mosomes found in the KB cells was absent in the third subline and some of its cells were endoreduplicated. A hypothesis of genomic selection is discussed.

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On the Synthesis of Taurine from Sulfate by the Chick: I. Influential Dietary Factors.* (31588)

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The sulfate requirements of many animals are met through the degradation of the sulfur amino acids. Some investigations have indicated that young chicks can utilize dietary sulfate in the synthesis of taurine. Lowe and Roberts(1) reported that within 36 minutes after administration of sulfate-S^{35} to a chick embryo, labeled taurine could be detected. Sulfate-S^{35} was not found in cystine, cysteine or methionine in the chick embryo(1), and we have found little or no S^{35} in cystine of the tissues examined in the chick after sulfate dosing(2). Recent reports have shown the universal presence of taurine in practically all tissues and have shown that the functions of taurine in the different tissues were diversified and essential(3,4,5,6,7,8). In view of these reports, the conversion of sulfate to taurine in the animal becomes increasingly significant and of interest.

The over-all purpose of the investigation reported in this manuscript was to study the utilization of sulfate-S^{35} in the chick, specifically the synthesis of taurine from sulfate as it is influenced by the nutritional state of the animal.

Experimental. One-day-old White Leghorn cockerels were maintained on purified basal diets(9) with supplements for a period of time, usually 14 days, as specified in each trial. Groups of 4 or 5 chicks were then orally dosed with 20 microcuries of carrier-free $H_2S^{35}O_4$ by means of a one ml pipette inserted through the mouth into the crop and emptied by gravity flow. The C^{14}-labeled compounds were administered by subcutaneous injection.

Blood, bile and liver samples were taken at various times after dosing. The blood from each group was pooled, allowed to coagulate, then centrifuged. The serum was assayed for total and protein bound S^{35} activity.

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TABLE I. Incorporation of Orally Dosed Sulfate-S^{35} into Taurine in the Various Tissues of 14-Day-Old Chick Fed a Purified Basal Diet.

Tissue	Hr after dose	Taurine-S^{35}, cpm/g tissue	Total taurine, μg/g tissue	S.A., cpm/μg
Heart	1	900	100.0	9.00
	2	2,500	125.0	20.00
	3	4,700	100.0	47.00
	4	19,400	121.5	159.67
	6	17,400	121.5	143.21
Spleen	1	300	47.3	6.34
	2	750	59.9	12.52
	3	2,800	56.3	50.62
	4	6,550	45.0	145.55
	6	12,850	56.3	228.24
Kidney	1	462	57.5	8.03
	2	1,122	43.8	25.62
	3	1,584	54.5	29.06
	4	7,260	51.3	141.52
	6	7,260	44.5	163.15
Liver	1	1,100	58.7	18.74
	2	2,750	65.0	42.31
	3	5,600	63.3	88.47
	4	13,100	54.0	242.59
	6	27,150	54.0	502.77

The bile fluid was chromatographed in butanol which had been equilibrated with acetic acid (1:1, 1-butanol:3% acetic acid) on Whatman No. 4 paper that had previously been dipped in 70% acetic acid solution and dried. Liver homogenates (20%) were prepared with distilled water and the deproteinized supernatant was chromatographed on Whatman No. 4 paper in an 80% aqueous phenol solvent system. Other tissues were handled in a manner similar to that of liver. The radioactivity of the chromatograms was counted in a liquid scintillator using a PPO: dimethyl-POPOP:toluene (5 g:.1 g:liter) scintillation solution. Total taurine concentrations of the liver and other tissues were determined by the method of Pentz *et al*(10).

Results and discussion. The extent of sulfate-S^{35} incorporation into the taurine of the various tissues in the chick was determined. The taurine-S^{35} concentration of liver, heart, kidney, and spleen was measured (Table I). *In vitro* studies with chick liver enzymes resulted in the production of labeled compounds similar to those found in the chick body indicating that intestinal microbial synthesis was not an interfering factor. The highest concentration of total taurine occurred in the heart. The results indicated that sulfate-S^{35} was incorporated into taurine-S^{35} in all of the tissues analyzed as early as one hour after the dose and the concentrations continued to increase with time. From these data, it appears that each organ tested is equipped with the necessary enzymes for conversion of sulfate-sulfur into taurine. The taurine-S^{35} of the heart was higher than that of either the spleen or kidney but the high total taurine concentration of the heart caused the specific activities of these tissues to be similar. The taurine-S^{35} of the liver was higher than that of the other tissues, especially at the 6-hour interval, and consequently liver taurine had the highest specific activities of the above tissues.

Methionine supplementation of a diet low in methionine and other sulfur amino acids was associated with enhanced growth and increased incorporation of sulfate-S^{35} into liver taurine (Table II). Methionine can be utilized to fulfill sulfur requirements and hence could exert a "sparing action" on the utilization of inorganic sulfur to supply these needs. Thus, more sulfate-S^{35} would be available for the synthesis of taurine with a subsequent increase in taurine-S^{35} concentrations.

Investigators have known for some time that methionine sulfur is used in the synthesis of cysteine. A low organic sulfur diet sup-

TABLE II. Influence of Methionine Supplementation to the Purified Basal Diet on Incorporation of Orally Dosed Sulfate-S^{35} into Taurine of Chick Liver.

Supplement to basal diet*	Avg wt, g/chick	Hr after $S^{35}O_4$ dose				
		1	2	3	4	6
		(Net cpm taurine-S^{35}/μg taurine/g liver)				
None	92.0	9.4	21.2	44.3	121.3	344.0
Methionine (0.3%)	120.0	248.8	299.6	263.8	35.6	19.9

* The basal diet used in these studies was the simplified ration of Martin and Patrick(8) minus methionine.

TABLE III. Influence of Cysteine HCl Supplementation to the Purified Basal Diet on Incorporation of Orally Dosed Sulfate-S^{35} into Taurine of Chick Liver.

Supplement to basal diet	Hr after $S^{35}O_4$ dose				
	1	2	3	4	6
	(Net cpm taurine-S^{35}/μg taurine/g liver)				
None	26.4	60.0	105.0	105.0	112.2
Cysteine HCl*	118.5	181.1	210.3	251.0	257.5

* Supplement equimolar to 0.1% taurine.

TABLE IV. Influence of Taurine Supplementation to the Purified Basal Diet on Incorporation of Orally Dosed Sulfate-S^{35} into Liver Taurine in the Chick.

Supplement to basal diet	Hr after $S^{35}O_4$ dose				
	1	2	3	4	6
	(Net cpm taurine-S^{35}/g liver)				
None	1730	1200	1800	3900	3700
Taurine (0.1%)	550	1700	2250	2100	2500
	(Net cpm taurine-S^{35}/μg taurine/g liver)				
None	26.4	60.0	105.0	105.0	112.2
Taurine (0.1%)	11.3	21.8	33.8	37.2	41.9

plemented with cysteine enhanced growth as well as the utilization of sulfate-S^{35} in the synthesis of taurine. Liver taurine radioactivity increased, total taurine concentration decreased, and thus the specific activities increased when cysteine was added (Table III). When methionine and cysteine were both supplemented, total taurine concentration of the liver was not repressed as it was when cysteine was added alone. The lowered total taurine concentrations may indicate repression of key enzymes in the organic pathway of taurine formation. Pasternak *et al* (11) working on the control of sulfate reduction in bacteria reported that when *E. coli* or *B. subtilis* are grown on cyst(e)ine instead of sulfate, the ability of extracts to synthesize PAPS is repressed. They found that in low concentrations, cysteine repressed sulfate activation by repressing the enzymes adenosine triphosphate sulfate-adenyltransferase and adenosine triphosphate adenyl-sulfate 3-phosphotransferase. With slightly altered PAPS formation and markedly repressed taurine concentration, the specific activity of liver taurine-S^{35} would still be higher. Mason *et al*(12) have suggested that taurine can be used to synthesize cysteine in the hen. Thus, in the presence of optimal concentrations of cysteine, feedback inhibition of phosphoadenosine phosphosulfate formation is conceivable. The possibility of enhanced phosphoadenosinephosphosulfatase activity due to enzyme activation must also be considered.

Preformed taurine supplemented to the chick ration effected a decreased incorporation of sulfate-S^{35} into taurine-S^{35} (Table IV). Some incorporation still occurred despite the presence of adequate amounts of preformed taurine in the diet, although dietary taurine reduced the need for synthesis in the animal. The presence of labeled intermediates in the liver homogenates similar to those obtained during *in vitro* studies with purified liver enzymes diminished the possibility of simple ion exchange of $S^{35}O_4$ and the sulfur of taurine.

Supplementation of a low-methionine diet with serine, glycine, ethanolamine or sodium isethionate enhanced sulfate-S^{35} incorporation into liver taurine (Table V). The presence of supplementary serine or glycine in the diet resulted in increased sulfate utilization for taurine synthesis when compared to animals on the non-supplemented ration. Supplementation with ethanolamine was associated with a rapid incorporation of S^{35} into taurine. The data of Table V indicate a 2-to 3-fold increase of taurine-S^{35} specific activity over that of the non-supplemented chicks up to one hour after dosing. This could indicate a sparing action for serine by ethanolamine at this early time.

TABLE V. Influence of Amino Acid Supplementation to the Purified Basal Diet on the Specific Activities of Liver Taurine of Chicks.

Supplement to basal diet*	Hr after $S^{35}O_4$ dose					
	.5	1	1.5	2	4	6
	(Net cpm taurine-S^{35}/μg taurine/g liver)					
None	10.9	8.3	11.6	8.4	20.8	30.8
Serine	4.0	16.3	26.0	36.3	50.6	45.1
Glycine	5.5	6.9	10.5	17.5	59.1	100.6
Na isethionate	6.3	10.6	34.9	29.8	61.3	69.4
Ethanolamine	16.9	27.5	13.7	13.2	27.7	22.7

* Supplements equimolar to 0.1% taurine.

TABLE VI. Influence of Sulfur Anion Supplementation to the Chick Purified Diet on Incorporation of Orally Dosed Sulfate-S^{35} into Liver Taurine and Bile Taurocholate of Chicks.

Supplement to basal diet	Hr after $S^{35}O_4$ dose				
	1	2	3	4	6
	(Net cpm taurine-S^{35}/g liver)				
None	1700	2200	5100	5300	5400
Thiosulfate (0.3%)	4700	2300	4300	7900	21400
Sulfate (0.3%)	500	1500	2600	6600	7500
Sulfite (0.3%)	2200	4400	2100	6400	5700
	(Net cpm taurocholate-S^{35}/ml bile)				
None	16,720	19,760	24,252	43,860	65,760
Thiosulfate (0.3%)	13,200	22,016	64,680	61,408	23,716
Sulfate (0.3%)	3,748	34,048	21,980	70,080	130,080
Sulfite (0.3%)	10,640	26,800	32,832	41,400	52,728

Other trials have indicated a graded increase in incorporation of sulfate-S^{35} with increased supplementation of glycine to the ration. The interconversion between glycine and serine must be considered as these results indicate that these amino acids play a prominent role in the sulfate to taurine reactions.

Supplementation of the purified sulfur deficient diet with the sodium salts of thiosulfate, sulfate or sulfite effected increased taurine-S^{35} concentrations in the liver as well as increased taurocholate-S^{35} activity in the bile (Table VI). Sodium sulfate supplementation increased the bile volume, enhanced chick growth, and increased sulfate-S^{35} incorporation into taurine. Gordon and Sizer(13) indicated that the chick can satisfy part of its total sulfur requirement with inorganic sulfate. This was verified by our observation that supplementation at 0.7% of the diet enhanced the sulfate-S^{35} to taurine reactions. Machlin (14) previously reported optimal growth in chicks with sulfate supplementation of 0.5% of the diet. This supplementation appears to provide a sulfate pool of optimal substrate level for the enzymes involved in sulfate metabolism without producing a level where repression might occur. The total taurine of chick liver increased significantly with supplemental sulfate to the basal diet (unpublished data). The reduction of a S^{35}-labeled pool due to added sulfate to the diet does not necessarily detract from an increased synthesis of taurine-S^{35} since the more optimal substrate level may enhance total utilization, and also the formation of more taurine.

Various C^{14}-labeled compounds were subcutaneously injected into chicks and incorporation of radioactivity into liver taurine was measured. The labeled carbon of β-alanine-1-C^{14} was not used in the synthesis of taurine. Incorporation into taurine occurred from the carbons of cysteine, alpha alanine, pyruvate, and ethanolamine, but the greatest incorporation occurred when serine-3-C^{14}, serine-C^{14}-UL, and glycine-1-C^{14} were injected (Table VII). Whenever unlabeled glycine was supplemented to the diet, the serine-3-C^{14} appeared to be utilized at a more rapid rate in the synthesis of taurine (Table VIII). These results indicated that serine and glycine were closely related to the re-

TABLE VII. Specific Activity of Liver Taurine After Subcutaneous Injection of Various C^{14}-Labeled Compounds into Chicks Fed Purified Basal Diets.

Compound injected	Hr after injection .5	1	2	3	4
Basal diet	(Net cpm taurine-C^{14}/μg taurine/g liver)				
Serine-3-C^{14}	22.95	29.06	23.33	—*	16.67
Serine-C^{14}-UL	—*	—*	42.02	—	48.32
Serine-1-C^{14}	.00	.00	.00	—	.00
β-Alanine-1-C^{14}	3.20	.00	.00	—	.00
L-Alanine-C^{14}-UL	27.31	27.30	5.81	—	2.53
Ethanolamine-1-2-C^{14}	5.92	7.86	12.50	—	19.41
Cysteine-3-C^{14}	7.20	13.40	12.10	13.80	18.40
Casein diet† and 1.0% glycine					
Serine-3-C^{14}	48.18	30.19	2.66	—*	3.33
Serine-3-C^{14}‡	8.07	4.78	14.69	—	76.70
Glycine-1-C^{14}	9.77	10.79	11.80	3.33	—*
Pyruvate-2-C^{14}	4.29	7.78	4.21	1.63	—

* Samples not obtained at these times.
† Basal diet with protein supplied as isolated soybean protein (5%) plus casein (20%) and 62% cerelose.
‡ No glycine added.

TABLE VIII. Influence of Glycine Supplementation to the Purified Diet on Incorporation of Injected Serine-3-C^{14} into Liver Taurine of 17-Day-Old Chicks.

Supplement to casein diet	Hr after dose	Taurine-C^{14}, cpm/g liver	Total taurine, μg/g liver	S.A., cpm/μg
None	.5	250	50.8	4.92
	1	300	62.8	4.78
	2	550	37.5	14.69
	4	3950	51.5	76.70
Glycine (1.0%)	.5	2650	55.0	48.18
	1	1700	56.3	30.19
	2	150	56.3	2.66
	4	200	60.0	3.33

actions leading to the synthesis of taurine and completely agreed with the results obtained from the dietary studies utilizing sulfate-S^{35}. Perhaps the carbon moiety of taurine is a compound that originates from glycine or serine. In a study designed to determine which carbons of serine were utilized most readily in the synthesis of taurine, incorporation of the 3-carbon was evident in the liver taurine as early as one hour after injection and little or no radioactivity from the 1-carbon of serine was found in liver taurine (Table VII). Good incorporation of the carbons of uniformly labeled alanine-C^{14} was observed at the 0.5 and 1 hour intervals while the carbons of ethanolamine-1-2-C^{14} were more evident after 2 and 4 hours.

The chromatographic analysis of labeled compounds in the liver of the chicks after administration of the radioisotopes indicated several peaks of radioactivity. From time studies, possible pathways of taurine synthesis from sulfate might include cysteinesulfinic acid, isethionic acid, hypotaurine, or mercaptoethylamine all of which have tentatively been identified chromatographically. Little or no activity was observed at areas comparable to cystine, cysteine, or methionine even though the chick does convert C^{14}-cysteine into taurine (Table VII).

Summary. Many tissues in the chick are equipped with the enzymes necessary for conversion of sulfate-S^{35} into taurine. Supplementation of a low sulfur amino acid diet with serine, glycine, ethanolamine, sodium isethionate, methionine, or cysteine enhanced sulfate-S^{35} incorporation into liver taurine. Addition of the sodium salts of thiosulfate, sulfate, or sulfite to the ration increased liver taurine-S^{35} and bile taurocholate-S^{35}. The

optimum level of sodium sulfate supplementation was 0.7% of the diet. Incorporation into taurine of labeled carbons occurred when cysteine-3-C^{14}, alanine-C^{14}-UL, ethanolamine-1-2-C^{14}, serine-3-C^{14}, serine-C^{14}-UL, and glycine-1-C^{14} were administered.

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Reversal of Contact-Inhibition in Primary Amnion Cultures by Hydrocortisone.* (31589)

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Surface contact by diploid cells in primary cultures results in certain alterations which in aggregate are given the term "contact-inhibition." Physical changes noted early after cells come into contact include cessation of motion of the undulating cytoplasmic membrane, decreased cellular motility and later, cementation of the intercellular membranes(1). Biological changes associated with these physical phenomena are decreased mitotic activity(2) and decreases in protein, RNA and DNA metabolism with disappearance of free ribosomes from the cytoplasm(3).

Confluent monolayers of primary human amnion cells are relatively stable in cellular density over long periods of maintenance *in vitro* under conditions where incubation is provided at 37°C in stationary racks and culture medium is replaced every 48-72 hours. That replication is present in such monolayers is difficult to determine, the mitotic index approaching 0.1% or less(4).

Reversal of contact-inhibition and growth induction were observed in amnion monolayers when hydrocortisone was added to the culture medium.

Material and methods. Amnion cultures. Cells were obtained throughout from fresh, clean amniotic membranes using versene 0.1% and trypsin 0.25%, explanted into stationary roller tubes or 3 oz prescription bottles in Eagle's minimal essential medium (EMEM) with 20% mammalian serum (10% human, 10% bovine) and incubated at 37°C. These primary cultures became confluent after periods of 5-7 days, at which time maintenance medium was added, containing 10% equine serum. In early experiments, media were completely replaced every 72-96 hours; in later experiments, every 48 hours.

Cell and nuclei counts. Initially, cells were enumerated in suspensions prepared by treating the monolayers first with 0.1% versene and then with 0.2% trypsin, which rendered

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