Metabolic Fate of S^{35}-Labeled Sulfate in Baby Pigs.* (23757)

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Machlin *et al.*(1) reported that a small amount of S^{35} from labeled sulfate is incorporated into the cystine of egg albumin. Some of the sulfur administered in sulfate form to growing chickens was incorporated into taurine, cystine and methionine(2,3). The incorporation of sulfate sulfur into taurine by developing chick embryos was also demonstrated(4). Dziewiatkowski(5) demonstrated incorporation of S^{35} from sulfate into cystine to a small extent by the young rat and pointed out that highest concentration of S^{35}-labeled cystine occurred in the fraction containing the intestinal tract and its contents, suggesting that the bacteria of the intestinal tract were involved in synthesis of labeled cystine. Dziewiatkowski and DiFerrante(6) demonstrated S^{35}-labeled cystine and methionine in sera of rats that had been injected intraperitoneally with S^{35}-labeled sulfate. The conversion of sulfate sulfur into cystine and methionine sulfur by the ruminant has been demonstrated(7,8).

Incorporation of S^{35} from labeled sulfate by the rabbit(9-11) results in highest uptake in cartilaginous tissues. Heath *et al.*(12) reported that when labeled sulfate was fed to a one-year-old boar, a large amount of the S^{35} appeared in urine. The urinary S^{35} level dropped rapidly during the first 2 weeks. Sulfur^{35} values in blood, sperm and seminal plasma were also reported. The purpose of the present study was to obtain further information about the metabolic fate of S^{35} sulfate administered to pigs with particular attention to conversion of sulfur into organic forms.

Methods. A single dose containing 1 millicurie of S^{35}-labeled sulfate together with 0.2 mg of carrier sodium sulfate was administered by stomach tube to each of three 17-day-old litter-mate Landrace gilts. The baby pigs were individually caged and fed pasteurized cows' milk 4 times daily for 4 days preceding and during the 4-day collection period. Selected tissues were processed to determine the concentration of S^{35} present(11). Intestinal tract contents were removed, dried at 60°C for 24 hours and then analyzed for S^{35}. In addition, 1 g samples (air-dry basis) of intestinal tract contents from each of the pigs, as well as samples from pig No. 3 of liver, skin, heart and red bone marrow were hydrolyzed by refluxing for 2 hours in a mixture containing 20 ml of 90% formic acid and 40 ml of 6 *N* hydrochloric acid. (Guranani *et al.* 13). The samples were evaporated to dryness under a partial vacuum, below 50°C. Distilled water was added and the samples were again evaporated to dryness. The residue was dissolved in 20 ml of distilled water. A 10 ml aliquot was fractionated by a modification of the procedure described by Mueller *et al.*(14). Resin columns which were 0.9 cm in diameter and 5 cm in height were used. An additional 100 ml of 6 *N* hydrochloric acid after 10 ml were used as indicated in the published procedure. The column was washed with distilled water until the effluent was neutral, and then with an additional 20 ml of distilled water and 40 ml of 1 *N* ammonium hydroxide. When a solution containing sulfate, methionine and cystine was put on the column, all sulfate was eluted by water in the first fraction, methionine was eluted by 0.8 *N* hydrochloric acid in 55% ethanol and cystine by 6 *N* HCl. However, in the case of the hydrolysates of the intestinal tract contents, all of the S^{35} was not eluted by 110 ml of 6 *N* hydrochloric acid, and from 1.2 to 1.5% of the S^{35} was eluted in the final fraction for which 40 ml of 1 *N* ammonium hydroxide was the elutrient. In order to characterize the forms in which the S^{35} occurred in the hydrolysate of the intestinal tract contents, fractionation on a 0.9 x 100 cm Dowex—50 x 12

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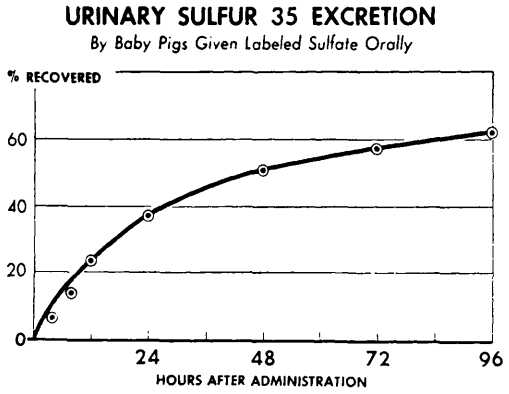

FIG. 1. Cumulative urinary S^{35} excretion curve.

columns in the ammonium cycle was carried out with the sample from pig No. 3. An aliquot of the hydrolysate representing 150 mg of dry matter was put on the column. About 64 ml of pH 2.0, 0.1 *M* ammonium formate was used to elute the column, followed by 196 ml of pH 3.4, 0.1 *M* ammonium formate, 194 ml of pH 4.3, 0.1 *M* ammonium acetate, 141 ml of pH 5.0, 0.1 *M* ammonium acetate and 69 ml of 1 *N* ammonium hydroxide. The flow rate ranged from 3.5 to 4.4 ml/hour. The use of pH 2.0, pH 4.3 and pH 5.0 buffers was suggested by the research of Davison(15).

Results. The urinary S^{35} excretion data are presented in Fig. 1. Most of the labeled sulfur was rapidly absorbed and then excreted by the renal pathway. Within the first 4 hours after dosing an average of about 7% of the dose was excreted in the urine. Table I presents data obtained by analysis of intestinal tract contents. Only a small part of the dose, less than 2%, appears in the intestinal tract contents, as compared with about 62% of the dose which was excreted in the urine. Fractionation of hydrolysates of intestinal contents from the 3 pigs by use of the 5 cm high columns as outlined above revealed that from 45 to 63% of the S^{35} present was in organic form. Most of this S^{35} was in the fractions eluted by the 0.8 *N* hydrochloric acid in 55% ethanol and the 6 *N* hydrochloric acid. However, from 1.2 to 1.5% of S^{35} recovered appeared in the final fraction, which was eluted by 1 *N* ammonium hydroxide. From 35 to 55% of S^{35} was in the sulfate-containing first fraction which was eluted by distilled water. The results of fractionation of S^{35} in the hydrolysate of intestinal tract contents from pig No. 3 by means of the 100 cm resin column revealed a well defined sulfate peak which contained 52% of total S^{35}. This was in general accord with the finding that 51% of total S^{35} from another aliquot of this hydrolysate which was run on the 5 cm resin column was contained in the first fraction, eluted by water. A cystine peak containing about 2.9% and a methionine peak containing about 13.3% of total S^{35} were eluted from the 100 cm resin column by pH 4.3 buffer from the 100 cm resin column. Identity of these peaks was confirmed by paper chromatography. A third peak, unidentified, was eluted by the pH 5.0 buffer and contained about 6.4% of the total S^{35}. In addition, about 14.7% of total S^{35} was eluted by 1 *N* ammonium hydroxide in a fourth peak.

The tissue S^{35} concentration values are presented in Table II. Highest S^{35} uptake occurred in ear cartilage. This is similar to findings in studies in which labeled sulfate was administered to rabbits(9-11). The fractionations by means of ion exchange revealed that almost all tissue S^{35} was in the sulfate-containing fraction. In the case of the hydrolysates of heart and red bone marrow samples, no organic-S^{35} was detected. About 0.01% of organic-S^{35} was detected in skin hydrolysates and 1.45% in liver hydrolysates.

The finding that a large part of the S^{35} in the intestinal tract contents had been converted to organic form while only a small amount of organic-S^{35} could be detected in tissue samples suggests that the microbial population of the intestinal tract was probably responsible for the incorporation of S^{35}

TABLE I. Intestinal Tract Contents of Baby Pigs 4 Days after Oral Administration of Labeled Sulfate.

Pig No.	1	2	3	Avg
Wt (g)	8.4	10.0	4.9	7.8
% dose/g	.15	.19	.16	.17
% dose in total	1.25	1.90	.78	1.31
% of S^{35} in sulfate-containing fraction	55.2	36.6	50.7	47.5
% of S^{35} as in organic sulfur fraction	44.8	63.4	49.3	52.5

TABLE II. Tissue S^{35} Concentration in 3-Week-Old Pigs 4 Days after Oral Administration of Labeled Sulfate.

Tissue	Pig 7 (wt 3995 g)		Pig 2 (3445 g)		Pig 3 (4035 g)		Avg (3825 g)	
	% dose*/g $\times 10^3$	% dose in organ	% dose*/g $\times 10^3$	% dose in organ	% dose*/g $\times 10^3$	% dose in organ	% dose/g $\times 10^3$	% dose in organ
Liver	4.50	.47	2.84	.29	4.12	.38	3.82	.38
Kidneys	3.75	.07	2.29	.05	2.55	.06	2.86	.06
Heart	2.03	.04	1.52	.03	1.45	.03	1.67	.03
Gastrocnemius muscle	1.65		.81		1.13		1.20	
Ear cartilage	83.50		47.64		61.92		64.35	
Skin	8.89		6.54		6.87		7.43	
Brain	3.01		2.18		2.61		2.93	
Red bone marrow	46.86		39.71		43.27		43.29	
Femur shaft	13.14		9.41		8.95		10.50	
Aorta	24.27		16.80		19.30		20.12	
Serum	4.10		3.31		4.20		3.87	

* Values standardized to body wt of 3700 g.

into organic form. Dziewiatkowski's research with the rat(5) suggests that a similar situation exists with this species.

Summary. The metabolic fate of S^{35} administered by stomach tube as sulfate to baby pigs has been studied. About 62% of the dose was excreted in urine within 4 days. Of tissues analyzed, ear cartilage, red bone marrow and aorta showed highest concentrations of S^{35}, and brain and muscle lowest concentrations, 4 days following dosing. About half of the S^{35} in intestinal tract contents was in organic form, while only very small amounts of organic-S^{35} were detected in tissues examined.

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