CCXXXVI. THE BASIC AMINO-ACIDS OF WOOL.

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ONLY two determinations have been made of the basic amino-acids present in wool, Marston [1928] using the indirect Van Slyke method found a constant proportion of basic nitrogen in several samples of wool analysed. His figures for histidine, arginine and lysine were 6.9, 10.2 and 2.8 % respectively. Vickery and Block [1930], in a careful series of determinations by direct isolation from a sample of wool, found 7.8 % of arginine, 2.3 % of lysine and only 0.66 % of histidine. In their paper they comment upon the fact that other keratins such as human hair have been found to contain about 0.5 % of histidine [Vickery and Leavenworth, 1929], whereas Marston's figures are over ten times as great.

Marston's figures were reported with some reservation, owing to the well-known difficulty of obtaining trustworthy results by the Van Slyke method, particularly when the protein under examination is one containing a large proportion of cystine.

With regard to the amount of arginine in wool, Marston mentioned that "the estimation of arginine by silver precipitation and by gravimetric means utilising flavianic acid as a precipitating agent yielded figures which were quite constant and at considerable variance with the figures yielded by the above methods" (Van Slyke determinations).

In order to clear up the discrepancy, it was thought advisable to verify Vickery and Block's results by a repetition of the determinations using the modified procedure described by Calvery [1929] for the quantitative isolation of the basic amino-acids from small quantities of protein (5 g.).

In the present investigations a single sample of wool has been used, successive duplicate determinations by the Calvery method being carried out and also a direct colorimetric determination of histidine by the Hanke and Koessler [1920] procedure.

The figures obtained by both methods were in close agreement, averaging 0.5 %, and thus confirming those of Vickery's analyses of wool and of human hair.

EXPERIMENTAL.

A fleece from a West Australian 2 years old Merino ewe was scoured by warm steeping in soap and water, thoroughly washed with liberal quantities of water in a hydro-extractor and finally run through a small carding machine

which not only removed much foreign material but also tended to mix the sample. After hand-carding it was picked free from any residual vegetable matter *etc.*

From the 250 g. of wool so prepared, 1 g. samples (duplicate) were taken for ash and 0.1 g. portions (triplicate) for total nitrogen determinations.

Moisture determinations were carried out upon 1 g. samples dried to constant weight at 105° in a stream of dry air.

	Moisture %	Ash %	Total nitrogen*
	10.66	0.62	16.50
	10.63	0.62	16.43
	—	—	16.37
Mean	10.65	0.62	16.43

* Corrected for ash and moisture.

It was found in a preliminary trial that when 5 g. of wool were boiled with 25 cc. of 20 % hydrochloric acid, maximal amino-nitrogen liberation was attained within 12 hours. There was no change up to 24 hours' boiling, the proportion of nitrogen present in the amino-form being, at this stage, 62.5 % as determined by formaldehyde titration and 82.47 % by reaction with nitrous acid ($\frac{1}{2}$ hour in the Van Slyke apparatus).

It is of interest to note that the non-amino-nitrogen calculated on the assumption of 4.4 % of proline, 1.8 % of tryptophan, 7.8 % of arginine, and 0.6 % of histidine works out at 16.23 % in good agreement with the 17.5 % calculated from the Van Slyke figures.

Determination of the bases by means of their silver salts etc.

Hydrolysis was effected by boiling 5 g. wool with 50 cc. of 20 % hydrochloric acid for 18 hours, after which Calvery's procedure was followed. Whenever possible loss of material was guarded against by testing washings of precipitates, crude fractions *etc.*, by means of the appropriate colour reactions—Sakaguchi's test for arginine, the diazo-reaction for histidine and the ninhydrin colour test for amino-acids in solution.

In this way the importance was realised, as emphasised by Calvery, of keeping a strongly acid reaction whilst adding silver oxide and in making distinctly alkaline to phenolphthalein when precipitating the arginine silver salt.

Some inconvenience was caused by the occasional precipitation of silver sulphide arising from the partial decomposition of some cystine in the presence of silver hydroxide. It was removed by filtration.

Of the crude histidine fraction, 50 cc. in all, 25 cc. were taken for total nitrogen, 24 cc. for isolation of the flavianate and 1 cc. diluted to 10 cc., of which 1 cc. aliquots were used for colorimetric histidine determinations by the Hanke and Koessler method.

The Congo red-methyl orange standard was checked against pure histidine hydrochloride and was entirely satisfactory.

As noticed by the other workers, the figures for total nitrogen of the purified fractions were in excess of the quantities of the bases actually isolated. The impurity in the first lysine fraction was particularly large.

Results of determinations.

Exp. No.	Histidine (%)		
	By total nitrogen	By flavianate	By colorimetric determination
1	0.736	0.59	0.54
2	0.733	0.62	0.51

Exp. No.	Arginine (%)	
	By total nitrogen	By flavianate
1	6.64	5.60
2	6.0	5.97

Exp. No.	Lysine (%)	
	By total nitrogen	By picrate
1	7.23	(1.86)*
2	2.24	2.8

* Portion lost.

Histidine by colorimetric determination.

2.5 g. of wool were boiled for 24 hours with 50 cc. of 20 % hydrochloric acid. The hydrolysate was made up to 100 cc. and total nitrogen determined on 2 cc. aliquots. The procedure outlined by Hanke and Koessler was then followed exactly, the final solution of basic phosphotungstate being diluted to 500 cc. Colorimetric determination was carried out upon 1 cc. samples.

Results.

	Histidine
	0.55 %
	0.55 %
Mean	0.55 %

As there was only 0.04 g. of histidine flavianate available for isolation, the values given by the colorimetric method are probably the more reliable.

DISCUSSION.

No claim is made that the proportions of the basic amino-acids present do not vary from wool to wool, since variations as great as 18 % are known to occur in the cystine content of Australian Merino wool [Barritt and King, 1929]. It is clear however that the percentage of histidine is of the order found by Vickery and Block; namely, 0.5-0.6 %.

Since, up to the present, all attempts to discover variability in the amino-acid content of different wools have been confined to cystine alone, the

realisation is important that this variation may well be influenced by variation in any other amino-acid present in the keratin. It would be of interest to carry out a comparative series of determinations of histidine and other easily determined amino-acids in a series of wools of different origin. Constancy of composition is hardly to be expected, nor is it required by the hypothesis put forward by Astbury and Woods [1931] as to the nature of the linkage of amino-acids in wools.

SUMMARY.

1. The basic amino-acids in a fleece of Australian wool have been estimated by Calvery's method. The values for histidine have been verified by Hanke and Koessler's colorimetric method.

2. This wool gave 0.55 % histidine, 6 % arginine and 2.2 % lysine.

3. These figures are in agreement with those given by Vickery and his co-workers for wool and for human hair and support his opinion "that Marston has been led to an erroneous conclusion with respect to the proportion of histidine yielded by wool."

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