CXVIII. THE DISTRIBUTION OF SULPHUR IN GOAT HAIR.

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(Received May 5th, 1933.)

DURING some recent experiments on the influence of various swelling agents on protein fibres it was noticed that, when treating hairs with concentrated solutions of sodium hydroxide in the cold, the cortex could be dissolved completely and the medulla, as far as microscopical appearance could tell, left intact. Human, horse and goat hairs could all be stripped of their cortex by this means, and it was therefore decided to make a sulphur analysis of the separated cortex and medulla. Although the absence of sulphur from the medulla is now generally accepted [King, 1933] and has been demonstrated by qualitative microchemical tests [Bekker and King, 1931] or inferred as a result of comparing data for medullated and non-medullated wool [Barritt and King, 1931], actual direct analysis of the two morphological parts of the hair shaft has not previously been recorded.

Goat hair was chosen as the material for the work since a large supply was available. The hair had previously been washed with a large quantity of clean tap-water and dried and degreased with acetone. A qualitative test on white hairs made on short cut pieces of the hair showed that, by soaking overnight in a solution of sodium cyanide and then adding sodium nitroprusside, a pink colour developed in the cortex while the medulla remained colourless. This suggested that the cystine group was mainly, if not exclusively, confined to the cortex.

A preliminary trial to select the most suitable concentration of sodium hydroxide for decorticating the hairs gave the following results (a large amount of reagent was used to a small amount of hair):

Concentration of NaOH solution	Condition after 5 hours	Condition after 24 hours
10 and 8 N	Cortex dissolved; medulla falling apart into discs	—
6.4 and 5.1 N	Hairs slightly coiled; cortex very swollen and medulla broken into segments	Cortex dissolved; medulla not in good condition
4.1 N	Hairs slightly coiled; cortex very swollen and medulla broken into segments	Cortex dissolved; medulla in good condition
3.3 N	Hairs violently coiled	Hairs just decorticated, medulla swollen
2.8 N	Hairs violently coiled	Hair in curly tangle which breaks on touching; cortex violently swollen; medulla broken
2.1 N	Hairs violently coiled	Cortex very swollen; bulging in places
1.7, 1.35 and 1.1 N	No change	Cortex swollen but intact

In order to effect the best separation of medulla and cortex while keeping down as much as possible the risk of damaging the medulla, 4*N* sodium hydroxide was taken. 11.31 g. of goat hair (containing 7.5 % of moisture) were mixed into 375 cc. 4*N* NaOH. A strong smell of ammonia developed. The mixture was left standing overnight at room temperature. The following morning a microscopic examination showed that the cortex had been completely dissolved while the medulla appeared to be intact. The medulla, buoyed up by air bubbles, was floating as a slippery mat at the surface of the solution. The containing vessel with its contents was, therefore, placed under a vacuum-pump to draw off the air bubbles. On returning to normal atmospheric pressure, the medulla residue left the surface of the liquid and became submerged. It did not settle to leave a clear, supernatant fluid. Solution and residue were separated by centrifuging. The supernatant solution was poured off, the residue washed twice with distilled water and separated each time by centrifuging. It was then washed with 50 % alcohol, centrifuged, washed with 70 % alcohol and again centrifuged. The residue now settled readily and completely in the wash fluids; it was washed twice with absolute alcohol and twice with ether. The residue was finally dried in the water-oven.

The dry weight of the medulla residue was 1.41 g. A portion was taken for nitrogen determination by Kjeldahl's method and a further portion for a sulphur determination by oxidation with bromine and fuming nitric acid and determination as barium sulphate. In carrying out the sulphur determinations the following method has been used.

When a strongly alkaline solution is under examination, it is advisable first to reduce the alkalinity, but to guard against loss of sulphur, should sulphides be present, by ensuring that the p_H of the solution does not fall below 10. Bromine water is added so that excess is present and fuming nitric acid is then added. Usually 5 cc. are sufficient. The liquor is evaporated slowly to dryness in a beaker over a water-bath and when thoroughly dry, a further addition of 5 cc. fuming nitric acid is made. A clock-glass is placed over the beaker and treatment on the water-bath continued until dryness is reached. In some cases, a further addition of nitric acid is required. Care is necessary to see that any residue left on the sides of the beaker comes in contact with the fuming acid. The dry residue is then treated with plenty of hot water, a small addition of hydrochloric acid made and after bringing to the boil the solution is filtered. The filtrate is brought again to the boil and the sulphur precipitated with barium chloride in the usual way.

The presence of a small quantity of flocculent material in the solution before filtering is of no moment as this, in our experience, is free from sulphur. The method is liable to error should barium compounds be present in the original solution. Should barium be present, filtering the solution after dissolving in water must be omitted, but the error will be very small if the original solution is free from insoluble inorganic material. A fairly large volume of water must be used and additions of hydrochloric acid are necessary should calcium be present so that the calcium sulphate is dissolved.

For solid materials, the protein should be moistened with bromine water and sufficient fuming nitric acid added to cover the material. The procedure then follows that given above.

The above method gives values for the total sulphur, which are reproducible, and which are sensibly the same as those obtained by the Denis-Benedict method. In our experience, the method is more reliable than this latter method and more suitable for fibrous substances.

The total volume of the alkaline solution of the cortex was taken as 375 cc. Nitrogen was determined on this fraction, after removing the free ammonia

under reduced pressure at 40–45°. From this determination, the percentage of non-amide-nitrogen in the cortex was obtained and, after allowing 13.5 % as amide-nitrogen, the total nitrogen of the cortex was calculated. The total nitrogen of the original hair was also determined directly and from this figure, after allowing for the nitrogen of the medulla, the total nitrogen of the cortex was also calculated.

The following results were obtained:

Percentage composition of goat hair by weight:

Cortex	86.5	100
Medulla	13.5	

Sulphur content as percentage by weight:

Whole hair	3.0	By direct determination
	3.2	Calculated

Cortex	3.6	By direct determination
Medulla	0.23	By direct determination

Nitrogen content as percentage by weight:

Whole hair	17.3	By direct determination
Cortex	17.0	Calculated from nitrogen content of solution of cortex
	17.6	Calculated from nitrogen of original hair
Medulla	12.7	By direct determination
	15.3	Allowing for 13 % loss of N as amide

The small quantity of sulphur found in the medulla is almost certainly due to small traces of cortex left clinging to the medullary discs. It was difficult to give the medullary residue a thorough washing, partly on account of the strong alkali used and partly on account of the lengthy centrifuging required at the early stages.

The figure of 3.6 % for the content of sulphur in the cortex of goat hair is very close to that of 3.82 [Barritt and King, 1926] for non-medullated wool. King [1933] gives figures for merino wools (non-medullated) from 3.46 to 4.11 %.

A rough calculation (possibly a little premature) indicates the ratio of sulphur in keratin to the nitrogen of the backbone of the keratin molecule. This can be made by assigning 13.5 % of the total nitrogen to amide groups (Marriott [1931] gives 13.5 % for ox hair, a figure since confirmed; Van Slyke [1911] gives 10 % for dog hair, but ox is more nearly related to goat so the former figure has been preferred), and allowing for the guanidine groups of the arginine residues of keratin and the free amino-groups of the lysine residues. The yield of hair hydrolysates in arginine has been taken as 8 % and in lysine as 2 % approximately. This gives the following rough approximation:

100 g. cortex contain 17.6 g. nitrogen.

Amide-nitrogen = 2.4 g.

Guanidine-nitrogen = 1.9 g.

Free amino-nitrogen = 0.2 g.

Nitrogen from *R* groups¹ = 4.5 g.

Hence, nitrogen from backbone of the molecule = 13 g.

Sulphur can be taken as approximately 4 g.

Hence there is 1 S atom to every 7 N atoms from the backbone.

Astbury and Street [1931] have suggested that in natural keratin the backbone is thrown into folds and that the unit -N-C-C- belonging to each individual amino-acid residue is repeated in blocks of 3. One sulphur atom to every two

¹ "R" group indicates the residual portions of the amino-acids after allowing for the -N-C-C- portion which forms the "backbone" of the protein molecule.

blocks would, therefore, give an atomic ratio of N to S of 6:1. Since the 7:1 ratio obtained by calculation can only be looked on as exceedingly rough, it seems not impossible that, on the average, keratinisation leads to a protein formed by 1 cysteine residue to every 5 other amino-acid residues or 1 double residue of cystine to every 10 other residues.

SUMMARY.

1. The cortex can be separated from the medulla of goat hair by treatment with 4*N* NaOH.
2. The percentage content of sulphur in the separated cortex was 3.60 and in the medulla 0.23. It is suggested that the medulla is substantially free from sulphur.
3. It is suggested that keratin may contain 1 cysteine residue to 5 other amino-acid residues.

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