A Radiochemical Assay for Glycine *N*-Acyltransferase Activity

SOME PROPERTIES OF THE ENZYME IN RAT AND RABBIT

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We have developed a sensitive radiochemical assay of glycine *N*-acyltransferase activity, using phenylacetyl-CoA as the acyl donor and glycine as the acceptor. This assay measures formation of the product, phenylacetyl-glycine, instead of disappearance of the substrate, phenylacetyl-CoA, as did earlier assays. The subcellular location and some properties of the conjugating activity were determined in liver and kidney of the rabbit and the rat. Rabbit lung and intestine were also tested for activity.

Conjugation of carboxylic acids with amino acids is usually considered to be a detoxication reaction and is very widespread in the animal and plant kingdoms (James *et al.*, 1972; Andreae & Good, 1957). The amino acid used varies with species and carboxylic acid substrate. The mechanism of the reaction in mammals has been established by Schachter & Taggart (1954) and Moldave & Meister (1957) for exogenous carboxylic acids, and by Elliot (1956) for endogenous bile acids, and was found to be the same in both cases. The first step is formation of the CoA derivative of the acid, which then reacts with a specific amino acid. In the case of bile acids, the reactions occur in the microsomal fraction (Elliot, 1956; Schersten, 1967), whereas for exogenous carboxylic acids the reactions occur in both mitochondrial and cytosol fractions (Schachter & Taggart, 1954; Moldave & Meister, 1957; Brandt *et al.*, 1968; Webster *et al.*, 1976). The present investigation concerns the reaction of phenylacetyl-CoA with glycine catalysed by preparations of liver and kidney from rabbit and rat. The procedures previously used to study this reaction have been of two types. The assay method used by Schachter & Taggart (1954), and subsequently by others (Moldave & Meister, 1957; Tischler & Goldman, 1970; Webster *et al.*, 1976), used the change in absorbance at 280nm due to the cleavage of the thioester bond of the acyl-CoA as a measure of the amount of amino acid conjugate produced. This method is useful when dealing with purified enzyme preparations, but is not reliable with crude preparations, which may also have high hydrolytic activity and indeed no conjugating activity. In addition, the product is never quantified when this

method is used, although in each case the authors demonstrated product formation qualitatively. Others (Irjala, 1972; Dixon *et al.*, 1976) have assayed transferase activity using radioactive carboxylic acid, e.g. benzoic acid, 4-aminobenzoic acid, salicylic acid, and appropriate cofactors including ATP, CoA and amino acid. In these cases, the radioactive amino acid conjugate was quantified, but this method can only be used to study the entire reaction sequence and is of limited value in studying the properties of (amino acid) *N*-acyltransferase.

Our reason for using phenylacetyl-CoA as the acyl donor was that different amino acids are used for conjugation of phenylacetic acid by different species and we are interested in comparative metabolism. It is generally thought (Schachter & Taggart, 1954; Forman *et al.*, 1971) that in rodents the same enzyme catalyses conjugation of benzoic acid and related compounds, and phenylacetic acid and related compounds, with glycine. This enzyme is termed glycine *N*-acyltransferase (EC 2.3.1.13).

A preliminary report of the results given in this paper was presented at the 59th Annual Meeting of the Federation of American Societies for Experimental Biology and Medicine in Atlantic City (James & Bend, 1975).

Experimental

Materials

Phenyl[1-¹⁴C]acetyl-CoA (chemical purity 95 ± 5%, radiochemical purity >99.3%, specific radioactivity 4.38 mCi/mmol) was purchased from New England Nuclear, Boston, MA, U.S.A. Phenylacetyl-CoA (lithium salt, mol.wt. 939.6) was purchased from P-L Biochemicals, Milwaukee, WI, U.S.A. Other chemicals were purchased from commercial sources,

Abbreviation used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

and were A.R. grade or better. Phenylacetylglycine used as a chromatographic standard was synthesized from phenylacetyl chloride and glycine as described previously (James *et al.*, 1972).

Plates for t.l.c. were purchased from Analtech, Newark, DE, U.S.A., or Quantum/Kontes, Fairfield, NJ, U.S.A., and were silica gel G or GF on glass (250 μ m layer). Strip-Mix_{TM} was purchased from Applied Science Laboratories, State College, PA, U.S.A.

Tissue preparation

Animals used were New Zealand White rabbits (2.5–3.0 kg) and Sprague–Dawley rats (180–250 g) bred at the National Institute of Environmental Health Sciences (NIEHS). Male rabbits and rats were used to establish the assay conditions. The rabbits were killed by air embolism and the rats by cervical dislocation between 08:00 and 09:00 h. Livers and kidneys were removed immediately and placed in ice-cold 0.15 M-KCl/0.02 M-Hepes, pH 7.4, which was used throughout for preparation and resuspension of subcellular fractions. All subsequent operations were carried out at 0–4°C. Weighed portions of the minced organs (9 g for rabbit kidney, 18 g for rabbit liver) were homogenized with 3 vol. of the buffered 0.15 M-KCl solution in a glass vessel by four passes of a motor-driven Teflon pestle. The suspensions were centrifuged in a SM24 head (DuPont/Sorvall) at 600g for 10 min to precipitate whole cells, nuclei and cell debris. A refrigerated (0–4°C) Sorvall RC5 centrifuge was used. Except in experiments to determine the subcellular localization of glycine *N*-acyltransferase activity, the 600g pellet (nuclei) was discarded. The 600g supernatant was centrifuged at 13300g for 20 min in the same centrifuge to precipitate a mitochondrial fraction. In subcellular localization studies, the 13300g supernatant was centrifuged at 176000g for 45 min to precipitate the microsomal fraction. A Beckman LS50 centrifuge was used with a 50Ti or 60Ti rotor. The microsomal and nuclear pellets were resuspended in the buffered 0.15 M-KCl to protein concentrations of about 20 mg/ml. The unwashed mitochondrial pellets were resuspended in the buffered 0.15 M-KCl to protein concentrations of about 5 mg/ml for kidney and 10 mg/ml for liver. Buffered hyperosmotic KCl has been used extensively as a medium for preparing subcellular fractions from liver homogenates by investigators in the field of xenobiotic metabolism (see Jerina *et al.*, 1968; Pandov & Sims, 1970; Forman *et al.*, 1971; Ionnides & Parke, 1973; Marcucci *et al.*, 1976; Fabacher & Hodgson, 1976; Howland & Bohm, 1977), and the fractions obtained appear to have distribution of enzymes similar to those in fractions prepared in sucrose after centrifugation at identical speeds. In each medium, the fraction precipitated from the 600g supernatant by centrifuging at 13300g con-

tained lysosomes and microsomal particles as well as mitochondria. Rat and rabbit lung appear to be separated into subcellular fractions more effectively when homogenized in buffered 0.15 M-KCl than in sucrose (Witschi, 1975; Devereux & Fouts, 1973; Matsubara & Tochino, 1971; Seidegard *et al.*, 1977). We have designated our liver and kidney fractions nuclear, mitochondrial, microsomal and cytosol.

In most experiments, mitochondrial suspensions were treated with Triton X-100 at a final concentration of 0.33% (v/v). The resultant suspension was centrifuged at 13300g or 176000g for 30 min and the supernatant (Triton X-100-solubilized preparation) was used in assays.

In some experiments, portions of the initial homogenized suspension, or of the mitochondrial suspension, were subjected to 10 s bursts of ultrasonic vibrations with a sonifier/cell disruptor (Heat Systems–Ultrasonics, Plainview, NY, U.S.A.) at 0–2°C. In experiments where the homogenized suspension of whole kidney or liver was sonicated, the 176000g supernatant (cytosol fraction) and the mitochondrial fraction were prepared and assayed for glycine *N*-acyltransferase activity. Sonicated mitochondrial suspensions were recentrifuged at 13300g or 176000g for 30 min, and the pellet and supernatant fractions were assayed separately.

Assays

To assay glycine *N*-acyltransferase activity, duplicate tubes were set up containing 0.05 ml of 1 M-glycine/NaOH buffer, pH 9.3 (final concn. 200 mM-glycine), 0.05–0.15 ml of tissue sample containing 0.1–1.0 mg of protein and water to 0.20 ml. The non-enzymic reaction was assayed with boiled tissue or resuspension medium in place of tissue. The tubes were preheated at 37°C and the reaction was started by adding 0.05 ml of phenyl[1-¹⁴C]acetyl-CoA (final concn. 2 mM, 5×10^5 d.p.m.). After 5 min, the reaction was terminated by the addition of 0.1 ml of 40% (w/v) trichloroacetic acid. Incubation mixtures were then extracted with 2 $\times$ 2 ml of ethyl acetate and the extracts pooled. Preliminary experiments demonstrated that phenylacetyl-CoA was not extracted into ethyl acetate, but that phenylacetic acid and phenylacetylglycine were readily extracted from aqueous solution at pH less than 3. A 0.5 ml portion of each pooled ethyl acetate extract was transferred to a scintillation vial and 10 ml of scintillation fluid, prepared as follows, was added: 16.5 g of 2,5-diphenyl-oxazole and 0.5 g of 1,4-bis-(5-phenyloxazol-2-yl)-benzene were dissolved in 2 litres of toluene, and 1 litre of Triton X-100 was added. Samples were counted in a Nuclear–Chicago Isocap 300 liquid-scintillation counter or a Beckman LS-335. The proportion of the total ¹⁴C extracted into ethyl acetate was calculated for each sample. Portions of the ethyl acetate extracts containing about 10^4 d.p.m.

were spotted on to thin-layer silica-gel GF or G plates and, as reference compounds, ethyl acetate solutions of phenylacetic acid and phenylacetyl-glycine. The plates were developed in butan-2-one/toluene/acetic acid (10:10:1, by vol.). The R_f values for phenylacetic acid and phenylacetyl-glycine are 0.80 and 0.45 respectively.

The plates were dried, viewed under u.v. light at 254 nm and the positions of the reference compounds, which quenched the fluorescence of the silica gel GF, were marked. Alternatively, the enzymically produced phenylacetic acid and phenylacetyl-glycine were located by radiochromatogram scanning, with a Packard model 7201 scanner. The plates were then treated with Strip-Mix and left to dry. The silica-gel strip was peeled off and areas of the silica gel corresponding to phenylacetyl-glycine and phenylacetic acid were cut out and placed in scintillation vials. The area around the origin and two non-radioactive areas were also placed in vials and scintillation fluid added to each. The amount of phenylacetyl-glycine formed could thus be quantified and the specific activity (nmol conjugated/min per mg of protein) calculated.

Optimum assay conditions were established by varying in turn phenylacetyl-CoA concentration, glycine concentration, pH, enzyme concentration and time of reaction. When glycine concentration was varied a constant amount of Hepes buffer, pH 9.0 (200 mM final concn.), was included. Tris buffer could not be used as it reacted with the phenylacetyl-CoA.

In some experiments, rabbit lung and small intestine were assayed for glycine *N*-acyltransferase activity. Homogenized suspensions of whole lung or washed small intestine were used as the enzyme source, and incubations were carried out in the presence and absence of Triton X-100.

Succinate-cytochrome *c* reductase activity was measured by the method of Yamashita & Racker (1969), and NADPH-cytochrome *c* reductase activity by the method of Phillips & Langdon (1962). Glutamate dehydrogenase activity was measured by the method of Fahien & Cohen (1969).

Protein concentrations were determined by the method of Lowry *et al.* (1951), with bovine serum albumin fraction V (Metrix, Chicago, IL, U.S.A.) as standard.

Statistical analysis

Pairwise comparisons of means were made by Mann-Whitney U tests (Hollander & Wolfe, 1973). *P* values of less than 0.05 were taken to represent significant differences between mean values.

Results

Assay procedure

The assay procedure described for measuring

glycine *N*-acyltransferase activity gave reproducible results for the amount of product (phenylacetyl-glycine) formed after incubation of phenylacetyl-CoA, glycine and tissue sample. Usually phenylacetic acid and phenylacetyl-glycine were the only radioactive ethyl acetate-extractable components, and phenylacetyl-CoA was the only component left in the aqueous phase. Ethyl acetate extracts of some incubation vials containing liver preparations or rat kidney microsomal fractions contained small amounts of an unidentified radioactive compound which remained at R_f 0.00–0.08. The extraction procedure removed more than 99.5% of phenylacetic acid and more than 99% of phenylacetyl-glycine from an acidified aqueous solution to the ethyl acetate phase.

Properties of glycine *N*-acyltransferase

A preliminary study of the distribution of glycine *N*-acyltransferase activity in subcellular fractions of rat and rabbit kidney showed that specific activities were highest in the mitochondrial fractions and next highest in the cytosol fractions, which may contain protein from the matrix of the mitochondrion. Similar distributions of conjugating activities were found in liver fractions from the rat and the rabbit, but overall hepatic activities were lower, especially in the rabbit.

Table 1. *Effect of agents that disrupt mitochondrial membranes on rabbit renal glycine N-acyltransferase activity*

Fraction	Activity
Expt. 1	
Mitochondria	1.5
Mitochondria+equal volume of distilled water	1.8
Mitochondria assayed 1 day after preparation	2.3
Sonicated mitochondria	5.3
Mitochondria+Triton X-100	16.5
Expt. 2	
Mitochondria	6.3
Sonicated mitochondria	16.4
176000g supernatant of sonicated mitochondria	33.4
176000g supernatant of sonicated mitochondria+Triton X-100	33.8
Mitochondria+Triton X-100	38.0
176000g supernatant of Triton X-100-treated mitochondria	68.5

The effects of several different agents that disrupt mitochondrial membranes on rabbit renal glycine *N*-acyltransferase activity are shown in Table 1. Treating mitochondrial suspensions with equal volumes of distilled water or storing the mitochondrial suspensions in the refrigerator for 24h caused a slight increase in activity, but a more marked increase was effected by sonication of the mitochondrial suspension or by treatment with Triton X-100 (final concn. 0.05%, v/v) in the incubation tube. Activity was increased by the same factor in the presence of 0.05–1.0% (v/v) Triton X-100.

If either sonicated or Triton X-100-treated mitochondrial suspensions were subsequently centrifuged at 13300g or 176000g for 30min, conjugating activity was localized in the supernatant, and little or no activity was associated with the pellet, especially from Triton X-100-treated mitochondria. The supernatant, obtained by centrifuging the Triton X-100-treated mitochondrial suspensions, had about ten times the conjugating activity found in untreated mitochondrial suspensions.

Addition of Triton X-100 to the solubilized preparation from sonicated mitochondria did not cause a further increase in activity. Addition of Triton X-100 to microsomal or cytosolic fractions did not affect

their glycine *N*-acyltransferase activities. About 60% of the mitochondrial protein was solubilized from Triton X-100-treated or sonicated rabbit renal mitochondria.

Succinate-cytochrome *c* reductase activity was assayed as a marker enzyme for inner mitochondrial membranes. Activity was found in mitochondrial suspensions, but was very low in the Triton X-100-solubilized preparations.

If the homogenized suspensions of whole liver or kidney were sonicated for 10s before differential centrifugation, the activities of cytosol fractions increased to about double the activities found in cytosol fractions from unsonicated suspensions, but some glycine *N*-acyltransferase activity remained associated with the mitochondrial fractions.

In some experiments, one kidney was frozen for 4 days and the other kidney was used immediately for preparation of mitochondrial and Triton X-100-solubilized preparations. The mitochondrial preparations from previously frozen kidneys had about twice the conjugating activity of the mitochondrial preparations from the freshly excised kidneys, whereas Triton-solubilized preparations from the frozen and thawed kidneys had less than half the

Fig. 1. Influence of pH on rabbit renal activity ●, Activity (nmol conjugated/min) at each pH by 0.12mg of protein, corrected for non-enzymic reaction; ○, activity (nmol conjugated/min) at each pH in the absence of protein. Tubes were incubated for 5min in the presence of 2mM-phenylacetyl-CoA and 200mM-glycine.

Fig. 2. Time course of glycine *N*-acyltransferase activity in rat renal (●) and hepatic (○) preparations Duplicate tubes containing 2mM-phenylacetyl-CoA and 200mM-glycine, pH9.3, were incubated at 37°C. Samples (0.25 ml) containing 0.125 mg of protein were removed at each time and assayed for phenylacetyl-glycine.

Table 2. *Activities of glycine N-acyltransferase and some marker enzymes in fractions of rat kidney*

Minced rat kidneys were homogenized with 3 vol. of cold 0.15 M-KCl/0.02 M-Hepes, pH 7.4, and the resulting suspension was fractionated in refrigerated centrifuges at 4°C under the conditions shown. These results are from one experiment, which was repeated twice with similar results. Enzyme activities were measured by standard procedures (see the Experimental section).

Fraction	Protein yield (mg/g of tissue)	Glycine <i>N</i> -acyltransferase		Succinate- cytochrome <i>c</i> reductase		Glutamate dehydrogenase		NADPH- cytochrome <i>c</i> reductase	
		(nmol/ min per mg of protein)	(μ mol/ min per g of tissue)	(nmol/ min per mg of protein)	(μ mol/ min per g of tissue)	(nmol/ min per mg of protein)	(μ mol/ min per g of tissue)	(nmol/ min per mg of protein)	(μ mol/ min per g of tissue)
Whole homogenate	122.0	8.6	1.05	19.7	2.40	13.1	1.60	4.4	0.54
Nuclear (0–600g pellet)	49.8	6.4	0.32	19.9	0.99	9.9	0.49	4.8	0.24
Mitochondrial (600–13 300g pellet)	29.0	13.3	0.39	57.8	1.68	24.2	0.70	8.6	0.25
176 000g supernatant of Triton X-100-treated 600–13 300g pellet	19.0	105.5	2.01	7.2	0.14	359.9	6.84	8.5	0.16
Microsomal (13 300–176 000g pellet)	3.4	0.8	0.003	7.3	0.02	0.0	0.0	18.8	0.06
Cytosol (176 000g supernatant)	34.1	9.9	0.34	0.0	0.0	9.0	0.31	0.8	0.03

Table 3. *Hepatic and renal glycine N-acyltransferase activities in rabbit and rat*

In each case subcellular fractions were prepared as described in the text and assayed for glycine *N*-acyltransferase activity, which was expressed as nmol conjugated/min per mg of protein. This Table shows the mean activity $\pm$ S.D. for numbers of individuals indicated in parentheses, except in the two cases where cytosol fraction from only one animal was assayed. Neither species showed a statistically significant sex difference for activities of the supernatant of Triton-treated mitochondria.

Species	Sex	Organ	Mitochondrial fraction	Supernatant of Triton-treated mitochondrial fraction	Cytosol
Rabbit	Male (6)	Liver	1.2 $\pm$ 0.6	3.1 $\pm$ 1.4	0.16
		Kidney	10.5 $\pm$ 1.2	98.0 $\pm$ 27.8	3.4 $\pm$ 0.2
	Female (4)	Liver	1.7 $\pm$ 0.2	4.1 $\pm$ 0.8	0.7 $\pm$ 0.3
		Kidney	12.5 $\pm$ 2.7	97.0 $\pm$ 10.9	3.0 $\pm$ 0.5
Rat	Male (13)	Liver	11.6 $\pm$ 5.2	36.2 $\pm$ 11.4	3.4
		Kidney	17.6 $\pm$ 1.7	71.9 $\pm$ 21.9	5.7 $\pm$ 1.1
	Female (5)	Liver	—	37.0 $\pm$ 5.4	3.4 $\pm$ 1.1
		Kidney	—	51.0 $\pm$ 2.5	11.9 $\pm$ 3.3

activity found in similar preparations from freshly excised kidneys.

With Triton X-100-solubilized preparations from kidney mitochondria, the pH-activity profile showed a peak from pH 8.8 to pH 9.5 (Fig. 1). Above pH 9.5, a high rate of non-enzymic conjugation was observed. The reaction in Triton-solubilized preparations of renal mitochondria is linear with protein concentration up to about 1 mg per tube, but at that protein concentration, about 80% of the substrate was conjugated with glycine in 5 min. Conjugating activity

was linear with time for up to 5 min for Triton-solubilized preparations from rabbit renal mitochondria (results not shown) or rat hepatic or renal mitochondria (Fig. 2).

By using the Triton-solubilized renal preparation from rabbit, the apparent K_m for phenylacetyl-CoA in the presence of 400 mM-glycine was $150 \pm 30 \mu M$ and the apparent V_{max} was 84 ± 4 nmol conjugated/min per mg of protein (means $\pm$ S.D., $n=3$). The apparent K_m for glycine in the presence of 2 mM-phenylacetyl-CoA was 20 ± 2 mM and the apparent V_{max} was

83±10nmol conjugated/min per mg of protein (means±s.d., $n=3$). The kinetic constants were determined by the method of Cleland (1963).

Subcellular localization

Table 2 shows the subcellular localization of glycine *N*-acyltransferase activity, compared with succinate-cytochrome *c* reductase, a mitochondrial marker (Kalina *et al.*, 1971), NADPH-cytochrome *c* reductase, a marker for endoplasmic reticulum (Phillips & Langdon, 1962) and glutamate dehydrogenase, a marker for the mitochondrial matrix (Sottocasa *et al.*, 1967). Activities of both glycine *N*-acyltransferase and glutamate dehydrogenase were highest in the Triton-solubilized preparation of the mitochondrial fraction. Similar results were obtained with hepatic fractions. Our mitochondrial fractions were contaminated with microsomal particles, as judged by the specific activity and activity per g of kidney of NADPH-cytochrome *c* reductase, whereas the microsomal fractions were relatively free of mitochondrial contamination, having less than 1% of the total succinate-cytochrome *c* reductase activity.

Comparison of activities in rat and rabbit

Glycine *N*-acyltransferase activities measured in mitochondria, Triton-solubilized preparations of mitochondria and cytosol fractions prepared from livers and kidneys of male and female rats and rabbits are shown in Table 3. Hepatic activities in Triton-solubilized preparations tended to be lower in male rabbits compared with females, but the difference was not statistically significant.

Other organs

Homogenized suspensions of whole rabbit lung, alone or in the presence of Triton X-100, had no measurable glycine *N*-acyltransferase activity above the non-enzymic rate, and the rate of formation of phenylacetyl-glycine in the presence of homogenized suspensions of rabbit small intestine, alone and in the presence of Triton X-100, was very low, about 1.5pmol conjugated/min per mg of small intestine. Suspensions of both lung and small intestine were very active in hydrolysing phenylacetyl-CoA to phenylacetic acid.

Discussion

The assay procedure described is specific for the product, phenylacetyl-glycine, unlike assays previously reported for this enzyme (Schachter & Taggart, 1954; Tischler & Goldman, 1970; Forman *et al.*, 1971). We found several instances where the specificity of this assay prevented erroneous assignment of glycine *N*-acyltransferase activity, since the thioester bond of phenylacetyl-CoA was cleaved, but the product was not phenylacetyl-glycine. Microsomal fractions of rat kidney, and to a lesser extent, liver and

rabbit hepatic mitochondrial fractions all catalysed formation of unidentified radioactive products during incubation with phenyl[1-¹⁴C]acetyl-CoA. The method may be adapted for use with other substrates, provided the labelled CoA derivative of the acid is available, or for other amino acid acceptors by modifying the chromatography system.

Freezing kidney before use resulted in a 50% loss in activity. Thus freshly excised tissue was always used in our study of the properties of glycine *N*-acyltransferase.

Our results indicate that in rat and rabbit kidney, glycine *N*-acyltransferase is located in the matrix of the mitochondrion and is probably not membrane bound. Others (Brandt *et al.*, 1968; Kielley & Schneider, 1950; Schachter & Taggart, 1954) have provided evidence that the enzyme is mitochondrial, but did not study its submitochondrial location. We found that in rat kidney fractions (Table 2), the properties and localization of glutamate dehydrogenase activity paralleled those of glycine *N*-acyltransferase. The initial steps of the procedure used by Fahien & Cohen (1969) to isolate glutamate dehydrogenase, widely accepted as a marker enzyme for the mitochondrial matrix (de Duve *et al.*, 1955; Sottocasa *et al.*, 1967), are very similar to those we use to solubilize the glycine-conjugating enzyme. In all, our subcellular fractionation studies and experiments that show the latency of the enzyme in mitochondria provide strong evidence that glycine *N*-acyltransferase is located in the mitochondrial matrix, at least in rat and rabbit kidney. The activity found in the cytosol during normal preparation of tissue may be due to leakage of enzyme from damaged mitochondria. Similarly, autolysis of membranes in stored mitochondrial suspensions probably accounts for the apparent increase in conjugating activity of supernatants of mitochondrial suspensions which were stored in the refrigerator for 2 days. Our results support those of Webster *et al.* (1976), who studied glycine and glutamine *N*-acyltransferases in rhesus monkey and man.

Our finding that glycine *N*-acyltransferase activity in the small intestine of rabbit was very low agrees with the study of Strahl & Barr (1971), who demonstrated a low rate of glycine conjugation of [¹⁴C]-benzoic acid in the 'tissue compartment of rat intestinal slices and everted intestinal preparations.' The significance of the high activities of phenylacetyl-CoA hydrolase found in preparations of lung and small intestine is not yet apparent.

In the presence of 2mM-phenylacetyl-CoA, the K_m for glycine is high (0.02M), and if extrapolated to the situation *in vivo* suggests that the concentration of glycine in the matrix of the mitochondrion must be high for maximum rates of conjugation. Others (Schachter & Taggart, 1954; Forman *et al.*, 1971) have reported similar high K_m values for this enzyme

in the rat, using different assay systems. These results support the evidence (Amsel & Levy, 1969) that the concentration of glycine available *in vivo* limits the rate of conjugation and hence elimination of carboxylic acids such as benzoic acid. More detailed studies of the kinetics of the glycine N-acyltransferase reaction await purification of the enzyme.

Purification of this enzyme, and those that catalyse conjugation of phenylacetic acid with other amino acids in other species, could lead to the elucidation of the reaction mechanism and perhaps to an explanation of the species differences in the amino acid used for conjugation of phenylacetic acid and related carboxylic acids during biotransformation *in vivo* of these non-nutritive acids.

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