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The Incorporation of $S^{35}O_4$ into Bile of Chicks.* (30792)

W. G. MARTIN AND H. PATRICK
Department of Agricultural Biochemistry, West Virginia University, Morgantown

The utilization of inorganic sulfate by the chick to synthesize taurine is of interest since it has been previously reported that cysteine-sulfur does not arise from sulfate(1). Further, over one-half of the sulfate-S^{35} administered to the 24-hour embryo was found in the taurine of the day-old chick(2).

The bile acids, cholic and derivatives of it, conjugate with taurine in the chick liver and are passed into the gall bladder. Cholic acid is the limiting factor in formation of taurocholate in dogs(3), and continued feeding appeared to force the synthesis of taurine(4). Feeding cholic acid to young chicks stimulated the incorporation of sulfate-S^{35} into taurocholate(5).

The purpose of this investigation was to ascertain the influence of taurine and the cholic acid derivatives on incorporation of sulfate-S^{35} into the bile of chicks.

Methods. Day-old chicks were fed *ad libitum* a simplified basal ration of cerelose (glucose-hydrate) and isolated soybean protein with supplements of vitamins, minerals, and corn oil. These birds were maintained on this diet with the taurine and bile acid supplements indicated in the respective Tables for 14 days or as long as indicated. Following the feeding period the isotope was administered and the chicks sacrificed as described in the Tables.

Sulfate-S^{35} was administered orally by means of a 1 ml pipette or injected subcutaneously. Each dose contained 20 μc of carrier free $H_2S^{35}O_4$ in dilute HCl solution. Bile fluid was removed from the gall bladder or the chicks and pooled for each treatment.

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TABLE I. Influence of Taurine on Incorporation of S^{35} from Injection Sulfate-S^{35} into Bile Fluid of New Hampshire Chicks. The isotope was injected subcutaneously at 1 day of age. Each value is pooled average of 5 chicks.

Supplemental taurine to diet	Days after subcutaneous injection of sulfate-S^{35}								
	1	2	5	7	9	12	14	16	19
	% S^{35} dose/ml bile fluid								
—	1.92	2.16	2.01	1.33	.94	.62	.31	.24	.18
.1%	1.31	1.15	1.19	1.02	.60	.44	.28	.20	.17

TABLE II. Effect of Dietary Taurine on Weight, Feed Efficiency, Protein Efficiency Ratio (PER), Quantity of Bile, and Concentration of S^{35} in Bile of 14-Day-Old New Hampshire Chicks. These chicks were sacrificed 24 hours after oral dose of $S^{35}O_4$.

Dietary taurine (%)	14-day wt (g)	Feed efficiency, gain/feed	PER	Avg bile vol (ml)	% orally dosed S^{35}/ml bile
—	121.6	.66	3.29	.06	1.02
.1	142.2	.78	3.92	.15	.44

From these pooled samples, an aliquot was diluted with distilled water, 0.1 ml of fluid was dried on stainless steel planchets and counted under gas flow geiger tube. Dosing standards were counted and all S^{35} activity reported as per cent of the S^{35} dose.

Whole and hydrolized bile samples were chromatographed to ascertain the presence of taurine-S^{35}. Activity was observed at the R_f of commercial taurine in three different solvent systems (80% aqueous phenol; butanol-acetic acid-water, 50:12:50 v/v; and collidine-lutidine:water, 1:1:1 v/v). When whole bile and n-butanol extracted bile was chromatographed, activity was observed at the R_f of commercial taurocholate (butanol-3% acetic acid, 1:1; Whatman No. 4 paper pretreated in 70% acetic acid).

Results and discussion. The supplementation of taurine to the diet diminished the amount of S^{35} present in chick bile fluid at periods during the first 14 days after injection (Table I). The S^{35} activity was about one-half that of the chicks not receiving dietary taurine during the first 5 days, suggesting that taurine synthesis from sulfate occurs even in the presence of exogenous taurine. When sulfate-S^{35} was given orally to the chicks which had been fed the basal ± taurine for 14 days (Table II), the concentration of bile-S^{35} was again decreased by dietary taurine. The bladder volume of bile was increased by dietary taurine as was the body weight, feed efficiency and protein efficiency ratio (PER). This is probably due, in part, to the lower level of dietary methionine from the soybean protein, since taurine can be formed from methionine.

Interestingly, when chicks were fed a casein-purified diet with optimal supplementations, little growth response to taurine or cholic acid was observed. When the soybean protein purified diet was fortified with methionine, less growth promotion attributed to taurine or cholic acid supplementation was observed. However, the presence of methionine supplied as the free amino acid or the feeding of casein as the only nitrogen source did not prevent or retard the use of sulfate to synthesize taurine in the chick liver.

Feeding cholic acid alone (Table III) further enhanced the bile-S^{35} concentration while cholic with taurine equaled that of the control chicks. The marked difference in bile volume, a 2-fold increase with taurine or taurine plus cholic, and a 3-fold increase with cholic, reflects a highly significant increase in the bile-S^{35} per chick receiving cholic acid. Those fed cholic with taurine had a 2-fold increase over the control of taurine supplemented chicks. This trial shows that cholic acid, and to a lesser extent taurine, stimulate the synthesis of the sulfur-containing bile-fluid components.

Realizing that the bile volume could fluctuate due to the individual metabolic pattern, a trial was made to ascertain bladder volume at hourly intervals after oral adminis-

tration of sulfate-S^{35} to the chick. The amount of sulfate-S^{35} and of taurocholate-S^{35} was measured in these bile samples (Table IV). The volume of bile with time appears relatively constant. Since consumption of food or the placement of substance in the digestive tract often effects the release of the bile fluid into the intestine, the chicks were usually deprived of food a few hours before dosing and sacrifice.

Even with the continual efflux of bile fluid, the amount of S^{35} in the bile increases through 6 hours after dosing. After dosing, an increasing quantity of the total S^{35} is incorporated in organic molecules as evidenced by the increased amount of taurocholate-S^{35}.

With the chick, addition of cholic, dehydro-, and deoxycholic acids was associated

TABLE III. Effect of Dietary Taurine, Cholic Acid or Both on Incorporation of S^{35} from Oral Doses of Sulfate-S^{35} into Bile of Chicks. Subsequent to sacrifice, 24 hours after dosing, the bile from each chick per diet group was pooled. Each dietary group contained 25 chicks.

Supplement to basal	14-day body wt (g)	Avg vol bile fluid/chick (ml)	% S^{35} dose/ ml bile fluid	% S^{35} dose in bile/chick
—	118	.07	2.12	.15
Taurine (.1%)	126	.15	1.07	.16
Cholic acid (.34%)	124	.22	4.98	1.10
Taurine (.1%) + cholic acid (.34%)	125	.17	1.99	.34

TABLE IV. Incorporation of Orally Administered Sulfate-S^{35} into n-Butanol Solution-S^{35} of Bile Fluid from 14-Day-Old Chicks at Short Intervals After S^{35}-Dosing. Chicks were dosed at 8 A.M. and deprived of food between dosing and time of sacrifice.

	Hours after dosing					
	1	2	3	4	5	6
Avg vol of bladder-bile (ml)	.07	.10	.08	.08	.09	.11
n-butanol soluble-S^{35} (% of total bile-S^{35})*	8.74	9.52	10.38	11.67	11.93	13.49

* Taurine and conjugated taurine are separated by n-butanol extraction as taurocholate is selectively absorbed by the alcohol(6).

TABLE V. Influence of Bile Acid and Taurine Supplementation on Distribution of Sulfate-S^{35} in Liver and Bile of Chicks 6 Hours After Oral Administration of the Isotope.

Supplement to basal*	Avg 14-day wt	Liver wt (g/100 g body wt)	% S^{35} dose in 1 g liver	% S^{35} activity in liver soluble in n-butanol†	Avg vol bladder bile/chick (ml)	% S^{35} dose in 1 ml bile	% S^{35} activity in bile soluble in n-butanol†
—	92.1	4.51	.12	1.92	.07	.87	13
Cholic	99.5	4.47	.06	10.34	.20	1.89	32
Lithocholic	72.8	11.28	.23	.94	.02	.25	17
Dehydrocholic	99.2	4.54	.19	3.09	.15	2.62	29
Deoxycholic	114.2	4.32	.08	6.25	.18	1.43	25
Taurine	96.2	5.03	.06	3.57	.13	.49	14
Cholic and taurine	121.6	4.26	.02	7.69	.21	.43	23
Lithocholic and taurine	77.8	12.29	.12	8.93	.03	.17	14
Dehydrocholic and taurine	91.2	4.57	.04	11.76	.17	.46	26
Deoxycholic and taurine	126.2	4.45	.05	6.25	.26	.39	23

* Taurine supplemented at 0.1% of diet; bile acids were supplemented equimolar to taurine
† Taurocholate is selectively absorbed by n-butanol(6).

with a slight growth response and enhanced bile-S^{35} concentration over the control while lithocholic acid was growth depressing and toxic at the same dietary level (Table V). The livers of the birds fed lithocholic acid were abnormally large and contained more S^{35} per gram than did the livers of the other dietary groups of chicks. The liver taurocholate-S^{35} 6 hours after dosing was highest when cholic and deoxycholic were fed alone and when dehydrocholic was supplemented with taurine.

Studies are in progress concerning the chick liver reactions whereby sulfate-sulfur is converted to taurine-sulfur without passing through cysteine, which is a normal reaction pathway of mammals.

Summary. Chicks utilize sulfate-S^{35} to synthesize taurine. Dietary cholic acid, taurine or both are associated with increased volumes of bile fluid. Cholic acid feeding enhances sulfate-S^{35} incorporation into the bile while taurine decreases such reactions.

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Cycasin Excretion in Conventional and Germfree Rats. (30793)

M. SPATZ, E. G. MCDANIEL AND G. L. LAQUEUR
Laboratories of Experimental Pathology and of Nutrition and Endocrinology, National Institute of Arthritis and Metabolic Diseases, Natinal Institutes of Health, U. S. Department of HEW, Bethesda, Md.

The glycoside cycasin, β-D-glucosyloxyazoxymethane, isolated from *Cycas circinalis* L (1,2) was previously reported to be hepatotoxic and carcinogenic in rats(3), guinea pigs (4) and mice(5). It was demonstrated furthermore that the aglycone of cycasin, methylazoxymethanol, produced acute and chronic toxic manifestations in rats similar to those observed with cycasin(6), and metabolic studies of cycasin *in vitro* had shown that a β-glucosidase was responsible for the hydrolytic cleavage of the aglycone from cycasin (7). The absence of signs of acute toxicity after intraperitoneal injections of the intact glycoside and its excretion in the urine suggested that cycasin, in order to produce acute toxic affects, required oral administration and that its cleavage most likely occurred in the alimentary tract(8,9,10). It was of some importance, therefore, to examine the question whether the enzymatic breakdown of cycasin was dependent on the presence of bacterial flora in the intestine.

The availability of germfree rats offered the opportunity to investigate this question. Preliminary observations in which germfree rats had been fed large amounts of the glycoside for 20 consecutive days indicated that such animals grew well without showing evidence of toxicity as judged from food intake, body weight gain and the microscopic appearance of the tissues. This was in striking contrast to conventional rats of the same strain in which food intake and body weight gain were reduced and in which severe liver necrosis and a high mortality commencing with the ninth day of the experiment were found(3).

The purpose of this report is: (1) to quantitate cycasin excretion in germfree animals and compare it with that in conventional animals; (2) to determine the time required for the disappearance of cycasin in urine and feces after discontinuation of feeding in these two groups of rats; and (3) to present a preliminary report on tumor development in cycasin-fed germfree rats.

Materials and methods. Thirty-five Sprague-