The Intermediary Metabolism of Some Sulfur-Containing Compounds Related to Cystine

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INTRODUCTION

The fact that the two naturally occurring sulfur-containing amino acids, cystine and methionine, appear to behave similarly in many biological processes has led several workers to propose mechanisms by which a conversion of methionine to cystine may take place. Most of these proposed mechanisms have no biochemical analogy and some no chemical analogue. One suggested mechanism involves the behavior of S-carboxymethylcysteine in cystinuric and normal individuals. Since a large number of the previous studies of sulfur metabolism have been made using rabbits, this species has been selected as the experimental animal for a further study of S-carboxymethylcysteine.

Although thiourea is not usually considered a substance of physiological occurrence or significance, work has appeared, from time to time, in which its metabolism has been studied. Earlier workers were interested in its possible function in the detoxication of phenol and in the ability of the animal organism (rabbit) to oxidize the sulfur portion of the molecule. These workers concluded that the sulfur of thiourea was oxidized in part as evidence by an increased excretion of ethereal sulfates after subcutaneous injection. The evidence is, however, not convincing. More recently,

a study of the oxidation of the sulfur of thiourea in the human being was reported and again an increase in ethereal sulfates was noted, but there was also an increase in the cystine excretion. This interesting finding also presented several unusual aspects of sulfur metabolism and studies of thiourea were therefore undertaken in which newer and more accurate methods were applied to the problem. In connection with these studies, it was also necessary to consider urinary sulfur partitions during fasting, since thiourea was somewhat toxic and its administration resulted in some loss of appetite and a refusal of food. Since the reports of fasting studies in the rabbit were found to be infrequent, some attention to this aspect of sulfur metabolism was necessary.

HISTORICAL

Cystine and Methionine

There are two sulfur-containing amino acids known to occur in the protein molecule, cystine, which was first observed as a constituent of urinary calculi by Wollaston in 1810 (1), and methionine, which was isolated from casein by Mueller in 1922 (2). Because of the lapse of more than a century between their discoveries, much of the earlier metabolic work on sulfur and cystine is open to the criticism that methionine was not considered. Studies of the metabolism of sulfur compounds have included: (a) the oxidation of the sulfur portion by the organism, (b) the utilization of the compound or compounds for growth, (c) the absorption of the compounds from the gastrointestinal tract, and (d) the utilization of the sulfur for the formation of detoxication products.

In early investigations of the oxidation of sulfur use was made of the methods for urine analysis proposed by Salkowski (3). He recognized two types of sulfur, the oxidized and the suboxidized, and proposed the name "neutral sulfur" for the suboxidized form. In 1906 Folin (4)

published a critical evaluation of existent sulfur determinations in biological fluids and described the methods for inorganic and sulfate sulfur which are used today. This report was soon followed by a now classical paper (5) which dealt with the distribution of urinary sulfur, and demonstrated that the organic sulfur fraction ("neutral sulfur") was usually relatively constant in amount. This was interpreted as proof that the organism could oxidize, readily and nearly completely, the sulfur-containing amino acids of the protein molecule.

The extent of biological oxidation of the sulfur in cystine has been studied by many workers. Wohlgemuth (6) reported the excretion of considerable amounts of thiosulfate when this amino acid was fed to rabbits; this finding, however, has not been universally confirmed and most workers have demonstrated a ready oxidation of the sulfur of cystine to sulfate upon oral or parenteral administration to rabbits (7), (8), (9), (10), (11) and dogs (8), (12), (13), and after ingestion by humans (14), (15). Likewise, cysteine has been found to be easily oxidized in the animal body (15), (16), (18) and by isolated tissues (17).

The administration of methionine to rabbits (11), (19), dogs (20), (21), (22), (23), and humans (15), (24) indicates that the degree of oxidation of its sulfur is

comparable to that of cystine in that two thirds or more of the administered sulfur is oxidized and is excreted in the urine as sulfate. After the administration of methionine, Virtue and Lewis (11) in experiments with rabbits, Vars (25) with a phlorhizinized dog, and Chase and Lewis (26) with rats reported the presence of a substance in the urine, which gave a disulfide test. Further, Pirie (17) incubated tissues with methionine *in vitro* and found a disulfide-like compound in the fluid in which the tissue was immersed. Since Butz and du Vigneaud (27) had been able to convert methionine into homocystine *in vitro*, it was suggested that methionine might be converted to homocystine in the body. Du Vigneaud, Loring, and Craft (19) studied the oxidation of homocystine in rabbits and found that its sulfur was oxidized to sulfates. These experiments suggested the attractive theory that methionine is converted into homocystine before the sulfur is oxidized. Brand has attempted to throw light upon the mechanism by which this conversion takes place through a study of the metabolism of S-carboxymethylcysteine.

S-Carboxymethylcysteine

$HOOCCH_2-S-CH_2CH(NH_2)COOH$

In 1936 Brand and associates (28) reported the results obtained after the administration of S-carboxymethylcysteine to two cystinuric and two normal individuals.

This compound like S-methylcysteine (29) does not support the growth of rats on a sulfur deficient diet, which indicates that cystine is not formed in the course of its metabolism. When S-carboxymethylcysteine was administered to a normal human, the sulfur excreted in the urine was oxidized to the extent of 40 per cent, and there was found in the neutral sulfur fraction a disulfide material which gave a cyanide-nitroprusside reaction, whereas the Sullivan cystine test was negative. The sulfur of S-methylcysteine was oxidized by the cystinuric individual (30) whereas only about 15 per cent extra sulfur was excreted as sulfate sulfur after ingestion of S-carboxymethylcysteine. A disulfide was found in the neutral sulfur fraction after S-carboxymethylcysteine administration to the cystinuric which possessed the same properties as the material which was present in the urine of the normal individual after S-carboxymethylcysteine ingestion. They suggested the possibility of thioglycolic acid formation, but their cystinuric urine yielded no ether-soluble fraction containing a disulfide compound; however, after the urine was reduced with zinc and hydrochloric acid, it contained an ether soluble fraction which gave an ammonia-nitroprusside reaction and a thioglycolic acid test. Since both thioglycolic acid and dithioglycolic acid are ether soluble, the urinary material was probably not one of these

compounds. Thus, from these facts, it was concluded that the metabolism of S-carboxymethylcysteine may possibly proceed with the formation of a mixed disulfide formed by the union of thioglycolic acid and cysteine

$$HOOCCH_2S\text{-}SCH_2CH(NH_2)COOH.$$

Previous studies made with cystinuric individuals have shown that cysteine and methionine behave differently than cystine; also, homocysteine behaves differently than homocystine. Cystine, when fed to the cystinuric, is oxidized to sulfates (31), (32), (33), (34), (35), (36), (37) whereas cysteine and methionine are not completely oxidized (35), (36), (38), (39) and lead to extra cystine excretion. Likewise, homocystine is almost completely oxidized by the cystinuric (36), but homocysteine is excreted largely as extra cystine and partly as homocystine (40).

From a consideration of the results obtained with S-carboxymethylcysteine, methionine, cysteine, and homocysteine in the cystinuric individual, Brand proposed a mechanism for the conversion of methionine to cystine. Methionine is demethylated to give homocysteine.

$$CH_3SCH_2CH_2CH(NH_2)COOH \longrightarrow HSCH_2CH_2CH(NH_2)COOH.$$

The homocysteine reacts with amino acrylic acid, yielding S-carboxyaminopropylcysteine,

$$HSCH_2CH_2CH(NH_2)COOH + CH_2{=}C(NH_2)COOH \longrightarrow S\begin{matrix}\diagup CH_2CH_2CH(NH_2)COOH \\ \diagdown CH_2CH(NH_2)COOH\end{matrix}$$

which in turn is believed to be split to yield cysteine.

$$S\begin{matrix}\nearrow CH_2CH_2CH(NH_2)COOH \\ \searrow CH_2CH(NH_2)COOH\end{matrix} \longrightarrow HSCH_2CH(NH_2)COOH$$

As evidence for the existence of such a compound, Brand cites the publication of Küster and Irion (41) in which they report the isolation of S-carboxyaminopropylcysteine after treating wool with sodium sulfide. The occurrence of this as a primary product seems questionable in view of the drastic preliminary treatment of the wool.

Thiourea

$(NH_2)_2C = S$

Thiourea was first prepared by Reynolds in 1869 (42) by the thermal rearrangement of ammonium thiocyanate, a reaction similar to Wöhlers (43) classical synthesis of urea from ammonium cyanate. Lange (44) made one of the first studies of thiourea with regard to its toxicity and reported that it was not toxic to rabbits. Pohl (45) reported that upon the injection of thiourea into a rabbit the greater portion of the administered material was excreted unchanged in the urine; a portion was apparently used for the synthesis of a compound possessing a garlic-like odor which could be smelled on the breath of the animal. By comparing the odors of various sulfides, he concluded that the material was probably diethyl sulfide. By

the use of a modification of Abel's method (46) he found that three to four milligrams of this sulfide were exhaled by the rabbit in twenty-four hours.

In 1895 Tauber (47) pointed out the importance of sulfur compounds in the detoxication of phenol; these findings led to a study of thiourea as a source of sulfur for detoxication. Sato (48) injected thiourea into rabbits which had received phenol orally and Kojo (49) carried out similar experiments with dogs as the experimental animal. Both of these workers reported an increase in ethereal sulfates from which they concluded that some of the sulfur of the thiourea was oxidized. Sato (48) also reported one experiment in which thiourea was injected into rabbits without the administration of phenol; he again found an increase in ethereal sulfates. A more extensive study of the metabolism of thiourea in rabbits without the administration of phenol was carried out by Masuda (50) who observed an increase in ethereal sulfates as well as neutral sulfur from which he concluded that a portion of the sulfur of thiourea must have been oxidized. Lewis (51) reported that after subcutaneous injection of thiourea into rabbits, the sulfate sulfur fraction remained unchanged while the neutral sulfur increased in proportion to the sulfur injected as thiourea. The urine gave a strong thiourea test by the method of Sato (52). Medes (15) studied the oxidation

of thiourea in the human after oral administration and her findings were in agreement with those of Masuda and Sato, in that the total sulfate excretion remained unchanged while the neutral sulfur and ethereal sulfates increased. The urine contained thiourea as evidenced by the test of Grote (53); there was also present a material giving a test with the Sullivan reaction (54) for cystine. Cystine must be in an uncombined form to give a positive Sullivan test, so Medes pointed out the possibility that a conjugated product of cysteine and thiourea may have been formed in the body, which, because of its extreme instability could hydrolyze to give cysteinesulfenic acid and thiourea.

$NH_2(NH^{=})C\text{-}S\text{-}S\text{-}CH_2CH(NH_2)COOH + H_2O \longrightarrow HOS\text{-}CH_2(NH_2)COOH + (NH_2)_2C{=}S$

The cysteinesulfenic acid ultimately underwent dismutation to give cystine and cysteinesulfinic acid.

$HOSCH_2CH(NH_2)COOH \longrightarrow HSCH_2CH(NH_2)COOH + HO_2SCH_2CH(NH_2)COOH$

$$HSCH_2CH(NH_2)COOH + HOSCH_2CH(NH_2)COOH \longrightarrow \begin{matrix} SCH_2CH(NH_2)COOH \\ | \\ SCH_2CH(NH_2)COOH \end{matrix}$$

Thus, it is possible to account for the positive Sullivan test. Evidence for the occurrence of these reactions *in vitro* has been given by Pirie (55) and Toennies (56).

Reppel (57) has reported the growth of Aspergillus niger upon media containing thiourea and from the amount of sulfate formed he concluded that small amounts of thiourea

must be used. Cystine behaved similarly.

In vitro, a 3 per cent solution of thiourea is reported to prevent the coagulation of blood, whereas urea has no effect (58). This is of interest when we consider work by Sterner and Medes(59) who studied the effect of cysteine and methionine on blood coagulation. In vitro only cysteine delayed the coagulation time while methionine in a concentration of 1 per cent remained without influence. In vivo cysteine had the effect of preventing coagulation in less than an hour, while methionine displayed its maximum effect two to four hours after administration.

Inanition

The general metabolism of the animal organism during inanition has interested various workers, but the results obtained with regard to the sulfur metabolism are not uniform. Particularly divergent results are observed with different species of animals. Benedict, in a study of two human subjects (60), (61) found a slight increase in the nitrogen to sulfur ratio during inanition as compared with values found when the person was assimilating food. Cathcart (62) has reported similar results. This suggests that the sulfur is held more firmly than nitrogen when the organism is receiving no food. In similar studies with dogs, Morgulis

(63) found a nearly constant nitrogen to sulfur ratio which indicates that the nitrogen and sulfur are metabolized in the same proportion as when the animal is receiving food. Wolf and Osterberg (64) reported that the sulfur was retained with more tenacity than the nitrogen which would result in a higher nitrogen to sulfur ratio. Lewis (65), however, observed that the nitrogen to sulfur ratio increased when dogs were fed and decreased upon fasting. This suggests a sulfur retention greater than the nitrogen retention after fasting, which can be interpreted as being due to a lag in sulfur excretion or to a specific demand for sulfur to build up sulfur rich proteins. Jacobson (66) found a variable nitrogen to sulfur ratio, depending upon the stage of starvation. It is of interest that Gaebler (67) found, upon injection of antuitrin, an anterior pituitary growth preparation, an increased nitrogen to sulfur ratio. In steers, Carpenter (68), (69) demonstrated a nearly constant nitrogen to sulfur ratio during inanition, while Hutchinson and Morris (70) felt that they had evidence for an increased nitrogen to sulfur ratio in the case of cows, as well as sheep and goats. Later work has confirmed this observation (71) and increases in the nitrogen to sulfur ratio of 3 to 10 times that of the pre-fasting days were reported, the greatest increase being in the case of sheep. Further, sheep on a phosphorus deficient diet (72)

showed an increased nitrogen to sulfur ratio which resulted from a decreased sulfur excretion. They suggest that this may show a possible utilization of sulfur for phosphorus, but they present no other experimental evidence. The problem of sulfur metabolism during inanition in rabbits has not been studied extensively and the results are not conclusive. Terroine and Razafimahery (73), (74) found that if the period of inanition was preceded by a period of privation of proteins, there was an increased nitrogen to sulfur ratio in the excreta during the early stages of inanition, but a large diminution just before death. Yamada* (75) fasted rabbits twelve days, analysing the urine every third day, and found a decrease in the sulfur excretion during the third and sixth days, but an increase on the ninth and twelfth days; however, he carried out no nitrogen determinations so it is difficult to compare his work with that of others. Wilson (76) believes a sulfur-containing fraction of the protein is the first to be synthesized and destroyed, which might explain a portion of the above findings, but not all of them. Wilson's experiments were carried out on humans.

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* The journal which contains this study was obtained from Chemical Abstracts, Columbus, Ohio, through the courtesy of Dr. E. J. Crane.

EXPERIMENTAL

Procedure

The sulfur compounds administered were thiourea, cystine, and S-carboxymethylcysteine.

Commercial thiourea (Eastman) required recrystallization from alcohol before a satisfactory analysis could be obtained.

Analysis	Found per cent	Theoretical per cent
Sulfur	41.79	42.02
Nitrogen	36.55	36.81

Melting Point 178-179° C. (corr.)

The cystine was prepared from hair and gave a satisfactory analysis and optical rotation.

Analysis	Found per cent	Theoretical per cent
Sulfur	26.66	26.67
Nitrogen	11.51	11.67

$$[\alpha]_D^{20^\circ} = -219.4^\circ$$

S-carboxymethylcysteine had been prepared by Dickens (77) through the action of cysteine hydrochloride, barium hydroxide, and monoiodoacetic acid, the barium being

removed as barium sulfate. Michaelis and Schubert (78) found that the reaction of cysteine hydrochloride and monochloroacetic acid would yield the same derivative and the isolation of the free acid could be accomplished by precipitation at its isoelectric point. A more direct method was found, however, in which cysteine hydrochloride, which is difficult to obtain in a pure state, is not involved. Such a synthesis has been accomplished through the action of sodium upon cystine in liquid ammonia (79) followed by condensation with monochloroacetic acid.

Anhydrous ammonia was passed through a liquifying column cooled by a bath of solid carbon dioxide and ether. The liquid ammonia was collected in a reaction flask fitted with a stirring mechanism and having two openings, one for the introduction of liquid ammonia and the other for the addition of sodium metal. The flask was surrounded by a Dewar flask. An approximately 5 per cent solution of cystine in liquid ammonia was prepared and sodium metal was added until a blue color persisted. Slightly more than the theoretical amount of redistilled monochloroacetic acid was then added slowly and after the reaction was complete the ammonia was allowed to evaporate at room temperature. The residue was dissolved in 25 cc. of water and the pH adjusted to neutrality to litmus with glacial acetic acid. The precipitate which formed upon standing in the ice chest

consisted for the most part of cystine and was removed by filtration. Concentrated hydrochloric acid was added to the filtrate until it was just acid to congo red paper and the solution was allowed to stand overnight in the ice chest. The crystalline precipitate which formed was filtered off, dissolved in the smallest amount of boiling water possible, and again set on ice. Six-sided plates separated out. The yield was 55 per cent of the theoretical.

The product described by Dickens had a melting point of 84° C. Michaelis and Shubert report 175-176° C. for their preparation, while the material prepared by this method has a melting point of 191-192° C. This was identical with a sample furnished by Dr. Erwin Brand.*

Analysis	Found per cent	Theoretical per cent
Sulfur	17.75	17.88
Nitrogen	7.78	7.83

Male rabbits of 2 to 4 kilograms body weight were kept in the usual metabolism cages and fed on one of three diets, depending upon the particular experiment. These diets were of three types: (1) commercial rabbit chow (Vitality Rabbit Chow), (2) cabbage, (3) cabbage and chow. The type and amount of diet were selected so that the

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* Appreciation is expressed to Dr. Erwin Brand who so generously furnished the carboxymethylcysteine used in the preliminary experiments.

rabbits would eat all of their food on the days when sulfur derivatives were administered. The urine was collected at regular intervals by applying pressure to the abdomen. Chloroform was used as a preservative for the urine samples. The sulfur compounds were administered orally by the use of a rubber catheter or were injected subcutaneously.

Thiourea was administered in aqueous solution; S-carboxymethylcysteine and cystine in aqueous solution as their sodium salts. Four to eight days were allowed between experiments in order to obtain satisfactory normal control values.

The total nitrogen of the urine was determined by the Kjeldahl method, the urea nitrogen by the method of Van Slyke and Cullen (80) using the glycerol extract of urease suggested by Koch (81), the creatinine by Folin's micro-method (82), the inorganic sulfate and total sulfate sulfur by the methods of Folin (4), and the total sulfur by the Denis modification (83) of Benedict's method (84). The ether-soluble sulfur was the total sulfur extractable from the urine by ether in a Griffith extractor (85). Disulfide linkages were determined by the colorimetric methods of Shinohara and Padis (86) and of Folin and Marenzi as modified by Rimington (87), the iodometric methods of Lavine (88), and of Virtue and Lewis (89), and the cuprous mercaptide

precipitation method of Medes and Padis (90). The Sullivan reaction (54), (91) was used for cystine determinations. The semi-micro method of Clark (92) was used for determining acetyl groups. The Goddard and Michaelis test was used to detect thioglycolic acid (93), and the nickel ethylenediamine nitrate was employed in the test for thiosulfates (94).

Results and Discussion

S-Carboxymethylcysteine

The distribution of the extra sulfur in the urine following administration of S-carboxymethylcysteine is summarized in Table I. The complete analyses are shown in Tables VIII, IX, X, and XI in the Appendix.

An examination of the sulfur partition in the urine after carboxymethylcysteine administration suggests a slight oxidation of the sulfur of this compound when it is ingested orally, but no oxidation after subcutaneous injection. This would indicate that the intestinal flora may have played a role similar to that found by Salkowski when taurine was fed to rabbits (95), (96).

Brand and coworkers (28) reported that a normal human oxidized the sulfur of carboxymethylcysteine after feeding to the extent of 40 per cent and cystinurics about 15 per cent.

Table I

Distribution of "Extra" Sulfur Excreted in Urine by the Rabbit in 24 Hour Period Immediately Following Administration of S-Carboxymethylcysteine.

The carboxymethylcysteine was administered in amounts equivalent to 400 mg. sulfur. Extra sulfur values are expressed as per cent of the total sulfur administered.
All experiments bearing the same letter were carried out with the same animal.

Exp. No.	Mode of administration	Extra sulfur excreted			"Cystine" Lavine	Ether sol. S acid urine	
		Total	Total sulfate	Organic			
		per cent	per cent	per cent	mg.	mg.	per cent
A-1	Orally	94	11	83	-	-	-
A-3	Orally	93	17	76	-	-	-
A-2	Inject	77	0	77	-	-	-
A-4	Inject	76	1	75	-	-	-
B-1	Orally	65	-5	70	161	-	-
B-3	Orally	76	11	65	159	-	-
B-2	Inject	60	0	60	71	-	-
B-4	Inject	64	-7	71	83	-	-
					"Cystine" Shinohara		
C-3	Orally	64	14	50	35.5	105	26
C-4	Inject	86	-3	89	2.5	49	12
C-8	Orally	-	-	-	-	67	17

Since they were unable to rule out the action of the intestinal flora, the oxidation may not have been carried out by the cells within the organism proper, but in the intestine by the action of microorganisms. It should also be pointed out that in man, taurine is not oxidized after oral ingestion (96) so the intestinal flora may not be as important in this species as in some animals. Another fact which brings out the difference in metabolism associated with the mode of administration is that no cyanide-nitroprusside reaction could be obtained in the urine when carboxymethylcysteine was injected in rabbits, nor did the Shinohara method show an increase when disulfide was determined quantitatively; however, the cyanide-nitroprusside reaction was strong and there was an appreciable amount of disulfide after oral administration. Pure S-carboxymethylcysteine did not give a cyanide-nitroprusside test or a color in the Shinohara method for cystine.

Brand reported that when the urine excreted after carboxymethylcysteine administration was reduced with zinc and hydrochloric acid, an ether-soluble fraction could be obtained which gave a strong nitroprusside reaction and a thioglycolic acid test. In the present series it was found that rabbit urine excreted after oral administration and treated in the same manner gave a strong nitroprusside reaction, but neither a positive thioglycolic acid test nor

a Sullivan reaction was obtained. Furthermore, Brand found no ether-soluble fraction in the urine of humans excreted after carboxymethylcysteine administration, but after oral administration to the rabbit, an ether-soluble fraction giving a strong cyanide-nitroprusside reaction, cuprous mercaptide precipitate and titration with iodine could be obtained, indicating that there must be a difference between the metabolism of carboxymethylcysteine in humans and rabbits. After parenteral administration to rabbits the urine contained an ether-soluble fraction, but it gave a negative cyanide-nitroprusside reaction. Brand postulates the formation of a disulfide between thioglycolic acid and cysteine, but did not isolate this compound. Tarver and Schmidt (97) suggested that carboxymethylcysteine might be excreted as the acetyl derivative in view of the discovery of Stekol (98) that S-benzylcysteine was acetylated before excretion. When the urine excreted after oral administration of carboxymethylcysteine was acidified and extracted with ether, the amount of acetyl groups present in this extract was not appreciably greater than the amount obtained from normal urine similarly treated.

In summary, it can be said that when S-carboxymethylcysteine was injected subcutaneously in rabbits, the sulfur portion of the molecule was not oxidized. There was a slight oxidation of the sulfur after oral administration,

but this can probably be attributed to the action of the intestinal flora. The manner in which this compound was administered was important with regard to its path in metabolism, as shown by the presence of a disulfide-like substance in the urine after oral ingestion but not after subcutaneous injection. These results fail to confirm the observations of Brand and associates in man.

Thiourea

Earliest studies which dealt with thiourea metabolism were carried out in an attempt to show whether oxidation of the sulfur of thiourea occurred, rendering it available as ethereal sulfates for detoxication purposes. The results obtained in these studies were interpreted as indicating an oxidation of the sulfur. An examination of the results given in Tables II, III, and IV in the text and in Tables XII, XIII, and XIV in the Appendix, shows that there was a decided decrease in the inorganic and total sulfate sulfur, regardless of the mode of administration or the nutritive state of the animal, indicating that there is probably no oxidation of the sulfur of thiourea. If thiourea were acting merely as a toxic substance, which was inhibiting general metabolism, one would expect to find a decrease in urea nitrogen excretion paralleling the decrease in sulfate sulfur. This was not the case as can be seen from the urea

Table II

A Typical Experiment to Show the Effect of Thiourea Administration on the Sulfur Partition in the Urine

Periods 1,3, and 5 represent the average normal excretion.

Experiment B-13 0.475 grams Thiourea (200 mg. S) Orally

Experiment B-14 0.475 grams Thiourea (200 mg. S) Injection

Period	Exp. No.	Urea nitrogen	Sulfur				Urea-N / Total SO_4-S	Urea-N / Inorganic SO_4-S
			Total	Sulfate				
				Total	Inorganic	Ethereal		
		mg.	mg.	mg.	mg.	mg.		
1		808	149	101	96	5	8.0	8.4
2	B-13	778	278	60	54	6	13.0	14.4
3		922	150	107	100	7	8.6	9.2
4	B-14	750	274	71	66	5	10.6	11.4
5		788	170	110	105	5	7.2	7.5

Table III

Distribution of "Extra" Sulfur, and Decrease in Oxidized Sulfur Excreted in the Urine by Rabbits Fed During the Period of Thiourea Administration

In experiments B-12, B-13, B-14, and D-4 thiourea equivalent to 200 mg. of sulfur was administered, and, in all other experiments, the equivalent of 400 mg. of sulfur was administered. Extra sulfur is expressed as per cent of total sulfur administered and decrease in total sulfate sulfur is calculated from the average daily excretion of sulfate sulfur and from the expected excretion of sulfate sulfur as calculated from the urea nitrogen to total sulfate sulfur ratio. All experiments bearing the same letter were carried out with the same animal.

Exp. No.	Mode of Admin.	Extra sulfur		Decrease in Total Sulfate S					
				Calc. from aver. daily excretion		Calc. from Urea-N / Total SO_4-S		Ether sol. sulfur	
		Total	Organic						
		per cent	per cent	mg.	per cent	mg.	per cent	mg.	per cent
B-5	Orally	70	83	50	37	-	-	-	-
B-6	Orally	62	79	70	42	-	-	275	69
B-7	Orally	68	83	66	40	48	33	270	67
B-10	Orally	47	78	124	75	116	74	215	54
B-12	Orally	58	82	48	47	37	41	67	34
B-13	Orally	65	86	42	41	30	33	77	39
C-6	Orally	50	83	132	71	-	-	-	-
D-1	Orally	77	83	26	16	-	-	-	-
B-8	Inject.	50	71	84	51	64	44	222	56
B-9	Inject.	50	75	110	67	84	60	231	58
B-14	Inject.	63	78	31	30	15	18	45	23
C-5	Inject.	83	97	55	29	-	-	-	-
C-7	Inject.	43	63	80	53	-	-	-	-
D-4	Inject.	33	75	83	64	61	56	103	52

Table IV

Distribution of "Extra" Sulfur and Decrease in Oxidized Sulfur Excreted in the Urine by Rabbits Fasted During the Period of Thiourea Administration

In experiment B-18 thiourea equivalent to 400 mg. of sulfur was administered. In all other experiments the equivalent of 200 mg. of sulfur was administered each day during two day periods. Extra sulfur excreted is expressed as per cent of total sulfur administered and decrease in sulfate sulfur is calculated from the average daily excretion of total sulfate sulfur and from the expected excretion as calculated from the urea nitrogen to total sulfate sulfur ratio. All experiments bearing the same letter were carried out with the same animal.

Exp. No.	Mode of Admin.	Extra sulfur		Decrease in Total Sulfate S					
				Calc. from aver. daily excretion		Calc. from Urea-N / Total SO_4-S		Ether sol. sulfur	
		Total	Organic						
		per cent	per cent	mg.	per cent	mg.	per cent	mg.	per cent
B-18	Orally	78	80	155	77	81	63	272	68
B-19	Orally	75	90	147	73	82	60	95	48
B-20	Orally	98	89	129	64	51	41	79	40
D-9	Orally	67	83	122	79	63	66	115	58
D-10	Orally	91	95	127	83	57	68	112	56
D-11	Inject.	67	85	125	81	64	69	93	47
D-12	Inject.	98	98	119	77	50	59	65	33

nitrogen excretion and the urea nitrogen to total sulfate sulfur ratios recorded in Table II and in the Appendix, or from the decreases in total sulfate sulfur shown in Table III. In Table III the decrease in sulfate sulfur is calculated from values for the average daily excretion of total sulfate sulfur and from the "predicted" total sulfate sulfur excretion. The predicted total sulfate sulfur excretion is the calculated total sulfate sulfur which would be excreted on experimental days, if the total sulfate sulfur and urea nitrogen were excreted in the same proportion regardless of the state of general metabolism.

If thiourea acted merely to reduce the general metabolism of the organism, the decrease in total sulfate sulfur should be proportional to the decrease in urea nitrogen and the ratio of urea nitrogen to total sulfate sulfur would remain constant. Thus, if we calculated the total sulfate sulfur excretion by dividing the urea nitrogen excretion on the experimental day by the average ratio of urea nitrogen to total sulfate sulfur during the normal periods, the value obtained should be the same as the value for total sulfate sulfur excretion determined experimentally. This was never found to be the case, although the values for decrease in total sulfate sulfur obtained in this manner were always lower than those calculated from the average daily excretion of total sulfate sulfur. There was some variation in the

ethereal sulfur excretion, but the values never varied more than one might expect from experimental error, when it is recalled that the value for ethereal sulfate must be obtained by difference from the determined values for inorganic and total sulfate sulfur.

Sato (48) reported one experiment which is comparable to experiments B-14 and D-4, in which thiourea was injected without feeding phenol. His conclusion, that oxidation took place, was based upon an increase of only 9 mg. in ethereal sulfate sulfur and a decrease in the ratio of the inorganic sulfate sulfur to ethereal sulfate sulfur. The inorganic sulfate sulfur excretion decreased 180 mg. in comparison to an increased excretion of ethereal sulfate sulfur of 9 mg. It is evident that the decrease in the ratio of inorganic sulfate sulfur to ethereal sulfate sulfur was much greater due to the decrease in inorganic sulfate sulfur, than to the increased excretion of ethereal sulfate sulfur which makes it difficult to understand the conclusion that oxidation took place. Masuda (50) studied this problem under conditions similar to Sato's, but the results were not uniform. The change in ethereal sulfate sulfur varied from 0 to +14 mg. per day and the inorganic sulfate sulfur excretion, from -78 to +13 mg., with three out of the four experiments showing a decrease in inorganic sulfate sulfur. Sato also used as a basis for his conclusion that thiourea

was oxidized, the change in the ratio of inorganic sulfate sulfur to ethereal sulfate sulfur, assuming that a decrease meant an increased ethereal sulfur excretion. As pointed out previously, a decrease in inorganic sulfate sulfur would cause a decrease in this ratio, so this is not a reliable index for oxidation.

When the methods which these workers used are considered, the small changes in ethereal sulfate sulfur observed lose their significance. The method of Salkowski (3) for ethereal sulfate sulfur requires precipitation of the inorganic sulfate sulfur, removal of this precipitate by filtration, hydrolysis of the filtrate and precipitation of the ethereal sulfate sulfur as barium sulfate. It was necessary to weight approximately 20 mg. of barium sulfate in most of the determinations these two workers reported, so there was undoubtedly some experimental error which may account for the increases in ethereal sulfate sulfur excretion after thiourea administration.¶ In some of the experiments with thiourea it was noted that after its administration the rabbits refused a portion of the chow of the diet; however, they always ate all of the cabbage. The question arose whether the decrease in sulfate sulfur was not the effect of fasting, since as will be discussed later, fasting causes a similar change in the sulfur distribution in the urine. In order to eliminate this factor, experiments were carried out

in which the diet consisted of cabbage alone. The animals ate all of this diet on experimental days and again a decrease in oxidized sulfur was observed. These experiments were B-12, B-13, B-14, and D-4. The animals did not eat all of the chow of the diet in experiments B-5, B-6, B-7, B-8, B-9, B-10, C-5, C-6, and C-7, so there is some question as to the absolute magnitude of the changes in urinary sulfur excretion.

The presence of thiourea in the urine after its administration could be demonstrated qualitatively. As an index to the quantitative excretion, the ether-soluble sulfur fraction in the urine was determined. Thiourea is decomposed in weak acid and alkali to give hydrogen sulfide, carbon dioxide, and an ammonium salt; in high concentrations of base there is formed ammonia and thiocyanate and sulfide salts (42). To prevent this, the extraction was carried out in neutral solution, but the recovery from known solutions was never over 85 per cent of that added and there was considerable variation between determinations on the same sample. Thus, the results must be considered as an approximate estimation of the ether-soluble fraction. When the amount of extra sulfur excreted as total sulfur was compared with the amount of sulfur in the ether-soluble fraction, it was found that these amounts were usually about the same.

In an attempt to determine the nature of the ether-soluble fraction, a 24 hour sample of urine collected after oral administration of one gram of thiourea was extracted with ether in a continuous extractor for about 16 hours; the ether was evaporated and about 300 mg. of a white solid remained which was dried over phosphorus pentoxide and re-crystallized several times from absolute alcohol. This material gave a Grote test, a precipitate with mercuric chloride, and when heated, gave a thiocyanate test with ferric chloride. The melting point was the same as for thiourea and there was no change in the melting point when the material was mixed with pure thiourea. Analysis for sulfur on two different products isolated gave 41.41 and 41.00 per cent sulfur as compared to the theoretical value of 42.02 per cent. This demonstrates that at least a portion of the thiourea administered must be excreted as such in the urine.

The only indication that there was an increase in the disulfide fraction after thiourea administration, as suggested by Medes (15), was a pink color which developed when the urine was allowed to stand several hours with sodium cyanide and sodium nitroprusside. Thiourea will give this same test due to its decomposition in alkaline solutions, as pointed out by Pavolini (99). A positive cystine test could not be obtained on the urine with either the regular Sullivan

reaction or the modification of Prunty which calls for reduction with zinc and hydrochloric acid. A precipitate could be obtained when the urine excreted after thiourea administration was treated with cuprous chloride as described in the procedure by Medes (90), but this precipitate contained no material which gave a Sullivan reaction. Thiourea is precipitated with cuprous chloride under these conditions, so the precipitate which formed in the urine was probably the cuprous mercaptide of thiourea. If the rabbit is capable of forming a disulfide between thiourea and cysteine similar to that reported by Medes (15) in humans, the feeding of thiourea with cystine might be expected to decrease the oxidation of the cystine by removing it in the urine as the cysteine complex.

$$(NH_2(NH=)C\text{-}S\text{-}S\text{-}CH_2CH(NH_2)COOH)$$

Thiourea was administered orally with cystine and the results obtained are given below. Extra sulfur excreted is expressed as the amount in excess of the normal excretion.

Sulfur administered as		Extra sulfur			
			Total		Ether
Thiourea	Cystine	Total	sulfate	Organic	soluble
mg.	mg.	mg.	mg.	mg.	mg.
0	200	110	82	28	--
200	200	251	76	173	107

Since it has been shown that the sulfur of thiourea was not oxidized by the rabbit, a consideration of the extra total sulfate sulfur excretion suggests that the sulfur of cystine was oxidized to about the same extent when it was fed with thiourea as when it was fed alone. However, it has been shown that thiourea depresses the normal oxidation of sulfur, so if this occurs when cystine and thiourea are fed together, the results could be interpreted as indicating a greater degree of oxidation of the sulfur of cystine when fed with thiourea than when fed alone. In any event, there was no indication that there was a decreased oxidation of the sulfur of cystine due to the presence of thiourea.

Two other possible paths for the metabolism of thiourea suggest themselves: the formation of thiocyanates and the formation of thiosulfates, but the increase in the amount of these ions on experimental days was negligible when compared to the tests on normal days.

A rabbit was fed thiourea and placed in a chamber through which air could be forced; this air was then passed through concentrated sulfuric acid in an attempt to trap the sulfide which Pohl (45) reported was present in the expired air of animals injected with thiourea. No precipitate could be detected in the sulfuric acid when a solution of iodine in potassium iodide was added. As a test for this method of detecting the sulfide, diethylsulfide was prepared

chemically (100) and when this compound was dissolved in concentrated sulfuric acid to which an iodine solution was added, a precipitate formed. The failure to secure positive results with the rabbit may have been due to the experimental set up. Pohl cannulated the trachea of the rabbit and thus there was not the large volume of air passing through the absorption bottles which was necessary when the rabbit was placed in the chamber.

Inanition

Since the change which was produced in the urinary excretion of nitrogen and sulfur when thiourea was administered was not unlike the change which might be expected during inanition, a study of urinary sulfur and nitrogen partition during inanition was thought advisable.

An examination of Table V shows that, in general, the effect of inanition on the urinary excretion of oxidized sulfur and urea nitrogen is very similar to the changes brought about by thiourea administration. These same changes are observed in the results given in Tables XII and XIV of the Appendix, with the exception of the values for creatinine, and neutral and ethereal sulfur. During fasting the creatinine value was always found to decrease, but this has been shown before in inanition experiments (101), (102). On the second day of the fast the ethereal sulfate sulfur tends to

Table V

Distribution of Sulfur Excreted in the Urine by Rabbits During Inanition

Experiments B-11 and B-17 were 24 hour fasts, while B-15 and B-16, D-2 and D-3, and D-7 and D-8 show the results obtained by analysing the urine each 24 hours during 48 hour fasts, B-16, D-3, and D-8 being the second day in each fast. Decrease in total sulfate sulfur is calculated from the average daily excretion of total sulfate sulfur and from the expected excretion as calculated from the urea nitrogen to total sulfate sulfur ratio. All experiments bearing the same letter were carried out with the same animal.

Exp. No.	Decrease in total sulfur		Decrease in total sulfate sulfur: Calc. from aver. daily excretion		Decrease in total sulfate sulfur: Calc. from Urea-N / Total SO_4-S	
	mg.	per cent	mg.	per cent	mg.	per cent
B-11	164	71	128	73	72	61
B-15	168	62	117	58	51	38
B-16	196	72	147	73	63	54
B-17	149	55	103	51	39	28
D-2	157	76	95	73	49	58
D-3	171	83	106	82	59	71
D-7	112	58	90	58	52	45
D-8	145	75	119	77	47	57

decrease, but Müller (103) showed in man, and Sherwin and Hawk (104) in dogs, that there was a decrease in indican in the urine, especially during prolonged fasting, so this probably accounts for the decrease in ethereal sulfate sulfur during fasting. The neutral sulfur excretion decreases markedly during inanition, which is of interest when compared with the results Folin reported after a study of metabolism at various levels of protein intake (5). When the diet was changed from a high to a low protein intake, the neutral sulfur excretion remained nearly constant, suggesting that there may be an entirely different process of sulfur oxidation in complete inantion, as compared to a condition when there is just a lowered food intake.

The most interesting change produced during inanition was in the ratios of urea nitrogen to total sulfate sulfur and to inorganic sulfate sulfur as shown in Table VI. The ratio of urea nitrogen to total sulfate sulfur was normally about 6.0 on a cabbage diet, but during the first day of inanition, it increased to 14 and on the second day of a consecutive fast it increased to 20. The urea nitrogen to inorganic sulfate sulfur ratio increased from about 6.5 to 17 the first day and to 26 the second day. In order to study the effect of diet, a diet of chow and cabbage was substituted for cabbage alone during the normal periods; these results are shown in Table VII. In this case the average increase

Table VI

Sulfur Partition in the Urine of the Rabbit During Inanition

During the normal periods the animal received a diet consisting of cabbage.

Date	Exp. No.	Urea nitrogen	Sulfur			Urea-N / Total SO_4-S	Urea-N / Inorganic SO_4-S
			Total	Sulfate			
				Total	Inorganic		
		mg.	mg.	mg.	mg.		
1939							
11/11-12		720	225	144	137	5.0	5.3
11/13		642	200	130	123	5.0	5.2
11/14	D-2	488	49	35	29	13.9	16.9
11/15	D-3	480	35	24	19	20.0	29.9
11/16-17		696	145	103	98	6.8	7.1
11/18-19		679	143	94	90	7.2	7.5

Table VII

Sulfur Partition in the Urine of the Rabbit During Inanition

During the normal periods the animals received a diet of chow and cabbage.

Date	Exp. No.	Urea nitrogen	Sulfur			Urea-N / Total SO_4-S	Urea-N / Inorganic SO_4-S
			Total	Sulfate			
				Total	Inorganic		
		mg.	mg.	mg.	mg.		
Rabbit B							
1939							
12/22		2202	226	193	183	11.4	12.0
12/23		2261	275	202	192	11.2	11.8
12/24-25		2375	269	203	189	11.7	12.5
12/26	B-15	1482	104	85	73	17.5	20.3
12/27	B-16	1281	76	55	49	23.3	26.2
12/28		2192	239	193	182	11.3	12.0
12/29-1/1/40		2205	259	198	184	11.1	12.0
1/2-3		2377	278	210	197	11.3	12.1
Rabbit D							
1940							
1/30-31		1800	181	141	131	12.8	13.7
2/1		1750	200	150	138	11.7	12.7
2/2		1924	192	144	133	13.4	14.5
2/3	D-7	1272	80	64	51	19.9	25.2
2/4	D-8	906	47	35	24	25.9	37.8
2/5		1535	189	137	129	11.2	11.9
2/6		1535	196	147	141	10.4	10.9
2/7-8		1721	195	150	137	11.5	12.6

of sulfate sulfur ratio was from 12 on normal days to 19 on the first day of inanition and to about 25 on the second day. Also, the urea nitrogen to inorganic sulfur ratios increased from a normal value of about 12 to 22 the first day, and to about 30 the second day. This demonstrates that the previous diet is of importance, but that it is not the primary cause for the change in the proportion of nitrogen to sulfur seen during inanition. Ratios of the magnitude obtained during fasting can be produced by feeding rabbits on a low sulfur diet such as sugar and milk, as is shown in a paper by Hill and Lewis (105).

The importance of the selection of a palatable diet in studies of sulfur metabolism is readily evident from the above results. The prediction of sulfur excretion, based upon nitrogen to sulfur ratios, is valid only when there is constant food intake. From experiments D-2, D-3, D-7, D-8, D-11, B-15, B-16, and B-17, shown in Tables VI, VII, and XII, it is definitely established that this ratio is not a constant during inantion. Under such conditions, calculations based upon a constant nitrogen to sulfur ratio might lead to conclusions which were far from what actually happened. From a consideration of experiment B-15, it may be seen that a compound fed at a level equivalent to 200 mg. of S might have been oxidized to the extent of 60 per cent, but if the animal refused to eat his food, the results might be

interpreted as indicative of no oxidation.

To further compare the effect of inanition and thiourea administration, thiourea was fed or injected into rabbits in the fasting condition. If experiment B-18, shown in Table IV, in which the fasted animal is given thiourea, is compared with B-16, shown in Table V, which is a comparable excretion during inanition, it can be seen that thiourea administration causes a larger decrease in oxidized sulfur than that found in simple fasting. Similarly, experiments B-19 and B-20 (Table IV), in which thiourea was administered, can be compared with the fasting levels of B-15 and B-16 (Table V), and also the same for D-9 and D-10 (Table IV) compared to D-7 and D-8 (Table V).

These results show that there is considerable similarity between the changes produced in the urinary sulfur and nitrogen partitions by inanition and by oral or parenteral administration of thiourea. The effects may be considered additive, since thiourea administered to a fasting animal produces a further lowering of the oxidized sulfur excretion in the urine.

SUMMARY AND CONCLUSIONS

1. Studies of the urinary partition of sulfur showed that the sulfur portion of S-carboxymethylcysteine was not oxidized in the organism of the rabbit when injected subcutaneously. There was a slight oxidation of the sulfur after oral administration, which was probably due to the action of the intestinal flora.

The manner in which this compound was administered was important with regard to its path in metabolism, as was shown by the presence of a substance in the urine excreted after oral administration, which gave a cyanide-nitroprusside test; no test could be obtained on the urine excreted after subcutaneous injection.

2. These results for S-carboxymethylcysteine with rabbits failed to confirm the observation made by Brand and associates, who observed some oxidation of the sulfur when this material was administered to normal and cystinuric men.

3. After oral or parenteral administration of thiourea to rabbits, there was no evidence from the urinary sulfur partition that the sulfur portion of the molecule was oxidized. The greater portion of the administered thiourea was probably excreted in the urine as such. This was shown

by isolation as well as by increases in the ether-soluble organic sulfur.

4. The presence of small amounts of cystine in the urine after thiourea administration as found by Medes in man could not be confirmed in the rabbit.

5. The administration of thiourea caused a decrease in oxidized sulfur, but no increase in ethereal sulfate sulfur excretion. This decrease in oxidized sulfur excretion is not believed to be due to a toxic effect, since the nitrogen metabolism was not proportionally changed as evidenced by the nitrogen to sulfur ratio.

6. Inanition in rabbits caused a decrease in the oxidized sulfur excretion without a proportional decrease in nitrogen excretion; a change similar to that found after thiourea administration. There was also a decrease in the excretion of neutral sulfur.

7. The oxidized sulfur excretion of the fasted animal was decreased still further by thiourea administration.

8. The importance of a constant food intake during sulfur metabolism studies is discussed.

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Food Chart

Experiment	Diet
A-1 through A-4	Chow
B-1 through B-11	Chow and Cabbage
B-12 through B-14	Cabbage
B-15 through B-20	Chow and Cabbage
C-1 through C-7	Chow and Cabbage
D-1	Chow and Cabbage
D-2 through D-6	Cabbage
D-7 through D-12	Chow and Cabbage

Table VIII

The Administration of S-Carboxymethylcysteine

Rabbit A. Initial weight 2.0 kg.

Date	Exp. No.	Total nitrogen	Creat-inine	Sulfur			N/S	N / Total SO_4-S
				Total	Total SO_4	Organic		
		mg.	mg.	mg.	mg.	mg.		
1937								
5/14		642	90	129	114	15	5.0	5.6
5/17-18		940	117	114	92	22	8.2	10.2
5/19-20		900	116	128	94	34	7.0	9.6
5/21		861	118	142	93	49	6.1	9.3
5/22	A-1	1137	146	502	138	364	2.3	8.2
5/23		966	129	154	101	53	6.3	9.6
5/24-25		877	120	123	90	33	7.1	9.7
5/26	A-2	1098	121	434	93	341	2.5	11.8
5/27		1042	154	189	93	96	5.5	11.2
5/28-29		1008	130	149	94	55	6.8	10.7
New food		-	-	-	-	-	-	-
5/30-31		982	131	103	62	41	9.5	15.8
6/1	A-3	1328	153	476	134	342	2.8	9.9
6/2		1106	130	102	63	39	10.8	17.5
6/8-9		1029	137	126	75	51	8.2	13.7
6/10-11		1129	129	98	66	32	11.5	17.1
6/12-13		1139	128	111	65	46	10.2	17.5
6/14	A-4	1472	134	408	70	338	3.6	21.0
6/15		1185	161	63	31	32	18.8	38.2
Died								

Key to Table VIII

All experiments bearing the same letter were carried out with the same animal.

Experiment No.	Sulfur administered as carboxymethylcysteine mg.	Mode of administration
A-1	400	orally
A-2	400	subcutaneous injection
A-3	400	orally
A-4	400	subcutaneous injection

Average normal values used in calculating extra sulfur elimination.

Experiment No.	Sulfur		
	Total mg.	Total sulfate mg.	Organic mg.
A-1 and A-2	128	94	34
A-3 and A-4	105	65	40

Recovery of sulfur calculated as per cent of sulfur administered.

Experiment No.	Sulfur		
	Total per cent	Total sulfate per cent	Organic per cent
A-1	94	11	83
A-2	77	0	77
A-3	93	17	76
A-4	76	1	75

Table IX

The Administration of S-Carboxymethylcysteine

Rabbit B. Initial weight 2.85 kg.

Date	Exp. No.	Total nitrogen	Creatinine	Sulfur			Cystine*	N/S	Total SO_4-S
				Total	Total sulfate	Organic			
		mg.	mg.	mg.	mg.	mg.	mg.		
1938									
10/24-5		1947	111	185	148	37	-	10.5	13.1
10/26-7		2165	156	232	180	52	-	9.4	12.1
10/28-9		2150	130	208	155	53	43	10.3	13.9
10/30-1		2183	143	228	177	51	34	9.6	12.4
11/1		2095	145	219	168	51	59	9.6	12.5
11/2	B-1	1434	125	496	157	339	206	2.9	9.1
11/3		1848	151	238	161	77	51	7.8	11.5
11/4-5		1883	151	246	191	55	41	7.7	9.9
11/6	B-2	1900	141	476	177	299	117	4.0	10.7
11/7		1929	152	248	189	59	51	7.8	10.2
11/8		1952	151	257	198	59	45	7.6	9.9
11/9		2011	150	282	212	70	32	7.1	9.5
11/10	B-3	1854	156	539	220	319	202	3.4	8.4
11/11		1975	150	276	203	73	39	7.2	9.7
11/12-3		1942	147	244	183	61	21	8.0	10.6
11/14		1875	150	225	167	58	17	8.3	11.2
11/15		2100	157	243	184	59	35	8.6	11.4
11/16		2000	151	240	182	58	34	8.3	11.0
11/17	B-4	1880	156	490	150	340	118	3.8	12.5
11/18		1911	150	239	163	76	43	8.0	11.7
11/19		1873	150	224	167	57	33	8.4	11.2

* Iodometric (Lavine (88))

Key to Table IX

All experiments bearing the same letter were carried out with the same animal.

Experiment No.	Sulfur administered as carboxymethylcysteine mg.	Mode of administration
B-1	400	orally
B-2	400	subcutaneous injection
B-3	400	orally
B-4	400	subcutaneous injection

Average normal values used in calculating extra sulfur elimination.

Experiment No.	Sulfur		
	Total mg.	Total sulfate mg.	Organic mg.
B-1, B-2, B-3, and B-4	236	178	58

Recovery of sulfur calculated as per cent of sulfur administered, and extra "cystine" calculated from iodometric titration (Lavine).

Experiment No.	Sulfur excreted as			Extra "cystine"
	Total per cent	Total sulfate per cent	Organic per cent	mg.
B-1	65	-5	70	161
B-2	60	0	60	71
B-3	76	11	65	159
B-4	64	-7	71	83

Table X

The Administration of S-Carboxymethylcysteine

Rabbit C. Initial weight 1.9 kg.

Date	Exp. No.	Total nitrogen	Cystine	
			Lavine	F.M.
		mg.	mg.	mg.
1938				
12/6		1968	32	19
12/7		1858	26	14
12/8		1865	24	14
12/9		1720	29	9
12/10		1790	27	11
12/11		1600	22	14
12/12	C-1	1338	25	88
12/13		2049	23	18
12/14-5		1673	22	21
12/16		1631	18	18
12/17		1639	20	17
12/18	C-2	1553	23	55
12/19		1553	21	16
12/20		1770	25	19

Key to Table X

All experiments bearing the same letter were carried out with the same animal.

Experiment C-1: 2.235 grams of carboxymethylcysteine (400 mg. S) were administered orally. Extra "cystine" by iodometric titration (Lavine (88)) was 74 mg. and by the colorimetric method (Folin Marenzi (87)) 0 mg.

Experiment C-2: 2.235 grams of carboxymethylcysteine (400 mg. S) were administered by subcutaneous injection. Extra "cystine" by iodometric titration (Lavine (88)) was 38 mg. and by the colorimetric method (Folin Marenzi (87)) 0 mg.

Table XI

The Administration of S-Carboxymethylcysteine

Rabbit C. Initial weight 3.15 kg.

Date	Exp. No.	Total nitrogen	Creatinine	Sulfur					Cystine*	N/S	N / Total SO_4-S
				Total	Total sulfate	Organic	Ether soluble Acid urine	Ether soluble Red. urine			
		mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.		
1939											
7/1		-	-	-	-	-	3	4	-	-	-
7/2	C-8	-	-	-	-	-	73	73	-	-	-
7/5		1632	172	171	135	36	-	-	4.5	9.5	12.1
7/6		1358	170	135	111	24	6	9	-	10.0	12.2
7/7		1496	186	158	135	23	-	-	-	9.5	11.1
7/8-9		1521	177	168	137	31	-	-	-	9.1	11.1
7/10		1470	169	172	139	33	-	-	6.9	8.6	10.6
7/11	C-3	1492	176	414	181	231	111	106	41.6	3.6	8.2
7/12		1338	177	196	148	48	22	21	5.1	6.8	9.0
7/13-4		1443	175	162	128	34	-	-	3.5	8.9	11.3
7/15-6		1663	174	198	158	40	-	-	-	8.4	10.5
7/17	C-4	1656	171	503	114	389	55	53	8.5	3.3	14.5
7/18 Died		1362	184	105	68	37	6	6.5	6.1	13.0	20.0

* Colorimetric (Shinohara (86))

Key to Table XI

All experiments bearing the same letter were carried out with the same animal.

Experiment No.	Sulfur administered as carboxymethylcysteine	Mode of administration
	mg.	
C-8	400	orally
C-3	400	orally
C-4	400	subcutaneous injection

Average normal values used in calculating extra sulfur elimination.

Experiment No.	Sulfur		
	Total	Total sulfate	Organic
	mg.	mg.	mg.
C-3, C-4	159	127	32

Recovery of sulfur calculated as per cent of sulfur administered.

Experiment No.	Sulfur excreted as		
	Total	Total sulfate	Organic
	per cent	per cent	per cent
C-3	64	14	50
C-4	86	-3	89

Table XII

The Effects of the Administration of Thiourea and Inanition

Rabbit B. Initial weight 3.2 kg.

Date	Exp. No.	Nitrogen				Sulfur							N	Urea-N	Urea-N
							Sulfate				Ether				
		Total	Urea	Urea of total	Creatinine	Total	Total	Inorganic	Ethereal	Organic	soluble	N/S	Total SO_4-S	Total SO_4-S	Inorganic SO_4-S
		mg.	mg.	per cent	mg.	mg.	mg.	mg.	mg.	mg.	mg.				
1938															
12/8		2434	-	-	197	289	222	-	-	67	-	8.4	11.0	-	-
12/9-12/10		1808	-	-	179	207	150	-	-	57	-	8.7	12.0	-	-
12/11		1630	-	-	178	202	142	-	-	60	-	8.1	11.5	-	-
12/12		1573	-	-	178	201	141	-	-	60	-	7.9	11.2	-	-
12/13-12/14		1513	-	-	173	180	128	-	-	52	-	8.4	11.8	-	-
12/15	B-5	1971	-	-	174	476	85	-	-	391	-	4.1	23.2	-	-
12/16		1525	-	-	164	176	99	-	-	77	-	8.7	15.4	-	-
12/17-12/18		1539	-	-	169	222	158	-	-	64	-	6.9	9.7	-	-
12/19		1479	-	-	166	183	131	-	-	52	-	8.1	11.3	-	-
1939															
4/22-4/23	*	1527	-	-	186	209	164	152	12	45	5.9	7.3	9.3	-	-
4/24		1498	-	-	187	199	161	151	10	38	5.8	7.5	9.3	-	-
4/25	B-6	1720	-	-	178	465	95	83	12	370	278	3.7	18.1	-	-
4/26		1520	-	-	194	211	154	146	8	57	27	7.2	9.9	-	-
5/7		2295	2020	87	-	288	210	190	20	78	-	8.0	10.9	9.6	10.6
5/8		2032	1784	87	-	232	184	166	18	48	-	8.7	11.0	9.7	10.7
5/9		2196	1929	87	-	263	214	198	16	49	-	8.3	10.3	9.0	9.8

Table XII - continued

5/10		2198	1891	86	181	246	196	182	14	50	5.3	8.9	11.2	9.7	10.4
5/11	B-7	2005	1470	83	181	485	99	85	14	386	275	4.1	20.3	14.9	17.3
5/12		1930	1672	87	184	216	150	139	11	66	24	8.9	12.9	11.1	12.0
5/15		2193	1907	87	184	244	199	186	13	45	-	9.0	11.0	9.6	10.2
5/16		2270	2007	88	182	242	196	183	13	46	-	9.4	11.6	10.2	11.0
5/17	B-8	1921	1452	84	182	416	81	73	8	335	228	4.6	23.7	18.0	20.0
5/18		1805	1571	87	187	202	139	129	10	63	20	8.9	13.0	11.3	12.2
5/22-5/23		2225	1949	88	187	224	172	161	11	52	-	9.9	12.9	11.3	12.1
5/24		2241	1948	87	188	238	190	179	11	48	-	9.4	11.8	10.3	10.9
5/25		2200	1922	87	189	252	202	191	11	50	-	8.7	10.9	9.5	10.1
5/26-5/28		2052	1798	87	187	246	195	183	12	51	-	8.4	10.5	9.2	9.8
5/29	B-9	1878	1386	83	183	419	55	48	7	354	236	4.5	34.2	25.2	28.8
5/30		1862	1579	85	188	248	166	156	10	82	16	7.5	11.2	9.5	10.1
5/31		1755	1521	87	183	223	178	166	12	45	-	7.9	9.9	8.6	9.2
6/1-6/2		1969	1714	87	184	247	193	182	11	54	-	8.0	10.2	8.9	9.4
6/3-6/4		2118	1840	87	187	229	178	168	10	51	-	9.2	11.9	10.3	10.9
6/5	B-10	2119	1571	82	193	404	41	34	7	363	215	5.2	51.5	38.4	46.2
6/6		1754	1495	85	192	214	135	124	11	79	36	8.2	13.0	11.1	12.1
6/7		1663	1453	87	186	193	151	141	10	42	-	8.6	11.0	9.6	10.3
8/4		2093	1761	84	198	224	172	-	-	52	-	9.3	12.3	11.2	-
8/5-8/6		2379	2032	85	204	240	179	165	14	61	-	9.9	13.3	11.3	12.3
8/7	B-11	1513	1339	89	200	68	47	34	13	21	-	22.2	32.2	28.5	39.4
8/8		2006	1720	84	202	194	147	136	11	47	-	10.6	14.0	11.7	12.6
10/3-10/4		1132	964	85	204	138	93	90	3	45	-	8.2	12.2	10.4	10.7

Table XII - continued

10/5-10/6	**	958	841	88	197	131	86	82	4	45	-	7.3	11.1	9.7	10.2
10/7-10/9		962	853	89	192	150	99	95	4	51	nil	6.4	9.7	8.6	9.0
10/10-10/11	B-12	1097	787	90	192	264	54	50	4	210	67	4.2	20.3	14.6	15.8
10/12-10/13		908	758	84	193	144	96	91	5	48	8	6.3	9.5	7.9	8.3
10/14-10/15		965	859	89	193	153	105	100	5	48	-	6.3	9.2	8.2	8.6
10/16-10/17	B-13	1079	778	91	168	278	60	54	6	218	77	3.9	18.0	13.0	14.4
10/18-10/19		1079	939	87	187	180	103	97	6	77	nil	6.0	10.5	9.1	9.7
10/24-10/25		1162	1028	89	172	165	123	114	9	42	-	7.0	9.4	8.4	9.0
10/26-10/27		900	800	89	162	135	96	91	5	49	-	6.7	9.4	8.3	8.8
10/28-10/29	B-14	1032	750	91	166	274	71	66	5	203	45	3.8	14.6	10.6	11.4
10/30-10/31		902	776	86	163	181	105	99	6	76	-	5.0	8.6	7.4	7.9
11/1-11/2		895	801	89	162	170	114	110	4	56	-	5.3	7.8	7.0	7.3
12/22		2538	2202	87	205	266	193	183	10	73	-	9.5	13.1	11.4	12.0
12/23	***	2598	2261	87	214	275	202	192	10	73	-	9.4	12.8	11.2	11.8
12/24-12/25		2680	2375	88	214	269	203	189	14	66	-	10.0	13.2	11.7	12.5
12/26	B-15	1759	1482	84	195	104	85	73	12	19	-	16.9	20.7	17.5	20.3
12/27	B-16	1490	1281	86	197	76	55	49	6	21	-	19.6	27.1	23.3	26.2
12/28		2497	2192	88	211	239	193	182	11	46	-	10.4	12.9	11.3	12.0

Table XII - concluded

12/29-1/1/40		2544	2205	87	213	259	198	184	14	61	-	9.8	12.9	11.1	12.0
1/2-1/3		2763	2377	86	215	278	210	197	13	68	-	10.0	13.2	11.3	12.1
1/4	B-17	1709	1507	88	191	123	99	83	16	24	-	13.9	17.3	15.2	18.2
1/5	B-18	1870	1398	87	190	387	47	36	11	340	272	4.8	39.7	29.8	38.8
1/6-1/7		2170	1856	86	203	254	173	163	10	81	-	8.6	12.5	10.7	11.4
1/8-1/9		2401	2110	88	209	271	201	189	12	70	-	8.9	12.0	10.5	11.1
1/21-1/22		2490	2185	88	222	298	220	203	17	78	-	8.4	11.3	10.0	10.8
1/23-1/24		2637	2301	87	222	274	206	192	14	68	-	9.6	12.8	11.1	12.0
1/25	B-19	1821	1490	82	200	254	55	41	14	199	95	7.2	33.1	27.1	36.4
1/26	B-20	1658	1353	82	192	272	73	62	11	199	79	6.1	22.7	18.6	21.8
1/27-1/28		2197	1898	87	214	267	197	185	12	70	-	8.2	11.1	9.6	10.3
1/29		2455	2145	87	217	249	189	175	14	60	-	9.8	13.0	11.4	12.3

* Weight 3.65 Kg.
** Weight 3.75 Kg.
*** Weight 3.95 Kg.



Key to Table XII

All experiments bearing the same letter were carried out with the same animal.

Experiment No.	Sulfur administered as thiourea mg.	Mode of administration
B-5	400	orally
B-6	400	orally
B-7	400	orally
B-8	400	subcutaneous injection
B-9	400	subcutaneous injection
B-10	400	orally
B-11	---	fasted
B-12	200 each day for two days	orally
B-13	200 each day for two days	orally
B-14	200 each day for two days	orally
B-15	---	fasted
B-16	---	fasted
B-17	---	fasted
B-18	400	orally
B-19	200	orally during fast
B-20	200	orally during fast

Average normal values used in calculating sulfur elimination during thiourea administration.

Experiment No.	Sulfur			Urea-N
	Total	Total sulfate	Organic	Total SO_4-S
	mg.	mg.	mg.	
B-5	196	135	61	-
B-6 through B-10	218	165	53	10.0
B-12, B-13, and B-14	149	102	47	8.7
B-18 and B-20	76	202	21	10.9
B-19	104	202	19	10.9

Recovery of sulfur calculated as per cent of sulfur administered; decrease in total sulfate sulfur calculated from the average daily excretion of sulfur and from the expected excretion of sulfur as calculated from the average Urea-N/Total SO_4-S ratio.

Exp. No.	Sulfur excreted as		Decrease in total sulfate sulfur	
	Total	Organic	Calc. from aver. daily excretion	Calc. from Urea-N Total SO_4-S
	per cent	per cent	mg.	mg.
B-5	70	83	50	-
B-6	62	79	70	-
B-7	68	83	66	48
B-8	50	71	84	64
B-9	50	75	110	84
B-10	47	78	124	116
B-12	58	82	48	37
B-13	65	86	42	30
B-14	63	78	31	15
B-18	78	80	155	81
B-19	75	90	147	82
B-20	98	89	129	51

Average normal values used in calculated sulfur elimination during inanition.

Experiment No.	Sulfur		Urea-N
	Total	Total sulfate	Total SO_4-S
	mg.	mg.	
B-11	232	175	11.3
B-15, B-16, and B-17	272	202	10.9

Decrease in total and oxidized sulfur during inanition as calculated from the average daily excretion and the Urea-N/Total SO_4-S ratio.

Experiment No.	Decrease in total sulfur	Decrease in sulfate sulfur	
		Aver. daily excretion	Urea-N / Total SO_4-S
	mg.	mg.	mg.
B-11	164	128	72
B-15	168	117	51
B-16	196	147	63
B-17	149	103	39

Table XIII

The Administration of Thiourea

Rabbit C. Initial weight 2.95 kg.

Date	Exp. No.	Total nitrogen	Creat-inine	Sulfur			N/S	N / Total SO_4-S
				Total	Total sulfate	Organic		
		mg.	mg.	mg.	mg.	mg.		
1939								
2/28-3/1		2000	155	269	197	72	7.4	10.2
3/6		2065	159	262	195	67	7.9	10.6
3/13		1710	159	247	190	57	6.9	9.0
3/14-15		1750	158	254	194	60	6.9	9.0
3/16-17		1829	158	276	212	64	6.6	8.3
3/18-19		1832	154	286	221	65	6.4	8.3
3/20	C-5	1919	148	505	130	375	3.8	14.8
3/21		1912	157	322	185	137	5.9	10.3
3/22-23		1698	158	256	189	67	6.6	9.0
3/25		1653	157	246	177	69	6.7	9.4
3/26-27		1646	157	250	183	67	6.6	9.0
3/28	C-6	1556	155	373	62	311	4.2	25.5
3/29		1854	160	321	176	145	5.8	10.5
3/30-31		1522	154	206	153	53	7.4	10.1
4/1-2		1575	155	193	144	49	8.2	10.9
4/5		1735	157	223	161	62	7.8	10.8
4/6	C-7	1390	151	332	56	276	4.2	24.8
4/7		1681	150	256	168	88	6.6	10.0

Key to Table XIII

All experiments bearing the same letter were carried out with the same animal.

Experiment No.	Sulfur administered as thiourea	Mode of administration
	mg.	
C-5	400	subcutaneous injection
C-6	400	orally
C-7	400	subcutaneous injection

Average normal values used in calculating sulfur elimination.

Experiment No.	Sulfur		
	Total	Sulfate sulfur	Organic
	mg.	mg.	mg.
C-5 and C-6	248	185	63
C-7	208	152	56

Recovery of sulfur calculated as per cent of sulfur administered; decrease in total sulfate sulfur calculated from the average daily excretion of sulfur.

Experiment No.	Sulfur excreted as		Decrease in total sulfate sulfur
	Total	Organic	
	per cent	per cent	mg.
C-5 1st day	64	78	55
2nd day	83	97	55
C-6 1st day	31	62	123
2nd day	50	83	132
C-7 1st day	31	55	96
2nd day	43	63	80

Table XIV

The Effects of the Administration of Thiourea and Inanition

Rabbit D. Initial weight 1.8 kg.

Date	Exp. No.	Nitrogen				Sulfur							N / SO_4-S	Urea-N / SO_4-S	Urea-N Inorganic / SO_4-S
							Sulfate				Ether soluble				
		Total	Urea	Urea of total	Creatinine	Total	Total	Inorganic	Ethereal	Organic		N/S			
		mg.	mg.	per cent	mg.	mg.	mg.	mg.	mg.	mg.	mg.				
1938															
11/21		2084	-	-	89	236	178	-	-	58	-	8.9	11.7	-	-
11/22		1953	-	-	88	226	171	-	-	55	-	8.7	11.4	-	-
11/23-11/24		2068	-	-	89	214	164	-	-	50	-	9.7	12.6	-	-
11/25		1967	-	-	85	215	165	-	-	50	-	9.1	11.9	-	-
11/26-11/27		1468	-	-	84	203	150	-	-	53	-	7.3	9.8	-	-
11/28		1502	-	-	84	205	151	-	-	54	-	7.3	10.0	-	-
11/29	D-1	1848	-	-	83	525	138	-	-	387	-	3.5	13.4	-	-
11/30		1306	-	-	84	193	141	-	-	52	-	6.8	9.3	-	-
12/1-12/2		1543	-	-	84	226	170	-	-	56	-	6.8	9.1	-	-
1939															
11/11-11/12	*	830	720	87	162	225	144	137	7	81	-	3.7	5.8	5.0	5.3
11/13		734	642	88	159	200	130	123	7	70	-	3.7	5.7	5.0	5.2
11/14	D-2	579	488	84	156	49	35	29	7	14	-	11.8	16.5	13.9	16.9
11/15	D-3	572	480	84	147	35	24	19	6	11	-	16.4	23.8	20.0	25.9
11/16-11/17		772	696	90	148	145	103	98	5	42	-	5.3	7.5	6.8	7.1

Table XIV - continued

11/18-11/19		772	679	88	152	143	94	90	4	49	nil	5.4	8.2	7.2	7.5
11/20-11/21	D-4	886	628	88	141	272	47	42	5	225	103	3.3	18.9	13.4	15.0
11/22-11/23		914	805	88	150	165	99	93	6	66	nil	5.5	9.2	8.1	8.7
11/24-11/25		848	753	88	154	187	124	120	4	63	-	4.5	6.8	6.0	6.3
11/26-11/27		823	718	87	158	202	130	125	5	72	-	4.1	6.3	5.5	5.8
12/3-12/4		851	758	89	154	198	130	121	9	68	-	4.3	6.6	5.8	6.3
12/5-12/6		864	784	91	155	167	108	100	8	59	-	5.2	8.0	7.3	7.8
12/7-12/8	D-5	845	770	91	157	315	212	208	4	103	-	2.7	4.0	3.6	3.7
12/9-12/10		889	791	89	153	218	132	127	5	86	-	4.1	6.7	6.0	6.2
12/11-12/12		875	798	91	150	239	141	135	6	98	-	3.7	6.2	5.7	5.9
12/13	D-6	982	756	89	151	457	206	199	7	251	107	2.2	4.9	3.7	3.8
12/14-12/15		742	667	90	154	204	118	112	6	86	-	3.6	6.3	5.7	6.0
12/16-12/17		904	831	92	150	222	141	137	7	81	-	4.1	6.4	5.9	6.1
1940															
1/30-1/31		2019	1800	89	175	181	141	131	10	40	-	11.1	14.3	12.8	13.7
2/1		1962	1750	89	165	200	150	138	12	50	-	9.8	13.1	11.7	12.7

Table XIV - concluded

2/2		2116	1924	91	165	192	144	133	11	48	-	11.0	14.7	13.4	14.5
2/3	D-7	1422	1272	89	156	80	64	51	13	16	-	17.8	22.2	19.9	25.2
2/4	D-8	1041	906	87	157	47	35	24	11	12	-	22.2	29.8	25.9	37.8
2/5		1668	1535	92	173	189	137	129	8	52	-	8.8	12.2	11.2	11.9
2/6		1708	1536	90	175	196	147	141	6	49	-	8.7	11.6	10.4	10.9
2/7-2/8		1941	1721	89	172	195	150	137	13	45	-	10.0	12.8	11.5	12.6
2/9	D-9	1349	1043	77	154	214	32	21	11	182	115	6.3	42.1	32.6	49.7
2/10	D-10	1193	920	77	152	228	27	18	9	201	112	5.2	44.2	34.0	51.1
2/11		1759	1541	88	167	228	142	134	8	86	-	7.7	12.4	10.9	11.5
2/12-2/13		1788	1600	90	167	205	155	147	8	50	-	8.7	11.5	10.3	10.9
2/14-2/15		1819	1632	90	171	220	165	154	11	55	-	8.3	11.0	9.9	10.6
2/16-2/18		1888	1708	90	170	216	161	152	9	55	-	8.7	11.7	10.6	11.2
2/19	D-11	1271	1019	80	152	214	29	18	11	185	93	5.9	43.9	35.2	56.7
2/20	D-12	1181	929	79	148	243	35	26	9	208	65	4.9	33.8	26.5	35.7
2/21		1825	1593	87	171	247	160	150	10	87	-	7.4	11.4	10.0	10.6
2/22-2/23		1822	1600	88	173	228	164	150	14	64	-	8.0	11.1	9.8	10.7
2/24-2/25		1846	1660	90	170	218	168	157	11	50	-	8.5	11.0	9.9	10.6

* Weight 3.15 kg.

Key to Table XIV

All experiments bearing the same letter were carried out with the same animal.

Experiment No.	Sulfur administered as thiourea mg.	Mode of administration
D-1	400	orally
D-2	---	fasted
D-3	---	fasted
D-4	200 each day for two days	subcutaneous injection
D-5	Cystine (200 mg.S)	orally
D-6	Cystine (200 mg. S) Thiourea (200 mg. S)	orally
D-7	---	fasted
D-8	---	fasted
D-9	200	orally during fast
D-10	200	orally during fast
D-11	200	subcutaneous injection during fast
D-12	200	subcutaneous injection during fast

Average normal values used in calculating sulfur elimination.

Experiment No.	Sulfur			Urea-N
	Total	Total sulfate	Organic	Total sulfate-S
	mg.	mg.	mg.	
D-1	218	164	54	-
D-2 through D-6	206	130	76	5.8
D-7 and D-8	192	154	-	11.0
D-9 and D-11	80	154	16	11.0
D-10 and D-12	47	154	12	11.0

Recovery of sulfur calculated as per cent of the sulfur administered; decrease in total sulfate sulfur calculated from the average daily excretion of sulfur and from the expected excretion of sulfate sulfur as calculated from the average Urea-N/Total SO_4-S ratio.

Experiment No.	Sulfur excreted as			Decrease in total SO_4-S	
	Total	Total sulfate	Organic	Calc. from aver. daily excretion	Calc. from Urea-N Total SO_4-S
	per cent	per cent	per cent	mg.	mg.
D-1	77	--	83	26	-
D-4	33	--	75	83	61
D-5	55	41	14	-	-
D-6 (calc. for cystine-S)	63	38	25	-	-
(calc. for total S admin.)	63	19	44	-	-
D-9	67	--	83	122	63
D-10	91	--	95	127	57
D-11	67	--	85	125	64
D-12	98	--	98	119	50

Decrease in total and oxidized sulfur during inanition as calculated from the average daily excretion and Urea-N/Total SO_4-S ratio.

Experiment No.	Decrease in total sulfur	Decrease in sulfate sulfur	
		Calc. from aver. daily excretion	Calc. from Urea-N / Total SO_4-S
	mg.	mg.	mg.
D-2	157	95	49
D-3	171	106	59
D-7	112	90	52
D-8	145	119	47