Synthesis of Taurine from Sulfate by the Chick.* (31265)

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The synthesis of taurine in the animal is of importance due to the key roles of this compound. That it is an essential component of the bile salts of some species has been known for some time. More recently, taurine has been found in nerve tissues and, also, in the cell membrane of the organism(1). It has been theorized that taurine may act as a cation accumulator in the cell membrane after its deamination to isethionic acid.

Ample evidence has been presented(2,3) that cystine can be converted to taurine in most species and that SO_4 is used by some organisms to synthesize taurine(4). The actual pathway whereby sulfate ion is incorporated into taurine by the chick is being investigated and preliminary findings presented here.

Experimental. The initial approach to the problem was *in vivo* studies with chicks which were fed a low sulfate containing diet prepared from purified soybean protein and glucose-hydrate with variation in supplements of minerals, vitamins, and various compounds thought to be associated with synthesis of taurine. In later trials, other proteins and protein combinations were incorporated into the purified diet such that amino acid deficiencies would be effected. After a 14-day feeding period, each chick was administered 20 μc of carrier free $H_2S^{35}O_4$ orally by introducing a 1 ml pipette into the crop and allowing the isotopic solution to drain gravimetrically into this part of the digestive tract. The chicks were then returned to the chick batteries and maintained on their respective rations until they were sacrificed by decapitation at various time intervals. Four or five chicks were randomly selected from each dietary group to be sacrificed at each of the time intervals and the blood serum, bile, and liver were sampled and assayed in the following manner. The blood was allowed to coagulate, then centrifuged, and the serum collected and assayed for total S^{35} activity and protein-bound S^{35}. The bile fluid was chromatographed (1-butanol equilibrated with equal volume of 3% acetic acid) on Whatman No. 1 paper previously soaked in 70% acetic acid. The liver was homogenized with distilled water and the deproteinized supernatant was chromatographed (80% phenol) on Whatman No. 4 paper. Total taurine was determined from the liver homogenate by formation of the dinitrophenol derivative after extraction with the acid form of Dowex 50(5). The S^{35} activity was determined by liquid scintillation.

Results and discussion. The chicks which received the low sulfate basal diet incorporated $S^{35}O_4^=$ into taurine as evidenced by the presence of taurine-S^{35} in the liver (Fig. 1, Table I) and taurocholate-S^{35} into the bile (Table II). When the specific activity of liver taurine was plotted *vs* time after $S^{35}O_4^=$ administration (Fig. 1), a sloped plateau was reached after 2 hours. Blood studies indicated that between one and two hours of time is required to establish the labeled sulfate pool in chicks fed this type of purified ration.

Chicks fed the purified sulfur-deficient diet supplemented with methionine, cysteine, thiosulfate, sulfite or sulfate gave a growth response when methionine or cysteine were fed; a slight increase with sulfate, no response to sulfite, and a growth depression with thiosulfate. The supplementation of the sulfur-deficient basal with sulfur compounds had a variable effect on the $S^{35}O_4^=$ utilization for taurine synthesis during the first 3 hours (Table I). After 4 and 6 hours, however, addition of the inorganic sulfur compounds enhanced the ratio of taurine-S^{35} to total liver-S^{35}, with methionine having a lesser influence after 6 hours. This may be due, in part, to the elevation of the inorganic sulfur concentration to a more optimum substrate level.

The inclusion of cholic acid in the purified

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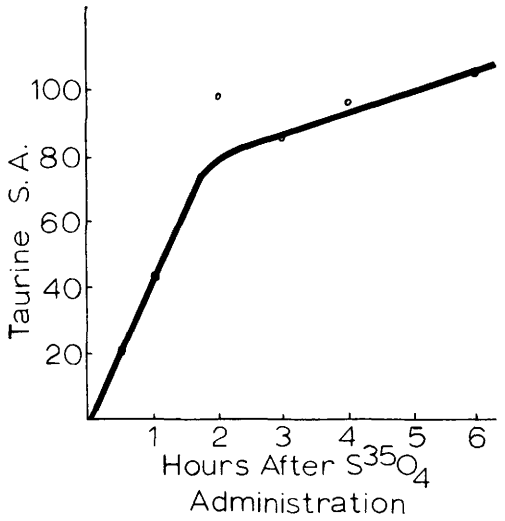

FIG. 1. Specific activity of chick liver taurine from birds fed the non-supplemented basal diet and sacrificed at various times after oral administration of $S^{35}O_4^=$.

basal diet enhanced the conversion of sulfate into taurine. Dietary cholic acid is also associated with increased bile volumes in chicks as measured by the volume of bladder bile fluid. The supplementation of cholic acid to the low sulfate purified basal diet effected a slight growth depression. The chicks in these studies were maintained on the basal diet for 10 days, then supplemented with cholic acid for 4 days before administration of the $S^{35}O_4^=$. The establishment of a S^{35}-labeled sulfate pool appeared to have been effected within one or two hours after administering the $S^{35}O_4$ to the chicks fed the basal diet (Table II). These data are consistent with blood serum-S^{35} findings in that they show that a

TABLE I. Percent of Total Liver-S^{35} as Taurine-S^{35} at Time Intervals After Oral Administration of $S^{35}O_4^=$ to Chicks Supplemented with Various Sulfur Compounds.

Supplement to basal*	Hr after oral administration of $S^{35}O_4^=$ 1	2	3	4	6
None	3.09	6.85	5.49	6.27	10.70
Methionine	3.67	2.65	6.96	7.18	18.50
Cysteine	2.46	1.48	4.07	6.38	10.03
Thiosulfate	4.15	3.26	5.72	14.51	21.71
Sulfite	2.46	7.12	4.68	15.80	25.68
Sulfate	.93	3.00	6.07	10.14	27.67

* Methionine was supplemented to the basal diet at a rate of 0.5% and the other compounds were added such that sulfur was equal.

plateau of total S^{35} activity had been reached by the second hour after dosing. Methionine, taurine or cysteine supplementation were associated with smaller taurocholate-S^{35} concentrations in the bile fluid than the basal dietary group. Cholic acid supplementation, alone or in the presence of methionine, appeared to enhance the level of taurocholate-S^{35} in the bile, but the presence of dietary taurine and cystine was associated with little change in taurocholate concentration when in the presence of cholate. Supplementation of sulfate repressed the achievement of the level found in the basal group, and after 4 and 6 hours they were equal. The low concentration of taurocholate from chicks fed cysteine apparently was derived from the lower levels of total taurine in the livers of these chicks. The specific activities of liver taurine substantiates the fact that the chick uses $S^{35}O_4$ to synthesize taurine (Table III). Dietary sulfate ap-

TABLE II. Ratio of Taurocholate-S^{35} per ml to Total Bile-S^{35} per ml of Bile Fluid in Chicks Sacrificed at Various Times After Dosing $S^{35}O_4$.

Supplement to basal diet*	Time (hr) 1	2	3	4	6
	% Taurocholate-S^{35} of total bile-S^{35}				
None	20.67	23.44	21.78	20.44	26.57
Methionine	10.75	13.83	14.35	14.98	17.23
Cholic acid	25.70	8.72	17.40	25.57	41.14
Methionine + cholic	29.45	32.55	21.02	21.49	17.41
Taurine	3.61	16.55	17.42	19.69	11.05
Taurine + cholic	9.04	11.26	14.53	16.86	18.94
Cysteine	15.02	18.26	14.73	12.69	2.44
Cysteine + cholic	3.13	9.94	12.34	15.73	3.04
Sulfate	0.51	14.78	14.95	20.38	27.74

* Methionine was added at the rate of 0.5% and the other sulfur compounds added such that the S was equal. Cholic acid addition was 0.34%.

pears to enhance the conversion of sulfate-S^{35} into taurine when considering that only tracer amounts of $S^{35}O_4^=$ were administered. Also, methionine effects a more rapid attainment of the specific activity equivalent to that of the basal group while cysteine, at this dietary level, continually enhanced the specific activity of taurine. This is probably due to similar labeling of taurine with S^{35} from sulfate and a much lower total taurine value.

When chicks were supplemented with glycine, serine, or alanine to ascertain the utili-

TABLE III. Specific Activity of Liver Taurine from Chicks Sacrificed at Various Times After Administration of $S^{35}O_4$.

Supplement to basal*	Time after dosing (hr) 1	2	3	4	6
	(S.A. = net cpm taurine-S^{35} per μg taurine in one g liver)				
None	39.8	101.8	85.7	98.1	103.8
Na_2SO_4	22.9	12.8	32.3	74.9	84.1
Methionine	104.1	113.1	130.9	111.0	114.9
Cysteine	98.0	105.8	121.7	143.5	156.3

* Supplements made such that the amount of S was equal to 0.5% methionine.

zation of these amino acids in the synthesis of taurine, the results presented in Table IV were obtained. The chromatographic separation of the S^{35}-labeled compounds of the liver indicated that at least 6 metabolites were present. In this trial, one-half of each amino acid group received no vitamin B_{12} supplement since previous experimentation had shown a possible involvement of this cofactor in the metabolism of sulfate(6).

When glycine was added to the basal diet, an enhanced rate of taurine synthesis was observed by 15 to 30 minutes after dosing. Little activity was found at the R_f equivalent to methionine and just slightly more at the R_f of cysteic acid. The chromatographic area of cystine appeared to have small amounts of activity one hour after dosing and thereafter. The conversion of $S^{35}O_4^=$ into taurine appeared to be rapid and showed good incorporation of the isotope from 30 minutes through the sample taken 4 hours after dosing. The activity shown at R_f 0.30 showed marked increases after the taurine accumulation and is at the R_f equivalent to isethionic acid. Chick liver, heart, spleen, and kidney can actively convert taurine to this acid. It appears from the results of Table IV that $S^{35}O_4^=$ does not pass *via* cysteine or cystine en route to its conversion to taurine and, that after the attainment of a certain level of taurine, the conversion to isethionate ensues. It is interesting to note that Mason *et al*(7) presented data which indicated that taurine may be an intermediate in the conversion of sulfate-sulfur to amino acid sulfur in hens.

Trials in progress indicate that under some dietary conditions considerable amounts of S^{35} activity appear at R_f 0.90 on the phenol chromatograms. Mercaptoethylamine has a

TABLE IV. Chromatographic Separation of S^{35} Activity in Chick Liver from Birds Fed the Purified Diet ± Vitamin B_{12} and Supplemented with Glycine, Serine, or Alanine. The paper was Whatman No. 4 and the solvent was 80% phenol.

Supplement to basal*	R_f equivalent to	Dietary vit B_{12}	Time after oral $S^{35}O_4$ dose (hr) .25 +	.25 −	.5 +	.5 −	1 +	1 −	2 +	2 −	4 +	4 −
			Net cpm per 100 λ protein-free liver homogenate									
None	Cysteic acid		12	4	5	7	9	9	12	21	11	19
	Cystine		6	5	8	5	27	25	34	30	42	40
	R_f 0.30 ± 0.05		13	3	11	20	49	37	61	56	113	127
	Taurine		25	78	95	115	244	126	229	164	258	146
	Methionine		1	1	0	4	4	1	1	3	2	4
Glycine	Cysteic acid		4	6	0	7	5	12	10	6	18	14
	Cystine		3	7	3	12	10	13	20	18	24	47
	R_f 0.30		2	9	4	5	18	68	26	38	186	176
	Taurine		109	69	181	142	267	124	241	146	254	165
	Methionine		0	1	0	0	0	1	4	1	2	0
Serine	Cysteic acid		3	0	5	5	9	14	15	14	20	15
	Cystine		5	4	0	2	5	12	17	29	41	27
	R_f 0.30		3	3	7	7	23	31	60	60	126	75
	Taurine		37	47	43	87	216	156	281	147	153	142
	Methionine		2	0	2	2	0	11	2	7	3	0
Alanine	Cysteic acid		1	0	4	2	4	2	8	0	5	9
	Cystine		2	0	2	0	6	6	14	6	45	39
	R_f 0.30		6	0	4	5	5	9	37	16	115	44
	Taurine		14	12	56	42	79	61	190	125	300	170
	Methionine		1	0	2	0	3	1	4	0	6	3

* Supplements were equimolar at 0.5% for glycine.

TABLE V. Total Liver Taurine from Chicks Fed the Purified Basal Diet ± Cysteine and Sacrificed at Various Times After Oral Administration of $S^{35}O_4$.

Supplement to basal	Hr after $S^{35}O_4$ dose 1	2	3	4	5
	μg taurine per g liver				
None	64.0	49.0	52.4	55.0	54.6
Cysteine*	38.5	40.4	41.3	37.6	32.3

* .3% cysteine HCl.

similar R_f in this solvent. The amount of activity found at this spot very often parallels that of the taurine spot, with lesser amounts present at the R_f of hypotaurine. The compound found at R_f 0.90 with the S^{35} is a nitroprusside positive compound.

The observation that cystine supplementation was associated with lower total taurine in the liver (Table V) is also being investigated further. A low liver taurine and a smaller concentration of bile taurocholate-S^{35} with cystine supplementation suggests that the chick may not use cysteine freely in the production of taurine. Pasternak *et al*(8) indicated that cysteine acts as a repressor of the enzymes utilizing sulfite in *E. coli* and that sulfite represses the sulfate-activating enzymes. These workers have postulated a mechanism of differential repression by varying members of the sulfate metabolic pathway in bacteria. Further, they report that the rate of cysteine formation determines how many enzymes are repressed, enzymes associated with sulfate activation, reduction, and sulfite reduction being included. Further research now in progress with C^{14}-labeled compounds indicates that only small amounts of labeled taurine are found in the chick after injection with cystine-3-C^{14}.

Therefore, we would suggest that the chick uses sulfate, after activation and reduction, to synthesize taurine. This compound is formed by the conjugation of activated sulfate and a carbon acceptor with or without the amine group. The latter appears more likely since our preliminary findings with C^{14}-labeled amino acids show good incorporation of C^{14} into taurine and taurocholate after injection into the chick. Taurine formation would, therefore, not pass *via* total reduction of sulfate sulfur into cysteine sulfhydryl followed by oxidation. In fact, it appears that in the chick, as in some bacteria, the formation of taurine from sulfate may be mediated by the cysteine level due to enzyme repression or feedback, which would suggest that cysteine may be formed from taurine. One possible pathway compound found frequently in our chromatographic separations of S^{35}-labeled compounds from chick liver appears to be mercaptoethylamine.

Summary. The young chick actively converts sulfate-sulfur into taurine. The presence of optimum levels of inorganic sulfate in the diet appears to enhance the reaction more than with organic sulfur. Dietary cysteine represses the formation of total taurine in the liver and taurocholate-S^{35} in the bile fluid. These preliminary data suggest that one or more of the nonessential amino acids may combine with activated sulfate to form taurine without passing *via* cysteine. Components of one carbon metabolism and vitamin B_{12} appear to be associated with the overall reaction.

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