✦ ✦ ✦ ✦

CYSTINE CONTENT OF SHEEP WOOL AS AFFECTED BY THE PROTEIN CONTENT OF THEIR DIET

Janet E. Long, V. G. Heller and A. E. Darlow
Oklahoma A. and M. College

INTRODUCTION

Cystine has been shown by Freudman to be di- B- thio alpha mino propionic acid. As early as 1907, Hans Buchtala (1) obtained cystine from different horn substances in a percentage of 14 from human hair and 2 from pigs hoofs. In 1907 Aberhalden and Voitinovici (2) hydrolized keratin from horn and wool with acid and analyzed solutions, obtaining the amino acids. Again in 1911 Buchtala (3) determined the percentage cystine of keratin from various hairs and hoofs of animals by calculating the amount of sulphur obtained from the cystine precipitate. Thus, early workers have shown the presence of a sulphur-containing amino acid, cystine, in the epidermal tissues of animals; tissues such as hair, horns and claws. Since cystine seems to be such a prominent amino acid in the animal body and is important in the growth of the animal, there is a possibility that the percentage of cystine in the diet if increased would affect the growth of hair.

The metabolism of sulphur in the body has been studied in various animals and the relationship between cystine content of diet and maintenance of the growth of the animal has been investigated. Lewis (4), investigating the relationship between the cystine content of diet and efficiency in the maintenance of nitrogenous equilibrium in dogs, has studied the nutritional value of casein, a protein low in cystine content, and of serum albumin, a protein high in cystine content. He has shown that serum albumin under conditions of low protein intake is more effective in maintaining nitrogenous equilibrium in dogs than is casein; but if casein is supplemented by cystine in the diet, it is just as efficient. Cystine is shown here to be essential for maintenance as well as for growth of the animal.

Lightbody and Lewis (5) presented evidence to show that the cystine and sulphur content of the hair of the young white rat varied with the cystine content of the diet of the animals.

Beadles, Braman, and Mitchell (6) have shown that the addition of cystine to a diet, whose protein content is so low as to be a limiting factor in growth and which is deficient in this amino acid, increases its value in promotion of hair growth in the albino rat.

Smuts, Mitchell, and Hamilton (7), in working on the relation between dietary cystine and the growth and cystine content of hair in the rat,

conclude that an addition of cystine to a cystine deficient diet corrects its deleterious effects on body growth, hair growth, and hair composition.

The variability of sulphur content of rabbit wool from type to type has been established by King (8) and by Barritt and King (9).

Up-to-date, specific work on the exact relationship of protein diet of sheep and the cystine content of wool has not been done. Sulphur has been shown to be present in wool. Rimington (10) examined wools differing in sulphur content from 3.34 to 4.08 per cent. He found that fine and coarse samples of Turkey Mohair are all capable of yielding their entire sulphur as cystine when hydrolyzed by acids. He found no evidence of any other sulphur containing compound entering into their constitution. Thus, in the study of the relation between protein diet and sulphur in the wool of sheep, practically all the sulphur may be considered to be present in the form of cystine. The problem then is to determine the percentage of cystine in samples of wool from sheep fed on differing diets.

Early workers determined the sulphur content of the wool and from that calculated the percentage of cystine. This was a long and laborious process. Cystine itself was actually isolated and determined as in Aberhalden's and Voitinovici's method. A quick, accurate and simple method of determination was deemed advisable. Colorimetric methods were therefore resorted to in order to obtain this desired end.

Max Sullivan (11) in 1926 and later in 1929 has given us a distinctive test for cystine and cystine determination based on the Beta-Napthoquinone Reaction. It is shown that cystine reacts with 1.2 naptho quinone-4- sodium sulphonate in an alkaline reducing atmosphere to give a red color which is so specific for cystine that it requires three free groups, SH, NH_2, and COOH for a positive outcome. The test is applied to estimations of cystine which on reduction give cystine. By the use of sodium cyanide as a reducing agent to convert, it was found possible to determine cystine quantitatively in mixtures containing other amino acids and thiocompounds provided the standard was cystine similarly treated.

Folin and Looney (12) also give us a colorimetric method for the estimation of cystine in proteins. This method is based upon the development of a blue color of reduced uric acid reagent; but it is said not to be specific. Folin and Marenzi (13) give a modified method for determining cystine based on the Folin and Looney method. In our investigation the Sullivan method proved most satisfactory.

EXPERIMENTAL

The sheep used in this study were those obtained from a ranch in West Texas. They were sheared in November of 1930, and these samples, delivered in October, have been designated as Series A.

Diets of varying high and low protein content were then fed designated sheep, starting December 1930. The samples of wool sheared from these sheep in April 1931 were referred to as Series B; and samples sheared from them in December 1931 after a longer period on the same diets were labelled Series C.

The sheep were divided into six lots, as described by Darlow (14), each lot being fed an assigned diet over the entire period. The corn and prairie hay used in the rations were finely ground, and two pounds of the mixture were fed per day in order to insure the same nutritive ratio over the entire period.

In the fall of the year 1930, wool samples, obtained from the sheep directly off the range before treatment with any prescribed diet, were analyzed for cystine. The cystine values of this series, previously designated as Series A, were determined by the Sullivan method.

Each sample of 10 to 15 grams was placed in a 250 cc. Erlenmeyer flask and covered with absolute alcohol. The dehydrated wool, after being wrung out and partially dried, was placed in a Soxhlet extractor and extracted with absolute ether for 48 hours. The defatted wool was then thoroughly washed with 95 per cent alcohol, transferred to a large Erlenmeyer flask and shaken with fifteen changes of tap water. This procedure frees the wool from the bulk of the adherent dirt particles. The remainder of the insoluble materials was then separated by combing the fibres. The sample was then returned to the flask and allowed to stand over night in 500 cc. of 0.01N HCl. This latter procedure insures a constant salt formation with the protein and removes practically completely the alkaline metals, most of the potassium and sodium, which are firmly bound to the fiber. This equilibrium procedure insures a constant sulphur percentage of the samples of wool from the same fleece. The sample was then washed with several changes of distilled water, allowing it to stand for an hour or so in each washing. It was then dried as much as possible in an oven at 100° C., then washed in absolute alcohol and completely dried for 96 hours at 100° C., then placed in air tight sample tubes and kept in a dessicator until needed for analysis. Analyses of the samples were carried out according to the method outlined by Max Sullivan (11).

Broadly speaking, the test depends upon the theory that cystine reacts with 1.2 naptho quinone-4- sodium sulphonate and alkali to give a red color which is not discharged by reducing agents, such as anhydrous hyposulfite ($Na_2S_2O_4$).

Samples of sheep wool known as Series B, obtained from sheep which had been placed on diets of varying high or low content for 130 days, were next analyzed. Cystine determinations were made using the above described Sullivan method and also a second method outlined by Folin and Looney.

The following tables sum up the percentage cystine of the samples of Series B as determined by both the Sullivan method and the Folin and Looney method.

PERCENTAGES OF CYSTINE

Sample No.	Sheep No.	Sullivan Method	Folin & Looney	Coeff
1	126	10.0246	10.9831	.95
2	127	9.2870	11.9113	.77
3	128	10.6076	10.0785	1.05
4	129	10.8231	10.6404	1.01
5	130	9.9631	11.0091	.90
6	131	9.4704	12.6401	.75
7	132	12.3279	11.2676	1.09
8	133	10.7865	11.7995	.91
9	135	10.8966	9.8000	1.11
11	137	11.5805	12.5984	.91
12	138	9.4511	10.4366	.905
13	139	11.2948	11.0616	1.02
14	140	11.1914	10.8602	1.03
15	141	12.1701	11.3141	1.07
16	142	11.5169	12.1212	.95
17	144	12.1701	11.3141	1.07
18	145	9.5683	10.0032	.95
19	146	11.6090	14.2514	.84
20	147	11.2009	11.9354	.94
21	148	10.0156	11.2939	.89
22	149	10.0313	11.2481	.89
23	150	10.9729	10.9826	.95
24	151	11.8251	12.6315	.93

Upon comparison of the results listed in the above table it would appear that there exists no proportionality in the percentages obtained by the two methods. The results obtained by the Folin and Looney method

are neither consistently higher or lower than those obtained by the Sullivan method. However, new trials checked those of the Sullivan method.

In the fall of 1931 a new series, C, of wool samples were furnished us. These samples were from sheep which had been on the prescribed diet for twelve months. The cystine determinations were made by the Sullivan method.

The following table summarizes the percentages of cystine in the three series:

SUMMARY OF PERCENTAGES OF CYSTINE OF THE THREE SERIES

Diet	Sheep No.	Series A	Series B	Series C
	127	11.1200	9.2870	
Normal	135	12.0000	10.8966	12.1827
	138	10.4347	9.4511	
	150		10.9729	12.3175
Average		11.1849	10.1519	12.2501
	144	9.6000	12.1700	14.3758
High Protein	129	8.7388	10.8231	
Linseed Oil Meal	126	8.6642	10.0246	
	180	12.8000	11.1321	11.8811
Average		9.9508	11.0374	13.1284
	147	10.6666	11.2009	14.6790
Cottonseed	137	10.0292	11.4805	
Meal	140	13.1883	11.1914	11.7073
	132	12.1855	12.3279	
Average		11.5172	11.5502	13.1932
	148	9.8000	10.0156	13.5237
Gluten	124		9.6930	10.1933
	131	11.1111	9.4704	12.3116
	146	11.5161	11.6090	
Average		10.8091	10.1970	12.0095
	128	8.9727	10.6076	8.8888
High	152			
Carbohydrate	151		11.8751	11.6788
	141	11.4285	12.1701	
Average		10.2006	11.5509	10.2838
	145 Half Fed	12.4332	9.5683	10.6202
Normal	136 Series C			
	149 Half Fed	12.8345	10.0313	11.8252
	130 Series B	10.6664	0.9631	
Average		11.9780	9.8542	11.2227
	142 Half Fed	8.4809	11.5169	9.7567
High	143 Series C		9.6684	
Carbohydrate	133 Half Fed	11.5394	10.7865	8.6331
	139 Series B	12.8342	11.2948	
Average		10.9515	10.8166	9.1949

Since no comparative results have been obtained when determining cystine in samples of Series B by the Folin and Looney method, it was thought well here to run parallel determinations on some samples of Series B, using both the Sullivan and the improved Folin and Marenzi methods. A comparison of the cystine determinations as given by the Sullivan method and by the Folin and Marenzi method is shown in the following table:

PERCENTAGES OF CYSTINE

Sheep No.	Sample No.	Sullivan Method	Folin & Marenzi Method
128	3	9.0745	12.4353
129	4	9.5301	12.6635
142	16	9.8891	10.4567
126	1	8.6886	10.4575
128	3	8.9220	12.9165
147	20	11.9305	12.3449

From a comparison of these values it would seem that the Folin and

Marenzi method gives from 2 to 3 per cent higher values for cystine than is obtained by the Sullivan method.

CONCLUSIONS

1. The Folin and Looney method of cystine determination gives no comparable results with the Sullivan method.
2. The Folin and Marenzi method of cystine determination gives values from 2 to 3 per cent higher than those obtained by the Sullivan method.
3. The analysis would indicate that cystine, like most of the constituents of the body, is not easily changed within all normal limits of feeding.

LITERATURE CITED

1. Buchtala, Hans. Cystine in Different Horn Substances. Z. Physiol. Chem., 52:424-81 (1907).
2. Aberhalden, Emil, and Voitinovici, Arthur. Cystine. Z. Physiol. Chem., 52:348-67 (1907).
3. Buchtala, Hans. Cystine. Z. Physiol. Chem., 69:310-12 (1911).
4. Lewis, Howard B. The Relation Between the Cystine Content of Proteins and Their Efficiency in the Maintenance of Nitrogenous Equilibrium in Dogs. J. Biol. Chem., 42:289-96 (1920).
5. Lightbody, Howard D., and Lewis, Howard B. The Relation of Protein and Cystine Content of the Diet to the Growth of the Hair in the White Rat. J. Biol. Chem., 82:485-97 (1929).
6. Beadles, Jessie P., Braman, Winfield, and Mitchell, H. H. Relation Between Cystine Deficiency in Diet and Growth of Hair in White Rat. Biochem. J., 88:623-7 (1930).
7. Smuts, P. B., Mitchell, H. H., and Hamilton, A. A. The Relation Between Dietary Cystine and the Growth and Cystine Content of Hair in the Rat. J. Biol. Chem., 95:283-95 (1932).
8. King, A. T. Some Chemical Aspects of Wool Research. J. Textile Industry, 1:361-8T.
9. Barritt, J., King, A. T., and Richard, J. N. The Effect of Cystine on the Keratin Composition in Rabbit Wool. Biochem. J., 24:1061 (1930).
10. Rimington, Claude. The Relation Between Cystine and Total Sulphur in Various Animal Hairs. Biochem. J., 23:726-29 (1929).
11. Sullivan, Max R. Studies on the Biochemistry of Sulphur. II. Further Studies on the Distinctive Reaction for Cysteine and Cystine. Treas. Dept. U. S. Public Health Service Reprint No. 1291 from Public Health Reports, 44:1421-28 (1929).
12. Folin, Otto, and Looney, Joseph M. Colorimetric Methods for the Separate Determination of Tyrosine, Tryptophane, Cystine in Proteins. J. Biol. Chem. 51:421-54 (1922).
13. Folin, Otto, and Marenzi, A. D. An Improved Colorimetric Method for the Determination of Cystine in Proteins. J. Biol. Chem., 83:103-109 (1929).
14. Darlow, A. E., Felton, W., and Heller, V. G. The Effect of Wool Production of Fine Wool Wethers. Proc. Southern Agricultural Workers, 1932.

✦ ✦ ✦ ✦