitts *et al.* (1970) observed ^{14}C transfer also from serine to glycine. Serine hydroxymethyltransferase catalyses a freely reversible reaction (Huennekens &

Osborn, 1959) and isotope exchange would be possible without any net chemical conversion. [Aqvist (1951) demonstrated that, in the rat, ^{15}N is readily transferred from leucine, isoleucine and valine into glutamate; also, that these branched chain amino acids receive ^{15}N from glutamate, despite the fact that there is no net biosynthesis of these dietary essential amino acids. These observations simply confirm the easy reversibility of most transamination reactions involving 2-oxoglutarate/glutamate]. Even so, considering all the pertinent findings (the normal kidney extraction of glycine and production of serine, the formation of serine from glycine with kidney slices observed in the present work and the ^{14}C transfer from glycine to serine) there can be little doubt that glycine is an important precursor of serine in the kidney. And it seems likely that glycine cleavage supplies, in part at least, the 'hydroxymethyl' groups postulated by Pitts *et al.* (1970) and Pitts & MacLeod (1972) as the source of the C-3 of the serine formed. This is not to say, however, that the production *in vivo* of serine from the kidney can be wholly explained by the metabolism of glycine extracted from the circulation. Even if all this glycine were converted into serine by means of methylene groups derived from another source this would not be enough to account for the serine output, in the dog (e.g. see Shalhoub *et al.*, 1963) or in the rat (Aikawa *et al.*, 1973).

The formation of serine from glycine in the kidney throws some light on the route by which glycine is converted into glucose *in vivo*; a role for glycine that has been long recognized (see e.g. Ringer & Lusk, 1910). Kidney itself, judging by the results of the present slice experiments, is not able to form glucose from glycine or serine. There was no evidence, in fact, of any catabolism of serine in the kidney and the likely fate of serine formed there, it appears, is export into the circulation. It is well established, however, that serine can be converted into glucose in liver (Krebs *et al.*, 1966; Ross *et al.*, 1967; Exton & Park, 1967; Krebs *et al.* 1976). Thus serine produced from glycine in the kidney and transported to the liver will make a contribution to body glucose formation. It remains to be investigated to what extent liver acting alone can promote gluconeogenesis from glycine via serine. With hepatocytes, glycine utilization, as indicated by $^{14}\text{CO}_2$ liberation from $[1-^{14}\text{C}]$ glycine, was greater per g of tissue than with rat kidney slices by about 25% and, for added glycine, urea formation ($\mu\text{mol/h}$ per g tissue) in hepatocytes was comparable with ammonia formation in kidney slices. Since the total mass of the liver is much greater than that of the kidneys its potential share in rat glycine catabolism appears to be the major one.

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