I. THE DISTRIBUTION AND ORIGIN OF SULPHUR IN WOOL.

I. METHIONINE IN WOOL.

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(Received November 9th, 1933.)

THE distribution of sulphur in protein has long been a subject of interest, and the work of Osborne and numerous other investigators on the sulphur in vegetable proteins indicated the probable presence of at least two forms of organic sulphur; further, Dakin [1920] in his amino-acid analysis of gelatin found sulphur compounds other than cystine. The isolation of methionine [Mueller, 1921] and its synthesis [Barger and Coyne, 1928] clearly established the existence of at least one other sulphur-containing amino-acid. Pirie [1932] has isolated methionine from caseinogen in 1 % yield, corresponding to approximately 50 % of the total sulphur; generally it may be said that this amino-acid appears to enter into the composition of proteins to a considerable degree and may have far-reaching effects in nutritional work.

Scleroproteins, particularly wool and hair, contain a large proportion of cystine which accounts for as much as 70 % of the total sulphur [Barritt, 1927].

Subsequently Marston [1928], Rimington [1929, 1, 2] and Barritt and Rimington [1931], using colorimetric methods devised by Sullivan [1926] and Folin and Marenzi [1929], were able to show that substantially all the sulphur in wool and hair could be accounted for as cystine, though it must be noted that 0.2 % of methionine has been isolated from wool [Mueller, 1923].

The possible non-specificity of the Folin-Marenzi reagent for cystine has been discussed, and the recent experiments of Jones and Gersdorff [1933] on the rate of liberation of amino-acids during the hydrolysis of caseinogen are of interest in that they indicate sources of possible error in the determination. A rapid initial increase in the depth of colour developed, the maximum being reached after 45 minutes corresponding to 0.53 % cystine, then an abrupt drop occurred and finally a gradual falling away to a constant value of 0.33 % after 18 hours. Using the Sullivan [1926] reagent, the cystine rose steadily to a constant value of 0.33 % after 6 hours. It appears that a reasonable time for the hydrolysis is necessary when using the Folin-Marenzi technique.

Isolation methods for the quantitative estimation of methionine are difficult and much loss of material occurs.

Baernstein [1932, 1] has developed a technique for the estimation of methionine in proteins based on methods described by Kirpal and Buhn [1915] and Pollack and Spitzer [1922] for the determination of methylthiol groups. In this method methyl iodide liberated by digesting the protein with hydriodic acid is estimated by passing into alcoholic silver nitrate and determining the silver iodide. Baernstein assumes that this volatile iodide arises solely from the methylthiol group of the methionine, the assumption being supported by the

facts that no methoxyl or *N*-methyl group has been shown to occur in proteins, no other compound containing a methylthiol or similar group has been isolated and lastly that the amino-acids other than methionine do not liberate volatile iodide when treated with hydriodic acid. Such determinations together with those of sulphhydryl and disulphide sulphur have enabled Baernstein [1932, 2] to account for the total sulphur in a large number of proteins, though keratins were not studied. In many instances the methionine content is high and with caseinogen methionine-sulphur accounts for 84 % of the total sulphur. The validity of the assumption that methionine is the sole source of volatile iodide may be questionable, but such determinations are not without significance in that they appear to give at least an upper limit for methionine content.

A series of determinations of methionine on various types of wool was made, a detailed account of the modifications found necessary being given below.

Table I.

	Quality*	Total sulphur on dry wt. %	Methionine on dry wt. %	% of total sulphur as methionine- sulphur
Welsh Mountain S. 64	58s	3.97†	0.44	2.4
Welsh Mountain Kemp	Very coarse	3.29	0.64	4.1
Romney Corriedale	36s	2.96†	0.66	4.8
Camel Hair	80s	3.37	0.67	4.3
Scotch Blackface (fine)	56s	3.73	0.57	3.2
Scotch Blackface (coarse)	Very coarse	3.10	0.51	3.4
Alpaca	60s	3.68	0.54	3.2
Mohair	36s	3.20	0.63	4.3
Lincoln	36s	3.46	0.50	3.0
Western Australian	70s	3.73	0.55	3.2
New Zealand	36s	3.40	0.50	3.2

* The qualities of the samples were determined by comparison with standard wools, and the author is indebted to Miss A. L. Walker for these determinations. Qualities of 80s, 70s, 60s, 56s and 36s correspond to diameters of 1.92, 2.07, 2.24, 2.83 and 3.90×10^{-3} cm. respectively.

† Indicates sulphur by the Carius method, others by the Benedict-Denis.

EXPERIMENTAL.

Preparation of samples. The wool samples were removed from the fleece in locks, which were opened out and degreased by washing in three baths of benzene at 60°. Small particles of foreign matter were removed by hand-sorting and the wools given a final treatment with benzene. The samples were dried at room temperature and well washed in six changes of distilled water, air-dried, and allowed to attain a uniform moisture content. Samples for methionine, sulphur and moisture content determinations were weighed off. The dry weights of individual samples were obtained by reference to determinations of moisture content made on samples taken at the beginning and end of the sampling. The moisture determination on the reference sample was carried out by passing a stream of dry air through the wool contained in a special bottle, maintained at 106° in an electrically heated oven, the procedure being fully described in an earlier paper [Barritt and King, 1926].

Determination of methionine. The technique employed was substantially that due to Baernstein [1932, 1] though with wool minor modifications were necessary.

Using a single washing-tube containing cadmium sulphate and a suspension of red phosphorus, it was found that a little hydrogen sulphide escaped and passed over into the alcoholic silver nitrate. This difficulty probably arises because of the very high cystine content of the wool-keratin and was overcome

by inserting a small tube containing glass wool or paper pulp moistened with cadmium sulphate solution between the washing-tube and the first silver nitrate tube. As a further check on the efficiency of the removal of hydrogen sulphide, a cadmium sulphate test-paper was inserted immediately before the first silver nitrate tube; if any signs of yellowing of the paper occurred, the experiment was abandoned.

In order to prevent the boiling hydriodic acid sucking up the side-tube and into the sulphuric acid wash-bottle, a bulb 2–3 cm. in diameter was blown on the side-tube; it was found to be quite effective. The duration of the run was 7–8 hours, this being sufficient in the case of wool-keratin.

Alcoholic silver nitrate (10 ml. of the order of $N/10$) was distributed between the two absorption-tubes. At the end of the run the tubes were washed out into a small beaker and the liquid evaporated on the steam-bath to about 10 ml. and made up to 50 ml. The contents of the flask were filtered through a dry paper and 20 ml. aliquots titrated with potassium thiocyanate (order of $N/50$), 0.5 ml. ferric alum indicator being added. The amount of volatile iodide was readily obtained from these data.

The blank on the apparatus was calculated from the results of eight determinations on each wool, four using 0.5 g. and four using 1 g. samples, these being taken alternately. A typical run is shown in detail as follows:

Welsh Mountain Wool. Sheep S. 64.

No. of exp. ...	1	2	3	4	5	6	7	8
Wt. of wool taken (g.)	0.5867	1.0658	0.5154	0.9535	0.5342	0.9467	0.4770	1.0640
ml. of 0.01933 KCNS equivalent to AgI	1.40	1.91	1.28	1.97	1.21	1.76	1.15	1.99

Moisture: 0.9698 g. of conditioned wool on drying out lost 0.1194 g.

Combining the above results, the blank on the run corresponds to 0.55 ml. and the methionine content of the wool to 1.35 ml. KCNS per g., giving 0.44 % methionine on the dry weight of the wool.

The value of the blank so obtained was found to agree well with values obtained when no wool was added. Although the blank is high in relation to the amount of KCNS corresponding to the methionine it is felt that the method employed is satisfactory in determining with reasonable accuracy the methyl iodide produced from wool-keratin.

Determination of sulphur content of wool. This was determined either by the Carius method using samples of 0.5 g. [Barritt and King, 1926] or by the method of Benedict [1909] and Denis [1910].

Using the latter method a sample of 0.8 to 1 g. was hydrolysed for 2 to 4 hours with 20 ml. 6*N* hydrochloric acid, the hydrolysate being made up to 50 ml. Aliquots of 20 ml. were taken and the determination then proceeded with as described by Rimington [1930]. This method is somewhat inaccurate owing to the fact that on hydrolysis, particularly in the early stages, small quantities of hydrogen sulphide are liberated. It is hoped to deal more fully with the determination of total sulphur in keratins in a separate paper, but in view of the work of Gortner [1922], which shows that no appreciable decomposition of cystine occurs during the time necessary for a protein hydrolysis, it is suggested that this liberated hydrogen sulphide arises from the methionine or some unknown sulphur compound of wool-keratin.

DISCUSSION.

The results given in the above table for a fairly representative series of wools do not justify any rigorous correlation between the methionine content and the type of wool, as was possible with the total sulphur in wools, where it was shown [Barritt and King, 1926] that total sulphur is higher in the finer wools grown under similar conditions. The sample of camel hair is of interest in that only 94 % of its total sulphur could be accounted for as cystine-sulphur [Rimington, 1929, 2] but its methionine content is not abnormally high.

The methionine content of the wools examined lies between 0.44 and 0.67 % of the dry weight, the methionine-sulphur varying from 2.4 to 4.8 % of the total sulphur. This quantity of methionine-sulphur accounts for about 3-4 % of the total sulphur in wool and does not seriously affect the previous conclusion that sulphur in wool occurs almost wholly in the form of cystine, though the possibility of undiscovered sulphur-containing amino-acids must not be overlooked. A possible explanation for the somewhat high values recorded for cystine-sulphur in keratin using the Folin-Marenzi technique is afforded by the work of Butz and du Vigneaud [1932] on the hydrolysis of methionine. On hydrolysis with acids, especially sulphuric acid, methionine gives homocystine, the next higher homologue of cystine, which is reactive to the Folin-Marenzi reagent; thus it appears possible that some methionine-sulphur may have been returned as cystine-sulphur using the colorimetric method of Folin-Marenzi.

The actual synthesis of wool-protein by the sheep is somewhat outside the scope of the present work, but it is interesting to note that the form in which the sulphur is obtained has been the subject of much speculation. Many calculations have been made of the necessary cystine content of herbage [Rimington and Bekker, 1932; Henrici, 1932; King, 1933; Woodman and Evans, 1932; Robertson, 1928] based on the assumption that cystine as such is essential for ultimate wool synthesis. These calculated values vary widely, some falling within, others outside the values for cystine in grasses as determined by Aitken [1930] and Evans [1931].

In determining cystine values obtained on a grass hydrolysate, the probability of loss of cystine due to interaction with carbohydrate material must be considered, and recently Lugg [1933] has shown that considerable loss of cystine occurs when it is hydrolysed by hydrochloric acid in the presence of sucrose and glycine. Preliminary determinations (by the author) of cystine in straw and potatoes show very high values, using the Folin-Marenzi technique, but only small amounts with the Sullivan reagent, illustrating the difficulties which occur when cystine is directly determined in complex materials.

Assuming a deficiency of cystine in the herbage, Rimington and Bekker [1932] have suggested that inorganic sulphur is elaborated by micro-organisms of the intestinal tract into bacterial protein which is then absorbed and is available for cystine synthesis, some support being given to this hypothesis by the work of Voltz [1920].

The total organic sulphur in herbage [Aitken, 1930; Evans, 1931] is however well above the sulphur requirements of wool production, but its actual distribution is as yet undetermined, though the work of Miller and Chibnall [1932] suggests the presence of methionine. In view of the possible replacement of cystine by methionine in growth experiments [Weichselbaum *et al.*, 1932; Jackson and Block, 1932], the suggestion of Jackson and Block, that cystine and methionine form a freely interconvertible system of which only one member is indispensable to a limited degree, and that an adequate supplement

of the other may suffice to provide a common metabolite of the two, is of especial interest in a study of wool production, though their experimental data do not eliminate the possibility of alternative explanations.

SUMMARY.

1. The methionine content of various wools has been determined. It is shown that the amount present does not substantially affect previous work on the relation between total and cystine-sulphur in wool, which indicated that substantially all the sulphur in wool could be accounted for as cystine.

2. The suggestion is made that methionine occurs widely in feeding stuffs and grasses and may play an important rôle in the ultimate synthesis of wool- and hair-proteins.

The author wishes to express his thanks to the Council of the Wool Industries Research Association for permission to publish this account, and to Prof. A. T. King, formerly Head of the Chemistry Department, for the interest he has taken in the work. Throughout the course of the investigation, the author's assistant Mr F. F. Elsworth, has given valuable help.

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