CXX. THE RELATION BETWEEN TOTAL SULPHUR AND CYSTINE CONTENT OF CERTAIN SAMPLES OF RABBIT FUR.

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IN a recent study of the effect of diets supplemented with cystine upon the composition of the keratin of rabbit fur, large variations, which it was concluded were seasonal, were found to occur in the total sulphur content of the fur of both control and experimental animals [Barritt, King and Pickard, 1930].

It was considered to be of interest to ascertain whether these increased sulphur values were represented wholly by cystine, since it has been shown [Rimington, 1929, 1, 2] that almost the whole of the sulphur in wool and various animal hairs can be accounted for in this form.

The results indicate that within slight limits, possibly of experimental error, the sulphur in all the samples examined can be accounted for in this form.

Some observations made upon the technique of colorimetric cystine determination emphasise, as detailed below, the instability of this amino-acid and the necessity of observing certain experimental precautions if low values are to be avoided.

EXPERIMENTAL.

The samples of rabbit fur were obtained from a number of Angora rabbits fed on a normal diet to which various additions of cystine were made during an interval of 84 days [Barritt, King and Pickard, 1930]. The material was sampled and purified as previously described [Barritt and King, 1926, 1929].

Approximately 1 g. of fur was hydrolysed with 25 cc. of 20 % hydrochloric acid for 5½ hours. During the initial stages of hydrolysis there was a tendency for violent bumping to occur. Any slight loss of material was not, however, of serious moment since only comparative values were required of total sulphur by the modified Benedict-Denis method, and of cystine by the Folin-Marenzi [1929] method, in the hydrolysate. The values for total sulphur in the samples were obtained by the Carius method [Barritt, King and Pickard, 1930].

Two 5 cc. samples of the hydrolysate were taken for total sulphur determination as described by Rimington [1930, 1]. For determination of cystine a 5 cc. sample of the hydrolysate was diluted to approximately 42 cc. and 5 cc. of 20 % sodium hydroxide were added slowly with constant shaking, this being sufficient to reduce the acidity to approximately $N/10$. The mixture was made up to 50 cc., filtered and 2 cc. portions of this solution taken for the colorimetric procedure using urea to prevent turbidity [Rimington, 1930, 2].

The detailed results of Table I show that the increased total sulphur of the second clip can be accounted for by the higher cystine content of these furs.

Table I.

		Total sulphur (Carius)	Cystine in 5 cc. of hydrolysate (Folin-Marenzi) (mg.)	Cystine in 5 cc. of hydrolysate calculated from determination of total sulphur (Benedict- Denis) (mg.)	Differ- ence (mg.)	Differ- ence (%)
Controls	Rabbit					
	103 B	5.10	32.5	32.8	-0.3	-1.0
	103 A	5.05	—	36.3	—	—
	243 B	4.64	33.7	35.0	-1.3	-3.7
	243 A	4.98	37.4	36.7	+0.7	+1.9
	302 B	4.12	30.7	30.7	0	0
	302 A	4.79	41.1	41.75	-0.65	-1.6
0.1 g. cystine per day	63 B	4.93	47.1	46.0	+1.1	+2.4
	63 A	5.11	39.7	39.6	+0.1	+0.25
	235 B	4.77	32.3	32.2	+0.1	+0.3
	235 A	4.91	36.4	36.5	-0.1	-0.3
0.2 g. cystine per day	193 B	4.92	38.7	38.9	-0.2	-0.5
	193 A	5.02	41.1	41.8	-0.7	-1.7
	238 B	4.85	35.1	35.4	-0.3	-0.85
	238 A	5.05	37.4	36.4	+1.0	+2.7
	253 B	4.57	36.7	37.2	-0.5	-1.3
	253 A	4.89	37.6	38.1	-0.5	-1.3

Samples marked B were clipped on April 18th, 1929.

Samples marked A were clipped on July 11th, 1929.

The effect of concentration during the neutralisation of the hydrolysate.

It was found advisable to adhere carefully to the above procedure for the colorimetric determinations since, otherwise, anomalous results were obtained. In particular it is essential that the hydrolysate should be largely diluted before addition of the alkali since neutralisation of a concentrated solution, even if carried out with every precaution against rise of temperature, is liable to lead to very appreciable destruction of cystine. In a particular example a 10 cc. sample of the undiluted hydrolysate was treated with 5 cc. of 40 % sodium hydroxide, added drop by drop, the vessel being immersed in cold water and kept continuously agitated. The mixture was filtered and of the almost colourless filtrate 5 cc. were taken for a determination of total sulphur

whilst another 5 cc. were diluted to 50 cc. and used for the colorimetric determination.

The results obtained were as follows:

Total sulphur (Benedict-Denis) equivalent to 19.2 mg. cystine/5 cc. filtrate.

Cystine sulphur (Folin-Marenzi) equivalent to 13.1 mg. cystine/5 cc. filtrate.

Or expressed as cystine in the original hydrolysate:

Total sulphur (Benedict-Denis) equivalent to 28.8 mg. cystine/5 cc. hydrolysate.

Cystine sulphur (Folin-Marenzi) equivalent to 19.6 mg. cystine/5 cc. hydrolysate.

When the hydrolysate was diluted in the normal way, as described above, the following values were obtained which show good agreement between total sulphur and cystine sulphur:

Total sulphur (Benedict-Denis) equivalent to 28.5 mg. cystine/5 cc. hydrolysate.

Cystine sulphur (Folin-Marenzi) equivalent to 28.6 mg. cystine/5 cc. hydrolysate.

It would appear that concentrated sodium hydroxide acting upon the undiluted hydrolysate causes a very appreciable destruction of cystine.

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