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Note added in proof: Drs. P. M. COWAN AND R. E. BURGE have kindly pointed out an error in the assumed value of m, the molecular weight per turn of the proline helix (eqn. 5). Based on the coordinates of main chain atoms *alone* the helix contributions of Polyproline I and II should be $[\alpha]_D = +68°$ and $-77°$, respectively. If side chain atoms are included, $[\alpha]_D = +160°$ (I) and $[\alpha]_D = -310°$ (II).

STUDIES ON THE STRUCTURE OF KERATIN

III. THE REACTION OF WOOL AND HORN KERATINS WITH SOLUTIONS OF SODIUM HYPOCHLORITE

C. EARLAND AND D. J. RAVEN
Department of Textile Industries, The Technical College, Bradford (England)

The reactivity of the combined cystine in keratin has been the subject of considerable study, and from the behaviour of these residues towards a number of reagents it has been deduced that the cystine in wool may be divided into a number of fractions. PHILLIPS *et al.*[1] have divided the cystine of wool into two main fractions, one of which reacts more readily with reagents such as sodium bisulphite, alkalis, formaldehyde and thioglycollic acid, although BLACKBURN AND LEE[2] consider that in their reaction with alkalis, the cystine residues of wool show a gradual gradation in reactivity rather than a definite break. ALEXANDER *et al.*[3] also have divided the cystine residues of wool and horn into two fractions, which are unrelated to those of PHILLIPS. These workers consider that only 25 % of the cystine in wool and horn is capable of oxidation by solutions of sodium hypochlorite and potassium permanganate, whereas it may all be oxidized by peracetic acid and chlorine in acid solution. Contrary to the conclusions of ALEXANDER *et al.*, ELLIOT AND ROBERTS[4], from the microscopical examination of oxidized wools, consider that the oxidation of only a portion of the cystine by permanganate is due to the morphology of the fibre. In view of the importance which is attached to the reactivity of the cystine residues of keratin a further investigation as to the nature of the fractions described by ALEXANDER *et al.* has been made.

The reaction between permanganate and wool is very complex since it is accompanied by the deposition of manganese dioxide at the sites of reaction, and the present paper has therefore been restricted to a reassessment of the reaction between solutions of sodium hypochlorite and the keratins of wool and horn.

EXPERIMENTAL

Materials

Virgin Australian wool of three qualities was purified as described in Part I[5]. Cow's horn was reduced in a Sturtevant 8" Laboratory Disintegrator, and the ground material separated into

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different particle sizes by passing through B.S.S. sieves ranging from Nos. 30 to 150. In view of the irregular shape of the particles, their direct measurement was unsatisfactory and the sieve aperture was taken as a measure of particle diameter.

Oxidations with sodium hypochlorite

Wool or horn, equivalent to 1.00 g dry material, was reacted for 24 hours in a closed flask at 18°C, with initial agitation, with the NaOCl solution, corresponding to a known amount of available chlorine, made up to 80 ml with saturated borax solution of pH 9.5. The oxidized keratin was collected in a sintered glass crucible and washed successively with saturated $Na_2B_4O_7$ solution, 1% $Na_2S_2O_5$ solution to reduce residual active chlorine, distilled water and finally acetone. The weight loss was determined after drying the material over P_2O_5.

Cystine analyses

These were performed by the method of SHINOHARA[6], using the phosphotungstic acid reagent of FOLIN AND MARENZIE[7]. For analysis 0.4 g of the sample was hydrolysed with 8.0 ml 5 *N* HCl for 8 hours in the case of horn and 5 hours for wool in a sealed tube at 120–125°C. The reproducibility of the analyses was within ± 0.3%, the majority being within ± 0.2%.

Calculation of results

The precise method of calculating the cystine lost during a reaction has not always been given unambiguously in previous publications, and confusion arises as to whether the values given refer solely to the cystine content of the solid material remaining after reaction, or whether the total cystine lost from the reaction system has been given. Since weight loses of over 50% may be involved in reactions with hypochlorite and keratin, it is essential to distinguish between the two, and in Tables II and III the cystine oxidized is calculated in both manners. In these tables, values in column 5 are given by 100 $(a - b)/a$ and in column 6 by 100 $- b$ $(100 - c)/a$, where a = % of cystine in keratin before reaction, (b) = % of cystine in product after reaction and (c) = % weight loss on oxidation.

RESULTS

The cystine content of untreated wools

The variation in the cystine content of wool with fibre diameter is shown in Table I. In view of the fact that the data presented in Table II are highly dependent on an accurate value for the cystine content of the wools used, analyses were performed in triplicate, each one being performed on a different hydrolysate.

TABLE I

THE CYSTINE CONTENT OF WOOLS OF DIFFERENT DIAMETERS

Quality of wool	*Av. fibre diameter (10^{-4} cm)*	*% Cystine*	*Mean value*
40s	39	11.31 11.36 11.48	11.38
56s	26	11.78 11.33 12.02	11.71
80s	19	13.10 12.80 12.92	12.94

The cystine content of wool and horn after oxidation with hypochlorite

The cystine contents of wools of different fibre diameters and horn of different particle size, after reaction with varying amounts of hypochlorite, are given in Tables II and III.

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TABLE II

THE CYSTINE CONTENT OF WOOLS AFTER OXIDATION WITH SOLUTIONS OF SODIUM HYPOCHLORITE

Av. fibre diameter (μ)	*Avail. Cl_2 reduced (g/100 g wool)*	*wt. loss %*	*Cystine content of prod. %*	*Cystine oxidized (%)*	
				neglecting wt. loss	*correcting for wt. loss*
39	0	0	11.38	0	0
39	20	7.8	9.60	15.6	22.2
39	35	16.1	9.42	17.2	30.6
39	70	36.6	9.54	16.2	46.9
39	100	47.3	9.64	15.3	55.4
39	250	100	—	—	100
26	0	0	11.71	0	0
26	20	7.0	9.83	16.1	22.0
26	35	14.3	9.24	21.1	32.4
26	70	33.4	9.28	20.8	47.1
26	100	45.1	9.20	21.4	56.8
26	250	100	—	—	100
19	0	0	12.94	0	0
19	20	4.1	10.14	21.6	24.8
19	35	15.3	10.54	18.5	31.0
19	70	33.1	9.87	23.7	49.0
19	100	45.1	9.45	27.0	58.3
19	250	100	—	—	100
19*	20	9.1	11.22	13.3	21.2
19*	35	19.8	11.37	13.1	29.7
19*	70	37.3	10.93	15.5	47.1
19*	100	53.5	10.97	15.2	60.5

* These reactions were performed at a liquor to wool ratio of 300:1 with mechanical shaking

TABLE III

THE CYSTINE CONTENT OF HORN OF DIFFERENT PARTICLE SIZE AFTER OXIDATION WITH SOLUTIONS OF SODIUM HYPOCHLORITE

Av. particle diameter (μ)	*Avail. Cl_2 reduced (g/100 g horn)*	*wt. loss %*	*Cystine content of product (%)*	*Cystine oxidized (%)*	
				neglecting wt. loss	*correcting for wt. loss*
500	0	0	12.57	0	0
500	35	19.3	10.62	15.5	31.9
500	69	38.1	10.51	16.4	48.3
500	106	53.4	10.51	16.4	61.2
340	0	0	12.58	0	0
340	20	7.5	9.87	21.5	27.4
340	35	20.0	10.00	20.5	36.5
340	69	38.3	10.02	20.4	50.8
340	106	53.0	9.95	20.9	62.8
340	400	95.5	9.14*	21.2	96.5
190	0	0	12.34	0	0
190	20	2.7	8.91	27.8	29.8
190	35	18.2	8.98	27.2	40.4
190	69	42.5	9.12	26.1	57.6
190	106	62.8	9.17	25.7	72.4
190	400	99.0	—	—	> 99.0
130	0	0	11.22	0	0
130	35	16.8	8.34	25.7	38.1
100	0	0	9.07	0	0
100	35	24.7	6.72	25.9	44.1
100	100	65.5	6.37	29.8	75.1

* Different sample of horn, untreated 11.6% cystine.

The nature of the reaction between hypochlorite and keratin

Wool of any fibre diameter dissolved completely in 15 minutes when treated with 300 % available chlorine as hypochlorite. Back titration of the excess of hypochlorite showed that 250 % of available chlorine had reacted. Since oxidation of all the cystine residues in wool to cysteic acid residues by the reaction:

$$-S-S- + 5\ NaOCl + H_2O \longrightarrow -2SO_3H + 5\ NaCl$$

requires only 20 % of available chlorine, 230 % of available chlorine must react with groups other than the disulphide bond. This was confirmed by reacting α-keratose[8,9], which is a fraction obtained from wool by oxidizing all the cystine residues to sulphonic acid groups with peracetic acid, with hypochlorite in saturated borax solution, when it was found that in 2 minutes 150 % chlorine had reacted, and in 15 minutes 225 % chlorine had reacted. The oxidation of the disulphide bond therefore constitutes only a very small part of the overall reaction between keratin and hypochlorite solution.

DISCUSSION

Contrary to the statement of ALEXANDER *et al.*[3], who appear to have misinterpreted their experimental findings, that only 25% of the cystine in keratin can be oxidized by hypochlorite, this work shows that this reagent can oxidize all these residues. The value of 25% for the cystine oxidized is obtained only by ignoring the weight losses produced by the oxidation, and it is seen from Tables II and III that if allowance be made for these losses, the cystine is progressively oxidized until the keratin dissolves completely.

The fact that peracetic acid and chlorine in acid solution oxidize all the cystine in keratin before solution occurs is due to their specificity for attacking the disulphide bond. Peracetic acid is nearly completely specific for oxidizing this group, and 20% of chlorine at pH 2.0 oxidizes 73% of the cystine in wool[3] compared with 100% if the reagent restricted itself to attack on the disulphide bond. Furthermore, the oxidized material is insoluble in the reaction solution. Thus, provided, sufficient oxidant is present and the reaction is allowed to proceed to completion, the solid material remaining after reaction will be completely devoid of cystine residues.

In the case of hypochlorite, however, the reagent is completely non-specific for cystine. Thus, when wool reacts with 20% of its weight of chlorine as hypochlorite only 20% of the reaction is confined to oxidizing the disulphide bonds, and when 250% of chlorine is used only 10% of the reagent present attacks these groups. Furthermore, the oxidized material dissolves as the reaction proceeds. Under these conditions the solid material remaining after all the hypochlorite has reacted will contain unattacked cystine. It is reasonable to postulate that in view of the large amount of main-chain degradation which must be produced by the hypochlorite, the oxidized keratin dissolves when approximately 25% of the cystine residues have been oxidized, the soluble protein then undergoing further oxidation. This homogeneous reaction will be faster than the heterogenous reaction with wool. It was found that a soluble derivative of keratin, devoid of disulphide bonds, α-keratose, reacted with 150% of chlorine in two minutes. It is relevant to the argument put forward that permanganate, which also oxidizes only the 25% cystine fraction, is also completely non-specific for oxidizing

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disulphide bonds in keratin. The reaction mechanism proposed readily explains the fact that increasing the quantity of oxidant increases the total cystine oxidized but the residual cystine content of the insoluble material remains constant.

Further support for the view that the fractions are unrelated to the chemical structure of keratin is provided by the fact that the fraction of cystine oxidized, ignoring the weight loss, varies from 15 to 30% according to the diameter of the wool or the particle size of the horn used. This need not be completely inconsistent with the presence of two cystine fractions in keratin if it is assumed, in the case of wool, that the fractions are different in wools of different fibre diameter, and in the case of horn the grinding and sieving procedure has itself produced a fractionation of the material, the more friable portions having a different structure from the remainder. These two explanations do not appear very probable, however, as in both cases the oxidized fraction, as given in column 5 of Tables II and III, bears a direct relationship to the square root of the total surface area of the wool or horn being oxidized. The influence of the solution to keratin ratio on the size of these fractions shows again that they are dependent on reaction conditions.

It must be concluded that the experimental findings of ALEXANDER *et al.*, which have been confirmed and extended in the present investigation, are capable of more than one interpretation, and it is essential that further evidence for the cystine fractions of keratin proposed by these workers should be found before their existence can be regarded as established.

SUMMARY

Cystine analyses have been performed on wools of different fibre diameters and horn after being ground to different particle sizes, after reaction with solutions of sodium hypochlorite.

It is considered that the reaction of keratins with solutions of hypochlorite does not establish the existence of two cystine fractions of different reactivities and it is shown that these results may arise from the use of a non-specific oxidant for the disulphide bond, followed by solution of the oxidized protein.

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