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RECENT PROGRESS IN THE CHEMISTRY OF DISULFIDES*

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I. INTRODUCTION

THE WIDESPREAD occurrence in nature of compounds containing sulf-hydryl and disulfide groups forces one to accept these substances as being essential in the chemistry of living processes.   The important role of naturally occurring sulfhydryl compounds such as glutathione and cysteine in the oxidative processes taking place in living cells was emphasized by Hopkins and his co-workers at Cambridge University many years ago. The role of sulfur in intermediate metabolism and its implications to medicine have been ably summarized in the recent book of duVigneaud (1).   It has even been suggested that sulfur-containing compounds may play a critical role in photosynthesis (2) and in vision (3).

The large body of research on the chemistry of proteins carried out over the past half century has established that the disulfide linkage is an important structural element in proteins.   The liberation of sulfhydryl groups when proteins are denatured may indicate that disulfide groups are holding the protein structure together and that they are ruptured on denaturation (4).   The detailed studies of Sanger (5) have shown that the polypeptide chains of insulin are held together by disulfide bonds.   Keratin is particularly rich in disulfide bonds and, for the case of wool and hair, cystine is found in greater abundance than any other single amino acid.   Most of the chemical treatment of wool and hair is concerned with the rupture and re-formation of the disulfide bond.   It is obvious, therefore, that any rationalization of the process employed, for example, in permanent hair waving must require a complete understanding of the chemistry of the disulfide linkage.


It is the purpose of the present paper to re-examine the current ideas on the chemistry of the disulfide linkage in the light of the research which has been carried out in our laboratory. A more detailed account of our research will be treated in a series of papers to appear elsewhere. In particular, we hope to show that the concept that unstable sulfenic acids are formed during disulfide cleavage has no basis in fact. We then consider the spectral evidence which can be related to the strain and to the chemical reactivity of the disulfide linkages. For our studies, we have examined a wide variety of model disulfide compounds, with particular emphasis on cystine. The results obtained for cystine are directly applicable to the chemistry of the disulfide linkage in keratins. We shall review the chemical processes involved in hair waving taking into account our findings on the chemistry of the disulfide linkage.

II. CHEMISTRY OF THE DISULFIDE BOND

In the early 1930's Schöberl and co-workers (6, 7) noted in their studies of model disulfide containing compounds that many of these compounds on treatment with an alkali solution formed three substances: a mercaptan, H_2S, and a carbonyl-containing compound such as an aldehyde or ketone. What was particularly interesting about this reaction was the fact that the mercaptan produced accounted for about 50% of the product; the remaining 50% could very well be accounted for quantitatively as being equal to the sum of the carbonyl compound and H_2S produced. In view of the fact that RSOH (sulfenic acids) are known to be very unstable compounds, Schöberl postulated the following reasonable mechanism to account for the products formed on reaction of a disulfide with alkali:

$$R—CH_2S—S—CH_2—R + H_2O \xrightarrow{\text{OH}^-} RCH_2SH + RCH_2SOH$$
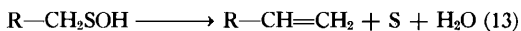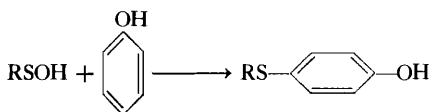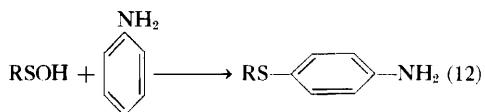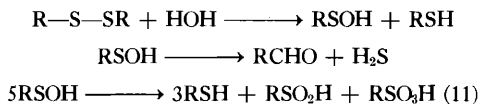$$RCH_2SOH \longrightarrow RCHO + H_2S$$
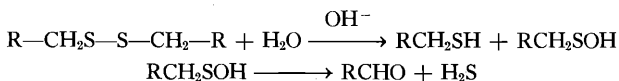
In this mechanism, hydrolytic cleavage, the attack of water upon the disulfide bond which is accelerated by the presence of OH^- ion, yields a mercaptan and a sulfenic acid as the primary step. The products ultimately isolated from the reaction were believed to result from the subsequent steps involving decomposition of the sulfenic acid to H_2S and an aldehyde. Whereas this mechanism appeared to account for the observed facts, no direct positive evidence in its support was forthcoming. On the contrary, some anomalous results were noted in the case of some secondary and tertiary disulfides (8), results which were explained as being the consequence of the operation of steric factors.

Nevertheless, Speakman (9) was soon to apply the concept of the hydrolytic cleavage of S—S to wool and other keratin proteins in an effort to explain the action of both steam and alkali upon these materials. As evidence of the universality of this phenomenon, Schöberl (10) himself sought

to interpret the chemistry of sulfur containing proteins and peptides such as insulin and glutathione in terms of the action of water upon the disulfide bond.

These original postulates of Schöberl and Speakman have acquired universal factual acceptance, as evidenced by the numerous references and published texts which contain their mechanism as a definitive explanation.

With the acceptance of the concept of hydrolytic disulfide cleavage as a basic tenet, the *sine qua non* of disulfide chemistry, all subsequent mechanisms offered to explain the chemistry of reactions of disulfides with a variety of reagents in aqueous solution required that a sulfenic acid and a mercaptan be formed by an attack of water on the disulfide bond. The final products obtained are pictured as resulting from the subsequent reaction of the reagent with the SOH (sulfenic acid) moiety. As a consequence an entire specialized chemistry involving reactions of sulfenic acids with various reagents had to be postulated, for example:

$$R-S-SR + HOH \longrightarrow RSOH + RSH$$

$$RSOH \longrightarrow RCHO + H_2S$$

$$5RSOH \longrightarrow 3RSH + RSO_2H + RSO_3H \quad (11)$$

$$RSOH + R'NH_2 \longrightarrow R-S-NH-R'$$

$$RSOH + R'SH \longrightarrow R-SSR' + H_2O$$

$$R-CH_2SOH \longrightarrow R-CH=CH_2 + S + H_2O \quad (13)$$

To our mind these reactions are purely figments of the imagination; there is not one piece of positive evidence in their favor. On the contrary, there exist many glaring anomalies which cannot be rationally accounted for by such machinations. Kharasch, who has made a serious study of sulfenic acid derivatives in his review (14) of the subject, had this to say:

"Since the actual structures of the sulfenic acids are not known with certainty, the mechanisms whereby various sulfenic acid derivatives are converted into products such as the disulfides, sulfinic acids, thiolsulfonic esters, etc., necessarily have only hypothetical status, and will probably warrant deeper consideration than has previously been given them in the literature."

Nevertheless mechanisms involving the reactions of sulfenic acids are categorically presented in attempts to explain the reactions that wool under-

goes during such processing as setting in steam, shrink-proofing, felting, bleaching, permanent waving, etc. (15, 16) as follows:

(1) $W—(CH_2)_2—S—S—(CH_2)_2—W + H_2O \longrightarrow$
$$W—(CH_2)_2SH + W—(CH_2)_2SOH$$

(2) $W—(CH_2)_2SOH + W—NH_2 \longrightarrow W—(CH_2)_2S—\overset{H}{N}—W$

(3) $W—(CH_2)_2SOH \longrightarrow W—CH{=}CH_2 + S + H_2O$

(4) $W—(CH_2)_2SOH \longrightarrow W—CH_2—CHO + H_2S$

(5) $W—CH_2—CHO + W—NH_2 \longrightarrow W—CH_2—C{=}NH—W$

Strain relaxation is stated to occur by the occurrence of reaction (1) whereas the setting or crosslinking step proceeds *via* steps (2) and (5). To quote Speakman on this point (17):
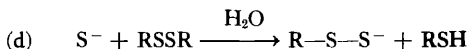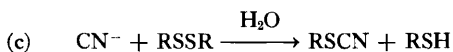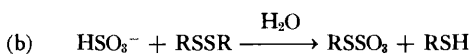
"No matter whether borax, sodium sulfite or sodium meta bisulfite is used as an assistant, the main reaction taking place when strained fibers are set in boiling water is — S — NH — bond formation between peptide chains." The profound conviction of the essential correctness of this mechanism is founded on the observations that either disulfuration or deamination of wool renders it incapable of undergoing permanent set. As a consequence it has been assumed that both —SOH and NH₂ participate in the crosslinking reaction. It is our feeling that the process of deamination in itself may be so disruptive as to render inoperative any normal relinking steps in which the sulfur might normally be involved. It has been noted (18) that peptides of cystine give anomalously high values when treated with the deaminating agent, nitrous acid, in the Van Slyke amino acid nitrogen determinations.

This practice of ascribing the crosslinkage mechanism directly to sulfenic acid or its decomposition products has continued despite the fact that many workers (19, 20) have painstakingly sought in vain to discover the —C═NH—, or —C—S—N—linkages both in wool and in model compounds.

Chemists subsequently have come to regard the mechanism of the attack of water on disulfides as being a displacement reaction of hydroxyl ion upon the disulfide linkage:

(a) $OH^- + R—S—S—R \xrightarrow{H_2O} R—SOH + RSH$
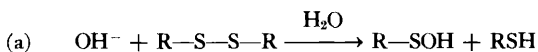
By analogy other ionic reagents which attack disulfides were visualized as proceeding by a similar nucleophilic attack on disulfide:

(b) $HSO_3^- + RSSR \xrightarrow{H_2O} RSSO_3 + RSH$

(c) $CN^- + RSSR \xrightarrow{H_2O} RSCN + RSH$

(d) $S^- + RSSR \xrightarrow{H_2O} R—S—S^- + RSH$

$$\text{(e)} \quad R'{-}S^- + R{-}SSR \xrightarrow{\quad H_2O \quad} RSSR' + RSH$$
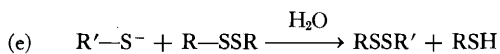
To test the validity of this concept we sought to ascertain whether other anions such as acetate, oxalate, fluoride, azide, cyanate, and sulfate, would function similarly. In no case did we find evidence of mercaptan formation. Hence no nucleophilic attack has occurred. It should be noted that those reagents which do successfully attack the di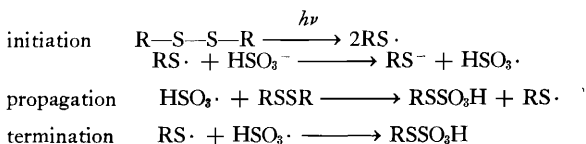sulfide (the reagents listed in equations a–e above), save the hydroxyl, have the property in common of being able to readily lose an electron in the presence of an electron acceptor to yield a free radical (21). Stated differently, all reagents effective in cleaving the disulfide bond are capable of readily undergoing one-electron transfer. Thus it would appear that the attacking moiety is essentially a free radical and the reaction which occurs is not ionic at all but a radical displacement reaction. If one reflects upon other known reagents that reduce disulfide, such as Zn and HCl or sodium metal in liquid ammonia, it would appear here as well that cleavage is occurring *via* the attack of a free radical.

$$R{-}SSR + 2H\cdot \xrightarrow[\text{HCl}]{\text{Zn}} 2RSH$$

$$R{-}SSR + 2Na\cdot \xrightarrow[\text{NH}_3]{\text{Na}} 2RSNa$$
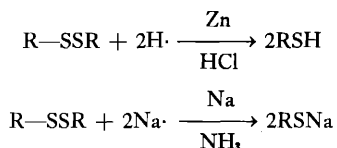
For the present, in writing the radical displacement reaction which is occurring, we prefer to be non-committal with regard to whether it is the thiyl radical, formed as a result of disulfide dissociation or as a consequence of a one-electron transfer on the part of the ion, which is attacking the disulfide linkage. The principal reaction products obtained by either approach are identical.

Speakman (22), Birch (23), Tobolsky (24), and others, have demonstrated that in the absence of oxygen, disulfide-containing compounds on exposure to either ultraviolet or diffuse daylight dissociate into thiyl radicals (RS·) which are capable of polymerizing vinyl monomers. Schönberg (25) has equally shown that many SS compounds will thermally dissociate into thiyl radicals. Let us for the moment visualize the consequences of the energized production of such radicals in the presence of ions capable of one-electron transfer.

$$\begin{aligned} \text{initiation} \quad & R{-}S{-}S{-}R \xrightarrow{\quad h\nu \quad} 2RS\cdot \\ & RS\cdot + HSO_3{}^- \longrightarrow RS^- + HSO_3\cdot \\ \text{propagation} \quad & HSO_3\cdot + RSSR \longrightarrow RSSO_3H + RS\cdot \\ \text{termination} \quad & RS\cdot + HSO_3\cdot \longrightarrow RSSO_3H \end{aligned}$$

The products resulting from these reactions are RS^- and $RSSO_3H$.

Once again let us consider a possible initiation mechanism wherein the

ion capable of one electron transfer, in the presence of oxygen, undergoes an auto-oxidation-reduction reaction to form radicals.

initiation $\quad SO_3^- + SO_3^- + \frac{1}{2}O_2 \longrightarrow -SO_3^-\cdot + SO_4^=$

propagation $\quad SO_3^-\cdot + RSSR \longrightarrow RSSO_3^- + RS\cdot$

$\quad\quad\quad\quad\quad RS\cdot + SO_3^= \longrightarrow RS^- + SO_3^-\cdot$

termination $\quad RS\cdot + RS\cdot \longrightarrow RSSR$
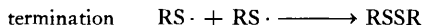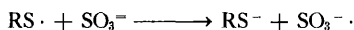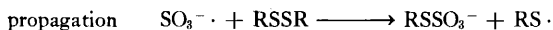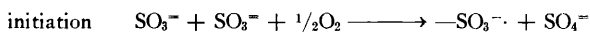
The products resulting from these reactions are identical with those obtained above. Clarke (26) had noted that sulfate is formed during the reaction of a sulfite with cystine which suggests that the latter mechanism is operative.

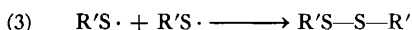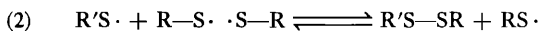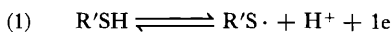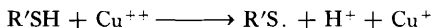As further evidence for the one-electron transfer concept, Karchmer (27) in polarigraphic studies of mercaptans, has shown that mercaptans readily undergo the following reaction:

$$R'SH \longrightarrow R'S\cdot + H^+ + 1e$$

One would predict that in the presence of an electron acceptor this step would proceed rapidly requiring almost no energy of activation. Thus in the presence of a simple disulfide, a mercaptan would be expected to rapidly undergo a radical displacement reaction resulting in a disulfide interchange equilibrium. Current work being done in our laboratory indicates that this is indeed the case, namely:

(1) $\quad R'SH \rightleftharpoons R'S\cdot + H^+ + 1e$

(2) $\quad R'S\cdot + R-S\cdot\ \cdot S-R \rightleftharpoons R'S-SR + RS\cdot$

(3) $\quad R'S\cdot + R'S\cdot \longrightarrow R'S-S-R'$

One-electron transfer would also account for the ease with which a mercaptan will oxidize in an alkaline medium in the presence of both oxygen and a one-electron metal catalyst such as Ag^+ or Cu^{++} or Fe^{+++}. For example:

$$R'SH + Cu^{++} \longrightarrow R'S.\ + H^+ + Cu^+$$

$$Cu^+ + O_2 + H^+ \longrightarrow Cu^{++} + H_2O_2$$

$$R'SH + H_2O_2 \longrightarrow R'SSR' + H_2O$$

If we accept the one-electron transfer-radical displacement theory as the mode of action of disulfide cleavage, how does one reconcile the fact that alkali or water, as Schöberl initially showed, is capable of cleaving the S—S bond? It should be remembered that the formation of hydroxyl radical from hydroxyl ion or from water under normal reaction conditions is energetically impossible. What is probably occurring is that the hydroxyl or alkoxy ion is performing in the role of a base by reacting with a proton in an acid-base relationship. Thus the hydroxyl ion is involved in a reaction

other than a direct attack on disulfide. What then is this other reaction which involves OH$^-$ and subsequently results in disulfide cleavage? We believe it is the following:

(1) $\quad$ OH$^-$ + R—C—S—S—C—R $\rightleftharpoons$ R—C—S—S—CH$_2$—R + HOH

(with H atoms on the carbon atoms as shown)

(2) $\quad$ R—C—S—SCH$_2$R $\longleftrightarrow$ R—C=S—SCH$_2$R

(3) $\quad$ R—C=S + $^-$S—CH$_2$R

(4) $\quad$ R—C=S $\xrightarrow{\text{H}_2\text{O}}$ RCHO + H$_2$S
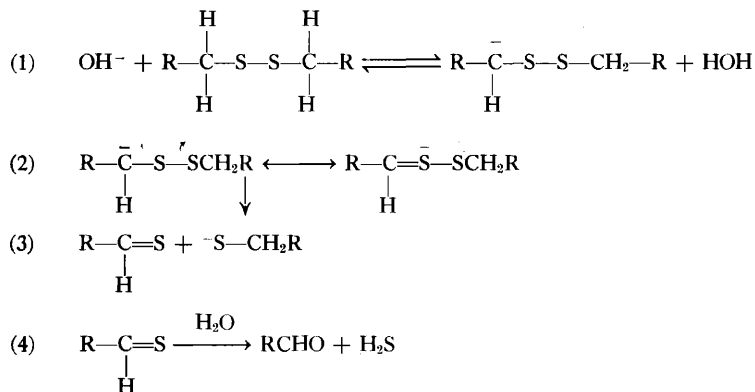
The attack of base upon a hydrogen atom joined to a carbon atom which is situated β to the site of cleavage is essentially a β elimination reaction (28). The analogy to a β elimination is quite valid in the sense 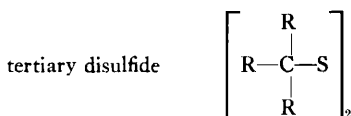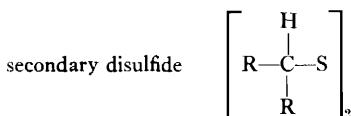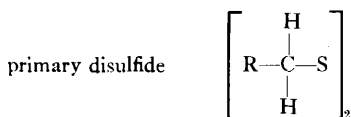that in this case also the group that cleaves in departing takes with it an electron pair while the entity which remains contains a double bond. In a typical β elimination cleavage occurs between carbon and sulfur or carbon and nitrogen; in this instance the cleavage is occurring between the sulfur-sulfur bond. It is well recognized that hydrogens on a carbon atom attached to a sulfide or sulfone have a tendency to be acidic (29). This ionizing tendency or hyperconjugation effect is greatly enhanced by the resonance stabilization of the anion thus formed through expansion of the sulfur octet to a valency of 10. Specific examples of this effect have been noted in the case of methyl vinyl sulfide, whereas the high Q value has been interpreted as signifying an apparent tendency on the part of the R—ĊH—S—CH$_3$ radical to expand the sulfur shell in order to achieve a resonance stabilization (30):

$$\text{R—ĊH—S—CH}_3 \longleftrightarrow \text{R—CH=Ṡ—CH}_3$$

Another example of this is the base catalyzed condensation of a diketone with thiodiglycolic acid to give substituted thiophenes (31).

Chemical evidence in favor of the β elimination mechanism is the fact that only primary and secondary disulfides are observed to give off hydro gen sulfide on attack by alkali. This corresponds with the fact that only primary and secondary disulfides possess hydrogen capable of being attacked. Schöberl, in addition, had noted that tertiary disulfides such as tetramethyl dithiodiglycolic acid are completely stable to alkali. To account for these differences in terms of his sulfenic acid hypothesis, Schöberl

was compelled to postulate that the evolution of H_2S was the exclusive property of primary and secondary sulfenic acids and not that of tertiary sulfenic acids.

primary disulfide $\left[\begin{array}{c} H \\ | \\ R-C-S \\ | \\ H \end{array} \right]_2$

secondary disulfide $\left[\begin{array}{c} H \\ | \\ R-C-S \\ | \\ R \end{array} \right]_2$

tertiary disulfide $\left[\begin{array}{c} R \\ | \\ R-C-S \\ | \\ R \end{array} \right]_2$

The conclusions that can be arrived at regarding the mechanism of disulfide cleavage, aside from thermal or photochemical dissociation of S—S into thiyl radicals which are capable of initiating polymerization, are that there are two distinct mechanisms capable of operating, one ionic and one radical in nature:

(1) Radical Mechanism

This reaction proceeds *via* a direct attack upon the S—S linkage, i.e., a typical radical displacement reaction similar to those observed by Tobolsky (32) and Stockmayer (33) to occur in the case of reaction of free radicals with both linear and cyclic disulfides. Included in this category are such supposed ionic reagents as: sulfite, cyanide, sulfide, and mercaptide. In addition such radical reagents as an active metal with an acid, sodium metal in liquid ammonia, or radicals resulting from growing polymer chains, or arising from the decomposition of radical catalysts, are also capable of undergoing radical displacement reactions on the disulfide bond.

(2) Ionic Mechanism

This mechanism which involves an indirect attack upon the S—S linkage results from a direct nucleophilic displacement on hydrogen by base, or it may be viewed simply as an ionization of an acidic hydrogen in the presence of base to form an anion which is resonance stabilized, one form of which involves an expansion of the sulfur shell to 10 electrons.

$$-\bar{C}H-S-S \longleftrightarrow -CH=\bar{S}-S-$$

This anion then proceeds to react *via* a β elimination to yield a mercaptan and a thioaldehyde. The latter compound in the presence of water readily

decomposes to give H_2S and an aldehyde. It is this mechanism which satisfactorily accounts for the results obtained by Schöberl without requiring the postulation of a sulfenic acid.

We shall now consider the spectral evidence for the dissociation of the β hydrogens, which is the basis for our mechanism for the attack of alkali on the disulfide bond.

III. STRUCTURE OF THE DISULFIDE BOND AND ITS SPECTRAL MANIFESTATIONS

Many of the unique properties associated with sulfur, either in the elemental state or combined with carbon in organic compounds may be interpreted in terms of the orbital distribution of its π electrons. These "uncommitted electrons" are readily polarizable and are easily unpaired when the molecule in which they are contained is introduced into an environment of free electrons or radicals, or when it is activated either thermally or photochemically. Thus, much of the spectral data as well as the chemical reactivity of sulfur compounds, as we shall indicate later, may be correlated on the basis of a consideration of these π electrons.

The angle which the S—S bond makes with an adjacent carbon atom is equal to 104°, as has been calculated from measurements made on such compounds as p,p'-dibromodiphenyl disulfide, dimethyl trisulfide, and elemental sulfur (34). The dihedral angle C—S—S—C as calculated from measurements taken on N,N'-diglycyl cystine has been shown to be 101° (35). This combined evidence suggests that a non-planar, almost right-angle skew distribution of valences about the S—S bond, is energetically preferred. Pauling has shown on the basis of theoretical calculations that repulsion of the π electrons on the sulfur atoms should lead to a chain structure for these molecules with dihedral angles C—S—S—C about 90–100° (36). He thus explained the stability of S_8 as compared to S_6 and S_{10} and other puckered rings by noting that in S_8 the dihedral angle appears to be closest to the optimum value as judged by the literature values available to date.

In compounds in which the bond angles of the disulfide bond deviate from those stated above, it is expected that the S—S linkage will be in a strained condition. These deviations may arise from such factors as steric hindrance of bulky groups situated on either side of the disulfide linkage, ionic interaction of charged groups within the molecule, a tendency on the part of the molecule to gain in total resonance stability, or as a consequence of electronic and angular distortions resulting from the formation of planar rings containing the S—S bond. However, in all cases of structures which have imposed strain there is a general approach to co-planarity of the C—S—S—C unit at the expense of the dihedral which concomitantly approaches zero. The resultant distortion of electronic orbitals should, as one

might predict, manifest itself in a greater ease of electronic perturbility. Thus one would expect that electromagnetic waves of longer wavelength, that is, light of lesser energy will be required to excite the π electrons to an activated state. This is what Calvin (37) essentially observed in his comparative study of the ultraviolet spectra of cyclic disulfide compounds as is shown in Table 1. Qualitative chemical evidence of strain associated with disulfide ring size was obtained by Affleck and Dougherty (38), who noted that the speed of polymerization of cyclic disulfides in the presence of AlCl₃ decreased with an increase in the number of atoms in the ring in the range of 5 to 7 in which they made their observations. Birch (39) had similarly noted that the 5-membered ring was only stable at 0°C. in the dark—in the presence of light it underwent immediate polymerization. Thus we may regard Calvin's data as indicating that the S—S linkage in cyclic disulfides is strainless when it absorbs at the lower wavelength (2500 Å.) and the strain is greater the greater is the wavelength of the absorption maximum.

From the above, it would appear that the relative strain existing in any disulfide might readily be determined by an observation of its ultraviolet spectrum. This, however, is not the case. The conclusions thus far stated apply only to cyclic disulfides of the type examined by Calvin. Many disulfides fail to display any specific ultraviolet absorption and are described as being "highly transparent" in this region. Furthermore some disulfide-containing compounds such as l-cystine have ultraviolet spectra which are highly sensitive to changes in pH. This is probably the reason why there are at least a dozen publications which contest the precise ultraviolet absorption of l-cystine.

In view of all these seemingly inconsistent and uncorrelated data we undertook an intensive study of the ultraviolet spectra of disulfide compounds with the view to answering the following questions:

1. Why is it that not all disulfide-containing compounds show absorption maximum in the ultraviolet?

2. What is the precise nature of the chromophoric group responsible for the ultraviolet absorption?

TABLE 1—ULTRAVIOLET ABSORPTION SPECTRA OF CYCLIC DISULFIDES (37)

Compound	No. of Atoms in Ring	No. of S Atoms	Wavelength, Å., Ultraviolet Max.
6,8 Thioctic acid	5	2	3340
Trimethylene disulfide	5	2	3340
5,8 Thioctic acid	6	2	2860
Tetramethylene disulfide	6	2	2865
4,8 Thioctic acid	7	2	2580
n-Propyl disulfide	Linear compound	2	2500

3. Can the concept of employing ultraviolet absorption to interpret disulfide strain be generalized to include compounds other than cyclic disulfides?

Among the compounds which we studied were *l*-cystine, oxidized glutathione, propyl disulfide, (*t*) butyl disulfide, penicillamine disulfide, dithiodiglycolic acid, dithiodisalicylic acid, dihydroxy dinaphthyl disulfide, dithiodimorpholine, dithiodimalic acid, β,β' diaminodiethyl disulfide and O,O' dibromodibenzyl disulfide. The ultraviolet spectra of these compounds were determined as a function of pH, i.e., examinations were made under acid, neutral, and basic conditions. The conclusions that were arrived at as a result of this investigation may be summarized as follows:

1. All disulfide-containing compounds, regardless of whether or not they showed an absorption or transparency under conditions of examination employed by previous workers, revealed absorption maxima either under alkaline or acid conditions. This generalization does not apply to tertiary disulfides.

2. This behavior of disulfide in alkali or acid media permits a general classification of disulfides to be made. First, there are those compounds such as the tertiary disulfides that give a non-specific absorption which is independent of pH. The remaining disulfide compounds will show absorption maxima either in acid or in alkali media.

3. The extinction coefficients of the observed maxima are continuous functions of pH. That is, if the maximum occurs in acidic medium—increased acidity will enhance the absorption. Likewise if the maximum occurs in alkali medium above a critical pH, the absorption is enhanced with increasing alkali.

4. The absorption spectra of those disulfides which show a response to changes in pH, exhibit three distinct phases: (a) non-specific absorption, (b) shoulders or inflections, and (c) absorption maximum. Phase "b" spectrum is an artifact in the sense that it can be shown to arise as a composite of the spectra of phase "a" and "c". Therefore there are essentially only two types of SS absorption—a non-specific and a specific absorption.

An interpretation of these data can be made if one assumes that it is

$$\overset{\displaystyle R}{\underset{\displaystyle |}{}}$$

doubly bonded sulfur =S— such as —C=S— which is behaving as the ultraviolet chromophore. Compounds such as tetra methyl thiourea which possess the —C=S group absorb at 250 mμ—coincident with the region in which simple unstrained disulfides such as *n*-propyl disulfide are observed to

$$\overset{\displaystyle R}{\underset{\displaystyle |}{}}$$

absorb. Implicit in the postulation of the —C=S— chromophore in disulfides is the assumption that some positive group such as a proton has

migrated from the carbon atom adjacent to the sulfur. The consequence of this step is to leave the carbon with an electron pair, i.e., a carbanion, with which it can form a double bond with the sulfur atom. This assumption seems justified in view of the fact that tertiary disulfides which have no hydrogens on the carbon joined to the sulfur only show non-specific ultraviolet absorption. Furthermore, compounds which show absorption maxima in alkali show only non-specific absorption identical with that of tertiary disulfides, when placed in acid medium.

It is interesting to note that regardless of whether alkali or acid is responsible for the generation of the ultraviolet absorption maximum of the disulfide compound, the absorption maximum always occurs in the region of 330 mμ. This is precisely the wavelength that Calvin has found to correspond to the absorption maxima of the very highly strained five-membered disulfide rings found in 6,8 thioctic acid and trimethylene disulfide. It can be shown from an analysis of all the possible structures in which these compounds can exist that the chromophore responsible for absorption at 330 mμ is the planar conjugated —C=S—S=C— grouping. Aromatic disulfide compounds readily assume this configuration (some in acid, others in alkali) as a consequence of an enhancement of resonance stabilization which results from an assumption of such a structure. Thus the energy required to deform the dihedral angle is more than compensated for by the gain in resonance stabilization of the final structure. Aliphatic disulfides having hydrogen atoms attached to the carbon which is joined to sulfur (these shall henceforth be designated as β hydrogens in conformity with our postulated β elimination mechanism) also show this absorption. This occurs only in very strong alkali and increases with increasing time. These hydrogens being less acidic are more reluctant to leave the carbon atom to which they are joined. In Calvin's strained cyclic 5-membered disulfide rings the β hydrogens are so acidic that they ionize readily in the presence of solvent to give the planar conjugated —C=S—S=C— with a dihedral angle approaching that of zero. The planar ring is apparently less strained than the 5-membered ring containing the 90° dihedral angle. It is of interest to recall that the essential feature of our postulation of the —C=S— group as being the fundamental ultraviolet chromophore is that we were required to assume that the loss of a proton occurred and that the resulting carbanion with its free electron pair was capable of donating the electron pair to sulfur to form the doubly bonded sulfur structure —C=S—.

According to independent views of Kimball (40) and Rothstein (41) theoretically, an electron pair may be donated to one of the unfilled 3d orbitals of the sulfur atom. It is therefore possible that molecules in which negatively charged sulfur atoms occur make definite contributions to the excited state.

What we are essentially measuring in our ultraviolet spectra studies of disulfide compounds is an acid-base phenomenon

$$BH \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B^- + H^+$$

In particular, in non-aromatic disulfides the dissociation in question is the following:

$$R-CH_2-S- + OH^- \rightleftharpoons R-\overset{-}{C}H-S- + HOH$$

$$\updownarrow$$

$$R-\overset{|}{C}H{=}S-$$

The resonance form involving doubly bonded sulfur is the ultraviolet chromophore. Here sulfur has taken an electron pair and expanded its octet to include the "d" orbitals. In this case a weak acid is dissociating at a very slow rate to yield a proton and an anion. The rate at which this process occurs was followed spectrophotometrically by measuring the increase in anion formation with time. Addition of large increments of base speeds up the attainment of equilibrium. In this manner we explain the series of curves obtained by the addition of alkali in increasing amounts to compounds such as dihydroxydinaphthyl disulfide, and dithiodiglycolic acid. A critical lower limit appears to exist with regard to the minimal amount of alkali that is required to produce a change in spectrum. This change is a change from the non-specific absorption spectra to the specific absorption spectra. The non-specific spectra correspond to the undissociated acid form, the specific spectra to that of the base or anion form.

With this as a background we feel we can answer the question as to why strain in cyclic disulfides manifests itself in the form of varying ultraviolet absorption maxima. With increasing strain there is a parallel increase in the tendency of the β hydrogens to ionize off in the presence of solvent leaving the base or anion form which is the ultraviolet chromophore. The pos-

TABLE 2—POSSIBLE ULTRAVIOLET DISULFIDE CHROMOPHORES

Acid Form	(Chromophore) Basic Form	Ultraviolet Absorption Max., Å.	Example Compound
—CH$_2$—S—	—CH=S—	2500	n-Propyl disulfide
—CH$_2$—S—S—CH$_2$	—CH=S—S=CH—	2800	Tetra methylene disulfide
—CH$_2$—S—S—CH$_2$—	Non-conjugated due to presence of the dihedral angle		
	—CH=S—S=CH—	3300	Trimethylene disulfide
	Conjugated, dihedral angle absent		

sibility of the C—S—S—C group to assume a linear conjugated structure of the form

$$-\overset{\underset{\displaystyle |}{H}}{C}=S-S=\overset{\underset{\displaystyle |}{H}}{C}-$$

would relieve the strain which is imposed on the member atoms of the cyclic disulfide as a consequence of the existence of the dihedral angle. The planar structure thus formed is capable of resonance stabilization. In this manner Calvin's empirical calibration of the ultraviolet spectra of cyclic disulfides may be rationally interpreted. In Table 2 are summarized the possible ultraviolet chromophores which could exist in strained and unstrained disulfides. This concept would explain why dimethyl disulfide gives an absorption maximum in the region of 2500 Å. whereas dithiodiglycolic acid under the same conditions shows a non-specific absorption spectra in the ultraviolet. If we recall that it is the ionization of a β hydrogen which permits a double-bonded sulfur to occur then both compounds should possess a finite rate at which hydrogen is ionized to yield the chromophore. However, in the case of dithiodiglycolic acid the presence of a stronger acid group within the molecule, namely the carboxyl group which itself dissociates to yield protons, effectively inhibits the β hydrogen dissociation so that no chromophoric absorption is observed. However, in the presence of strong alkali, dissociation of the β hydrogen is favored and a definite absorption maximum is observed.

IV. STRUCTURE OF CYSTINE

The amino acid cystine can be characterized by being termed "anomalous." Despite the fact that this was the first amino acid to be discovered, elucidation of its chemical structure required nearly a century before being positively established by independent synthesis. The many anomalous properties of cystine undoubtedly contributed to this delay. First of all, this amino acid is notoriously insoluble; it is soluble only to the extent of about 20 mg. per cent in water, and is very soluble only in either strong acids or alkali. It differs again from other amino acids in regard to the marked salting-in effect produced by the addition of certain selected salts such as calcium chloride (42). Another anomalous property of cystine is its extreme acidity; its first acid dissociation constant is comparable to the acidity of strong inorganic acids and it is about 10,000 times stronger than acetic acid.

The e.m.f. of the mercaptan-disulfide system *l*-cysteine-*l*-cystine does not conform to the standard Nernst relationship, but agrees well with a modified expression of the following type:

$$E_h = E_0 - \frac{RT}{F}\, pH - \frac{RT}{F}\, \ln\, [RSH]$$

in which the resulting e.m.f. is independent of the disulfide concentration (43).

A further remarkable property of cystine is the high value of its specific optical rotation, namely 220°. Most amino acids by comparison possess optical rotations of the order of 8 to 15° (44). Toennies and Lavine (45) have shown in the case of cystine, that whereas in the isoelectric region of pH 3 to 7, the optical rotation is constant and maximal, in strong acid the rotation drops slightly and in alkaline medium this drop in rotation is precipitous. One may also add to this list of singular attributes the fact that the disulfide linkage in cystine is quite resistant to most chemical attack in comparison with cystine combined in proteins. Furthermore, simple esters of cystine spontaneously decompose to an entire series of degradation products (46).

In view of these facts we have sought to interpret these anomalies of cystine in terms of a unique structure. Employment of molecular models of both the Fischer-Herschfelder and Catalin types indicate that the optically active cystine molecule in all likelihood exists as an approximate tridentate pincer or "closed-claw"-like structure. A skeletal molecular model of the cystine molecule revealed that the entire structure appeared to assume the form of a helix. This structure satisfactorily accounts for all the wealth of apparently unrelated data concerning cystine to be found in the literature.

The incentive to look for a unique structure in the case of cystine was provided by the evidence that lanthionine, which differs from cystine only in that one of the sulfurs is removed, and djenkolic acid which only differs in the respect that a methylene (CH_2) is introduced between the two sulfur atoms, both show lower and more normal values for the optical rotation. Yet it is not the presence of the asymmetric carbons nor the disulfide linkage alone that is responsible for making cystine unique. Penicillamine disulfide, which has the S—S linkage as well as the same asymmetric centers, and which differs from cystine only in that the β hydrogens are replaced by methyl groups (see Table 2) possesses the expected value for its optical rotation, namely 23°. Our models of penicillamine disulfide also revealed that the presence of bulky methyl groups in place of hydrogen so restricted the rotation about the C—S and S—S bonds that the "closed-claw" structure is prohibited. Instead, this resulting structure appears to assume a linear configuration in which the two amino acid groups are maintained rigidly apart by a collar of methyl groups. Further indication in favor of the unique structural configuration of d or l-cystine is that a wide number of enzymes which will attack either the amino or disulfide linkage in cystine will be ineffective against penicillamine disulfide (47).

As we have indicated in Section III, the restricted rotation in cystine is of the order of at least 13–15 kcal., and this favors the formation of the clawlike structure. In penicillamine disulfide the barrier to rotation about the S—S bond is so large as to probably approach the order of the bond strength

of C—S bond namely 45 kcal. It is indeed this finite restricted rotation in cystine which contributes to the formation and stability of the tridentate pincer that we have postulated.

A summary of the conditions responsible for the unique structure of cystine are the following:

1. There is a fairly high degree of restricted rotation about the S—S bond of the order of 15 kcal., resulting from the requirement of a 90–100° dihedral angle.

2. There are two identical asymmetric carbons which comprise the amino acid groups.

3. The amino acid groups exist in the zwitter ion form.

4. The amino acid groups are separated from the S—S bond by a methylene group.

The consequence of these four essential conditions is that cystine in crystalline form as well as in solution in the isoelectric regions exists as a rigid closed pincer structure. The pincer itself forms as a result of the specific bond angles and van der Waal radii of sulfur and carbon which

TABLE 3—OPTICAL ROTATION OF AMINO ACIDS CONTAINING SULFUR

Compound	Formula	Specific Optical Rotation
l-Cysteine	$HOOC$—$\overset{H}{\underset{NH_2}{C}}$—$CH_2$—$SH$	−10.1°
l-Methionine	$HOOC$—$\overset{H}{\underset{NH_2}{C}}$—$CH_2$—$S$—$CH_3$	+21.2°
l-Cystine	$\left[HOOC-\overset{H}{\underset{NH_2}{C}}-CH_2-S\right]_2$	−220°
l-Penicillamine disulfide	$\left[HOOC-\overset{H}{\underset{NH_2}{C}}-\overset{CH_3}{\underset{CH_3}{C}}-S\right]_2$	−23.8°
l-Djenkolic acid	$HOOC-\overset{H}{\underset{NH_2}{C}}-CH_2-S-CH_2-S-CH_2-\overset{H}{\underset{NH_2}{C}}-COOH$	−44.5°
l-Lanthionine	$HOOC-\overset{H}{\underset{NH_2}{C}}-CH_2-S-CH_2-\overset{H}{\underset{NH_2}{C}}-COOH$	+ 8.0°
l-Cystathionine	$HOOC-\overset{H}{\underset{NH_2}{C}}-CH_2-CH_2-S-CH_2-\overset{H}{\underset{NH_2}{C}}-COOH$	+23.7°
l-Homocystine	$\left[HOOC-\overset{H}{\underset{NH_2}{C}}-CH_2-CH_2-S\right]_2$	+77.0°

force the zwitter ion amino acid groups into close proximity of each other. This resulting rigid structure is essentially fixed in place by the restrained rotation about the S—S bond, the ionic interaction between NH_3^+ and COO^- ions as well as possible hydrogen bond formation between NH_3^+ and the π electrons on sulfur.

Skeletal molecular models of cystine reveal that the molecule as a whole exists in the form of a helix, as mentioned previously. A helical structure would be expected to rotate the plane of polarized light. As such, one should accordingly expect such a structure to possess optical activity. Thus the high optical rotation of cystine would presumably result from the combined contribution of the two asymmetric carbons and that produced by the helix itself. The observed optical rotation of quartz, which possesses no intrinsic asymmetric elements, is believed to be the result of a helical configuration of the molecules within the crystal lattice. It must be noted that in the case of cystine the helical structure is a function of the charge interaction of NH_3^+ and COO^- groups and as such is sensitive to changes in pH. In alkali, the positive charges are removed and the helix destroyed. As a consequence one might well predict that the optical rotation of cystine should radically drop on addition of alkali. Such an effect was indeed noted by Toennies and Lavine (45). Normally one would not expect the optical rotation of a compound to vary so markedly with pH, if at all. That the extremely high optical rotation of cystine is largely dependent upon helix formation, rather than as the consequence of the presence of the two asymmetric amino acid groups, may be judged from a consideration of the data in Table 3.

The order of magnitude of the optical rotation of simple amino acids has been observed to be ±7 to $\pm15°$ (48). L-cysteine, for example, has a rotation of $+11°$. If one visualized the union of two cysteine molecules by removal of the hydrogen on the sulfur atoms and if optical activities were additive one might predict that in the absence of any interaction the resulting optical rotation might be of the order of 22°. However, as is known, cystine has an optical rotation of 220°. If in cystine now one replaces the four β hydrogens with four bulky methyl groups the resulting compound, penicillamine disulfide, has none of the anomalous properties of cystine. Unlike cystine, penicillamine disulfide is very soluble in water and possesses an optical rotation of 23° (49). This is the value one would theoretically predict as being equal to the summation of the optical rotations of two equivalent isolated amino acid centers. Thus it would appear in the case of penicillamine disulfide that the presence of four methyl groups within the cystine molecule has effectively erected a barrier whose end result is to completely isolate one amino acid group from any interaction that it might exert upon the other. The presence of this barrier precludes any helical arrangement of the atoms within the molecule. Models reveal that in

this case a helical configuration is impossible and that the only possible configuration under these circumstances is a linear one in which the amino acid groups are as far apart as possible. These facts would satisfactorily account for the normal solubility and normal optical rotation observed for penicillamine disulfide. The marked loss in optical rotatory power involving a change from 220 to 22°, a decrease of 90 per cent, resulting from modifying cystine to penicillamine disulfide, enables one to assess the relative contribution that the helical structure itself contributes to the total optical rotation of cystine.

Another example which may be cited to illustrate the predominant importance of the helical configuration as contributing to the high optical rotatory ability of cystine, may be seen in a comparison with the amino acid cystathionine. Here the structure of cystine is modified such that a methylene group CH_2 replaces one of the sulfur atoms. The resulting structure lacks the disulfide linkage and is composed of two identical amino acid groups separated by four atoms consisting of three carbons and one sulfur. Here, too, the helical structure is lacking, and here too, the optical rotation has fallen from 220° to a value of 22°.

An examination of a skeletal molecular model of cystine reveals that three ionic groups (2 carboxylate and one protonated amino groups) form the prongs of the pincer. These lie within a plane forming the apices of a small triangle. External to this triangle and at a considerable distance away is the fourth group (an amino group) of the zwitter ion pair. A postulated structure of this nature satisfactorily explains the anomalous acidic and basic group dissociation constants of cystine, namely: $pK_1 < 1.00$, pK_2 1.7, pK_3 7.48, pK_4 9.02. It will be noted here immediately that two of these constants are values which are considered to be "normal" for amino acids and two of them are distinctly abnormal. Let us consider the abnormal dissociation constant pK_1 which is reported by several workers to be less than unity. This fact is indicative of a very strong acid whose dissociation is so great as to be incapable of any precise measurement—approximately the strength of inorganic acids. The positively charged atmosphere in the vicinity of the carboxylate site is so great as to render it almost impossible for a proton to approach this site and hence the pK is very low.

The pK_3 of one of the amino groups is also extremely low, that is, it is somewhat reluctant to acquire a proton. This would imply that the protonated amine is in a vicinity of negative charges which would be most conducive to the release of an acquired proton.

V. Disulfide Cleavage and the Chemistry of Hair Waving

Let us now consider some of the factors operative in disulfide cleavage. It is obvious that one of the factors is the tendency to release of strain im-

posed upon a disulfide bond. Prior to our ultraviolet spectral interpretation of disulfide strain there were two methods employed to measure this strain; at best these methods were little more than qualitative. The "alkali lability" method used by Clarke (50), Schöberl (51), and others, is based on the time required to produce a precipitate of lead sulfide when a sulfur containing compound is treated with an alkali solution of a soluble lead salt.

A second method employed by Affleck and Dougherty (52) in the case of cyclic disulfide was to observe the apparent ease with which polymerization of the disulfide compound occurred in the presence of $AlCl_3$. In our method not only have we been able to estimate the disulfide strain from ultraviolet spectra analysis, but we have been able to quantitatively interpret the spectra in terms of the specific chromophores responsible for the disulfide absorption in the ultraviolet. We have shown that the categorical assertion that alkali of itself cleaves the S—S bond is not necessarily true. Many tertiary disulfides are stable in alkali. Actually ultraviolet spectra do not reveal the strain existing in a disulfide bond as such, but rather they reveal the presence of a chromophore. This chromophore is produced as a consequence of the desire on the part of the strained structure to assume a non-strained configuration. The structure invariably assumed by strained disulfides is the conjugated linear form —C≡S— S≡C—. This particular chromophoric form will occur in either acid or alkali and depends solely on the structure of the disulfide compound in question.

In addition to the apparent strain imposed on the disulfide linkage by pH or structural distortion of the sulfur bond angles, there is another factor affecting S—S cleavage, namely, the steric factor. Here we will attempt to distinguish between internal and external steric factors. What we imply by an internal steric factor may be understood by the following consideration. When large or numerous bulky groups are present on both sides of an S—S linkage, these groups will serve to barricade the disulfide against attack by various disulfide-specific reagents. In the case of penicillamine disulfide, reagents which will normally cleave cystine such as sulfite, cyanide, etc., are now ineffective. This we interpret in terms of the presence of the four methyl groups. Schöberl (53) has similarly indicated in the cases of tetra methyl and tetra phenyl dithiodiglycolic acids, that these compounds are not attacked by reagents that ordinarily cleave S—S. Arnold (54) in addition, has reported that tertiary butyl disulfide is extremely difficult to reduce. In all instances cited here bulky groups within the molecule are preventing an attack upon the disulfide group.

The external steric factor in connection with disulfide cleavage is only manifest in three-dimensional structures such as proteins. The proteins, keratin, cortocin, and insulin, have the common feature that all contain

about 13 per cent *l*-cystine in addition to qualitatively possessing the same amino acids. These proteins differ in the sequence in which the amino acids occur along the peptide chains. The proteins most likely differ in the identity of the amino acid joined to the cystine peptide link at the site of the disulfide cross link. In the case of insulin, Sanger (5) has shown that one important sequence involving cystine is the tri-peptide unit—glycine-cystine-alanine-. In the case of keratin the large amounts of dicarboxylic acids present as aspartic and glutamic acids lead us to believe that in a good number of chains several of these dicarboxylic acids are joined to cystine at the site of the crosslink. There occurs in nature a tripeptide, glutathione, which consists of glutamyl-cystyl-glycine.

It has been established by Phillips and co-workers (55) that about 25 per cent of the combined cystine in wool keratin is resistant to the attack of chemical reagents such as sulfite, alkali, cyanide, permanganate, etc. This resistant portion has been designated as the D fraction. Reed and others (56) have reported that commercial alkaline thioglycolate solutions effectively reduce only about 75 per cent of the cystine present in hair. The remaining resistant fraction seems to correspond to the D fraction observed in wool. Studies of peptides derived from wool hydrolyzates reveal that there is a juxtaposition of glutamic and cystine residues (57). The presence of the bulky side chain group of the former amino acid residue may well serve as an effective barrier to chemical attack upon the disulfide bond. Benesch and Benesch (58) attribute the greater resistance of glutathione to oxidation as compared with cysteine as being due to the glutamyl residue with which the sulfur can hydrogen bond. If this proves to be the case then certain disulfide linkages in hair would be shielded by this external steric effect and hence may account for the D fraction.

Aside from the academic interest in the external barrier effect, what are the practical consequences of this phenomenon? It might well be that the essential success of the hair waving process is due largely to these few resistant bonds which make up the D fraction. These resistant disulfide linkages serve to maintain the skeletal structure of the hair when it is treated with reagents that cleave the other disulfide links. If these resistant links were not present, application of a reducing agent would then cleave all crosslinks and thus produce a material capable of undergoing plastic flow. Hence the hair will have lost its fiber properties. Severely damaged hair may well be characterized by a destruction of a good number of these resistant groups.

It may now be seen that the danger inherent in employment of too strong a thioglycolate solution or too long a processing time is that all crosslinking disulfide bonds will be broken, yielding a plastic non-fibrous material. It is thus not necessary and, in fact, potentially detrimental to cleave all S—S bonds. Just enough bonds of the non-resistant, non-skeletally important

type should be cleaved so as to make the hair pliable and permit it to as-
sume the shape of the curler.   This essentially is the advantage of using a
more controllable reducing agent, such as the mercaptan employed in cold
waving, rather than the harsh reducing agents such as sodium meta bi-
sulfite which cleave SS bonds indiscriminately.   Even partial reduction of
resistant SS bonds accompanied by parallel chain stoppage or increased
lateral chain separation raises the unhappy specter that an isolated partly
shielded mercaptan group may be formed within the hair.   In the subse-
quent oxidation step customarily employed to convert the mercaptan to a
disulfide crosslink either of the two following situations may arise:

   1.   The W—SH group finds itself as an isolated entity incapable of re-
linking with any neighboring mercaptan group.   Subsequent attack by a
strong oxidizing agent will succeed in oxidizing this mercaptan to combined
cysteic acid.   As a result the hair as a fiber is now intrinsically that much
weaker for having lost a formerly resistant disulfide linkage, or

   2.   The W—SH group might be too isolated to allow for its immediate
oxidation.   In such a case the remaining more favorably situated mercap-
tan groups are converted to disulfide links and this isolated mercaptan is
sealed into the interior of the hair.   With the passage of time and the
continual kinetic chain slippage and folding, this mercaptan group will find
itself within the immediate proximity of a disulfide crosslinkage.

   At such time the distinct possibility of disulfide-mercaptan interchange
arises.   Owing to the ease with which this interchange (59) can occur, this
isolated mercaptan will be capable of acting as an "internal plasticizer,"
resulting in a slow exchange of S—S and SH bonds along the protein chain
and producing stress relaxation or loss of the permanent wave.   This effect
is similar to what one observes when thiokol rubber under stress is subjected
to mercaptan vapor (60).   duVigneaud (61) has suggested that a similar
mechanism may be responsible for the progressive gradual inactivation of
insulin which occurs on the addition of small amounts of cystine to a solu-
tion of this protein.

   On the basis of the results of our "test tube" experiments we cannot, of
course, explain all the subtilities of the hair waving process.   What we
have tried to show in the last section of this article, however, is how infor-
mation obtained from these new studies on disulfide cleavage can be used
to explain the over-all chemistry of the hair waving process.   We hope that
our research may help to rationalize the empirical chemical procedures
which are now employed in hair waving and to suggest new and more effec-
tive methods for the waving of hair.

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