THE ROLE OF DISULPHIDES AND MERCAPTANS IN HAIR CHEMISTRY

By J. L. STOVES, PH.D., M.Sc., F.R.I.C.*

APART from a small amount of methionine, the whole of the sulphur present in keratin fibres can be accounted for as cystine, the disposition of this amino-acid within the fibre being such as to form a bridge between peptide chains:

$$>CH.CH_2.S.S.CH_2.CH<$$

Many estimations of the sulphur content of keratin fibres have been made and it is now definitely established that different animal fibres contain varying amounts of sulphur. Neglecting differences due to histological variations, the significance of which has been discussed elsewhere,[1,2] variation in the sulphur content of hair from the same species is now regarded simply as indicating variation in the number of cystine disulphide linkages between the peptide chains. An argument against the presence of such linkages in keratin has been based on the fact that chemical reactivity of protein sulphur is much greater than that of sulphur in free cystine. As already indicated,[3] however, this increased reactivity of protein sulphur can be readily understood in view of the nature of the groupings attached to the cystine linkage in hair.

* Chemical Consultant.

CYSTINE AND HAIR GROWTH

Since keratin contains a relatively high percentage of cystine (*c.* 15 per cent in human hair) it has been suggested, from time to time, that hair growth would be stimulated by supplying to the diet preparations rich in sulphur and cystine. Very conflicting results have been obtained when this form of therapy has been applied to human beings. Difficulties of experimental technique apart, the fact that cystine or sulphur deficiency is only one of several possible causes of poor hair growth goes far to explain these variable results. In the cosmetic field interest has long centred round the possibility of stimulating hair growth by external application of the essential constituents of keratin. Recent work on cutaneous absorption, e.g., Valette in Paris, Hermann, Sulzberger and Baer in U.S.A., has shown that under certain conditions "penetrasols" based on propylene glycol, xylol, an hydrotropic substance like antipyrine and a wetting agent of the sodium arylalkyl sulphonate types make it possible to convey the most varied substances through the epidermis. Penetration in all cases takes place via the hair follicles, passage through

the walls of which occurs in the region below the level of the keratinised layer.

Colloidal sulphur dissolved in oil is another substance which is readily absorbed, liposolubility being essential for penetration of substances through the layer of sebum lining the hair follicle.

So far as penetration of hair "nutrients" is concerned, therefore, this work opens up an interesting field for investigation. There still remains the question of assimilation of penetrants by the hair papilla, and here in particular there is room for strictly controlled experiment, possibly using radioactive isotopic markers. In this connection laboratory experiments have already shown that methionine can replace the dietary cystine necessary for growth of the rat, and can provide the sulphur which appears as cystine in keratin.[4] Toennies[5] suggested that conversion of methionine to cystine probably proceeds through the formation of a methyl sulphonium derivative of S-(β-amino-β-carboxyethyl)-homocystine by direct coupling of methionine with serine. This methyl sulphonium derivative, by elimination of methyl alcohol and subsequent cleavage would yield cysteine. The unsymmetrical thio-ether cystathionine which is the second stage in the Toennies mechanism, was prepared by Brown and du Vigneaud[6] and shown to be split *in vivo* preferentially to cysteine. The following year cysteine was also shown to be formed when homocysteine and serine were added *in vitro* to liver slices under anaerobic conditions, and the isolation by Stettin of cysteine containing a high concentration of nitrogen isotope from the tissues of rats fed on serine containing N^{15} added further confirmation to the theory. Kilmer and du Vigneaud[7] later prepared dl-methionine containing an excess of the stable isotopes S^{34} and C^{13}, the latter in the β and γ positions. When this was fed to rats the isotopic ratios in the cystine isolated from the fur indicated that 80 per cent approximately of the sulphur, but no significant amount of carbon, from the methionine found its way into the cystine. This observed transfer of sulphur agreed with the previous demonstration[8] using radioactive S^{35}, and it appears, therefore, that methionine provides its sulphur, but none of its carbon for the synthesis of cystine *in vivo*.

So far as the formulation of hair dressings and brilliantines is concerned it is true to say that more precise information on cutaneous absorption and assimilation of amino-acids and sulphur compounds in normal and pathological scalp conditions is desirable. Nevertheless, at the present time the incorporation of keratin hydrolysates, methionine or colloidal sulphur in such lotions seems to be amply justified on empirical grounds.

The cystine disulphide linkages of hair appear to be formed mainly during the process of keratinisation. Throughout the lower regions of the hair follicle where the fibre is actively growing, cortical cells are

polygonal and non-birefringent, while chemical tests show that the sulphur of the protein is present mainly as thiol (-SH) residues. In the shaft of the extruded, fully keratinised fibre, however, cortical cells are elongated, dehydrated, show birefringence which is indicative of molecular orientation and crystallinity, while the sulphur is chiefly in the form of cystine disulphide linkages bridging adjacent polypeptide chains. This formation of disulphide linkages by oxidation of thiol residues is catalysed by copper, a reaction which has been studied in some detail in the sheep. The new linkages confer considerable stability upon the fibre, which, in the fully keratinised state, has been likened to a vulcanised protein. Nevertheless, under certain conditions the cystine linkage exhibits marked reactivity, a fact of some significance in cosmetology where on occasions this reactivity is a desirable feature, e.g., in permanent waving and depilation, while at other times, e.g., during shampooing, bleaching and dyeing, the aim should be to cause only the minimum of cross-linkage breakdown. Various academic and technical aspects of this reactivity will now be considered.

Action of Alkalis

For some time there was considerable uncertainty as to the nature of the reaction occurring between cystine disulphides and alkalis. As early as 1906, Fromm suggested simple hydrolysis leading to the formation of sulphenic acid and mercaptan :

$$R.CH_2.S.S.CH_2.R \rightarrow R.CH_2.SOH + HS.CH_2.R.$$

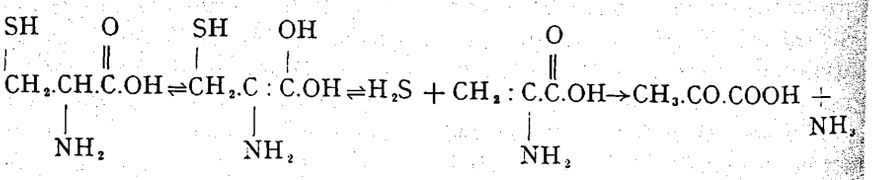
Bergmann and Stather,[9] however, treated dialanyl cystine dianhydride with sodium hydroxide and sodium ethoxide in alcoholic solution and obtained hydrogen sulphide, sulphur and a methylene diketopiperazine, which immediately polymerised, but which appeared to be identical with that obtained by the action of alkali on glycyl and alanyl serine. In hot alkaline solution cysteine and cystine break down to yield ammonia and pyruvic acid, sulphide also being formed. Nicolet[10], from a consideration of the reaction of β-ketonic sulphides with alkali

$$R.CO.CH_2.CHR.S.R \rightleftharpoons R.C(OH)CH:CH.R.S.R \rightleftharpoons R.CO.CH:CH.R + R.SH,$$

suggested that the alkaline decomposition of cysteine takes the course indicated below.

Support for this view was given by the synthesis of cysteine derivatives from mercaptans and unsaturated azlactones or the corresponding open-chain esters.

Meanwhile, during a study of the



oxidation of dithiodiglycollic acid in alkaline solution, Schöberl and Wiesner[11] isolated thioglycollic acid, oxalic acid and hydrogen sulphide. On quantitative grounds they concluded that a primary hydrolysis of the disulphide had occurred with formation of thioglycollic acid and a sulphenic acid. Decomposition of the sulphenic acid might then occur in one of two ways :

$$2R.CH_2.S.OH \rightarrow R.CH_2.SH + R.COOH + H_2S$$

$$R.CH_2.S.OH \rightarrow R.CHO + H_2S$$

From 1933 onward Schöberl *et al.* studied the reaction of many disulphides, and in 1939 Schöberl and Rambacher concluded that all cystine derivatives are decomposed by alkali into thiols and sulphenic acids. Subsequent reactions might involve liberation of hydrogen sulphide from thiol, if labile, or from the sulphenic acid to leave methylene and aldehyde groups respectively. The sulphenic acid might also give off sulphur leaving an alcohol. These reactions are summarised in the following equations :

$$R.CH_2.CH_2.SH \rightarrow R.CH:CH_2 + H_2S$$

$$2R.CH_2.S.OH \rightarrow R.CH_2.SH + R.COOH + H_2S$$

$$R.CH_2.S.OH \rightarrow R.CHO + H_2S$$

$$R.CH_2.S.OH \rightarrow R.CH_2.OH + S$$

Such a view explains the results of both the Bergmann and Schöberl schools and throws considerable light on the action of alkalis on keratin. Earlier work by Marriott and by Speakman suggested that in the case of hair the secondary reactions taking place after the initial disulphide hydrolysis can, under certain conditions, give rise to the formation of new, stable linkages between the polypeptide chains. In a study of the effect of *p*H on this rebuilding, Stoves[12,13] showed that at 35° C. only a small number of new bonds are formed in hair treated for 24 hours with alkali at *p*H values less than 10·5. Above this *p*H, solutions give rise to sufficient rebuilding to prevent supercontration of the hair in boiling bisulphite solutions. This investigation also clearly showed that the new linkages are of two types, one of which is stable to boiling for 4 hours in N/10 hydrochloric acid. The isolation of lanthionine[14] $HOOC.CH(NH_2).CH_2.S.CH_2.CH(NH_2).COOH$ by acid hydrolysis of wool which had been boiled for an hour in 2 per cent sodium carbonate solution, established the presence of a sulphide linkage as the acid stable type, a possibility originally suggested by Speakman and Whewell.[15] The mechanism of formation is not known, but in view of the observation by Nicolet and Shinn[16] that under the influence of alkali, serine may be converted to dehydroalanine which can react with benzyl mercaptan to give the compound $C_6H_5.CH_2.S.CH_2.CH(NH_2).COOH$, it is possible that an alcohol residue formed by loss of sulphur from sulphenic acid, loses the elements of water to form combined aminoacrylic acid which combines with a thiol residue to give a sulphide linkage :

$$>CH.CH_2.S.OH \rightarrow S + >CH.CH_2OH$$
$$\rightarrow >C:CH_2 + H_2O$$
$$\downarrow + HS.CH_2.CH<$$
$$>CH.CH_2.S.CH_2.CH<$$

Alternatively, the new linkage may be formed by loss of hydrogen sulphide from thiol groups, followed by an addition reaction between the $>C:CH_2$ groups so formed and other thiol residues. Phillips and Cuthbertson[17] have suggested that the sulphenic acid, formed by the initial hydrolysis of the cystine linkage, decomposes to form combined amino-acrylic acid and a hypothetical dihydrogen sulphoxide. The amino-acrylic acid residue is then thought to combine with a thiol group, while the sulphoxide forms a more complex inorganic sulphur compound, or decomposes into water and sulphur.

The second type of linkage resulting from the action of alkali on hair is believed to be of the type –N:CH– arising by condensation of an amino group with an aldehyde formed by loss of hydrogen sulphide from a sulphenic acid residue. Evidence supporting this hypothesis is described in detail elsewhere.[13] The final result obtained on treating hair with alkali, therefore, is determined by the magnitude of the primary disulphide hydrolysis, together with the extent to which the various secondary reactions have taken place which in turn is determined by the time of treatment, together with the temperature, concentration and *p*H of the solution used. Accordingly, it seems reasonable to conclude that in the older methods of high temperature permanent waving using alkaline assistants, the chemical mechanism involves formation of lanthionine and Schiff's base type of linkages rather than –SNH– bonds. This subject is at present under examination in the author's laboratory.

Action of Reducing Agents

According to Fruton and Clarke[18] the interconversion of cystine to cysteine is a thermodynamically reversible process, the redox potential of which is apparently characteristic of the general system :

$$R.S.S.R. + 2H \rightarrow 2R.SH$$

and is independent of the group R.

Reduction of cystine to cysteine can be effected in acid solution by means of tin or zinc, while in neutral or alkaline solution an excess of a second thiol compound, e.g., thioglycollic acid, may be used for reduction of the disulphide linkage in proteins :

$$R.S.S.R. + 2R'SH \rightarrow 2R.SH + R'.S.S.R'.$$

This reaction, analogous to that between thiophenol and 2-2′dinitrodiphenyl disulphide studied by Lecher in 1920, was used in the early 1930's by du Vigneaud and by Goddard and Michaelis for reducing the disulphide linkage of proteins such as hair, wool and feathers. In passing it is of interest to note that keratin is characteristically resistant to enzymolysis, but when the polypeptide chains of wool or hair are freed by reduction of the disulphide linkages, the protein is rapidly

hydrolysed by trypsin into its constituent amino-acids. Hair medulla, which has few if any disulphide linkages, is, on the other hand, readily digested by trypsin. Recently, however, it has been shown that treatment of fibres with reagents capable of forming certain types of new, non-sulphur containing linkages in the medulla can render these cells completely resistant to tryptic digestion.[20] Stabilisation of disulphide linkages can be achieved by thioglycollic acid reduction followed by alkylation with dihalides.[21]

A further form of disulphide linkage reduction is that brought about by the action of sulphites and bisulphites[22]

$$R.S.S.R. + Na_2SO_3 \rightarrow R.S.Na + NaO.SO_2.S.R.$$

$$R.S.S.R. + NaHSO_3 \rightarrow R.SH + NaO.SO_2.SR.$$

The sodium-S-cysteine sulphonate is unstable and decomposes when warmed with acids to give sodium bisulphate and cysteine, while alkali gives sodium bisulphite and sulphenic acid. Human hair fibres treated with bisulphite-sulphite solutions *p*H 3-11 at 22·2° C. showed maximum weakening at *p*H4·5 and 11.[23] When the extent of cystine linkage breakdown is not excessive, loss in resistance to extension of bisulphite treated hair can be completely restored by after-treatment with aqueous solutions of benzoquinone, or formaldehyde *p*H8. This restoration results from formation of new linkages by reaction of the quinone with thiol and amino groups, as well as from re-formation of cystine linkages by a partial reversal of the original disulphide breakdown.[13]

The work of Phillips *et al.*[24] on the action of bisulphites on wool suggests that the disulphide linkages are not all equally reactive, and the view has been expressed that the combined cystine of wool can be divided into four subfractions, termed A, B, C, and D differing under certain experimental conditions in their rate and mode of reaction with sodium bisulphite and thioglycollic acid. These differences in reactivity may arise from differences in side-chain environment, fraction (A+B) being associated with polar side-chains, whereas (C+D) is associated with non-polar side-chains.

Mercaptans as P.W. Assistants

Reduction of cystine disulphide linkages by means of sulphites or thiol compounds is of immediate interest to the hair cosmetologist owing to the use of these materials in permanent waving, while certain thiols find further use as depilatories. Practical factors governing the use of alkalis, sulphites and mercaptans as waving assistants have recently been discussed[25] and in view of the ever-growing use of thiol compounds in cold waving it seems desirable to limit the present consideration to this group. The predominance of high temperature waving up to the time of the 1930's was not due to lack of attempts to develop cold waving methods, but rather to an unfortunate choice of chemical reagents.

Alkaline assistants such as borax, carbonates, phosphates, ammonia or ammonium salts, in the concentrations used for high temperature waving were not satisfactory at lower temperatures unless used for long periods ranging from 4 to 24 hours. Attempts to use solutions of greater concentration resulted in excessive damage to the hair. Obviously then, a waving mechanism not depending on initial disulphide linkage hydrolysis is required for cold waving, and an indication of the correct approach was obtained when ammonium hydrogen sulphide, which brings about keratolysis by reduction of cystine linkages, proved to be a more speedy reagent. The malodorousness of this material, however, resulting from liberation of hydrogen sulphide by hydrolysis proved a serious disadvantage. Moreover, sorption of sulphide by hair gave rise to a disagreeable odour whenever the hair was re-wetted, while continued reduction of cystine linkages caused relaxation of the hair fibres so that the wave became less tight with each re-wetting. As already indicated, work in the biochemical and textile fields had demonstrated the use of mercaptans for reduction of the disulphide linkage in scleroproteins, and in 1940 cysteine hydrochloride at a *p*H not less than 10 formed the basis of a patent specification.[26] While of considerable theoretical interest this process does not appear to have received any wide application, probably owing to the fact that the material is costly, the solution is unstable, leaves a dusty deposit on the hair, and although the hair is well waved it is very dull.[27] McDonough,[23] in a U.S.P. specification filed in 1941, but not granted until 10 years later, then showed that satisfactory cold waving could be obtained using mercaptans of general formula R.SH, where R is an organic group such as ethyl or benzyl, in solutions of 1–10 per cent concentration and *p*H7-9·5. Moreover, substitution in the alkyl or aryl radical gave products with less objectionable odours. Such substituent groups may be polar (acidic or basic) or non-polar. For example :

(i) Carboxyl or sulphonic acids, nitro, chloro or halogenated groups.
(ii) Primary, secondary or tertiary amino groups.
(iii) Alcohol, aldehyde, keto, ester or ether groups.

In general, the polar compounds containing a carboxyl group give excellent waving results when used in the form of the ammonium salts. It has been found, however, that the corresponding sodium and potassium salts, even in ammoniacal solution, are much less satisfactory, and the same applies to ethanolamine salts. As will be considered in detail later, alkaline earth salts of certain of these thio-acids possess depilatory properties, e.g., calcium thioglycollate. In the case of mercaptans containing water solubilising non-polar groups, such as glyceryl mono-mercaptan, it is interesting, in view of present developments in one solution cold waving methods, to note that McDonough states that the removal of mercaptan is so complete by water

alone that excellent non-relaxing waves can be obtained without need for additional fixing or setting rinses.

The materials are used in concentrations of 3 to 8 per cent, the speed of waving increasing with concentration up to 15 per cent. By varying the concentration of mercaptan the necessary practical adjustments of waving speeds can be made to suit different systems of hot or cold waving as well as different types of hair. Treatment can be carried out in aqueous solutions of *p*H7 up to but *not* including 10, but in practice it is found desirable not to exceed *p*H9·5, otherwise the time in which it is possible to wave the hair is too short. Optimum alkalinity is *p*H9·2–9·5. In cold waving careful *p*H control is essential, speed of waving increasing with *p*H of the thiol solution. So far as *p*H regulation is concerned McDonough states that while all alkaline materials are satisfactory, bases with a dissociation constant less than 5×10^{-3} and preferably about 10^{-5} are much less destructive in action. For cold waving, volatile bases such as ammonia, methylamine or ethylamine are preferable to non-volatile alkalis since the former give tighter waves. Ammonia, in particular, is outstanding in its advantages since its odour blends pleasantly with that of mercaptans, the ionisation constant is satisfactory, and between *p*H 7–10 it is relatively non-irritating to the skin. These basic U.S. patents, together with 22 similar patents and patent applications in other countries, cover the use of mercaptans in cold waving and have recently resulted in the issue of world-wide licensing agreements.

Thioglycollates are the mercaptans most widely used, and formulated with resin dispersions produce cloudy or opaque cold wave preparations. Recently,[29] it was suggested that 0·3 to 0·5 per cent lanette wax SX or cetyl alcohol, distilled water and wetting agent (secondary sodium alkyl sulphate or alkyl aryl sodium sulphonate) be emulsified in the usual way and after cooling to room temperature, thioglycollic acid and ammonia be added.

Since early in 1949 thioglycerol has been widely used for home waving in France. Prepared as a 20 per cent solution from glycerol monochlorhydrin and sodium hydrosulphide, it is sold to the public as 5 per cent thioglycerol and 15 per cent ammonia.[27] This solution of *p*H 10·5 gives excellent waves, the setting time being at most 2 hours and even with difficult hair the waves last 6 months.[27] For saloon use, however, the speed of waving is too low, and while good results are obtained at high temperatures, liberation of hydrogen sulphide makes the process impracticable. Instability of an ammoniacal solution of thioglycerol necessitates addition of ammonia to the mercaptan solution immediately before use, which is a further manipulative disadvantage. Morelle[30] has, therefore, developed the use of thiolactic acid, formed by reaction of α-chloropropionic acid with sodium hydrosulphide, followed

by acidification of the alkaline thiolactate, extraction and distillation. Ammonium thiolactate is claimed to be non-toxic under normal use, furthermore, it reduces cystine disulphide linkages, but does not give rise to any appreciable fibre swelling, so that damage to the hair is minimal. Finally, it is stated that the wave produced has a strikingly natural appearance, and the hair is supple and glossy without the use of auxiliary agents.

In view of the early use of ammonium hydrogen sulphide for cold waving it is interesting to note that some two years ago an Austrian patent[31] claimed the use of 5 to 15 per cent monomethanolammonium sulphide *p*H 9–11, followed by a finishing rinse containing an oxidising agent. The reagent is stated to give good results with bleached and porous hair, while the odour is reported to be less unpleasant than that of the thioglycollates. Information on relative toxicity, however, was not given.

Until comparatively recently, cold waving involved the use of two solutions, the thioglycollate being followed by a "neutraliser" whose function is to remove excess mercaptan by oxidation to disulphide. At the same time depression of the fibre swelling resulting from treatment with thioglycollate occurs to a variable extent, while rebuilding of cross-linkages can increase fibre stability. Where the initial reduction process has caused appreciable modification of the hair it is important that rinsing should be thoroughly carried out in order to allow these last two reactions to occur, otherwise the hair will remain permanently damaged. Solutions of hydrogen peroxide, acidified with organic acids, such as tartaric, citric or acetic have been used, the acid peroxide oxidising the thioglycollate without bleaching the hair pigment. The amount and nature of the acid used in the oxidising rinses are factors exercising considerable influence on the efficiency of oxidation, citric acid, for example, being an acid to avoid.[32] A second type of oxidising agent is based on solutions of ammonium, alkali or alkaline earth iodate or bromate, potassium bromate in particular having widespread use in home permanent waving kits. Several cases of accidental or suicidal ingestion of such bromates have drawn attention to the potential dangers of these materials, and one manufacturer has replaced bromate by the much less toxic sodium perborate and sodium hexametaphosphate. α-keto acids such as pyruvic or benzyl formic acids (1·5 to 2 per cent, together with a wetting agent) have also been put forward as alternative rinses.[33]

Recently, cold waving solutions which do not require a "neutraliser" have appeared on the British and U.S. markets. The method of Beste and Reed[34] replaces the oxidising rinse by atmospheric oxidation catalysed by soluble salts of copper, manganese or iron, applied as a preliminary rinse or in the waving lotion. Advantages claimed for this process are better control of the

reducing reaction, minimising of injury risk, and avoidance of bleaching. This development opens up a new field of investigation for the manufacturers of permanent waving solutions, possible lines of approach to which have been considered elsewhere.[25]

Reducing Agents as Depilatories

Alkaline reducing agents constitute an important group of depilatories, calcium and strontium sulphides being long-established materials. In aqueous solution these compounds are hydrolysed to give hydrosulphide and hydroxide, the latter playing an important role in maintaining the solution near *p*H 12.

$$2CaS + 2H_2O \rightarrow Ca(HS)_2 + Ca(OH)_2$$

Hydrosulphide reduces the cystine disulphide linkages of keratin and in the presence of alkali, swelling and softening of the fibre takes place, so that after 4 to 5 minutes' treatment with a correctly formulated depilatory the hair can be wiped from the skin with a damp cloth. Alkaline solutions of mercaptans, such as benzylmercaptosulphonic acid or ethyl mercaptoaniline have been patented as depilatories,[35] while calcium thioglycollate[36] is the basis of several nationally advertised products. Calcium thioglycollate exists in two crystalline forms corresponding to the formulæ

$(SH.CH_2.COO)_2Ca$ and $S.CH_2.COOCa.\ 3H_2O$

The second form is preferable owing to lack of odour and absence of undesirable effects on perfumes.[37] It is used in concentrations of 4 to 10 per cent, the commercial products being brought to *p*H 12 by inclusion of free lime. Depilation times are of the order 5 to 10 minutes. Addition of pro-oxidants such as the soluble salts of copper, manganese and iron is stated to give better and safer thioglycollate depilatories.[34] All the products described so far have functioned in an alkaline medium of about *p*H 12, but a departure from this general class has been described in the Demuth patent which uses guanidine thioglycollate or calcium thioglycollate and guanidine, the *p*H of the reagent being kept below 10 by hydrazine sulphate.[38]

In conclusion, it remains to say that formulation of depilatories is beset with many difficulties, such as the choice of a satisfactory filler, selection of a suitable perfume, the prevention of separation and the inhibition of drying out. These matters, however, fall outside the scope of this discussion.

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