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Addendum added in proof (July 17th, 1957). Since submitting the above paper, we have seen the paper by J. H. BRUEMMER, P. W. WILSON, J. L. GLENN AND F. L. CRANE [*J. Bacteriol.*, 73 (1957) 113] also describing the preparation of small particles (called electron-transporting particle) from extracts of *A. vinelandii*. The DPNH oxidase activity of these particles (prepared by alcohol fractionation) is about the same as that of the WSP described in the above paper when correction is made for the different temperatures employed for the measurements.

THE MECHANISM OF THE REACTION BETWEEN CYSTINE IN KERATIN AND SULPHITE/BISULPHITE SOLUTIONS AT 50° C

PART I

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INTRODUCTION

Many experiments have been described in which a keratin fibre — wool, human hair, etc. — has been stretched and then treated in some way so that, after boiling slack in distilled water, its final length differs from its initial length. If the final length is greater than the initial length, the phenomenon has been called "permanent set"; if the fibre is shorter after the treatment, it has been called "supercontraction". The physical mechanism of these changes is well-established[1]: cross-linkages between the protein chains of the keratin are broken by chemical treatment, and the supercontraction or permanent set of the fibre after treatment depends on the degree to which cross-linkages are re-formed between the protein chains in the extended fibre. There is evidence that the cross-linkage breakdown is a dynamic equilibrium, and that under the conditions of the initial chemical treatment described in this paper, the number of unbroken cystine disulphide cross-linkages at any time is very small.

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Thus the degree of permanent set or supercontraction is uncomplicated by residual unbroken cross-linkages and depends only on the re-formed cross-linkages. The cross-linkages in the treated or untreated fibre may be covalent (cystine disulphide or lanthionine, for example) or electrovalent (the so-called "salt-linkages"—the attraction between oppositely charged groups on the side-chains). In addition, the protein chains are held together by strong hydrogen-bonding forces and intermolecular attraction. The difference between permanent set and supercontraction is a difference of degree only. Their magnitude is a function of (but not directly proportional to) the number of cross-linkages between the protein chains, with only an arbitrary reference to the original length of the fibre.

Experiments on the setting properties of fibres can thus throw light on the reactivity and environment of the cross-linkages. This paper deals with the reactivity of the cross-linkages in human hair with solutions containing Na_2SO_3, Na_2SO_4 and H_2SO_4 in 10% EtOH at 50°. In effect the reaction mixtures are sulphite/bisulphite solutions, and it has been shown analytically[2] that their main reaction is with the cystine disulphide linkage in keratin fibres.

Setting experiments with fibres extended 45%

Method: (i) A length of approximately 4 cm is cut from the root end of a purified human hair, mounted in a setting frame[3] and its dry length measured with a travelling microscope. (ii) It is extended 45% of this original length in approximately 3 min in distilled water at room temperature. (iii) The fibre is transferred to a sulphite/bisulphite reaction mixture at 50° in a thermostat. (iv) After 1 h in the reaction mixture the fibre is removed and rinsed twice in distilled water for 1¼ min in all, and then (v) immersed slack for 1 h in boiling distilled water which has previously been boiled vigorously for 20 min to expel dissolved oxygen. (vi) The fibre is removed from the boiling water, allowed to dry and its length measured so that the permanent set or supercontraction can be calculated as a percentage of the original length. (vii) It is then immersed slack in boiling 5% $NaHSO_3$ for 1 h and then (viii) rinsed twice in distilled water, allowed to dry and its length measured so that the new permanent set or supercontraction can be calculated.

This experimental sequence was repeated with different fibres using sulphite/bisulphite solutions with different pH values. The pH values were adjusted by adding varying amounts of H_2SO_4 solution to a Na_2SO_3 solution, and $[SO_4^{-2}]$ was kept constant by adding the appropriate volume of a Na_2SO_4 solution. The reaction mixtures were $M/2$ in Na_2SO_3 and $M/4$ in SO_4^{-2}; they differed only in their Na^+ and H^+ concentrations. All the solutions were made up in 10% EtOH as an anti-oxidant—in this way the experiments were in line with previous work[4]. After the experiments the reaction mixtures were cooled to room temperature and their pH values measured. The results are shown in curves B and B′ in Fig. 1: the curve B shows the permanent set or supercontraction after treatment with boiling distilled water, the lower curve B′ shows the results when this treatment is followed by boiling 5% $NaHSO_3$. Curves A and A′ in Fig. 1 show the results of experiments using an identical sequence, except that the fibres were in the sulphite/bisulphite reaction mixtures at 50° for 2 h instead of 1 h.

Alternative experimental sequence

By omitting steps (v) and (vi) from the experimental sequence—by taking the fibres from the reaction mixtures, rinsing them and immersing them slack in boiling 5% $NaHSO_3$ without the intervening step in boiling distilled water—the curve C in Fig. 1 was obtained. It has the same shape as the curves A′ and B′ and shows a maximum at the same pH value, but the numerical values of the permanent set or supercontraction are lower, showing that some re-formation of cross-linkages occurs during rinsing or in boiling distilled water before the fibre has contracted to its final length. Further experiments showed that unrinsed fibres when taken straight from the sulphite/bisulphite reaction mixtures to the boiling 5% $NaHSO_3$ solution gave the same results as rinsed fibres, and therefore any cross-linkages formed during rinsing are not stable in boiling 5% $NaHSO_3$ solution.

Difference curves—set owing to re-formation of cystine disulphide bonds

Fig. 2 shows the difference curves between the two curves A and A′ and the two curves B and B′ in Fig. 1.

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Boiling distilled water releases some hydrogen bonds and intermolecular attractions so that the permanent set or supercontraction a fibre acquires is largely a function of the number of covalent cross-linkages formed during the treatment. LINDLEY AND PHILLIPS[5] have shown that $NaHSO_3$ breaks at least 75% of the cystine disulphide bonds in wool. The products are S-cysteine sulphonate, the sulphenic acid and α-amino-acrylic acid (all incorporated in the protein chains), which are unlikely to give rise to cross-linkages in the conditions of the treatment used in the present experiments — boiling 5% $NaHSO_3$. Therefore the permanent set or supercontraction remaining after this treatment is a function of the number of covalent cross-linkages which are stable to the treatment. These will be called "bisulphite-stable" cross-linkages, giving rise to bisulphite-stable set. The problem can thus be divided into two parts: the factors governing the re-formation of cystine disulphide bonds, and the mechanism of the formation of bisulphite-stable cross-linkages.

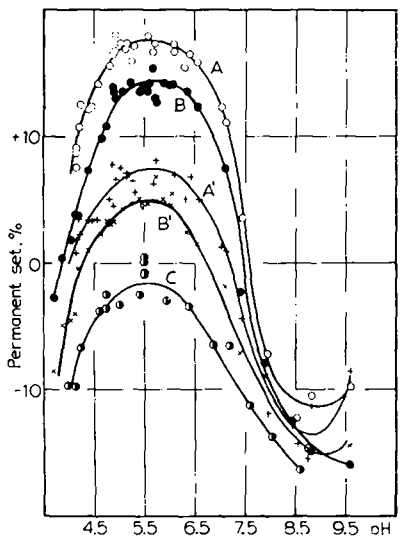

Fig. 1. Permanent set and supercontraction. Fibres extended 45% and immersed 2 h in sulphite/bisulphite mixture, followed by 1 h slack in boiling water (A); followed by 1 h slack in boiling 5% $NaHSO_3$ (A'). Fibres extended 45% and immersed 1 h in sulphite/bisulphite mixture, followed by 1 h slack in boiling water (B); followed by 1 h slack in boiling 5% $NaHSO_3$ (B'). Fibres extended 45% and immersed 1 h in sulphite/bisulphite mixture, followed by 1 h slack in boiling 5% $NaHSO_3$ (C).

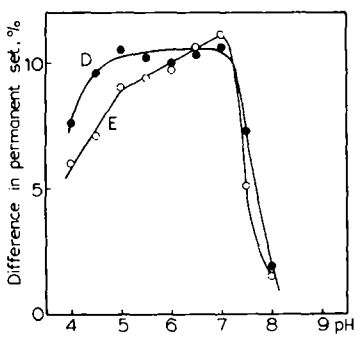

Fig. 2. Difference curves. Curve D: difference between curve A and curve A' in Fig. 1. Curve E: difference between curve B and curve B' in Fig. 1.

The difference curves in Fig. 2 represent, therefore, that part of the set acquired in the sulphite/bisulphite reaction mixtures (or subsequently) which is not stable in boiling 5% $NaHSO_3$ — the permanent set caused by re-formation of cystine disulphide bonds by the reverse reactions of the equilibria (1) to (4) below. In the sulphite/bisulphite reaction mixtures there are large excesses of the reagents in solution (one fibre in 200 ml of solution) so that, at equilibrium, the number of unbroken cystine disulphide bonds at any time will be negligibly small. The degree of supercontraction at the extremes of pH value investigated (pH 3.7 to pH 9.6) shows that over the whole range of pH values the cystine scission reaction is probably fast enough to allow equilibrium to be reached within 1 hour in the reaction mixture. Therefore, the re-formation of cystine disulphide bonds does not take place in the reaction mixtures, and this makes inappropriate an earlier theory[1] that re-formation occurs

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in the sulphite/bisulphite reaction mixtures when their pH values are within the "iso-electric region" of the protein. The disulphide re-formation occurs in the same conditions (while rinsing in water or immediately after immersion in boiling water) whatever the pH value of the sulphite/bisulphite reaction mixture. The level or nearly level portions of the curves in Fig. 2 between pH 5 and pH 7 are probably artifacts: a result of placing too much importance on the numerical value of the set before and after treatment with boiling 5% $NaHSO_3$.

Some of the broken cystine cross-linkages give rise to bisulphite-stable cross-linkages; the factors governing the reunion of the remaining broken disulphide bonds are obscured in the present experiments by the stronger, bisulphite-stable cross-linkages which are formed at the same time.

Effect of OH^- ions alone on fibres extended 45%

There is chemical evidence[6] for the conversion of cystine to lanthionine in the protein chains of wool in alkaline solutions and, parallel with this, it has been found that the formation of bisulphite-stable cross-linkages occurs in alkalis. This can take place in solutions far from the iso-electric region, which again suggests that salt-linkages holding the protein chains together are not a necessary condition, at least for this type of permanent set.

To study the effect of OH^- ions in the absence of SO_3^{-2}, HSO_3^-, etc., the previous experimental sequence (i) to (viii) was used, except that in this case the sulphite/bisulphite reaction mixture was replaced by a Na_2SO_4 solution of the same ionic strength ($3M/4$). The pH value of this solution was adjusted by adding 10 ml of an $M/4$ buffer (phthalate or phosphate) and a few drops of 1.0N HCl or 1.0N NaOH to 200 ml of the Na_2SO_4 solution. 10% EtOH solutions were used throughout. The results of these experiments are shown in Fig. 3: the higher curve shows the supercontraction after the fibres have been treated in the Na_2SO_4 reaction mixtures at 50° and immersed slack in boiling distilled water for 1 hour. The lower curve shows the supercontraction when this treatment is followed by immersion slack in boiling 5% $NaHSO_3$ solution for 1 hour. If the pH value of the Na_2SO_4 reaction mixture is below 7.0, supercontraction does not occur in the ensuing treatment with boiling distilled water. The diminished supercontraction obtained by treating the fibres for a further hour with boiling 5% $NaHSO_3$ shows that at pH values above about 5.5 the concentration of OH^- ions is sufficient to cause the formation of bisulphite-stable cross-linkages at 50° and that the degree of cross-linking increases as the pH value of the reaction mixture is increased.

Chemical reactions involved in bisulphite-stable setting and supercontraction

In any theory of permanent set or supercontraction significance cannot be attached to the numerical values of these quantities. In the following theory of the reaction mechanism, the most important experimental fact is the pH value of the sulphite/bisulphite reaction mixture in which fibres acquire the greatest set, and the effect on this "pH value of maximum set" of modifications of the experiments.

In the reaction mixtures there are four possible reacting species: H_2SO_3, HSO_3^-, SO_3^{-2} and OH^-. The ratio of their concentrations at any given pH value is governed by the first (K_1) and second (K_2) dissociation constants of sulphurous acid and the dissociation constant (K_w) of water. Cystine itself and cystine incorporated in the

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protein chains are not susceptible to acid hydrolysis, and therefore H^+ is not a possible reacting species. The reactions are therefore as follows; chemical evidence for the products has been found by ELSWORTH AND PHILLIPS[7].

$$RSSR + H_2SO_3 \rightleftharpoons RSSO_3H + RSH \quad (1)$$
$$RSSR + HSO_3^- \rightleftharpoons RSSO_3^- + RSH \quad (2)$$
$$RSSR + SO_3^{-2} \rightleftharpoons RSSO_3^- + RS^- \quad (3)$$
$$RSSR + OH^- \rightleftharpoons RSOH + RS^- \quad (4)$$

RSSR, $RSSO_3H$, RSH and RSOH stand for cystine, S-cysteine sulphonate, cysteine and the sulphenic acid respectively, incorporated in the protein chain.

$$RSSO_3^- + H^+ \rightleftharpoons RSSO_3H \quad (5)$$
$$RS^- + H^+ \rightleftharpoons RSH \quad (6)$$

Because the equilibria (5) and (6) are instantaneous, the products of the reaction of RSSR with either H_2SO_3, HSO_3^- or SO_3^{-2} are the same at any given pH value. If the rates of the forward reactions are r_1, r_2, r_3, r_4 and their rate constants k_1, k_2, k_3, k_4 respectively, then –

$$r_1 = k_1 [RSSR] [H_2SO_3]$$
$$r_2 = k_2 [RSSR] [HSO_3^-]$$
$$r_3 = k_3 [RSSR] [SO_3^{-2}]$$
$$r_4 = k_4 [RSSR] [OH^-]$$

The forward reactions of the equilibria (1) to (4) thus give three possible starting points for the formation of bisulphite-stable cross-linkages, $RSSO_3^-$ (or $RSSO_3H$), RSH (or RS^-) and RSOH. Cross-linking reactions must involve two side-chains, and several suggestions have been made for the formation of bisulphite-stable cross-linkages. It is known[6] that in alkaline solution RSH reacts with RSOH to form lanthionine, RSR. It has also been suggested[8] that RSOH first forms R'CHO which then combines with lysine ($R''NH_2$) to give $R'CH{:}NR''$. To explain setting in sulphite/bisulphite mixtures, –SNH– cross-linkages from RSOH and lysine have been put forward[9]. In the present paper the cystine derivative which gives rise to the cross-linkage is the main concern. In a following paper the nature of the bond formed will be considered.

Using sulphite/bisulphite mixtures at room temperature with wool, ELSWORTH AND PHILLIPS[7] observed the variation of $RSSO_3^-$ and RSH formed in the reaction as the pH value of the reaction mixture was varied. They found maximum amounts of these products when the pH value was approximately 5. The titration curve of sulphurous acid showed that at approximately this pH value $[HSO_3^-]$ is a maximum: on the acid side of pH 5 $[H_2SO_3]$ increases at the expense of $[HSO_3^-]$; on the alkaline side $[SO_3^{-2}]$ increases at the expense of $[HSO_3^-]$. ELSWORTH AND PHILLIPS concluded that HSO_3^- was the important reacting species, in other words that the forward reaction of (2) was very much faster than those of (1), (3) or (4). This explanation cannot be transferred to the present experiments to explain the pH value of maximum set. If HSO_3^- were the predominant reacting species maximum breakdown of cystine disulphide bonds would occur at the pH value where $[HSO_3^-]$ is greatest. But the present work suggests that the cystine scission reaction is an equilibrium, that in the conditions of the experiments very little unbroken cystine remains at equilibrium, and that equilibrium is reached within 1 hour of treatment. What is required is an explanation of the maximum *formation* of bisulphite-stable cross-linkages at or near the pH value where $[HSO_3^-]$ is greatest.

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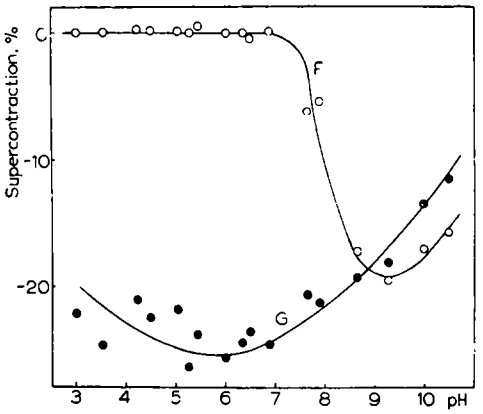

Fig. 3. Supercontraction in the absence of Na_2SO_3. Fibres extended 45% and immersed 1 h in a buffer solution without Na_2SO_3, followed by 1 h slack in boiling water (F); followed by 1 h slack in boiling 5% $NaHSO_3$ (G).

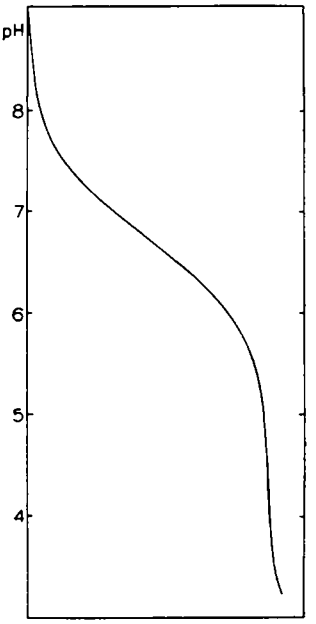

Fig. 4. Titration curve. Titration curve of Na_2SO_3 in 10% EtOH against H_2SO_4 in 10% EtOH at the ionic strengths used in the setting experiments whose results are shown in Fig. 1.

Fig. 4.

CECIL AND MCPHEE[10] found that cystine itself in solution reacts much more quickly with SO_3^{-2} than with HSO_3^- or H_2SO_3. This suggests an alternative explanation of the present work which is not incompatible with ELSWORTH AND PHILLIPS' results, although cystine combined in a protein need not necessarily react in the same way as uncombined cystine. The necessary assumptions are (i) that SO_3^{-2} and OH^- are the important reacting species; in other words, that the forward reactions of (1) and (2) are negligibly slow compared with those of (3) and (4); and (ii) that the products of the forward reaction of (3) give rise to bisulphite-stable cross-linkages very much faster than those of the forward reaction of (4). Since RSH is a product of both reactions, assumption (ii) is equivalent to assuming that $RSSO_3^-$ (with some other side-chain, RSH or $R''NH_2$, etc.) gives rise to bisulphite-stable cross-linkages very much faster than RSOH (again with some second side-chain).

The ratio of $[RSSO_3^-]$ to [RSOH] formed in the forward reactions of (3) and (4) (reactions which compete for the RSSR combined in keratin) is equal to the ratio of the rates, r_3 to r_4.

$$\frac{[RSSO_3^-]}{[RSOH]} = \frac{r_3}{r_4} = \frac{k_3}{k_4}\frac{[RSSR]}{[RSSR]}\frac{[SO_3^{-2}]}{[OH^-]} = \frac{k_3\,[SO_3^{-2}]}{k_4\,[OH^-]} = \frac{k_3}{k_4K_w}\cdot[SO_3^{-2}]\,[H^+] = \frac{k_3K_2}{k_4K_w}\cdot[HSO_3^-] \quad (7)$$

This equation shows that the ratio of $[RSSO_3^-]$ to [RSOH] will be greatest at the pH value where $[HSO_3^-]$ is a maximum—where $[H^+] = \sqrt{K_1K_2}$. Both reactants, SO_3^{-2} and OH^-, increase in concentration (following different laws) as the pH value of the solution is increased, and therefore the bond-scission reaction is most likely not to be complete when the fibres are in more acid solution. In Fig. 1 the curves A' and B' do not show any tendency to be closer together on the alkaline side, as might be expected if all the cystine disulphide bonds had not been broken on the

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acid side during the 1 hour treatment, so that more would be broken during the second hour, allowing further cross-linkage formation. This confirms that even at pH 3.7 cystine disulphide cross-linkage breakdown is virtually complete in 1 hour and equation (7) represents the true state of affairs.

Thus on assumption (i) ELSWORTH AND PHILLIPS' results have been explained: maximum $RSSO_3^-$ formation in a reaction mixture where $[HSO_3^-]$ is greatest. If the initial breakdown of disulphide cross-linkages is complete, and if assumption (ii) is correct, equation (7) would predict that the pH value of the reaction mixture which causes maximum set in a fibre would coincide with the pH value where $[HSO_3^-]$ is a maximum. Equation (7) shows that this result is independent of k_3 and k_4. (The equation would not predict the approximate coincidence of maximum $RSSO_3^-$ and maximum RSH formation found by ELSWORTH AND PHILLIPS, since RSH is formed in both the competing reactions. If equation (7) represents the facts, then RSH formed in the hydrolysis, $RSSR + OH^- \rightarrow RSOH + RSH$, reverts, of course, by the reverse reaction, to cystine under the conditions of the hydrolysis of the wool – boiling 5 *N* HCl for 4 hours.)

TARTAR AND GARRETSON[11] working on sulphurous acid at 25° have found $K_1 = 1.72 \cdot 10^{-2}$ and $K_2 = 6.24 \cdot 10^{-8}$. The geometric mean $\sqrt{K_1 K_2}$ is therefore equal to $10^{-4.48}$, and $[HSO_3^-]$ is a maximum in a sulphite/bisulphite mixture whose pH value is adjusted to 4.48. For several reasons this result is not strictly applicable to the present work. K_1 and K_2 vary with ionic strength, and TARTAR AND GARRETSON's values are for infinite dilution. Also, the values of K_1 and K_2 in water and in 10% EtOH will differ. For this reason the titration curve, Fig. 4, was determined at room temperature using the same concentrations of reagents in 10% EtOH as used in the setting experiments. It shows that the pH value where $[HSO_3^-]$ is a maximum remains close to 4.5 even in a solution of high ionic strength in 10% EtOH. K_1 and K_2 vary with temperature, and the pH values of the reaction mixtures are measured at room temperature. However, it is unlikely that the variation in K_1 and K_2 with temperature is large enough to cause an appreciable difference between the pH values of the mixtures at room temperature and 50°.

DISCUSSION

From a consideration of the general case where H_2SO_3, HSO_3^-, SO_3^{-2} and OH^- all react with RSSR the significance of the difference between the pH value where the set acquired by a fibre is greatest (5.7) and the pH value where $[HSO_3^-]$ is a maximum (4.5) can be inferred.

By the same reasoning as before, the concentrations of $RSSO_3^-$ and RSOH formed in the competing reactions at any pH value are in the ratio

$$\frac{[RSSO_3^-]}{[RSOH]} = \frac{k_1[H_2SO_3]}{} + \frac{k_2[HSO_3^-]}{k_1[OH^-]} + \frac{k_3[SO_3^{-2}]}{} =$$

$$= \frac{1}{k_1 K_w}\left(k_1[H_2SO_3][H^+] + k_2 K_1[H_2SO_3] + k_3 K_2[HSO_3^-]\right) \qquad (8)$$

In the special case considered in the last section, the first two terms in the bracket were assumed to be negligible and therefore the ratio $[RSSO_3^-]/[RSOH]$ was greatest in this case at a pH value where $[HSO_3^-]$ was a maximum. If the fibres are given more time to react with the sulphite/bisulphite solution this would give more time

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for $RSSO_3^-$ and RSOH to form cross-linkages. Fig. 1, curve A', shows the effect of doubling the time the fibres are allowed to react with the sulphite/bisulphite solutions at 50°. The fact that there is no shift in the pH value where the bisulphite-stable set is greatest (*cf.* curve B', the bisulphite-stable set after 1 hour) means that, in 2 hours, neither cross-linking reaction is complete. If assumption (ii) in the previous section is not strictly obeyed—if RSOH does give bisulphite-stable cross-linkages although slowly—then the effect would be to make the maximum on the setting curve less sharp, but it would leave it at the same pH value until it disappeared into a straight line, as it would if $RSSO_3^-$ and RSOH gave bisulphite-stable cross-linkages at the same rate. If the first assumption (i) were not strictly true, and H_2SO_3 or HSO_3^- gave $RSSO_3^-$ at a rate comparable with SO_3^{-2}, then the pH value where the bisulphite-stable set is greatest would be on the acid side of the pH value where $[HSO_3^-]$ is a maximum.

The effect of contributions from the equilibria (9), (10) and (11) must be considered.

$$RSOH + H_2SO_3 \rightleftharpoons RSSO_3H + H_2O \quad (9)$$

$$RSOH + HSO_3^- \rightleftharpoons RSSO_3^- + H_2O \quad (10)$$

$$RSOH + SO_3^{-2} \rightleftharpoons RSSO_3^- + OH^- \quad (11)$$

Because the equilibrium (5) is attained instantaneously, the forward reactions of the above equilibria give identical products at any given pH value. If there is a tendency for the forward reactions to go at an appreciable rate then the effect would be indistinguishable from RSOH itself giving permanent set. Similarly, if there is a tendency for the reverse reactions to proceed at a measurable rate in the rinsing water or in boiling distilled water, then the effect would be to lower the set obtained and also to make the maximum less sharp.

This leaves one possible explanation of the difference between the pH value of the solution in which the set acquired by a fibre is greatest and the pH value where $[HSO_3^-]$ is a maximum. ELSWORTH AND PHILLIPS' results show that the pH value of a sulphite/bisulphite mixture which causes maximum formation of $RSSO_3^-$ is, within the limits of experimental error, the pH value where $[HSO_3^-]$ is greatest, as required by equation (7). The present results of setting experiments show that the setting reaction involving the products of this breakdown of cystine disulphide bonds must be alkali-catalysed.

EXPERIMENTAL

The human hair was purified by extraction 12 h in ether and 12 h in EtOH, followed by rinsing overnight in running water and then in large quantities of distilled water.

In the setting experiments the reaction mixtures were contained in large boiling tubes and allowed 35 min to reach the temperature of the thermostat. Slight reductions in this time affect the reproducibility, perhaps because of incomplete reaction of dissolved oxygen with the reaction mixture. After rinsing, the fibres were immersed in boiling distilled water which was always boiled on the same hot-plate: again, slight differences in temperature affected the reproducibility because part of the cross-linking takes place immediately the fibre is immersed in the boiling water before it has contracted to its final length.

In the following step—immersion in boiling 5% $NaHSO_3$—the $NaHSO_3$ was weighed out and added to the calculated volume of boiling distilled water 1 min before the fibre is immersed. The volume was kept constant by adding small quantities of boiling distilled water from time to time during the hour.

AnalaR $Na_2SO_3 \cdot 7H_2O$, Na_2SO_4, and H_2SO_4 were used. Commercial $NaHSO_3$ was used. For the buffer solutions, AnalaR potassium hydrogen phthalate, KH_2PO_4, $Na_2HPO_4 \cdot 12H_2O$, NaOH and HCl were used.

The pH values of the solutions were measured with a Cambridge pH meter and Cambridge glass and calomel electrodes. The readings were reproducible to 0.05 pH unit.

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ACKNOWLEDGEMENT

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SUMMARY

The results of setting experiments show that SO_3^{2-} and OH^- are the main reactive ions when the cystine disulphide cross-linkages in human hair are attacked by sulphite/bisulphite solutions in 10% EtOH at 50°.

S-Cysteine sulphonate, $RSSO_3^-$, incorporated in the protein chain, is the most important source of bisulphite-stable set at the lower pH values (4 to 8) investigated. The cross-linkages involved are formed in the reaction mixtures at 50° and also when a treated fibre is immersed slack in boiling distilled water.

The cross-linking reaction is alkali-catalysed.

The second side-chain which combines with $RSSO_3^-$ to form a bisulphite-stable cross-linkage will be discussed in a following paper.

The sulphenic acid group, RSOH, incorporated in the protein chain also gives rise to bisulphite-stable cross-linkages; its effect becomes appreciable at pH values above 8. Viewed with other evidence[6], this suggests that the cross-linkages in this case are lanthionine, RSR, bonds.

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PHOTOCHEMISTRY OF CYTOSINE NUCLEOSIDES AND NUCLEOTIDES

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The effects of ultraviolet irradiation of nucleic acids and their derivatives have received considerable attention, largely because of their widespread occurrence in living organisms, their high selective absorption in ultraviolet light, and considerable evidence pointing to them as the immediate and principal receptors of radiation resulting in a variety of biological effects. Although it is well known that purines are considerably more resistant to irradiation than pyrimidines, there is a surprising paucity of quantitative data on this subject.

In most instances the photolysis of purine and pyrimidine derivatives is accompanied by a destruction of the absorption spectrum and the formation of a wide variety

References p. 364.