The Synthesis of Taurine from Sulfate
II. Chick Liver Phosphoadenosinephosphosulfate-Transferase[1] (34278)

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The ubiquitous presence of taurine in animal tissues and the recent indications of diverse biochemical and physiological functions for this compound encourage continued investigation into taurine biosynthesis in biological systems. Sulfate–sulfur can be incorporated into taurine by the young chick (1, 2). Before inorganic sulfate can be utilized it must be enzymatically activated in the form of phosphoadenosinephosphosulfate (PAPS) and subsequently transferred to an acceptor molecule. The synthesis of taurine from sulfate in the chick is initiated by sulfate activation reactions with subsequent transfer to an acceptor, catalyzed by a sulfotransferase. The purification of this transferase and information concerning the acceptor is presented.

Methods. One-day-old white leghorn cockerels were maintained on a purified diet which contained isolated soybean protein, dextrose, Wesson oil, and a complete mineral and vitamin premix (3). Supplements of methionine, methionine-hydroxyanalog (MH A) or cysteine in varying amounts (Table 3) were added to the basal diet and the chicks were fed these experimental rations for 14 days.

Dietary groups of 10 chicks received an isotope, either sulfate-^{35}S or methionine labeled in the methyl or carboxy position or uniformly labeled, by subcutaneous injection. The birds were sacrificed by decapitation at the time specified in each experiment (Table 3). More chicks were reared to 14 days of age on the complete basal diet to obtain sufficient quantities of liver for the isolation and purification of the activating enzymes and the sulfotransferase.

Partially purified ATP-sulfurylase (ATP: sulfate adenylyltransferase, EC2.7.7.4) and APS-kinase (ATP:adenylyl-sulfate 3′-phosphotransferase, EC2.7.1.25) were prepared according to procedures described elsewhere (4). PAPS-^{35}S was obtained in substrate quantities by enzymatic synthesis using chick liver enzymes and purification on an ECTEOLA-formate column with stepwise ammonium formate elution (5, 6). Two ml of the PAPS^{35}S preparation was added to a 1.3 × 50 cm ECTEOLA column, and was eluted with 120 ml each of 0.00, 0.01, 0.05, 0.10, 0.50, 0.75 and 1.00 *M* ammonium formate.

The isolation and partial purification of the PAPS-transferase (sulfotransferase, EC 2.8.2) was accomplished according to the following procedure. The supernatant of a 33% aqueous liver homogenate was saturated to 70% with dry ammonium sulfate. The suspension was centrifuged at 10,000 rpm for 15 min and the precipitate was discarded. The supernatant was dialyzed for 24 hr at 3° against at least four changes of cold distilled water. The pH of the solution was then adjusted to 5.5 with cold acetic acid (0.1 *M*) and the filtrate was discarded. The precipitate was redissolved in a small volume of phosphate buffer, pH 8.3. This active transferase fraction was further purified on an ECTEOLA column. The ECTEOLA was soaked in 1.0 *N* NaOH and washed with distilled water until the pH of the wash water was 7.0. The ECTEOLA was then equilibrated in 0.1 *M* phosphate buffer (pH 5.0) and the column was packed by gravity. The

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TABLE I. Determination of PAPS-Transferase Activity of Various Preparations of Chick Liver Homogenates *in Vitro* with Endogenous Acceptors (reaction time 15 min).

Fraction	Prot. conc of orig. solution (μg/ml)	Prot. conc of reaction mixture (μg/ml)	Taurine-^{35}S formed (cpm/ml)	Sp act (cpm/μg)	Fold purification
Whole liver supernatant	15,660	6260	1800	0.4	
70% $(NH_4)_2SO_4$ supernatant	400	160	950	5.94	14.8
Supernatant after adjustment to pH 5.5	200	80	1140	14.25	35.6
Precipitate after adjustment to pH 4.5	400	160	2400	15.00	37.5
ECTEOLA column					
Tube no. 30	6.67	2.67	1440	539	1348
31	6.67	2.67	1290	483	1208
32	6.33	2.53	1120	443	1107

sample was applied, eluted with phosphate buffer eluants of increasing pH (5.0, 6.0, 7.0, 8.0) and collected in 10.0-ml fractions. Each fraction was then assayed for protein concentration (7) and for transferase activity.

The PAPS-transferase activity was measured in Bicine (*N, N* bis-2-hydroxyethylglycine) buffer, pH 7.4, 0.2 *M*, (0.3 ml); purified PAPS-^{35}S, radioactivity 16,200 cpm/ml (0.3 ml); and the protein in the enzyme fraction (0.3 ml) varied, in a total volume of 1.20 ml. The boiled liver extract was prepared by heating a small volume of 33% aqueous liver homogenate in a boiling water bath for 2–3 min and centrifuging. The samples were reacted for 15 min at 37° and the reaction was terminated by submersion in a boiling water bath for 60 sec. The samples were centrifuged to remove the coagulated protein, the taurine was separated by paper chromatography (Whatman no. 4, 80% aqueous phenol), and the radioactivity of taurine and sulfate was determined by liquid scintillation.

The *in vitro* synthesis of taurine was accomplished by adding 15 μ*M* ATP, 10 μ*M* $MgCl_2$, 20 uc Na $_2$ $^{35}SO_4$, 200 uM bicine buffer (pH 7.4), and varying amounts of DL-methionine to an aliquot of undialyzed liver homogenate. Three mg of protein was present and the final volume of the reaction mixture was 1.0 ml. This mixture was incubated for 10 min at 37° and the reaction was terminated as before.

Results. PAPS-transferase from chick liver which appears to be associated with the *in vitro* synthesis of taurine has been purified (Table I). The transferase activity of the whole liver supernatant was increased by 70% $(NH_4)_2SO_4$ precipitation. Further purification was accomplished by pH adjustment to 5.5 with 0.1 *M* acetic acid. Finally, the transferase was precipitated at pH 4.5 and resuspended in phosphate buffer, pH 8.3. This fraction was further purified on an ECTEOLA column with phosphate buffer of increasing pH (Fig. 1).

The purified transferase enzyme was reacted with PAPS-^{35}S and the endogenous acceptors of the boiled liver extract. The same reaction with $^{35}SO_4$ as the sulfur donor was also performed (Table II). The incorporation of ^{35}S into taurine when $^{35}SO_4$ was the sulfur donor was approximately 0.02% compared to 10.0% when PAPS-^{35}S was reacted with the transferase. Thus, the sulfate moiety of PAPS

TABLE II. A Comparison of the Synthesis of Taurine from PAPS-^{35}S or $^{35}SO_4$ by the Partially Purified Transferase Enzyme.

Radioactive compound added[a]	Amount of activity per reaction mixture (net cpm)	Taurine-^{35}S synthesized (*N* cpm/ml of reaction mixture)	
		15 min	30 min
$^{35}SO_4$	44×10^6	90	130
PAPS-^{35}S	47×10^2	370	690

[a] The radioactive compound was added to: 0.3 ml of transferase fraction (400 μg of protein/ml); 0.3 ml of boiled liver extract; 0.3 ml of bicine buffer, pH 7.4 (200 μmoles); 2.1 ml total volume.

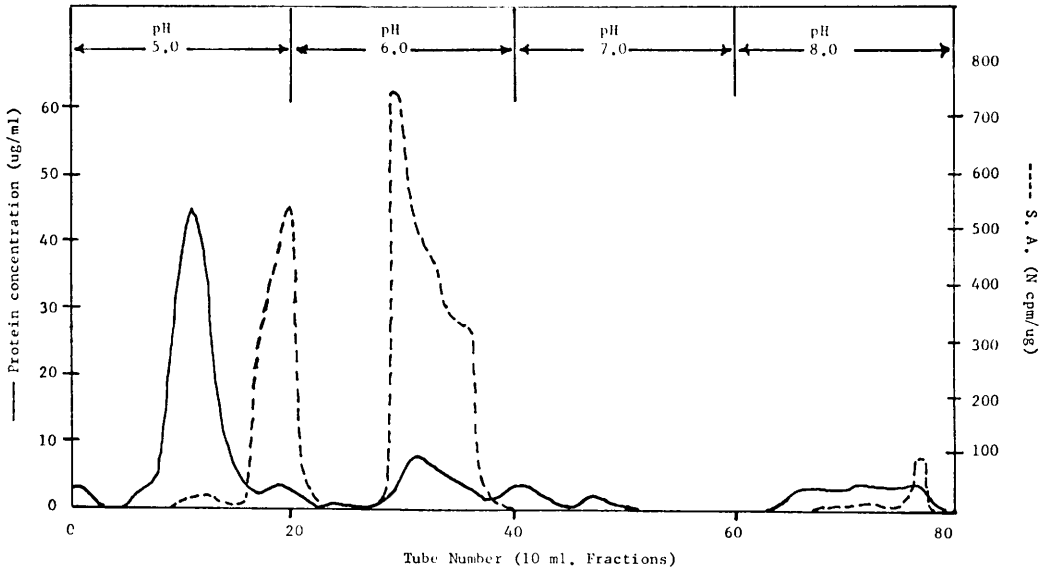

FIG. 1. Graph of protein concentration and PAPS-transferase activity of ECTEOLA column fractions eluted with phosphate buffer of increasing pH.

is added to a compound in the boiled liver extract which is converted into taurine.

Earlier experiments had indicated that DL-methionine favored the incorporation of sulfate into taurine. To test the influence of methionine on the synthesis of taurine, graded levels were added to the diet of young chicks and the liver taurine was assayed following $^{35}SO_4$ injection (Table III). Methionine supplementation to the 0.2% level resulted in increased incorporation of sulfate-^{35}S into liver taurine with no further increase at higher methionine concentrations. The total taurine of the liver increased linearly, reaching a maximum at 0.3% methionine, and then leveled off. In all cases the liver of chicks fed the supplemented diets exhibited better incorporation of labeled sulfur and a greater increase in total taurine than did those on the nonsupplemented diet. It should be noted that the sulfur of the added methionine did not decrease the utilization of inorganic sulfate for taurine synthesis of these levels. Furthermore, the specific activity of liver taurine was higher in all the methionine-supplemented groups than in the control birds.

As methionine enhanced the synthesis of taurine-^{35} from sulfate-^{35}S and cysteine retarded this conversion, the direct involvement of the methionine molecule was considered. When methionine was replaced by methionine-hydroxy analog in the purified diet, the chicks injected with sulfate-^{35}S and their livers assayed for taurine, a marked decrease in the incorporation rate was observed (Table III). Also, equimolar cysteine, as has been previously observed (8), effected less total taurine in the liver when supplemented at the three highest levels (Table III). The specific activity of the liver taurine from the cysteine-supplemented chicks was similar to those fed MHA at low concentrations, but then decreased at higher levels.

To examine the utilization of carbon from the methionine molecule in the synthesis of taurine from sulfate, methionine-^{14}C was employed. Chicks were fed the purified basal diet for 14 days and then injected with methionine labeled with ^{14}C in the methyl position, the carboxyl position, or uniformly labelled. The chicks were sacrificed 15, 30, 45, or 60 min after injection, and their livers were cleaned and homogenized. This liver preparation was deproteinized and then extracted with Dowex-50 and the taurine-^{14}C was separated by paper chromatography. No taurine-^{14}C was observed when methyl or

TABLE III. The Influence of Dietary Methionine, Methionine-hydroxy analog, and Cysteine on the Rate of Synthesis of Liver Taurine-^{35}S following the Injection of Sulfate-^{35}S.[a]

Dietary supplement	Amount (mmole/100 g)	Liver taurine (μg/g)	Taurine-^{35}S (cpm/mg)	Sp act cpm/g / μg/g
Methionine	0	42.0	3.0	71.4
	0.67	42.0	9.5	226.2
	1.33	46.3	16.1	347.8
	2.00	52.5	11.9	226.7
	2.67	51.0	11.1	217.6
	3.33	47.5	11.0	231.6
Methionine-hydroxy analog	0	42.0	2.9	69.1
	0.67	46.3	2.3	49.7
	1.33	52.1	1.4	26.7
	2.00	51.0	1.5	29.4
	2.67	53.8	3.2	59.5
	3.33	52.8	3.9	73.9
Cysteine	0	42.0	2.6	61.9
	0.67	40.0	2.1	52.5
	1.33	46.3	1.5	32.4
	2.00	47.3	1.3	27.5
	2.67	47.5	0.8	16.8
	3.33	45.0	0.9	20.0

[a] The 14-day-old chicks were sacrificed 1.5 hr after subcutaneous injection of the isotope (20 μCi).

carboxy-labeled methionine was injected, but 2800, 4025, 4550, and 3025 cpm of taurine-^{14}C/g of liver was obtained for each sampling time when methionine-^{14}C (U) was injected.

The *in vitro* synthesis of taurine in the freshly prepared liver homogenate was stimulated by the addition of DL-methionine. The 10-min incorporation rate of sulfate-^{35}S into taurine-^{35}S in the control (no methionine added) was approximately 12,000 cpm/ml of reaction mixture. When 10, 50, or 100 μmoles of methionine was added, the amount of taurine-^{35}S observed was 14, 21, and 22.5 $\times$ 10^3 cpm/ml, respectively.

The incorporation of ^{14}C into taurine by the chick liver homogenate was examined as described above. No radioactive taurine was detected in the homogenate when methionine-methyl-^{14}C or methionine-carboxyl-^{14}C was added, while good incorporation occurred with methionine-^{14}C (UL). The taurine-^{14}C observed after 30, 60, and 90 min of reaction with uniformly labeled methionine was 1780, 2900, and 1980 cpm/ml of reaction mixture, respectively.

Discussion. A PAPS-transferase in chick liver was purified and this enzyme fraction appears to be involved in the synthesis of taurine. The enzyme is specific for the sulfate moiety of PAPS and does not use inorganic sulfate as efficiently.

The carbon moiety of taurine is present in the liver. When the purified transferase fraction was added to the liver boiled extract and PAPS-^{35}S, taurine-^{35}S accumulated. Methionine added to a low-sulfur diet resulted in an enhanced incorporation of sulfate into taurine in the chick (8, 9). This result was not effected by the donation of methyl groups, as equimolar methyl donors gave no response (2). The synthesis of cysteine from methionine and then the oxidation of cysteine to form taurine appears to be an unlikely pathway for the conversion of sulfate to taurine. First, supplemental cysteine fed to chicks in a purified diet was associated with decreased liver taurine as compared to the control

chicks (8). Pasternak *et al.* (10) demonstrated that cystine will repress the synthesis of PAPS in *E. coli*. Second, only the sulfur of methionine would be observed in taurine arising from the intermediate cysteine.

These data suggested that the methionine molecule may be a source of carbon for the synthesis of taurine after demethylation and desulfurylation. Evidence supporting this view was obtained from methionine-^{14}C injection and *in vitro* experiments. Only ^{14}C from uniformly labeled methionine was observed in taurine when injected into the chick, no activity being observed when the carboxy-or the methyl-^{14}C methionine was used. When whole liver homogenate was reacted with DL-methionine and PAPS-^{35}S, or with methionine-^{14}C (UL) and PAPS, increased activity in taurine was observed over the control. Furthermore, the hydroxy analog of methionine appeared to enhance the liver taurine concentration but the specific activities were no higher than for the nonsupplemented chicks and were even depressed at the intermediate level.

The carbon compound which combines with the sulfur moiety of PAPS and is subsequently converted to taurine appears to be heat-stable as it is present in a boiled extract of liver. It appears to be derived from methionine, exclusive of the carboxyl and methyl carbon. Taurine of high specific activity has been obtained when ^{14}C-labeled serine, glycine, ethanolamine, or alanine was injected into the chicks (9). Minimal enzymatic alterations appear necessary since the transferase fraction in the presence of the boiled extract and PAPS effects the synthesis of taurine. Alternatively, the total enzyme requirement for taurine synthesis may be present in the transferase fraction, assuming that preformed quantities of carbon skeleton are not present in the boiled extract.

Summary. The existence of PAPS-transferase in chick liver was demonstrated and this enzyme was isolated and partially purified. The requirement of PAPS as a substrate for this transferase in the synthesis of taurine was demonstrated. Studies on the origin of the acceptor molecule and the carbon moiety which ultimately becomes taurine indicated that methionine may be directly involved in this synthesis. Studies showed that neither the carboxyl nor the methyl carbon of methionine became taurine carbon, while some of the remaining carbons of methionine appeared in the taurine molecule.

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