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Urinary Nitrogen and Sulfur Excretion in Dogs under Different Dietary Treatments [1,2]

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ABSTRACT Nitrogen balance studies with dogs were carried out to determine the relationship between urinary nitrogen and sulfur excretion, under different dietary treatments. The results indicate that the variation of urinary sulfur is slightly higher than that of urinary nitrogen excretion. Although urinary nitrogen and sulfur excretion varied among individual dogs, the N/S ratio was similar for all animals. A parallelism between urinary nitrogen and sulfur excretion was noted when the protein of the diet fed was of relatively high nutritional value, during a decrease in nitrogen intake and during protein depletion. Urinary nitrogen and sulfur excretion did not parallel each other when the animals were fed at a low level of a methionine-deficient protein and its supplementation with the amino acid. A decrease in the intake of calories caused an increase in urinary nitrogen excretion but did not affect urinary sulfur excretion. It was also observed that the supplementation of a protein deficient mainly in lysine and tryptophan with small amounts of good quality proteins decreased urinary nitrogen excretion but did not affect sulfur excretion. It is suggested that the determination of the urinary sulfur excretion helps in the interpretation of nitrogen balance studies, as related to diet and physiological condition of the animal.

Relatively few studies have reported the factors affecting excretion of urinary sulfur. Since methionine is an essential amino acid and component of proteins, investigations of urinary sulfur excretion would be helpful in obtaining more information on nitrogen metabolism as related to diet. Eckert (1) observed a linear relationship between absorbed sulfur and urinary inorganic sulfate sulfur, similar to that described by Allison and Anderson (2) for absorbed and urinary nitrogen and nitrogen balance. It has been suggested, therefore, that sulfur excretion can be used as a measure of protein quality. Other workers (1, 3–5), studying the relationship between urinary nitrogen and sulfur excretion, noted a close relationship between the 2 elements. Bressani et al.[4] also studied sulfur excretion in comparison with nitrogen excretion in amino acid imbalance feeding conditions. In these studies a difference in the pattern of excretion between sulfur and nitrogen in urine was reported. Recently, Miller and Naismith (6) and Miller and Donoso (7) studied the sulfur content of foods, as an indicator of the deficiency of total sulfur-containing amino acids in proteins and of the protein value of diets. They reported that the sulfur content of food could be used to predict protein value. More information on sulfur metabolism and its relation to nitrogen metabolism is needed to interpret more efficiently the newer modifications of protein quality evaluation (8, 9). Therefore, the results of several experiments in dogs carried out during the last 3 years are presented in this paper to show the behavior of urinary sulfur excretion in comparison with nitrogen excretion under different dietary treatments.

MATERIAL AND METHODS

In a series of experiments, factors affecting nitrogen balance were studied to determine whether urinary sulfur excretion would behave as urinary nitrogen excretion. The experiments were carried

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[4] Unpublished data, R. Bressani, L. G. Elías and J. E. Braham.

out in healthy, deparasitized dogs 3 to 8 months of age. The animals were housed in individual metabolism cages, weighed daily before feeding, and fed once a day at 8:00 A.M. Feeding was performed by weighing daily the amount of food according to body weight and adding to it 600 cm^3 of hot water and allowing to cool to 32° before feeding. Balance periods lasted 4 days. Urine was collected in dark bottles containing 1 cm^3 of concentrated acetic acid and both urine and feces were collected twice a day and stored at 4° until analysis was performed. Each 4-day collection of urine and of feces was homogenized, volume and weight noted and representative aliquots withdrawn for nitrogen analysis by the Kjeldahl method. Total inorganic sulfur was measured in urine using the gravimetric method of Folin (10). No attempts were made to determine ethereal sulfate and sulfur and organic sulfur excretion, since they have been shown to be independent of sulfur intake, are not influenced by diet and tend to remain nearly constant (1, 3, 11, 12).

Variability of urinary nitrogen and sulfur excretion. In the first experiment the variability in urinary nitrogen and sulfur excretion under constant nitrogen and calorie intake was studied. Four male dogs, 3 months of age, from the same litter were used. The initial weights were 4.35, 4.17, 4.76 and 4.01 kg. The dogs were fed approximately 6 g of protein and 130 kcal/kg body weight/day for 32 days with a casein diet and 32 days on a diet made with INCAP vegetable mixture 9 (13). The casein diet fed consisted of: (in per cent) casein, 25; DL-methionine, 0.3; hydrogenated vegetable fat, 10; mineral mixture (14), 2; cod liver oil, 1; cellulose, 2.7; sucrose, 15; dextrin, 7.4; and dextrose, 36.6; plus 5 ml of a complete vitamin solution (15) per 100 g. This diet contains around 22% protein and 418 kcal/100 g. To adjust the intake of calories to the required level, a nitrogen-free diet was fed in which casein and methionine in the diet described above were replaced by dextrin; the nitrogen-free diet contained 418 kcal/100 g. Vegetable mixture 9 was fed as such together with specific amounts of the nitrogen-free diet. In this experiment, 8 consecutive balance periods of 4 days' duration each, were analyzed for each protein source.

Effect of supplementation of casein with methionine at 3 levels of nitrogen intake. In the second experiment, 9 dogs divided in groups of three, were used. One group received 3.0 g of protein, the second 6.0 g and the third 7.2 g of protein/kg body weight/day. Calories were set at 120 kcal/kg/day. Data on three 4-day balance periods were obtained from each group of dogs with a casein diet equal in composition to the one described above but without added methionine, and also three 4-day balance periods for each group of dogs with the casein-plus-methionine diet described above. The group fed low protein had an average initial weight of 2.70, the second group averaged 4.02 and the third 8.08 kg and the ages ranged from 5 to 7 months.

Effect of decreasing nitrogen intake. In a third experiment, 3 dogs with an initial weight of 9.05, 9.48 and 9.04 kg were used. The dogs were fed the casein-plus-methionine diet described above. The protein intake for three 4-day balance periods was about 1.1 g/kg/day. It was then decreased to approximately 0.5 g, whereas the intake of calories was adjusted to 120 kcal/kg/day, by feeding small amounts of the nitrogen-free diet. The lower level of nitrogen intake was maintained for 8 additional 4-day balance periods.

Effect of protein depletion. The fourth experiment was designed to study urinary nitrogen and sulfur excretion during protein depletion followed by protein repletion. Four dogs, initially weighing 16.7, 11.4, 13.4, and 10.6 kg, were used. The animals were fed a nitrogen-free diet of the following percentage composition: cornstarch, 20; mineral mixture (14), 2; cod liver oil, 1; and 5 ml of a complete vitamin solution (15) per 100 g of diet. The diet contained 436 kcal/100 g and the dogs received an amount of food providing 130 kcal/kg of body weight/day. The nitrogen-free diet was fed for 6 balance periods of 4 days duration each. After the sixth period, or 24 days, the dogs were repleted by feeding vegetable mixture 9 (13) as the source of protein, and the nitrogen-free diet described above as the source of additional calories and other nutrients for 3 more 4-day balance periods.

Effect of calorie restriction. The effect of calorie intake was studied in the fifth experiment, in which 4 dogs were used, fed at a constant level of protein at 2 levels of intake of calories, 140 and 85 kcal/kg body weight/day. At the beginning of the study the animals weighed 8.41, 6.89, 8.69, and 6.64 kg. Vegetable mixture 9 (13) was fed as the source of protein, and the intake of calories was adjusted with the nitrogen-free diet described above. Protein intake at the low and high intake of calories was approximately 6.0 g/kg body weight/day. At the higher intake of calories, eight 4-day balance periods were analyzed, whereas at the lower level five 4-day balance periods were examined.

Effect of the supplementation of poor quality protein on urinary sulfur. Finally, to test the sensitivity of sulfur as a measure of supplementation of poor quality protein, a sixth experiment was carried out. In this study, 4 dogs weighing at the start 6.94, 5.73, 6.57, and 6.22 kg were used. For two 4-day balance periods, they were fed about 3.0 g of protein/kg body weight/day of a diet made up of the following: (in per cent) lime-treated corn flour, 78.0; corn gluten, 4.0; skim milk, 5.0; mineral mixture, 2.0; hydrogenated vegetable fat, 6.0; cod liver oil, 1.0; and vitamins. The milk in the diet was then replaced by an equivalent amount of corn gluten and the diet fed to the dogs for two 4-day periods. In the last stage, 4% fish flour replaced an equivalent amount of corn gluten protein and the diet was fed for two more 4-day balance periods. In all experiments, the handling of the dogs was the same.

RESULTS

Variability of urinary nitrogen and sulfur excretion. Table 1 presents the results of the first experiment. The coefficient of variation in urinary sulfur excretion was higher than that of urinary nitrogen excretion for both sources of protein. However, there was no statistical evidence that this difference was significant. It is probable that the higher coefficient of variation for sulfur excretion is due to the lower accuracy of the sulfur determination, as compared with the analysis of nitrogen in urine. Although urinary nitrogen and sulfur excretion varied among individual dogs, the N/S ratio was similar for all animals, within the diet fed.

TABLE 1

Variation[1] in urinary nitrogen and sulfur excretion in 4 dogs fed 2 types of protein[2]

Dog no.	Nitrogen			Urinary sulfur	Urinary N/S ratio
	Intake	Urine	Retention		
	mg/kg/day	*mg/kg/day*	*mg/kg/day*	*mg/kg/day*	
		Casein			
1	954	450	422	34.4	13.3
2	959	462	397	35.0	13.7
3	956	518	383	36.7	14.7
4	958	554	310	36.4	15.6
Avg	959	491	378	36.7	13.5
SD	34.5	36.4	35.8	3.5	2.5
C[3]	3.6	7.4	9.5	9.1	18.5
		Vegetable mixture 9			
1	936	335	320	19.7	17.0
2	940	443	245	24.5	18.0
3	943	340	322	19.5	17.7
4	940	450	267	25.0	18.1
Avg	940	392	288	22.2	17.8
SD	25.4	32.9	50.1	2.3	2.1
C	2.7	8.4	17.4	10.4	11.8

[1] Estimates of variability on a within dog basis.
[2] Each value represents the average of eight 4-day balance periods.
[3] Coefficient of variation.

TABLE 2

Effect of feeding casein at 3 levels with and without methionine on urinary nitrogen and sulfur excretion in 9 dogs

Diet fed	Balance no.[1]	Nitrogen Intake	Nitrogen Urine	Nitrogen Retained	Urinary sulfur	Urinary N/S ratio
		mg/kg/day	*mg/kg/day*	*mg/kg/day*	*mg/kg/day*	
Casein	1	497	453	− 4	16.8	26.9
	2	505	437	+ 7	13.3	32.8
	3	511	427	+47	9.7	44.0
	Avg	504 ± 12.0	439 ± 53.0	+16 ± 49.3	13.3 ± 3.6	
Casein + DL-methionine	1	534	361	126	10.3	35.2
	2	558	295	241	11.1	26.7
	3	561	269	250	12.8	21.0
	Avg	551 ± 57.7	308 ± 63.7	206 ± 69.7	11.4 ± 3.6	
Casein	1	998	578	375	16.1	35.9
	2	1006	563	400	14.5	38.7
	3	973	554	389	15.2	36.5
	Avg	992 ± 19.0	565 ± 37.0	388 ± 38.3	15.3 ± 1.4	
Casein + DL-methionine	1	953	539	384	23.4	23.0
	2	994	499	452	23.7	21.1
	3	976	518	426	22.7	22.8
	Avg	974 ± 20.0	519 ± 29.3	420 ± 40.3	23.3 ± 2.9	
Casein	1	1161	617	504	17.3	35.6
	2	1148	776	294	22.7	34.1
	3	1130	750	348	24.7	30.4
	Avg	1146 ± 23.0	714 ± 79.3	382 ± 105.0	21.6 ± 3.1	
Casein + DL-methionine	1	1134	705	375	24.2	29.1
	2	1120	746	323	34.0	21.9
	3	1106	642	418	27.2	23.6
	Avg	1120 ± 20.3	698 ± 72.0	372 ± 71.3	28.5 ± 7.8	

[1] Each balance period was of 4 days' duration.

Effect of supplementation of casein with methionine at three levels of nitrogen intake. Table 2 summarizes the results of the second experiment. At the low and intermediate level of nitrogen intake when no methionine was added, urinary nitrogen and sulfur excretion decreased with respect to time. At the high level, urinary nitrogen and sulfur excretion increased with respect to time. Addition of methionine at the low and intermediate level of nitrogen intake decreased urinary nitrogen excretion, whereas urinary sulfur remained essentially the same at the low level of intake and increased at the intermediate level. When methionine was added at the high level of nitrogen intake, urinary nitrogen and sulfur excretion showed no trend, although on the average, nitrogen excretion was less than with the unsupplemented diet, whereas sulfur excretion was higher. The N/S ratio with the unsupplemented diet increased at the low level of nitrogen intake and decreased at the high, with no change observed at the intermediate level. When the supplement was added, the N/S ratio decreased with respect to time at the low level of nitrogen intake but showed no trend at the other 2 levels studied. When averages per treatment were compared, the N/S ratios were higher when the diet was without the methionine supplement than when it was added.

Effect of decreasing nitrogen intake. Table 3 shows the effect of decreasing the level of nitrogen intake from approximately 1.0 to 0.5 g/kg/day. Urinary nitrogen and sulfur excretion was high at the higher level of nitrogen intake and decreased when the intake of nitrogen was decreased. From the fourth period on, both urinary nitrogen and sulfur tended to increase. The urine N/S ratio, however, remained constant.

Effect of protein depletion. Table 4 presents the results of protein depletion on urinary nitrogen and sulfur excretion. Both urinary nitrogen and sulfur excretion decreased as depletion progressed, from the third to sixth period of depletion when they became almost constant. Feeding of protein increased both urinary nitrogen and sulfur excretion. The N/S ratio, however, decreased during nitrogen deprivation, but was similar before and after protein depletion.

Effect of calorie restriction. The results on the effect of the intake of calories on urinary nitrogen and sulfur excretion is shown in table 5. At the high level of intake of calories both nitrogen and sulfur excretion were parallel and did not differ between periods. At the lower level of calorie intake, urinary nitrogen and sulfur excretion were also similar between periods, except in period 2 in which excretion of both was less. On the average, the low level of calorie intake resulted in increased nitrogen excretion in urine, but in no change in urinary sulfur when compared with the higher caloric intake level. The N/S ratio was also higher for the lower intake of calories.

Effect of the supplementation of poor quality protein on urinary sulfur. Table 6 presents the data obtained on the effect of

TABLE 3

Effect of decreasing nitrogen intake on the urinary nitrogen and sulfur excretion in dogs

Balance no.[1]	Nitrogen		Urinary sulfur	Urinary N/S ratio
	Intake	Urine		
	mg/kg/day	*mg/kg/day*	*mg/kg/day*	
Initial	1120±20 [2]	698±62	28.5±7.2	24.5
1	543±8	460±40	19.4±1.1	23.7
2	539±6	393±15	18.6±2.2	21.1
3	532±6	370±23	14.4±2.7	25.7
4	540±8	334±16	13.0±0.9	25.7
5	550±14	370±10	12.8±0.9	28.9
6	603±20	408±20	16.8±0.9	24.3
7	607±22	447±3	18.4±2.8	24.3
8	564±24	390±16	15.8±0.2	24.7

[1] Average of the 4-day balance periods of 3 dogs.
[2] SD.

TABLE 4

Effect of protein depletion and repletion on urinary nitrogen and sulfur excretion in 4 dogs

Balance no.[1]	Nitrogen		Urinary sulfur	Urinary N/S ratio	Avg wt
	Intake	Urine			
	mg/kg/day	*mg/kg/day*	*mg/kg/day*		*kg*
			Before depletion		
1	979±4	497±86	23.6±1.8	21.0	12.30±2.39
2	978±1	501±80	26.4±5.1	19.0	12.56±2.46
3	975±6	453±101	25.4±6.6	17.9	12.86±2.57
			Depletion		
1	—	143±21	11.6±1.4	12.3	13.05±2.72
2	—	114±21	9.9±1.5	11.5	12.93±3.34
3	—	89±20	8.1±2.3	11.0	12.76±2.70
4	—	84±19	7.8±1.1	10.8	12.71±2.68
5	—	82±5	7.7±0.9	10.7	12.70±2.76
6	—	87±29	7.1±1.5	12.2	12.63±2.52
			Repletion		
1	986±42	329±96	14.4±4.4	22.9	12.54±2.08
2	951±39	342±115	14.4±5.3	23.8	12.69±1.06
3	964±58	360±114	16.9±5.1	26.6	13.12±1.90

[1] Average of the 4-day balance periods of 4 dogs.

TABLE 5

Effect of intake of calories on urinary nitrogen and sulfur excretion in dogs (average 4 dogs)

Balance no.	Nitrogen			Urinary sulfur	Urinary N/S ratio
	Intake	Urine	Balance		
	mg/kg/day	*mg/kg/day*	*mg/kg/day*	*mg/kg/day*	
		Intake of calories, 140 kcal/kg/day			
1	940±28	404±71	276±53	23.2±13.1	17.5
2	939±23	386±68	312±41	22.4± 3.6	17.4
3	939±23	397±51	284±40	21.9± 3.2	18.1
4	941±28	382±72	282±65	21.3± 3.2	17.9
Avg	940±24	392±65	288±50	22.2± 5.8	17.7
		Intake of calories, 85 kcal/kg/day			
1	1005±4	485±69	305±61	21.2± 3.7	22.9
2	1006±24	423±155	358±112	18.9± 7.7	22.4
3	1014±17	536±131	241±94	23.4± 5.8	23.0
4	1016±22	594±120	179±87	24.1± 4.8	24.7
5	1014±17	581±95	219±66	23.3± 3.3	24.9
Avg	1011±17	524±114	260±84	22.2± 5.1	23.6

TABLE 6

Effect of supplementing a corn protein diet with skim milk and fish flour on urinary nitrogen and sulfur excretion (average for 4 dogs)

Additions to basal diet	Nitrogen			Urinary sulfur	Urinary N/S ratio
	Intake	Urine	Balance		
	mg/kg/day	*mg/kg/day*	*mg/kg/day*	*mg/kg/day*	
5% skim milk	484±14	272±16	95±19	20.4±1.9	13.3
None	494±10	307±38	60±97	19.8±1.7	15.5
4% fish flour	480±6	219±8	151±11	18.9±1.7	11.9

supplementing poor quality protein with proteins of good quality on the excretion of urinary nitrogen and sulfur. The addition of skim milk and of fish flour resulted in lower excretion of nitrogen, but urinary sulfur did not change. The N/S ratio for the unsupplemented diet was slightly higher than the N/S ratio resulting from supplementation.

DISCUSSION

The results observed in most of the experiments reported indicate that, in general, urinary sulfur excretion parallels the urinary excretion of nitrogen. In all phases of metabolism there appears to be a parallelism between nitrogen and sulfur. They occur together in the proteins of food and are stored together in body proteins. Although there are many compounds of significance in body economy which contain only one or the other of these 2 elements, the preponderance of both nitrogen and sulfur storage goes into the synthesis of protein. From the results presented, however, it is evident that factors which affect nitrogen excretion in the urine do not necessarily affect the excretion of sulfur in the same manner. This difference may be useful in understanding the metabolic interaction between different nutrients, and in the physiological state of the organism with respect to nitrogen and sulfur needs.

In the studies presented, only inorganic sulfate sulfur was determined in the urine, since it has been demonstrated in dogs (1, 11, 12) that excretion of inorganic sulfate sulfur is directly proportional to absorbed sulfur and that ethereal sulfate and sulfur and organic sulfur excretion are independent of sulfur intake. Similar results have been reported by Beach et al. (3) in studies of 8 normal children. These authors reported that inorganic sulfate sulfur in urine accounted for 84% of the total urinary sulfur which is similar to the value reported by Eckert (1). Beach et al. (3)

also stated that inorganic sulfate sulfur in urine depends primarily on the level of sulfur intake and the ethereal and organic or neutral sulfur are influenced less by diet and tend to remain more nearly constant.

Fecal loss of sulfur also appears to remain constant according to the results of Eckert (1), Beach et al. (3) and Wright et al. (5), and it is equal, or nearly so, to the inorganic sulfur content of the diet. Eckert (1) showed that fecal sulfur excretion did not increase in magnitude when casein sulfur was added to the diet, a result which suggested that all the protein sulfur was absorbed by the animal.

The N/S ratios in the present study under normal protein feeding conditions were similar to those reported by Lewis (4) in dogs, and Beach et al. (3) and Wright et al. (5) in children.

Lewis (4) observed in growing dogs that the N/S retention ratio is around 14.5 and showed that during fasting and consequent tissue catabolism a urinary N/S ratio of 13 to 16 was obtained. In the present investigation, decreasing nitrogen intake decreased the nitrogen and sulfur excretion and a parallelism between urinary nitrogen and sulfur excretion was observed. Feeding a protein-free diet, however, did not affect the N/S ratio and the decrease in absolute amounts of N and S were essentially the same proportion. Again a parallelism between the 2 elements was observed.

A difference in behavior between urinary nitrogen and sulfur excretion was also observed when calories became limiting. A reduction in calories increased nitrogen excretion but did not affect sulfur excretion, suggesting an economy by the animal of sulfur-containing amino acids, which are limiting in muscle protein. This observation was in agreement with the results of Allison et al. (16) and Swanson (17) who reported that methionine supplementation to protein-free diets fed to dogs and rats with full stores of nitrogen resulted in decreased excretion of nitrogen in urine.

Finally, urinary sulfur excretion was not affected significantly when a poor quality protein was supplemented with skim milk or fish meal. There are two possible explanations for this. 1) The poor quality protein, which in this case was corn protein, is not limiting in sulfur-containing amino acids, but rather is limiting in lysine and tryptophan. Therefore, nitrogen was more important than sulfur. 2) The supplements added are themselves relatively limiting in sulfur containing amino acids. When added to the low quality protein, even if they supplied small excesses of lysine and tryptophan, sulfur-containing amino acids were relatively low, and thus no change in sulfur excretion was observed.

The determination of sulfur excretion in urine is, therefore, important since it shows, not only the parallelism to N excretion, but also a difference in behavior which may explain differences in needs of nitrogen and sulfur as affected by dietary conditions and physiological states of the animal. The use of sulfur analysis in urine has been used by Bressani et al.[5] in studies of amino acid imbalance and niacin deficiency and by Bressani and Braham (18) in investigations of the effect of water on nitrogen metabolism. In cases of amino acid imbalances and niacin deficiency, sulfur in urine did not parallel nitrogen excretion, whereas changes in water intake did not affect sulfur excretion but affected urinary nitrogen.

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