The Synthesis of Taurine from Sulfate III. Further Evidence for the Enzymatic Pathway in Chick Liver[1] (36232)

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The pathway for taurine synthesis from cysteic acid (1) and cysteine-sulfinic acid (2), as well as from the degradation of the sulfur amino acids (3), has been elucidated. It has been reported that taurine may be synthesized *de novo* from inorganic sulfate (4–8). The utilization of inorganic sulfate requires its conversion to the active form, 3′-phosphoadenosine-5′-phosphosulfate (PAPS), after which it is enzymatically transferred to a suitable acceptor molecule by PAPS-transferase (EC 2.8.2) (9). This transfer has been shown both *in vivo* (10–11) and *in vitro* (12) to require pyridoxal phosphate. This paper presents evidence for the *de novo* synthesis of taurine from PAPS and serine by the chick.

Materials and Methods. Experimental animals were maintained on a basal (30% isolated soybean protein) semipurified ration until they reached the age of 14 days, at which time they were sacrificed; their livers were excised and chilled. The livers were washed three times in cold distilled water prior to the preparation of 25% homogenates made with cold distilled water. The homogenates were then centrifuged at 20,000*g* for 40 min and the pellet was discarded. The supernatant was made 35% saturated with dry $(NH_4)_2SO_4$. This solution was stirred for 30 min at 4° prior to centrifugation at 20,000*g* for 15 min. The precipitate was discarded and the supernatant was made 70% saturated with dry $(NH_4)_2SO_4$. This was stirred and centrifuged as described above, and the precipitate was retained for the enzymatic synthesis of PAP^{35}S. The resulting supernatant was then dialyzed for 18 hr against cold, running distilled water. Following dialysis, the enzyme solution was lyophilized and the dry powder was dissolved in 0.5 *M* Tris–HCl, pH 7.4. This enzyme preparation was used in the initial studies.

Further purification of the active fraction was obtained by applying the preparation to a carboxymethylcellulose (CMC) column, 2 × 40 cm, packed by gravity flow, and eluted with Tris–HCl buffer (pH 8.0) with a gradient ranging from 0.5 to 1.0 *M*. The samples were eluted in 10 ml fractions which were monitored for protein concentration on an Isco Model US-2 ultraviolet analyzer preset at 280 nm. Protein peak fractions were pooled and protein determinations were made by the method of Lowry *et al.* (13).

Enzyme assays were carried out using a basic reaction mixture (Table I). Taurine-^{35}S determinations were made by chromatographically separating 100 μl quantities of the clear reaction mixture supernatant on Whatman No. 4 paper eluted with a solvent system of ethyl acetate:formic acid:water (70:20:10). Strips corresponding to Ninhydrin-sprayed, spotted taurine standards were cut into 1 × 1 in squares and were placed into glass scintillation vials containing 10 ml of a liquid scintillation cocktail (5 g of PPO and 0.1 g of dimethyl-POPOP in 1 liter of toluene). These were counted on a Packard TriCarb liquid scintillation spectrophotometer previously standardized for ^{35}S. Pyruvic acid determinations were made using the

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TABLE I. Basic Reaction Mixture for Taurine Synthesis.[a]

Component	Quantity
Tris–HCl, pH 8.5	1000 μmoles
Enzyme preparation[b]	Varied
$PAP^{35}S$[c,d]	15,000 cpm, 30 μmoles
Substrate acceptor	50 μmoles
Pyridoxal phosphate[e]	Varied
Total vol (ml)	3.2

[a] Reactions were run at 38° for 10 min, halted by the addition of 0.3 ml of 25% trichloroacetic acid. The denatured protein was removed by centrifugation at 5000g for 5 min.

[b] The protein concentration varied between 75 and 100 μg.

[c] $PAP^{35}S$ was enzymatically prepared as reported elsewhere (14). The $PAP^{35}S$ contained approximately 30,000 cpm/ml with 60 μmoles of PAPS/ml.

[d] In assays for pyruvic acid production PAPS was replaced by an equal volume of distilled water.

[e] Pyridoxal phosphate was added in concentrations of 1.5×10^{-7} g/μg of protein.

method for keto-acids of Sayre and Greenberg (15).

Results. Previous reports from this laboratory indicated that certain ^{14}C-labeled compounds, when administered to chicks fed diets of low sulfur amino acid content, gave rise to taurine-^{14}C of high specific activity (9, 16). These compounds, as well as others which were theoretical precursors of taurine, were reacted with the supernatant of the 0.7 $(NH_4)_2SO_4$ saturated liver fraction using the mixture of Table I. These results (Table II) indicated that L-serine and its precursors possessed the greatest ability to be enzymatically converted into taurine by sulfate addition from PAPS, or to pyruvic acid by dehydration and deamination. These data support previous experiments that serine provided the carbon for taurine synthesis, thus L-serine was selected as the substrate for the PAPS-transferase assays.

Prior to further studies of this reaction sequence, the enzyme fraction was purified by CMC chromatography as described above. This resulted in the isolation of three discrete protein peaks from the supernatant of the 70% $(NH_4)_2SO_4$ fractionation. The elution pattern from this purification step is shown in Fig. 1. Enzymes assays for both taurine and pyruvic acid production, using the basic reaction mixture (Table I), were run for each of the protein peaks. The first protein peak, labeled P_1, contained 75% of the activity for taurine and pyruvic acid synthesis; peak two, about 15%; and peak three about 10%. The activity of taurine synthesis in the presence of $PAP^{35}S$ and that of pyruvate production in its absence were equal in each peak. Thus, the peak one fraction (P_1) was selected for all further experimentation.

Optimum reaction time trials were carried out using the basic reaction mixture. Reactions were carried out in separate Warburg vessels, to which were added 25% TCA at 5 min intervals. Figure 2 indicates that the optimum time for catalysis in this *in vitro* reaction occurs during an interval from 5 to 10 min from the onset of the reaction. The addi-

TABLE II. Enzyme Specific Activities with Varying Substrate Acceptor Compounds.[a]

Substrate (50 μmoles)	Sp act	
	Dehydrase[b] (μmoles/μg)	Transferase[c] (cpm/μg)
α-Aminobutyric acid	0.017	23.8
β-Aminobutyric acid	0.016	15.7
γ-Aminobutyric acid	0.016	10.2
α-Aminoisobutyric acid	0.016	8.4
β-Hydroxybutyric acid	0.016	13.7
o-Phosphoethanolamine	0.016	23.0
o-Phosphoserine	0.018	23.0
Phosphatidyl ethanolamine	0.166	39.7
Phosphatidyl serine	0.267	44.6
L-Serine	2.520	138.4
BLE[d]	1.700	37.50
Aminoethylphosphonic acid	0.031	7.2

[a] Enzyme contained in 70% $(NH_4)_2SO_4$ precipitation supernatant.

[b] Serine dehydrase specific activity was calculated (μmoles of pyruvic acid formed/μg of protein).

[c] PAPS-transferase specific activity was calculated (cpm of ^{35}S incorporated into taurine-^{35}S/μg of protein).

[d] BLE = boiled liver extract: prepared by boiling a small quantity of the 25% liver homogenate for 2 min and obtaining the clear supernatant by centrifugation.

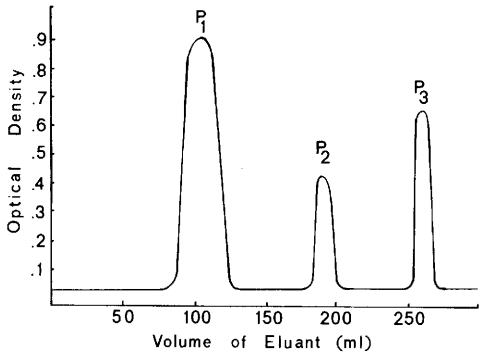

FIG. 1. Elution of taurine synthesizing proteins from semipurified fraction of chick liver homogenate from carboxymethylcellulose column eluted with Tris–HCl gradient (pH 8.0; 0.5–1.0 *M*). Ten milliliter fractions were collected.

tion of an amount of protein and pyridoxal phosphate equal to that originally included in the reaction vessel gave rise to a stoichiometric increase in the amount of the product formed, indicating that, at the end of 5 min of reaction, the enzyme was inactivated.

Determinations of optimum reaction pH were carried out using individual test tubes containing Tris–HCl buffer in which pH had been adjusted in 0.5 unit increments over a range from pH 6.0 to 9.5. The results (Fig. 3) indicated that maximum conversion of serine to either pyruvate or taurine occurs at pH 8.5. The factor determining whether taurine or pyruvate was the product to be formed

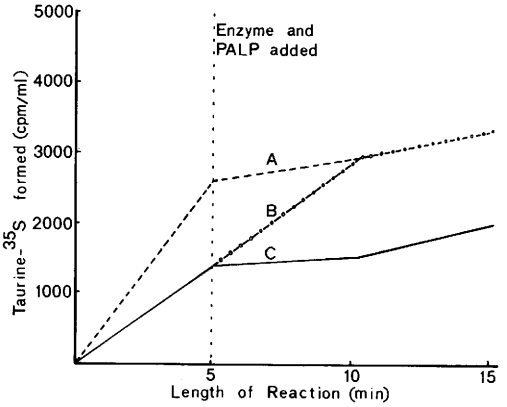

FIG. 2. Effect of enzyme supplementation on PAPS-transferase product formation (μg/ml of protein): (A) 5000; (B) 2500 + 2500 added after 5 min of reaction had proceeded; (C) 2500.

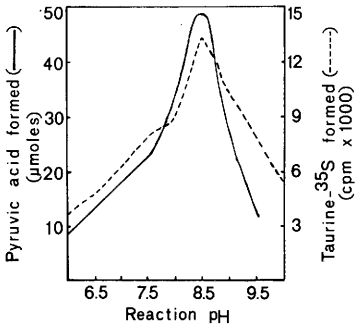

FIG. 3. Determination of the optimum reaction pH for serine dehydrase (P_3) and PAPS-transferase: pyruvate synthesis reactions were run in the absence of PAPS.

was the concentration of PAPS included in the reaction mixture. When 30 μmoles of PAPS was included, serine was converted to taurine. With decreasing quantities of PAPS, the concentration of pyruvate increased with a decrease in taurine production.

Serine-^{14}C-UL was incubated with carrier serine (50 μmoles) and unlabeled PAPS with a P_1 preparation in the basic reaction mixture. These reactions were carried out for time intervals of 0, 5, 10, 15, and 30 min in Warburg vessels containing filter paper wick saturated with 1.0 *M* KOH to trap the $^{14}CO_2$ evolved in the course of the reaction. The reactions were stopped by the addition of 25% TCA, the wicks removed and placed into glass scintillation vials. The reaction mixture supernatants were analyzed for taurine-^{14}C (Table III). These results substantiate the earlier experiment showing that the reaction ceases in 5 to 10 min. Also indicated is the conversion of serine to taurine and CO_2 without the loss of radioactivity due to conversion to other compounds.

As the conversion of serine to taurine appeared to be a direct enzyme sequence with no free intermediates being formed, a mechanism was postulated by which this could occur. This theoretical consideration was extrapolated from the work of Flavin (17). In this catalysis, serine is first dehydrated to α-aminoacrylic acid, which then becomes sulfonated by reaction with PAPS to form pyridoxal-bound cysteic acid, which is then decarboxylated to taurine. To test the

TABLE III. Distribution of ^{14}C Atoms from Serine-^{14}C-UL in Taurine Synthesis.

Time (min)	Activity (cpm/ml × 10^3)				Utilization (%)
	Serine-^{14}C	$^{14}CO_2$	Taurine-^{14}C	Total recovery	
0	101.6[a]	0[b]	0[a]	101.6[a]	0
5	35.1	21.0	45.3	101.5	65.4
10	33.4	21.3	48.8	103.5	67.7
15	33.4	20.9	48.8	103.2	67.7
30	34.6	20.8	48.9	104.3	66.9

[a] Determined as counts per minute per milliliter of reaction mixture following paper chromatographic separation on Whatman No. 4 paper eluted with ethyl acetate:formic acid:water (70:20:10).

[b] Determined (cpm) in filter paper wicks saturated with 1.0 *M* KOH and counted in dioxane liquid scintillation cocktail.

validity of this postulation, cysteic acid was incubated with the serine-^{14}C-UL reaction mixture as described above. The results (Table IV) show that, in the presence of cysteic acid, serine conversion was reduced by approximately 66%, indicating competition between serine and cysteic acid on the P_1 protein.

Further studies were carried out using cysteic acid as the sole substrate for taurine synthesis. Cysteic acid consumption and taurine production were determined at zero and after 5, 10, 15, and 30 min reaction time from chromatographs using a densitometer. With reaction time, the concentration of cysteic acid clearly decreased and that of taurine increased, indicating that the P_1 fraction was capable of catalyzing the decarboxylation of cysteic acid. This experiment was repeated using increasing concentrations of cysteic acid and in each case the taurine produced was increased.

Compounds selected as inhibitors were chosen on the basis of their structural similarities to the substrate (glycyl-serine, *o*-methylserine), the cofactor (deoxypyridoxine HCl), and from literature reports (CTP, *N*-ethylmaleimide, KCl). Each inhibitor was added to the basic reaction mixture in quantities from 0 to 50 μmoles after mixing with serine. Inhibition of taurine synthesis was observed by the presence of the substrate inhibitors glycyl-serine and *o*-methylserine (Table V) but the addition of deoxypyridoxine HCl was without effect. The addition of *N*-ethylmaleimide completely blocked enzyme activity through the binding of sulfhydryl groups required for activity. Identical results were obtained with the P_1 serine dehydrase activity (Table VI).

Discussion. The results of these experiments indicate that *de novo* taurine synthesis

TABLE IV. Effect of Cysteic Acid on the Fate of Serine-^{14}C-UL in Taurine Synthesis.

Time (min)	Activity (cpm/ml × 10^3)				Utilization (%)
	Serine-^{14}C	$^{14}CO_2$	Taurine-^{14}C	Total recovery	
0	63.1	0[b]	0[a]	63.1[a]	0
5	50.6	3.37	6.93	60.9	19.8
10	49.8	3.52	7.20	60.5	21.1
15	49.2	3.83	7.29	60.3	22.1
30	47.0	4.44	9.06	60.5	25.6

[a] Determined (cpm/ml) following paper chromatographic separation on Whatman No. 4 paper eluted with ethyl acetate:formic acid:water (70:20:10).

[b] Determined by liquid scintillation spectrophotometry of filter paper wicks saturated with 1.0 *M* KOH and counted in dioxane liquid scintillation cocktail.

TABLE V. Effect of Various Inhibitors on Taurine-^{35}S Synthesis by the P_1 Fraction.[a]

Compound tested	Conc (μmoles added)					
	0	10	20	30	40	50
Glycyl-serine	40.3 ± 0.7[b]	34.5 ± 0.3	29.1 ± 0.1	11.6 ± 0.2	9.73 ± 0.5	3.76 ± 0.2
Deoxypyridoxine HCl	42.6 ± 0.3	42.7 ± 0.1	42.5 ± 0.4	42.4 ± 0.3	42.1 ± 0.1	42.9 ± 0.7
o-Methylserine	56.0 ± 0.1	43.5 ± 0.1	40.5 ± 0.2	38.2 ± 0.2	30.9 ± 0.2	30.1 ± 0.1
KCl	9.1 ± 0.1	11.5 ± 0.1	12.0 ± 0.2	12.5 ± 0.1	15.6 ± 0.4	24.9 ± 0.5
N-Ethylmaleimide, 0.01 *M*	40.3 ± 0.7	—	—	—	—	00.0 ± 0.0
CTP (2.0 μg/ml)	19.7 ± 0.4	—	—	—	—	17.6 ± 0.1

[a] Results of three experimental assays run simultaneously using the same protein and PAPS preparation.

[b] Values expressed as PAPS-transferase specific activity (cpm of taurine/μg of protein).

[c] Reaction run only in the presence and absence of each compound which was added to the reaction mixture as 0.5 ml inclusions.

proceeds by the sulfonation of dehydrated L-serine by the sulfate group of PAPS in a pyridoxal-requiring system. The fact that this reaction proceeds in the direction of pyruvate formation in the absence of PAPS indicates that the first step in the catalysis is carried out by the enzyme serine dehydrase (EC 4.2.1.13), which is responsible for the formation of pyruvic acid from serine. This also implies a PAPS requirement by the enzyme for taurine synthesis. In comparing data obtained in this research with literature reports, it was found that the pH optimum for this PAPS-transferase preparation was 8.5, which is comparable to pH 8.3 for cystathionine synthetase and serine dehydrase (18–19). The data showing that this reaction ceases in five to 10 min is similar to that reported for serine dehydrase (19). The fact that deoxypyridoxine HCl possesses no inhibitory activity for the enzyme parallels reports which theorized that this was due to the lack of the PO_4 group on the cofactor (19). These comparisons support the hypothesis that PAPS-transferase may be coupled with serine dehydrase activity in the synthesis of taurine.

Following the addition of the sulfate moiety of PAPS, the pyridoxal-bound intermediate, hypothesized to be cysteic acid, is decarboxylated to form free taurine and CO_2. Studies using cysteic as the substrate and as a competitor for L-serine verify that cysteic acid is the intermediate formed in this synthesis. This implies that the enzyme fraction contains, in addition to PAPS-transferase and serine dehydrase, cysteic acid decarboxylase activity.

TABLE VI. Effect of Various Inhibitors on Serine Dehydrase Activity of the P_1 Fraction.[a]

Compound tested	Conc (μmoles added)					
	0	10	20	30	40	50
Glycyl-serine	0.73 ± 0.01	0.60 ± 0.01	0.39 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.06 ± 0.01
Deoxypyridoxine HCl	0.93 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	0.93 ± 0.01
o-Methylserine	1.05 ± 0.01	0.80 ± 0.01	0.55 ± 0.01	0.55 ± 0.01	0.55 ± 0.01	0.53 ± 0.01
KCl	1.00 ± 0.01	1.00 ± 0.01	1.06 ± 0.01	1.09 ± 0.01	1.12 ± 0.02	1.17 ± 0.02
N-Ethylmaleimide, 0.01 *M*	0.73 ± 0.01	—	—	—	—	0.00 ± 0.00
CTP (2.0 μg/ml)	0.40 ± 0.01	—	—	—	—	0.52 ± 0.05

[a] Results of three experimental assays run simultaneously using the same protein preparation.

[b] Values expressed as enzyme specific activity (μmoles of pyruvic acid formed/μg of protein).

[c] Reaction run only in the presence and absence of each compound which was added to the reaction mixture as 0.5 ml inclusions.

Through heat denaturation studies and supplemental purifications, it was seen that a net loss in protein content occurs with a substantial increase in enzyme activity. These factors support a hypothesis that the enzymes responsible for taurine synthesis from PAPS are present in a sulfur metabolizing complex capable of dehydrating and deaminating serine, sulfonating serine, and decarboxylating cysteic acid.

Therefore, it is theorized that taurine is synthesized from sulfate in the chick by the following pathway:

$$\text{ATP} + \text{SO}_4^{2-} \xrightarrow[\text{ATP-sulfurylase}]{\text{Mg}^{2+}} \text{APS} + \text{PPi},$$
(EC 2.7.7.4)

$$\text{APS} + \text{ATP} \xrightarrow[\text{APS-kinase}]{} \text{PAPS} + \text{ADP},$$
(EC 2.7.1.25)

$$\text{L-Serine} \xrightarrow[\text{Serine Dehydrase}]{\text{Pyridoxal PO}_4} \alpha\text{-aminoacrylic acid} + \text{HOH},$$
(EC 4.2.1.13)

$$\alpha\text{-aminoacrylic acid} + \text{PAPS} \xrightarrow{\text{Pyridoxal PO}_4} \text{cysteic acid} + \text{PAP},$$

$$\text{cysteic acid} \xrightarrow[\text{cysteic acid decarboxylase}]{\text{Pyridoxal PO}_4} \text{taurine} + \text{CO}_2.$$
(EC 4.1.1.15)

The significance of this alernate pathway of taurine synthesis is not known. It is known that increasing concentrations of cysteine repress the synthesis of taurine, as evidenced by the decrease in the total taurine content of chick liver (20). This inhibition is located at the level of ATP conversion to PAPS, thereby reducing the level of PAPS needed for the sulfonation of dehydrated serine. It is also known that the presence of dietary methionine in quantities to afford optimal growth enhances tissue taurine synthesis (21). Therefore, in the presence of optimal dietary cysteine, the pathway reported herein becomes inhibited and taurine is synthesized by the degradation of the sulfur amino acids. In the case of suboptimal levels of cysteine, however, synthesis of PAPS is not inhibited and a larger portion of the tissue taurine would arise by this pathway.

Summary. The purpose of this research was to determine the enzymatic pathway for the synthesis of taurine from inorganic sulfate in chick liver. The enzymatically active proteins were obtained in the supernatant fraction from an ammonium sulfate precipitate (0.7 saturation) of chick liver homogenate using carboxymethylcellulose (CMC)-ion exchange chromatography. Experiments designed to ascertain the acceptor of the sulfate from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) indicated that serine had the highest activity. Further, pyridoxal phosphate-dependent serine dehydrase activity was required in the reaction utilizing serine to form taurine. The formation of taurine was preceded by the intermediate being converted to enzyme-bound cysteic acid. Inhibition studies indicated that PAPS-transferase, serine dehydrase, cysteic acid decarboxylase activities were required for this reaction, as well as a sulfhydryl requirement.

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