CANINE CYSTINURIA. THE EFFECT OF FEEDING CYSTINE, CYSTEINE, AND METHIONINE AT DIFFERENT DIETARY PROTEIN LEVELS*

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The question whether cystinuria, long under study in man, also affects animals was answered affirmatively in 1935 by Morris, Green, Denkel, and Brand (1) who reported the removal of a cystine stone from an Irish terrier. Shortly thereafter the son of a male litter mate of the same dog was found to be cystinuric (2). By breeding the younger cystinuric dog with related females (non-cystinuric) Brand *et al.* (3–5) obtained more than 300 descendants of which twelve were definitely cystinuric, all males. Through the courtesy of Dr. Brand two of these cystinuric Irish terriers, designated as Dogs 32-T and 38-U, were placed at our disposal.

As reported by Brand, Cahill, and Kassell (6) the reaction of the fresh urine of these dogs was negative by the Sullivan direct method. After the urine had been aged for several days, the Sullivan reaction became positive. Brand *et al.* (2) believe that the reaction is negative owing to the presence in the urine of an unknown reducing substance which interferes with the color development. If the cystine in the urine was precipitated with cuprous chloride according to the method of Rossouw and Wilken-Jorden (7) and the copper removed from the washed precipitate by hydrogen sulfide, the filtrate, after concentration, contained free cystine determinable by the Sullivan method. The question as to why the Sullivan method is negative in the freshly voided urine will be considered in detail in a later paper. However, we have evidence (Howard and Sullivan (8)) that certain cysteine complexes of the thiazolidine type which are negative in the Sullivan reaction are quantitatively opened by precipitation with cuprous chloride and then react positively.

It is the conclusion of all workers that in human cystinuria the urinary cystine increases with increased protein intake. Brand, Cahill, and Harris (9) have postulated that the error of metabolism in the case of cystinuria is concerned with the ingested methionine and cysteine and not with the ingested cystine. Lewis, Brown, and White (10) also found that both methionine and cysteine stimulated the excretion of cystine in a human cystinuric but to a lesser degree than found by Brand *et al.* (9) and re-

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ported that the stimulation was more marked upon a lower protein diet than upon a high protein diet. Hess and Sullivan (11) in a study of two cases of human cystinuria found a stimulation of cystine excretion following the ingestion of both methionine and cysteine with one cystinuric but not with the other.

In their early study of canine cystinuria Brand, Cahill, and Kassell (6) confined their investigation to the determination of cystine, creatinine, total nitrogen, and sulfur distribution. Later (5) Kassell, Brand, and Cahill reported that with increasing protein (casein) intake the cystine excretion increases. In our investigation we have studied not only the effect of various levels of dietary protein upon the cystine excretion in two cystinuric dogs but also the effect of the administration of methionine, cysteine hydrochloride, and cystine upon the cystine excretion at the various levels of protein intake.

EXPERIMENTAL

The diet was that recommended by Dr. Brand and had the following percentage composition: casein[1] 25, sucrose 45, salt mixture[2] 1.5, bone ash[3] 3.5, yeast[4] 5, and lard 20. Daily, 5 cc. of cod liver oil were added to the diet of each dog. When the level of casein in the diet was changed, the sucrose was varied inversely. The diet was prepared fresh once a week. The amino acids fed, *l*-cystine, *l*-methionine, and cysteine hydrochloride, were analytically pure. The cysteine hydrochloride was prepared from the same lot of cystine as was used in the feeding experiments. The *l*-methionine was isolated from casein. The weighed amount of each amino acid was intimately mixed with the diet each day. Each dog was fed approximately 150 gm. of diet per day.

As communicated to us by Dr. Brand in an extension of the data of Brand *et al.* (6) Dog 4 was bred with a non-cystinuric female, Dog 2, to give a cystinuric male, Dog 4-19, and a non-cystinuric female, Dog 4-22. Dog 4 bred with female Dog 3 gave female Dog 9-31. The latter bred with Dog 4-19 gave Dog 32-T. Dog 4-22 bred with Dog 4-19 gave female Dog 17-A which bred to Dog 4-19 gave the cystinuric Dog 38-U. Dog 32-T was born April 25, 1939, and Dog 38-U was born May 11, 1939. The dogs were kept in separate metabolism cages. The urine, collected daily, was

[1] Casein Company of America, edible casein No. 453 (sulfate-free).

[2] The salt mixture prepared was as follows: ferric citrate 9 gm., potassium iodide 2.5 gm., potassium chloride 35 gm., calcium phosphate ($CaHPO_4 \cdot 2H_2O$) 39 gm., monobasic potassium phosphate 61 gm., magnesium citrate 163 gm., sodium chloride 190 gm. To this mixture are added 100 to 200 mg. each of copper tartrate, cobalt chloride, manganese phosphate, and zinc carbonate.

[3] Eimer and Amend, fine, Cupel, Michigan (sulfate 0.24 per cent).

[4] Fleischmann's dried yeast, 7.56 per cent N, 0.37 per cent S.

analyzed immediately for cystine, total nitrogen, total sulfur, total sulfate sulfur, and inorganic sulfate sulfur. Dog 32-T weighed 7 kilos and Dog 38-U weighed 9 kilos.

TABLE I

Average Daily Urinary Excretions during Control Periods on Various Protein Levels

Urine	Dog No.	Protein level			
		5 per cent	10 per cent	25 per cent	50 per cent
		gm.	*gm.*	*gm.*	*gm.*
Cystine	32-T	0.008	0.039	0.082	0.144
	38-U	0.008	0.042	0.082	0.144
Nitrogen	32-T	0.96	1.98	5.16	9.33
	38-U	0.95	2.04	5.21	9.39
Total S	32-T	0.096	0.138	0.249	0.462
	38-U	0.095	0.147	0.247	0.482
Neutral S	32-T	0.050	0.061	0.111	0.196
	38-U	0.045	0.066	0.119	0.233

TABLE II

Total Urinary Excretion for 4 Day Period during Ingestion of Amino Acids upon Various Protein Levels

Urine	Dog No.	Protein level									
		5 per cent	10 per cent	25 per cent	50 per cent	5 per cent	10 per cent	25 per cent	50 per cent	5 per cent	25 per cent
		2.0 gm. methionine				2.3 gm. cysteine hydrochloride				2 gm. cystine	
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Cystine	32-T	0.104	0.289	0.293	0.537	0.084	0.233	0.323	0.545	0.033	0.335
	38-U	0.137	0.355	0.405	0.560	0.092	0.381	0.370	0.518	0.038	0.333
Nitrogen	32-T	4.22	8.44	24.0	39.7	4.24	8.78	23.7	41.5	3.83	22.2
	38-U	4.05	8.41	23.2	39.9	4.51	9.40	23.3	43.3	3.94	21.4
Total S	32-T	0.627	0.776	1.166	2.253	0.819	1.068	1.558	2.193	0.807	1.406
	38-U	0.626	0.799	1.187	2.416	0.851	1.023	1.559	2.180	0.881	1.263
Neutral S	32-T	0.284	0.328	0.497	0.891	0.427	0.373	0.667	1.119	0.371	0.615
	38-U	0.319	0.291	0.576	0.883	0.381	0.339	0.626	1.073	0.367	0.590

The urine as collected contained no cystine sediment and, in fact, gave no reaction for free cystine by the Sullivan method and recourse was had to precipitation with cuprous chloride. An aliquot of the urine (25 cc. for the lower casein levels and 10 cc. for the 50 per cent level) was precipitated with cuprous chloride (6), the copper was removed from the complex with hydrogen sulfide, and the filtrate was concentrated to the original volume of the aliquot of the urine. During the concentration the cysteine was oxidized to cystine and in the Sullivan method cystine was employed as the standard.

The casein in the diet was varied in content from 5 to 50 per cent in four different levels, 5, 10, 25, and 50 per cent. Table I gives the average daily excretion of the principal substances determined in the urines of both dogs. The average value in each case is based upon a considerable number of determinations made both before and after the feeding of the various amino acids. Since the amount of diet furnished to each dog and eaten was the same each day, the values upon which the averages are based are, in all cases, close together.

Table II presents the data on the effect of feeding methionine, cysteine hydrochloride, and cystine on the same urinary constituents. Of the amount of each amino acid indicated, one-half was fed daily for 2 successive days and the urine was collected for these 2 days and also for the next 2 days to insure complete elimination of any cystine produced by the ingestion of the compound administered.

DISCUSSION

The two dogs do not differ greatly in their response to the different levels of casein in the diet. The greatest variation is in the neutral sulfur output on the 50 per cent casein diet (Table I). The effect of the different protein levels in the diet upon the excretion of urinary cystine is marked. In agreement with the findings of Brand *et al.* (5) there is a sharp increase in urinary cystine following each increase in the level of dietary protein. It may be noted that the increase in cystine output is proportionally greater than the increase in protein intake, especially upon the lower levels of protein. The excretion of nitrogen increases directly as the dietary protein is increased but such a proportionality does not occur in the neutral sulfur output.

The ingestion of methionine and cysteine hydrochloride by the dogs causes the excretion of far more extra cystine upon the 5 and the 10 per cent casein levels than upon the 25 and the 50 per cent levels. In fact on the higher levels of protein intake these amino acids cause little if any increase in cystine excretion over that on the basal diet. The actual increase in cystine output following the administration of any amino acid can be calculated by subtracting from the amount excreted in the 4 day period, as given in Table II, 4 times the daily average for the corresponding casein level as given in Table I. The results are given in Table III together with the actual percentage increase of the cystine excreted over the control period for each amino acid fed. In general Dog 38-U gives a more marked response to the amino acid fed than does Dog 32-T, particularly upon the lower casein levels. However, the trend with both dogs is the same.

Both the methionine and the cysteine hydrochloride exert their most marked effect upon the cystine excretion when the casein is fed at a 5 per cent level. Increasing the casein level to 10 per cent and feeding either methionine or cysteine hydrochloride produces an increased output of cystine but the increase is far less than on the 5 per cent casein level. When the casein level is further increased to 25 or 50 per cent, the increase or decrease in extra cystine excreted is well within the experimental error of the methods. Whether the absolute amount of cystine excreted is considered or the percentage increase over the normal, it is evident that the two lower levels of dietary casein permit methionine and cysteine hydrochloride to bring about the excretion of extra cystine in the urine, while the two higher levels do not. This finding with cystinuric dogs is in agreement with the finding of Lewis, Brown, and White (10) with human cystinuria that the excretion of cystine is stimulated by methionine and cysteine more on a higher protein intake than on a lower. In no case did the feeding of cystine have any influence upon the cystine output.

TABLE III

Urinary Excretion of Extra Cystine Following Ingestion of Amino Acids (4 Day Period)

Protein level	Dog No.	Methionine	Cysteine	Cystine
per cent		*gm.*	*gm.*	*gm.*
5	32-T	0.072 (225)*	0.052 (163)*	0.001 (3)*
	38-U	0.105 (328)	0.060 (188)	0.006 (19)
10	32-T	0.133 (85)	0.077 (49)	
	38-U	0.187 (111)	0.213 (126)	
25	32-T	−0.035 (−11)	−0.005 (−2)	0.011 (3)
	38-U	0.077 (23)	0.042 (13)	0.009 (2)
50	32-T	−0.039 (−6)	−0.031 (−5)	
	38-U	−0.016 (−3)	−0.058 (−10)	

* The figures in parentheses are the percentage increase in cystine excreted above the control.

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