Benzoyl-Coenzyme A:Glycine N-Acyltransferase and Phenylacetyl-Coenzyme A:Glycine N-Acyltransferase from Bovine Liver Mitochondria

PURIFICATION AND CHARACTERIZATION*

Dhirendra L. Nandi, Samuel V. Lucas, and Leslie T. Webster, Jr.

From the Department of Pharmacology, Northwestern University Medical School, Chicago, Illinois 60611, and the Department of Pharmacology, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106

Two closely related acyl-CoA:amino acid N-acyltransferases were purified to near-homogeneity from preparations of bovine liver mitochondria. Each enzyme consisted of a single polypeptide chain with a molecular weight near 33,000. One transferase was specific for benzoyl-CoA, salicyl-CoA, and certain short straight and branched chain fatty acyl-CoA esters as substrates while the other enzyme specifically used either phenylacetyl-CoA or indoleacetyl-CoA. Acyl-CoA substrates for one transferase inhibited the other. Glycine was the preferred acyl acceptor for both enzymes but either L-asparagine or glutamine also served. Peptide products for each transferase were identified by mass spectrometry. Enzymatic cleavage of acyl-CoA was stoichiometric with release of thiol and formation of peptide product. Apparent Km values were low for the preferred acyl-CoA substrates relative to the amino acid acceptors (10^5-10^6 M). Both enzymes were inhibited by high non-physiological concentrations of certain divalent cations (Mg^2+, Ni^2+, and Zn^2+). In contrast to benzoyltransferase, phenylacetyltransferase was sensitive to inhibition by either 10^-4 M 5,5'-dithiobis(2-nitrobenzoate) or 10^-5 M p-chloromercuribenzoate; 10^-4 M phenylacetyl-CoA partially protected phenylacetyltransferase against 5,5'-dithiobis(2-nitrobenzoate) inactivation but 10^-1 M glycine did not. For activity, phenylacetyltransferase required addition of certain monovalent cations (K^+, Rb^+, Na^+, Li^+, Cs^+, or (NH4)^+) to the assay system but benzoyltransferase did not. Preliminary kinetic studies of both transferases were consistent with a sequential reaction mechanism in which the acyl-CoA substrate adds to the enzyme first, glycine adds before CoA leaves, and the peptide product dissociates last. This process is physiologically important in detoxification and shows species specificity with respect to both the acyl and amino acid components of the peptides (1, 2). Although such specificity is probably conferred by a class of enzymes termed acyl-CoA:amino acid N-acyltransferases only one of these proteins has been purified to near-homogeneity (3) and much needs to be learned about their number, properties, intracellular loci, genetics, and evolution.

Schachter and Taggart first discovered acyl-CoA:amino acid N-acyltransferase activity in preparations of pig kidney cortex (4). Later, they showed that a partially purified enzyme from bovine liver mitochondria catalyzed acyl transfer from a wide variety of aliphatic and aromatic acyl-CoA substrates specifically to glycine (5). The activity of the bovine preparation with both benzoyl-CoA and phenylacetyl-CoA was hard to reconcile with the recent findings of Webster and co-workers who separated and partially purified the benzoyl- and phenylacetyltransferase activities in mitochondrial preparations from rhesus monkey and human liver (6). One enzyme fraction catalyzed benzoyl transfer from benzoyl-CoA specifically to glycine while the other catalyzed phenylacetyl transfer from phenylacetyl-CoA specifically to L-glutamine. The acyl-CoA substrates for one transferase fraction inhibited activity of the other. We now find that bovine liver mitochondria actually contain two closely related N-acyltransferases with acyl-CoA substrate/inhibitor specificities resembling those described above for rhesus monkey and man (6). Both a benzoyltransferase and a phenylacetyltransferase have been separated, purified to near-homogeneity, and characterized. Similarities and differences between these two enzymes are the subject of this report.

EXPERIMENTAL PROCEDURES

Chemicals and suppliers as well as methods for chemical syntheses, enzymatic assays, enzyme purification, protein electrophoresis, molecular weight determinations, and identification/quantitation of reaction products are given either in the attached miniprint supplement1 or in the legends of the tables and figures.

RESULTS

Results are condensed in this section whereas most of the primary experimental data is shown in the supplement.1 Table I summarizes the results of protein electrophoresis of the preparations of the bovine liver mitochondrial benzoyltransferase and phenylacetyltransferase. The protein bands were identified by the Coomassie Brilliant Blue R 250 stain and photographed. The results are shown in the supplement.1

1 Portions of this paper (including "Materials and Methods," Figs. 1S through 7S and Tables 1S through 8S) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full-size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20814. Request Document No. 78M-1895, cite author(s), and include a check or money order for $2.10 per set of photocopies.
Comparable results were obtained with phenylacetyltransferase—formation of benzoylglycine (hippurate), benzoylaspartokinase (Table IVS). Both enzymes were stereospecific for the substrate indicating that each enzyme consists of only a single polypeptide chain.

Physical Properties—By SDS\(^2\)-disc gel electrophoresis, each enzyme appeared as a single band located between carbonic anhydrase, 29,000, and glyceraldehyde-3-phosphate dehydrogenase, 36,000 (Fig. 2). The phenylacetyltransferase did not separate completely from the higher molecular weight protein marker whereas the benzoyltransferase did. Together the two enzymes produced a single broad band. By conventional disc gel electrophoresis, phenylacetyltransferase appeared as a heavy band located at the same position where the enzyme activity was found (Fig. 1S). Benzoyltransferase showed three protein bands which corresponded to three peaks of enzymatic activity (Fig. 2S); the anomalous electrophoretic behavior of this enzyme was not investigated further.

Both enzymes had molecular weights near 33,000 (Table IIIS). In contrast to SDS-disc gel electrophoresis, gel filtration gave a slightly higher molecular weight for benzoyltransferase than for phenylacetyltransferase. Both enzymes had identical molecular weights as judged by sucrose density gradient centrifugation. Agreement of the SDS-gel electrophoresis results with those obtained by the other two methods indicates that each enzyme consists of only a single polypeptide chain.

Reaction Requirements, Peptide Products, and Stoichiometry—Formation of benzoylglycine (hippurate), benzoylaspartate, and benzoylglutamine from benzoyl-CoA depended on active enzyme and the appropriate amino acid acceptor; comparable results were obtained with phenylacetyltransferase (Table IVS). Both enzymes were stereospecific for the forms of asparagine and glutamine. When enzymatically formed peptides were isolated by high pressure liquid chromatography, mass spectra of their methyl ester derivatives showed that the heavy band was located at the same position where the corresponding activity was found (Table IIS). The hydroxylapatite column was inactivated by dialysis or concentration by ultrafiltration.

Physical Properties—By SDS\(^2\)-disc gel electrophoresis, each enzyme appeared as a single band located between carbonic anhydrase, 29,000, and glyceraldehyde-3-phosphate dehydrogenase, 36,000 (Fig. 2). The phenylacetyltransferase did not separate completely from the higher molecular weight protein marker whereas the benzoyltransferase did. Together the two enzymes produced a single broad band. By conventional disc gel electrophoresis, phenylacetyltransferase appeared as a heavy band closely preceded by a very light band; the heavy band was located at the same position where the enzyme activity was found (Fig. 1S). Benzoyltransferase showed three protein bands which corresponded to three peaks of enzymatic activity (Fig. 2S); the anomalous electrophoretic behavior of this enzyme was not investigated further.

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Substrates, Inhibitors, Stimulators, and pH Optima—Findings under this heading are summarized in Table I. The benzoyltransferase catalyzed not only benzoyl, but also salicyl, acetyl, propionyl, butyryl, isobutyryl, isovaleryl, 3-methylcrotyl, and tiglyl transfer from the corresponding acyl-CoA to glycine (Table VIS). Malonyl-CoA did not serve as a substrate and only marginal activity was observed with methylmalonyl-CoA. The hydroxylapatite fraction having the highest transferase activity with benzoyl-CoA also showed the highest activity with the other active acyl-CoA substrates; this suggested that all activities were derived from a single enzyme. Phenylacetyltransferase utilized phenylacetyl-CoA and indoleacetyl-CoA as aryloxyacyl-CoA substrates; relative near-maximal rates were about 2.5 to 1 at 0.1 mM acyl-CoA and 0.2 mM glycine. Acyl-CoA substrates for one transferase did not serve as substrates for the other but instead those tested acted as competitive inhibitors with respect to the preferred acyl-CoA substrate (Fig. 3S). At high concentrations of glycine, apparent \( K_m \) values for the preferred acyl-CoA substrates of both enzymes were in the \( 10^{-5} \) range while they were even higher for nonpreferred acyl-CoA substrates of benzoyltransferase (Table VIIS).

Glycine was the preferred acyl acceptor for both enzymes but L-asparagine and L-glutamine were also active. With different preparations of benzoyltransferase, L-asparagine at 0.1 mM gave 10 to 20% of the rate observed with 0.1 mM glycine and 0.1 mM benzoyl-CoA; the corresponding rate with 0.1 mM L-glutamine was 5 to 7%. The apparent \( K_m \) values for L-asparagine and L-glutamine were appreciably higher than that of \( \sim 3 \times 10^{-4} \) mM found for glycine; substitution of these acceptors for glycine also raised the apparent \( K_m \) for benzoyl-CoA (Table VIIS). Phenylacetyltransferase also had higher apparent \( K_m \) values for L-asparagine and L-glutamine than for glycine even though its apparent \( K_m \) for glycine (20 mM) was higher than that of benzoyltransferase (Table VIIS). Other L-amino acids listed in the supplement under "Substrate Specificity," as well as D-alanine, D-glutamine, methylvamine, N,N-dimethylglycine, and aminomethylphosphonate, did not serve as acyl-CoA substrates.

Footnote 6, for column conditions. Fractions were assayed for benzoyltransferase activity, phenylacetyltransferase activity, and protein as described under "Materials and Methods" in the miniprint.
acceptors; the last three compounds acted as weak inhibitors of benzoyltransferase with respect to glycine (Fig. 4).

Both transferases were inhibited by divalent cations in a concentration-dependent manner which was specific for the particular cation (Table I, Table VIII). Nickel and zinc appeared to be more potent inhibitors than magnesium.

The enzymes responded differentially to sulfhydryl reagents. Preincubation of benzoyltransferase (2 min at pH 8 and 30°C) with either 10^{-4} M DTNB, 10^{-3} M p-CMB, 10^{-3} M iodoacetamide, or 10^{-3} M N-ethylmaleimide produced no inhibition of enzyme activity. Under identical conditions, phenylacetyltransferase activity was completely inhibited by p-CMB while DTNB reduced activity by 88 to 94%; neither iodoacetamide nor N-ethylmaleimide were inhibitory. Phenylacetyl-CoA (10^{-4} M) partially protected phenylacetyltransferase against DTNB inactivation, raising enzymatic activity to 65 to 70% of control.

Benzoyltransferase displayed maximal activity in the presence of the assay buffer (25 mM Tris-Cl, pH 8) whereas phenylacetyltransferase was nearly inactive (Table I, Table VIII). However, phenylacetyltransferase activity was stimulated by added potassium ions to a maximum observed at 0.1 M KCl; several other monovalent cations at 0.1 M also stimulated phenylacetyltransferase activity (Table VIII).

Both transferases had broad pH optima in the range of 8.4 to 8.6.

**Kinetics**—The results of preliminary bisubstrate and product inhibition kinetic studies are summarized in Table II. Both transferases showed similar kinetic patterns. Double reciprocal plots of initial velocities versus varying concentrations of either acyl-CoA or glycine at fixed concentrations of the other substrate revealed converging straight lines with a common intercept located to the left of the ordinate below or at the abscissa (Fig. 5). This location of the intercept below or at the abscissa is consistent with a sequential reaction mechanism and the converging lines eliminate a classical ping-pong mechanism where parallel lines would be expected (7).

Double reciprocal product inhibition plots reveal that the

![Fig. 2. SDS-disc gel electrophoresis of purified benzoyltransferase and phenylacetyltransferase. Gels left to right: benzoyltransferase alone; benzoyltransferase between glyceraldehyde-3-phosphate dehydrogenase (top) and carbonic anhydrase (bottom); phenylacetyltransferase alone; phenylacetyltransferase located extremely close to glyceraldehyde-3-phosphate dehydrogenase with carbonic anhydrase below; benzoyltransferase and phenylacetyltransferase together. Direction of migration was from top to bottom where a band corresponding to the tracking dye is seen in each gel. Molecular weights used for marker proteins were: carbonic anhydrase, 29,000; and glyceraldehyde-3-phosphate dehydrogenase, 36,000.

### Table I

**Properties of benzoyltransferase and phenylacetyltransferase**

| Parameter compared | Benzoyltransferase | Phenylacetyltransferase |
|--------------------|--------------------|-------------------------|
| Molecular weight   | ~35,000            | ~33,000                 |
| Substrates         | Benzoyl-CoA and salicyl-CoA; certain aliphatic acyl-CoAs | Phenylacetyl-CoA and indoleacetyl-CoA |
| Amino acid         | Glycine, L-asparagine, L-glutamine | Glycine, L-asparagine, L-glutamine |
| Inhibitors         | Phenylacetyl-CoA and indoleacetyl-CoA | Benzoyl-CoA and butyryl-CoA |
| Acyl-CoA            | Mg^{2+}, Ni^{2+}, Zn^{2+} | Mg^{2+}, Ni^{2+}, Zn^{2+} |
| Amino acid         | Relatively insensitive | Sensitive |
| Stimulators*       | None detected      | K^{+}, Rb^{+}, Li^{+} |
|                   |                    | Na^{+}, Cs^{+}, (NH_4)^{+} |
| Optimum pH         | 8.4-8.6            | 8.4-8.6                 |

* Mechanism of reaction “stimulation” not established.
peptide product acted kinetically as a competitive inhibitor with respect to the acyl-CoA substrate but a noncompetitive inhibitor with respect to glycine; the reduced CoA product produced a noncompetitive pattern with either the acyl-CoA or glycine as variable substrates (Figs. 6S and 7S). This pattern of inhibition is consistent with an ordered mechanism wherein the acyl-CoA substrate adds first and the peptide product dissociates last (7).

**Discussion**

The study of acyl-CoA:amino acid N-acyltransferases in liver or kidney mitochondria appears important for several reasons. First, it may provide an explanation at the molecular level for the wide species diversity found in the excretion of amino acid conjugates of organic acids (1). Second, it should yield insights into the evolution and genetics of these important detoxifying systems. Third, it may relate to the pathogenesis and possibly the treatment of certain organic acidaemias. Finally, it does provide a useful model for studying mechanisms of enzymatic transfer reactions.

The major contribution of the present study is the demonstration with nearly homogeneous preparations that the benzoyl-CoA:glycine N-acyltransferase and the phenylacetyl-CoA:glycine N-acyltransferase activities reflect the presence of two distinct enzymes in bovine liver mitochondria. Both activities previously had been ascribed to a single protein (5).

Characterization of these two closely related transferases revealed several properties that were either unknown or different from those described before. For example, enzymatic acyl transfer to glycine was not completely specific as previously reported (5, 8, 9). Both L-asparagine and L-glutamine served as weak acyl acceptors posing the question as to whether or not the L-glutamine acceptor activity might account for the traces of phenylacetylglutamine found in cow's milk (10). Inhibition of bovine benzoyl- or phenylacetyltransferase activity by high unphysiological concentrations of divalent metal ions has not been reported nor have two substrate and product inhibition kinetic studies been performed. The differential sensitivity of these two enzymes to sulfhydryl reagents is a new finding as is the selective stimulation of phenylacetyltransferase activity by certain monovalent cations.

Our findings complement and generally substantiate those of Lau et al. (11) who recently studied the photoaffinity labeling of the bovine liver benzoyltransferase with p-azidobenzoyl-CoA. These investigators obtained molecular weights of 36,000 by gel filtration on Sephadex G-100 and 34,000 by SDS-disc gel electrophoresis for a highly purified but nonhomogeneous preparation of this mitochondrial enzyme. Their value compares favorably with our previous estimate of 32,000 (Table III). These studies all indicate that the bovine benzoyltransferase consists of only a single polypeptide chain with, according to the method of Lau et al. (11), one active site.

Comparisons between corresponding acyltransferases of ox versus rhesus monkey or human liver mitochondria reveal similarities and differences which relate to the evolution of these proteins. Specificities for acyl-CoA substrates and inhibitors were similar between species suggesting, not only that further enzyme purification will not change the acyl-CoA specificity reported for the primate enzymes (6), but also that the pattern of acyl-CoA specificity was established before the evolutionary diversion of primates from ungulates and possibly other mammals. In contrast, the interspecies differences in transferase specificities for amino acids reveal that the high specificity of the rhesus monkey and human benzoyl- and phenylacetyltransferases for glycine and L-glutamine, respectively, occurred after primate evolution diverged from that of the ungulates. The finding that purified bovine phenylacetyltransferase can utilize both glycine or L-glutamine as substrates leads to the speculation that a single mitochondrial transferase in New World primates may account for phenylacetyl conjugation to both glycine and L-glutamine. In contrast to Old World primates and man which excrete benzoates conjugated only with glycine and arylacetates conjugated only with L-glutamine, New World monkeys excrete both phenylacetylglutamine and phenylacetylglutamic acid in the urine (13). The difference between the molecular weight of ~33,000 found for the two bovine transferases compared to ~24,000 for the rhesus monkey transferase (6) eliminates a single amino acid substitution and a monomer-dimer relationship as the basis for the interspecies dissimilarity between these enzymes. The small size of all of these proteins makes them attractive candidates for amino acid sequencing to study further the genetics and evolution of amino acid conjugation.

Acyl transfer from certain CoA esters could play a role in the pathogenesis of specific human organic acidaemias. Hartlitt and Gompertz have recently reported that CoA ester derivatives of certain alphabetic carboxylic acids implicated in these acidaemias may serve as substrates for partially purified preparations of benzoyltransferase from bovine liver mitochondria (14). The present study shows in addition that activity with these nonpreferred substrates is attributable to the benzoyltransferase rather than the phenylacetyltransferase which undoubtedly contaminated their preparation. Although activation to the acyl-CoA derivative is the first and probably rate-limiting step in the pathway of amino acid conjugation (12), it is conceivable that the transfer step might become rate-limiting in certain genetic disorders.

The preliminary two substrate and product inhibition kinetic studies are consistent with a sequential reaction mechanism wherein the acyl-CoA associates first with the enzyme, glycine adds to the enzyme prior to dissociation of the first product, CoASH, and the peptide product dissociates last. The kinetic data should, however, be interpreted with caution because insensitivity of current methods for product detection prevented our conducting experiments at acyl-CoA substrate concentrations well below their apparent Kₐ values. Intersecting lines on the primary double reciprocal plots are inconsistent with a double displacement or ping-pong mechanism (7) and this mechanism involving an acyl-enzyme intermediate also seems unlikely because Lau _et al._ reported that photoaffinity-labeled benzoyltransferase contained both the aroylacyl and the CoA moieties of p-azidobenzoyl-CoA (11). Lau's study also provides independent evidence for formation of a binary complex between the acyl-CoA and enzyme. This concept is supported by our earlier observation that benzoyl-CoA but not glycine protected benzoyltransferase from heat inactivation (15) and the present finding that phenylacetyl-CoA but not glycine protected phenylacetyltransferase from DTNB inhibition. The kinetic studies suggest that peptide products should protect these enzymes too. Apparent Kₐ values for substrates and products must eventually be compared with Kₐ values obtained directly from enzyme inactivation or spectroscopic studies. The marked differences in reaction rates of benzoyltransferase with different acyl-CoA substrates at near their kinetically saturating concentrations (Table VIS) argues against dissociation of CoA from the enzyme as being a final common rate-limiting step but detailed comparative kinetic studies with nonpreferred acyl-CoA substrates, _e.g._ butyryl-CoA, should be helpful in establishing this point.

The present purification procedure, perhaps combined with...
affinity chromatography (11), permits the preparation of sub-
strate quantities of at least the benzoyltransferase so that
formation of binary complexes or putative enzyme-bound
intermediates might be demonstrated directly or indirectly by
appropriate spectroscopic techniques. Nuclear magnetic res-
onance studies of reduced coenzyme A as well as the acyl-CoA
substrates and acyl-CoA inhibitors of both transferases have
already been performed to facilitate these alternative ap-
proaches to elucidating the reaction mechanism (16, 17).

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Additional references are found on p. 7235.
Benzyl- and Phenylacetyltransferases

**Materials and Methods**

Materials were purchased from the following suppliers: 1. Sigma Chemical Co., St. Louis, Mo. 2. Eastman Kodak Co., Rochester, N. Y. 3. F. D. Ross and Co., Chicago, Ill. 4. Fisher Scientific Co., Pittsburgh, Penn. 5. Sigma Chemical Co., St. Louis, Mo. 6. Taylor Manufacturing Co., Philadelphia, Pa. 7. J. T. Baker Chemical Co., Phillipsburg, N. J. 8. F. D. Ross and Co., Chicago, Ill. 9. J. T. Baker Chemical Co., Phillipsburg, N. J. 10. Fisher Scientific Co., Pittsburgh, Penn. 11. Eastman Kodak Co., Rochester, N. Y.

**Chemical Analysis**

pH measurements were made with a commercial pH meter. The concentration of acid or base required to bring the solution to neutrality was determined by titration with standard 0.1N alkali or acid.

**RESULTS**

**Assay of Transferrase Activity**

Two types of enzyme assays were used. Usually, the initial rate of acceptor-dependent substrate uptake was measured. In other cases, the amount of product formed at a given time was measured. The acceptor substrate used was either unlabelled or radioactively labelled. Incubation mixtures were transferred to paper disks and exposure to air was minimized. The absorbance of the paper disks was determined and the amount of product formed calculated.

**Substrate Specificity**

The transferrase activity was shown to be specific for transferrin. Assays were performed in the presence of different concentrations of transferrin. The amount of product formed was determined and the reaction rate calculated. The results showed that the transferrase activity was specific for transferrin.

**Purification of Enzyme**

The enzyme was purified from the liver of the horse by the method of Hamilton and Mano (1964). The enzyme was isolated from the liver by the method of Hamilton and Mano (1964) and the specific activity was determined.

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**TABLE 1**

**Purification of Remote C-14 Glycine N-acetyltransferase**

| Purification Stage | Protein (mg) | Units (Units/mg) | Activity (Units) |
|--------------------|--------------|------------------|-----------------|
| stage 1 | 250 | 100 | 2500 |
| stage 2 | 50 | 1000 | 50000 |
| stage 3 | 10 | 20000 | 200000 |

**Figures**

1. The specific activity of the enzyme at various pH values is shown in Figure 1. The activity was maximal at pH 7.0 and decreased at lower and higher pH values.
2. The Michaelis-Menten constant (Km) for the enzyme was determined and found to be 0.01 M.
3. The Lineweaver-Burk double-reciprocal plot for the enzyme is shown in Figure 2. The plot was linear and the values of Vmax and Km were determined.

**Tables**

1. The results of the experiment are shown in Table 1. The enzyme was purified to a specific activity of 200,000 units/mg.
2. The recovery of the enzyme was 95%.

**Data Analysis**

The data was analyzed using statistical methods to determine the significance of the results. The results were found to be statistically significant at p < 0.05.

**Conclusions**

The enzyme was successfully purified to a high specific activity and the properties were characterized. The enzyme was found to be specific for transferrin and had a Km of 0.01 M.

**ACKNOWLEDGMENTS**

The authors wish to thank Dr. J. F. Miescher for providing the liver tissue and Dr. C. L. Ehrman for helpful discussions. This work was supported by a grant from the National Institutes of Health, Bethesda, Md.

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### TABLE II S

| Fractionation stage | Volume (ml) | Specific activity (units/mg prot) | Units/ml |
|---------------------|-------------|---------------------------------|----------|
| Mitochondrial supernatant | 174         | 7.44                            | 0.044    |
| (NH₄)₂SO₄, 0-25% ³ | 37.1        | 44.0                            | 0.0084   |
| AL (NH₄)₂SO₄, 50% ⁴ | 12.5        | 1.84                            | 0.1168   |
| Bio-Gel P-200 ¹⁵ | 1.1         | 14.3                            | 3.56     |
| Bio-gel P-300 ⁵ | 1.5         | 2.0                             | 0.38     |
| Hydrosil B ⁷ | 9.1         | 0.32                            | 1.14     |

Notes: ³, ⁴, ⁵, ⁹, ¹⁵ as footnote 1, Table 3. Details of method are given on page 796 of this issue.

### TABLE III S

| Method | Experiment | Benzylic transference | Phenylacetyl transference |
|--------|------------|-----------------------|---------------------------|
| Durose density | 1      | 13000                | 13000                     |
| Gradient centrif. | 2       | 12100                | 12100                     |
| Ultracentrif. | 3       | 12730                | 12730                     |
| Gel filtration | 4      | 33370                | 33370                     |
| SDS disk gel | 5       | 13000                | 13000                     |

### TABLE IV S

| Experiment | System ¹ | Conjugate formed (mol) |
|------------|----------|------------------------|
| 1          | N-benzylglycine | 137                    |
|            | N-benzyllisopropylamine | 157                  |
|            | N-benzylphthalimide | 0.3                  |
| 2          | N-benzylglycine | 137                    |
|            | N-benzyllisopropylamine | 157                  |
|            | N-benzylphthalimide | 0.3                  |
| 3          | N-benzylglycine | 137                    |
| 4          | N-benzylglycine | 137                    |
| 5          | N-benzylglycine | 137                    |
| 6          | N-benzylglycine | 137                    |

¹ Preparations were performed as described under MATERIALS AND METHODS. Product formed by complete system is isolated directly into a lyophilized form. N, N'-disubstituted compounds were modified using the acid chloride method. Enzyme concentrations and incubation times were adjusted so that at least 30% of product was formed in each complete system.
Benzoyl-coenzyme A:glycine N-acyltransferase and phenylacetyl-coenzyme A:glycine N-acyltransferase from bovine liver mitochondria. Purification and characterization.

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*J. Biol. Chem.* 1979, 254:7230-7237.