Sulfhydryl-Disulfide Interchange

This biological chain reaction explains aspects of protein denaturation, blood clotting, and mitosis.

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Recently it has become evident that a variety of phenomena involving proteins and peptides possess certain rather unusual features consistent with the assumption that existing disulfide bonds have been disrupted and new disulfide bonds have formed. Unlike the previously known cases of disulfide modification in proteins by reduction of these bonds to sulfhydryl groups followed by reoxidation of the latter to new disulfide linkages, the more subtle disulfide rearrangements here described take place in the absence of added reducing and oxidizing agents and appear to be chain-type reactions initiated in most cases by very small amounts of sulfhydryl compounds. Under appropriate conditions, the initiating sulfhydryl reacts with a disulfide group to form a new disulfide linkage, at the same time generating a new sulfhydryl group capable of reiterating the process (Fig. 1). Thus, a single sulfhydryl initiator can bring about the reaction of a large number of disulfide groups, and the occurrence of such processes, often under rather mild conditions, can exert far-reaching effects.

This article summarizes the reported examples of such sulfhydryl-disulfide interchange phenomena. Although most of these observations are concerned with in vitro transformations of proteins and peptides, it now appears that similar reactions are involved in certain physiological processes as well. It is hoped that the concept of a sulfhydryl-disulfide chain reaction may prove of value in elucidating the mechanisms of still other biological phenomena.

Protein Reactions

Most observations of sulfhydryl-disulfide interchange reactions have been concerned with phenomena accompanying protein denaturation, chiefly those of aggregation. The first indication that protein sulfhydryl groups play a role in the aggregation of denatured protein came from observations of the remarkable influence of the single sulfhydryl group of bovine plasma albumin on the nature of the clot or coagulum formed when solutions of this protein are heated (*1*). Clots formed by the thermal denaturation of ordinary bovine plasma albumin at neutral *p*H are opaque, friable, and synerizing, whereas those formed from albumin which has had its sulfhydryl group destroyed or blocked by treatment with an appropriate "sulfhydryl reagent" are transparent, firm, and nonsynerizing. Moreover, in the absence of the sulfhydryl group, solid gels are formed in solutions of much lower albumin concentration than when the sulfhydryl group is present. On the basis of existing concepts of gel structure (*2*), it was concluded that in the coagulum formed from sulfhydryl-containing albumin, the denatured protein chains lie in close side-by-side association and that the albumin sulfhydryl group must in some way bring about this type of aggregation (*1*).

The manner in which a sulfhydryl group is able to promote cross linking of protein molecules became apparent during subsequent experiments on the denaturation of proteins by urea (*3*). The previously known property of proteins such as plasma albumin, fibrinogen, γ-globulin, and egg albumin to form clear firm gels when exposed to concentrated urea (*4*) was shown to depend on the presence of small amounts of protein sulfhydryl groups. Gelation in urea or in guanidine hydrochloride is favored by increased *p*H, inhibited by oxygen, eliminated by blockage of protein sulfhydryl groups, and restored to sulfhydryl-free protein by the addition of trace amounts either of sulfhydryl-containing proteins or of simple mercaptans. Since the observed phenomena appear to involve a stoichiometry quite different from that usually encountered in protein reactions, it was proposed that, under the conditions of protein denaturation, the sulfhydryl group initiates a chain reaction with disulfide groups in the manner illustrated in Fig. 1, leading to a regular three-dimensional gel network in the case of urea denaturation (and to side-by-side association of protein molecules in the case of thermal denaturation where no urea molecules are present to hold the protein units apart).

Subsequent measurements (*5*) of viscosity changes in more dilute bovine plasma albumin solutions clearly demonstrated that, in addition to the large immediate increase in viscosity following exposure of the protein to concentrated urea, there is a further gradual sulfhydryl-dependent viscosity rise which is influenced by the same factors as is the gelation in more concentrated albumin solutions, and which thus reflects the disulfide interchange reaction. Similarly, in the case of thermal denaturation, the results of viscosity and sedimentation measurements on dilute albumin solutions, as well as the effect of traces of mercaptans on the thermal coagulation of iodoacetamide-treated albumin, furnish strong support for the concept that lateral association by sulfhydryl-initiated disulfide interchange takes place when albumin solutions are heated (*6*).

These considerations of a chain-type sulfhydryl-disulfide interchange reaction operating during conditions of protein denaturation have been confirmed and extended by a number of investigators. As part of an extensive study of the effect of urea denaturation on the viscosity and optical rotation of protein solutions, Kauzmann and his collaborators (*7*) have shown that, with both ovalbumin and bovine plasma albumin, an exchange reaction between sulfhydryl and disulfide groups is an important cause of aggregation during denaturation, especially at *p*H values above neutrality. Subsequent investigations of solubility changes during the course of urea denaturation of bovine plasma albumin permitted Kauzmann and Douglas (*8*) to distinguish between intramolecular disulfide exchange, which diminishes protein solubility only slightly, and intermolecular disulfide exchange leading to aggregation and a larger decrease in solubility. From the

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ability of reducing agents to decrease the light scattering of solutions of albumin which had been denatured by heat, shaking, or exposure to ethanol or urea, Halwer (*9*) concluded that the denatured protein is cross linked, at least partially, by intermolecular disulfide bonds, this cross linking being most pronounced in the case of the alcohol-treated protein.

From the effect of *p*H and of sulfhydryl reagents on the molecular weights of proteins in 7*M* urea, McKenzie, Smith, and Wake (*10*) concluded that, with bovine plasma albumin, the only cause of aggregation is sulfhydryl-disulfide interchange, which occurs under alkaline but not under acid conditions. With ovalbumin, aggregation appears to take place both by disulfide exchange and by hydrogen bonding, the latter being somewhat more important. Kolthoff and his coworkers (*11*) have studied viscosity changes of dilute bovine plasma albumin solutions after treatment with guanidine hydrochloride, and, in agreement with previously mentioned findings with urea, they have observed a gradual prolonged increase in viscosity, resulting from protein aggregation through sulfhydryl-disulfide interchange. After prolonged exposure of bovine plasma albumin to guanidine hydrochloride, the same authors observed a decrease in the reactivity of the protein sulfhydryl group toward silver or mercuric ions, which they attribute to the production, by sulfhydryl-disulfide interchange, of a sulfhydryl group less accessible to silver or mercury than the original one. Toward ferricyanide, the reactivity relationship is reversed; Kolthoff and Anastasi (*12*) have shown that the original sulfhydryl group in native bovine plasma albumin is not oxidizable by ferricyanide, but that exposure to concentrated urea or guanidine hydrochloride produces a new sulfhydryl group which, after removal of the denaturing agent, can be oxidized by ferricyanide to form a protein dimer.

A number of observations confirm the occurrence of disulfide interchange during thermal denaturation of proteins. The effect of oxidizing and reducing agents on the viscosity and turbidity of heated β-lactoglobulin solutions led Zittle and DellaMonica (*13*) to conclude that the protein sulfhydryl groups promote lateral association of peptide chains, with resulting opacity, although the effect is less pronounced than in the previously mentioned case of bovine plasma albumin (*1, 6*). From the influence of sulfhydryl reagents on the sedimentation behavior of thermally denatured bovine plasma albumin and on its solubility in 83-percent acetic acid, Steinrauf and Dandliker (*14*) have shown that during the first 30 seconds at 100°C and *p*H 5.6, intramolecular rearrangement of hydrogen bonds takes place, after which a rapid intermolecular disulfide interchange commences, leading to polymerization. Somewhat similar conclusions were reached by Warner and Levy (*15*) working at the lower temperature of 65.7°C. Kinetic investigations, involving sedimentation studies of the intermediate products formed in this mild thermal denaturation of bovine plasma albumin in the presence and absence of sulfhydryl reagents, indicate that the initial reaction is an intramolecular sulfhydryl-disulfide interchange and that aggregation takes place subsequently, in part by an exchange mechanism of an intermolecular type.

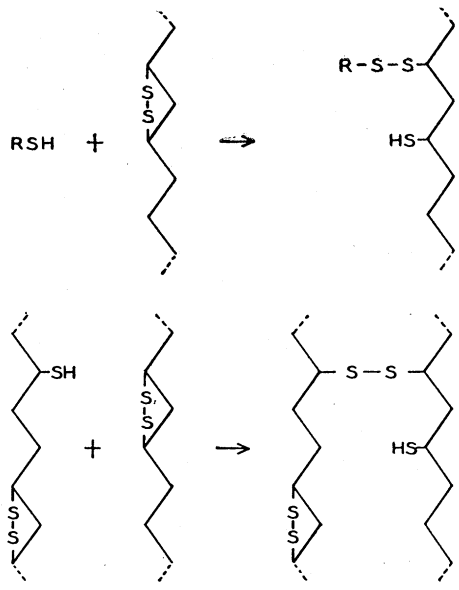

Fig. 1. Sulfhydryl-disulfide interchange in proteins.

Apparently, precipitation of a protein with alcohol is an especially favorable condition for disulfide interchange. In addition to the previously mentioned experiments with ovalbumin (*9*), Straessle (*16*) has reported that treatment of human plasma mercaptalbumin with cold aqueous ethanol of more than 60 percent alcohol content by volume can cause partial conversion of the protein to a dimer, a reaction which does not occur with iodoacetamide-treated albumin under similar conditions. This sulfhydryl-initiated cross-linking reaction provides an explanation for the earlier observation of Cohn, Hughes, and Weare (*17*) that aggregation of protein takes place during the methanol extraction of lipids from albumin. Recently, Rouser (*18*) has reported that nonprotein sulfhydryl compounds in plasma exert a marked influence on the alcohol precipitation of the protein. On the addition of ethanol, plasma from normal individuals gives a coarse granular precipitate, whereas plasma low in cysteine, obtained from chronic lymphatic leukemia patients, as well as solutions of commercial human plasma albumin, give a fine, difficult-to-filter precipitate which is slowly transformed to the coarse granular type upon addition of small amounts of cysteine.

The sulfhydryl-initiated transformation of intramolecular disulfide bonds to intermolecular linkages with accompanying gelation has been clearly demonstrated by Benesch and Benesch (*19*), who used a model protein, thiolated gelatin. This substance is prepared by treatment of gelatin, which itself contains no sulfhydryl or disulfide groups, with N-acetylhomocysteine thiolactone. Oxidation of the thiolated protein with ferricyanide in dilute solution gives a protein with intramolecular disulfide linkages, as indicated by the lack of change in its viscosity and sedimentation characteristics on oxidation. Treatment of a solution of the oxidized protein with a trace of mercaptoethylamine causes an immediate transformation into a firm, heat-stable gel similar to that produced by oxidation of the thiolated gelatin in concentrated solution where intermolecular disulfide bonds are formed directly.

In a practical application of sulfhydryl-disulfide interchange, Arnold (*20*) has cross linked a monomolecular layer of fibrinogen to afford a semipermeable protein membrane for use in model cell membrane studies. A thin layer of fibrinogen in saline is floated carefully on an aqueous surface, and the addition of cysteine to the water effects two-dimensional cross linking of the protein to form a stable film.

A somewhat different protein phenomenon considered to involve sulfhydryl-disulfide interchange is the long-range elasticity of wool. When a wool fiber is stretched in water by an amount greater than about 30 percent, irreversible structural alterations take place which have been postulated to result from the rupture of disulfide linkages (*21*). Since the sulfhydryl content of wool was found to be unaltered by the stretching process, Burley (*22*) has sug-

gested that stretching brings a sulfhydryl group into contact with a disulfide group whereupon interchange takes place to form a new disulfide linkage in a manner which relieves the mechanical strain. In support of this exchange mechanism, Burley observed that wool fibers with their sulfhydryl groups blocked by previous reaction with iodoacetamide or N-ethylmaleimide stretch at a much slower rate and to a lesser extent than do untreated, sulfhydryl-containing fibers under the same conditions. Moreover, untreated fibers which stretch readily in water are resistant to stretching in 0.1*N* hydrochloric acid, an environment unfavorable for the sulfhydryl-disulfide reaction.

Klotz and his coworkers (*23*) have studied the interaction between the sulfhydryl group of bovine plasma albumin and the disulfide-containing dyestuff 2, 2′-(2-hydroxy-6-sulfonaphthyl-1-azo)-diphenyl disulfide. In this case, only the first stage of the sulfhydryl-disulfide chain reaction appears to take place. To explain the stoichiometry observed, the authors propose a novel type of long-range intramolecular sulfhydryl-disulfide interchange in the albumin molecule, which involves electron transport by way of the hydration lattice of the protein.

Two examples of reversible protein aggregation in the absence of denaturing agents have been ascribed to sulfhydryl-disulfide interchange. These are the association of soluble feather keratin, which takes place as the protein concentration is increased (*24*), and the dimerization of bovine plasma albumin, which occurs when the *p*H is lowered to 3.4 or below (*25*). These phenomena differ from those previously described not only in that they are manifest in the absence of conditions which disrupt the protein structure but also in that the aggregation appears to be freely reversible by such relatively mild manipulations as dilution or raising of the *p*H to neutrality. Moreover, in the case of bovine plasma albumin, the aggregation is observed in a *p*H region where sulfhydryl-initiated disulfide interchange ordinarily does not occur. Although these examples may represent rather special cases of disulfide exchange, it would seem advisable to consider the alternative possibility that some other type of sulfhydryl-dependent aggregation may be involved.

It is now established that sulfhydryl groups can contribute to the association of protein units in ways other than through disulfide bonds. In addition to the well-known dimerization of albumin by reaction of sulfhydryl groups with divalent mercury (*26*), it appears that the sulfhydryl group itself can interact with some other protein group or groups to form a stable but reversible linkage. Participation of sulfhydryl in a thiazoline structure, first proposed by Linderstrøm-Lang and Jacobsen (*27*), has been demonstrated by Calvin (*28*) to exist under certain conditions in the peptide glutathione. Madsen and Cori (*29*) have observed a reversible, sulfhydryl-dependent aggregation of the enzyme phosphorylase. Deutsch and Morton (*30*) have reported an association of human serum macroglobulin units which appears to involve sulfur-containing groups, and Lorand (*31*) has described a somewhat similar aggregation for the fibrin stabilizing factor of blood plasma. Finally, the existence of a stable but reversible intramolecular "sulfhydryl bond" has been proposed (*32*) to account for several rather unusual properties of bovine plasma albumin. Thus, the multiple possibilities for the role of sulfhydryl groups in linking protein units together should be borne in mind when one is considering sulfhydryl-dependent aggregation phenomena in protein systems.

Peptide Reactions

In the foregoing examples, protein disulfide groups appear to participate in interchange reactions under conditions of denaturation. No doubt most disulfide groups in native proteins are either hindered or otherwise unreactive (*33*), so that some disruption of protein structure is necessary to make them available for reaction. Without this restriction, disulfide exchange reactions could take place indiscriminately in physiological systems, and chaos would ensue in the living cell. In the case of simpler peptides and amino acids, however, the disulfide groups are readily available, and interchange reactions often take place with amazing ease, even with peptides which contain no sulfhydryl groups as initiators.

This fact was first reported by Sanger (*34*) after observations that an acid hydrolyzate of insulin may contain many more cystine peptides than can be accounted for by any unique structure for the protein. In a subsequent study, Ryle and Sanger (*35*) demonstrated that disulfide interchange in peptide solutions at 37°C can take place either in neutral or in alkaline medium or else in strongly acid medium (7 to 12*N* HCl) with little or no exchange observed in moderately acid solutions. The former process is accelerated by an increase in *p*H or by the addition of mercaptans and inhibited by sulfhydryl-blocking reagents, whereas disulfide interchange in strongly acid solution is inhibited by added mercaptans. Cognizant of these facts, Sanger and his associates (*36*) were able to devise conditions for the hydrolysis of insulin so as to avoid disulfide interchange and the resulting artifacts among the peptides produced.

Additional examples of disulfide exchange reactions both in acidic and in alkaline media have been described by Schöberl and Gräfje (*37*), who also found that illumination with ultraviolet light promotes disulfide interchange. Although the latter observation suggests the possibility of disulfide interchange by a free radical mechanism, because of the *p*H dependence of the photochemically induced exchange, the authors consider it probable that the illumination in some way promotes the formation of mercaptide ions rather than initiating a free radical process.

Ressler (*38*) has observed that on standing at room temperature in sodium bicarbonate solution the cystine-containing peptide hormone oxytocin undergoes loss of biological activity accompanied by aggregation and decreased solubility. This behavior is ascribed to a disulfide interchange reaction which produces intermolecular disulfide linkages; the latter can be cleaved, and biological activity can be partially restored, by treatment of the inactivated hormone with cysteine, glutathione, or hydrogen sulfide. Since the inactivation takes place under the mild alkaline conditions ordinarily used for the preparation of dinitrophenyl derivatives of proteins and peptides, the need for caution is noted in the interpretation of results involving dinitrophenyl derivatives of disulfide-containing peptides.

Reaction Mechanisms

The sulfhydryl-initiated disulfide interchange illustrated in Fig. 1 undoubtedly is analogous to the intermediate step in the reduction of simple disulfides by mercaptans, a reaction which has been studied extensively (*39*) and which is known to proceed by nucleophilic attack of a mercaptide anion

(RS^-) on the disulfide bond. Unless they are strongly influenced by other factors, such as changes in electrostatic repulsive forces between the charged protein molecules (*1*), disulfide interchange reactions of this type would be expected to proceed at a more rapid rate as the *p*H, and thus the mercaptide ion concentration, is increased. In the protein experiments discussed above in which comparisons have been made, an increase in *p*H has been found to enhance phenomena ascribed to the disulfide interchange reaction.

In the case of peptides which contain no sulfhydryl groups, the disulfide interchange reaction in neutral or alkaline solution appears to proceed by a similar mechanism, the initiating sulfhydryl group being produced by the hydrolysis of a disulfide bond (*35*). In strongly acid medium, on the other hand, disulfide exchange takes place by a different mechanism, demonstrated recently by Benesch and Benesch (*40*) to involve electrophilic attack of a sulfenium cation (RS^+) on the disulfide bond. Under the action of strong hydrochloric acid, the following reactions occur:

$$R\text{-}S\text{-}S\text{-}R + HCl \rightleftarrows RSCl + RSH \quad (1)$$

$$RS^{\oplus}Cl^{\ominus} + R'\text{-}S\text{-}S\text{-}R' \rightleftarrows R\text{-}S\text{-}S\text{-}R' + R'SCl \quad (2)$$

The known reaction of mercaptans with sulfenyl chlorides (*41*) appears to be partially reversible, so that small amounts of sulfenyl chloride are formed from the disulfide (reaction 1). The sulfenyl chloride then reacts with a disulfide, in a manner also described by Moore and Porter (*42*), to form the mixed disulfide and to regenerate a new sulfenyl chloride which carries on the process (reaction 2). Marked enhancement of disulfide exchange is observed upon the addition of small amounts of sulfenyl chlorides or sulfenic acids (RSOH), which in strong acid can give rise to sulfenium ions, as well as of hydrogen peroxide, which reacts with disulfides to produce sulfenium ions. That the acid-catalyzed exchange is inhibited by the addition of mercaptans is readily understandable, since the presence of these substances reverses reaction 1 and thus depletes the amount of sulfenyl chloride, the concentration of which is already rate-limiting.

Physiological Processes

The foregoing examples of disulfide interchange reactions are in vitro processes which take place when a protein or peptide is subjected to chemical or physical manipulation. Whether similar interchange reactions take place in vivo is a question of considerable interest, and there are now strong indications that sulfhydryl-initiated disulfide interchange may play a role in certain important physiological processes.

One such phenomenon is the clotting of blood fibrinogen (*43*). It is well established by the work of Robbins (*44*) and of Lorand (*45*) that the fibrin clot formed from the action of thrombin on purified fibrinogen (fibrin-*s*) is different from the physiological clot obtained from the coagulation of blood or of recalcinated plasma (fibrin-*i*). Fibrin-*s* is soluble in *5M* urea or in weak acid or alkali, whereas fibrin-*i* is insoluble in these reagents and also possesses much greater mechanical strength. The formation of the insoluble type of fibrin requires the presence both of calcium ions and of a nondialyzable, heat-labile substance present in blood plasma, known variously as Laki-Lorand (L-L) factor, fibrin-stabilizing factor (FSF), or urea-insolubility factor. The properties of fibrin-*i* indicate that this clot possesses stable cross linkages not present in fibrin-*s*, whereas the fact that fibrin-*i* dissolves readily in a mixture of urea and thioglycolic acid (*46*) suggests that these additional cross linkages are disulfide bonds.

The observation that small amounts of sulfhydryl-blocking reagents prevent the formation of urea-insoluble fibrin clots led Loewy and Edsall (*47*) to propose that fibrin-*i* is cross-linked by intermolecular disulfide bonds produced through a sulfhydryl-initiated interchange of the disulfide groups of fibrin-*s*. With the subsequent isolation of the fibrin stabilizing factor in purified form (*31, 48–50*), and the demonstration that this substance contains from one (*49*) to two (*50*) equivalents of titratable sulfhydryl per 100,000 grams of protein, sulfhydryl which is essential for FSF function, this factor would appear to possess the necessary qualifications to be an initiator of disulfide interchange.

During the clotting process, sulfhydryl groups are produced whose reactivity appears to be considerably increased over that of the original FSF sulfhydryl (*49*). Inactivation of FSF by pretreatment with silver ions requires ten times the silver concentration necessary to prevent fibrin-*i* formation when the silver is present during the clotting process, whereas iodoacetamide will inhibit fibrin-*i* formation but will not inactivate FSF on pretreatment. These observations of Loewy's are reminiscent of Kolthoff's (*11, 12*) studies on aggregated plasma albumin after guanidine hydrochloride treatment, and they are consistent with the involvement of a sulfhydryl-disulfide interchange mechanism in the formation of fibrin-*i*.

Whether the sulfhydryl groups of FSF are the only such groups which can initiate the conversion of fibrin-*s* to fibrin-*i* is not certain. Earlier findings that simple mercaptans (*47*) or plasma albumin (*51*) could promote this transformation have been attributed by Lorand and Jacobsen (*52*) to a regeneration of inactivated FSF tenaciously bound to the protein employed, since, with their fibrinogen preparations, neither cysteine nor plasma albumin showed any FSF activity. However, Loewy (*49*) has prepared fibrinogen which by immunological criteria appears to be free of bound FSF, and preliminary experiments with this material indicate that simple mercaptans do show FSF activity when present in rather high concentration. Thus, further study is required before agreement can be reached as to the specificity of the FSF as the reaction initiator. In any case, the concept that FSF, augmented in some way by calcium ions, effects cross linking of fibrin molecules through a sulfhydryl-disulfide interchange reaction provides a reasonable explanation for the formation of the physiological fibrin-*i* clot.

A second biological phenomenon which may involve sulfhydryl-disulfide interchange is that of cell division. It has long been known (*53*) that during mitosis the acid-soluble sulfhydryl (glutathione) content of the sea urchin egg exhibits a cyclical variation; it decreases after fertilization, reaches a minimum at about the time of spindle formation, and returns to its original level prior to cleavage. After observations that the protein sulfhydryl content of the sea-urchin egg likewise varies, but with an inverse relationship to the variation of the glutathione level, and also that the isolated mitotic apparatus is soluble in sodium thioglycolate, Mazia (*54*) proposed that disulfide bonds are important structural features of the mitotic apparatus and that the formation of this entity may involve the polymerization of small protein molecules through a glutathione-initiated disulfide interchange reaction. The construction of the mitotic apparatus is considered to involve both gelation of protein through intermolecular disul-

fide bond formation and orientation of the gel structure through secondary bond production; the orientation but not the gelation process is prevented by the mitotic inhibitor, colchicine (*55*). The participation of sulfhydryl groups in mitosis is further indicated by the reversible blockage of the mitotic cycle of sea-urchin eggs by the addition of small amounts of mercaptoethanol (*56*), whereas the ability of mercaptoethanol to induce twin formation, when present during a particular stage of the cleavage cycle of *Dendraster* eggs, suggests that the simple mercaptan competes with protein sulfhydryl groups in the formation of disulfide bonds involved in interblastomere linkages (*57*). Although many details of the complex physiological process of mitosis remain to be elucidated, the intriguing suggestion that sulfhydryl-disulfide interchange plays an important role affords a promising approach for further investigation.

Eldjarn and Pihl (*58*) have reported that cystamine and cysteamine administered to a mouse rapidly become incorporated into the blood proteins, apparently by a sulfhydryl-disulfide exchange. This finding suggests that disulfide interchange reactions with body constituents may be intimately concerned with the protective action of these sulfur compounds against ionizing radiation.

Whether sulfhydryl-disulfide interchange plays a role in other biological phenomena remains to be investigated, although certain additional suggestions along these lines have been put forward. In view of the previously mentioned inactivation and reactivation of oxytocin, presumably by a disulfide exchange mechanism, Ressler (*38*) has suggested the possibility that disulfide-containing peptide hormones, such as oxytocin and vasopressin, may exist physiologically in an inactive form, with activation taking place at appropriate sites under the influence of sulfhydryl compounds such as glutathione. After observations of what appears to be a long-range intramolecular sulfhydryl-disulfide interchange in bovine plasma albumin mediated through the bound water lattice, Klotz (*23*) pointed out that such a mechanism could furnish a means of electron transport in oxidation-reduction reactions involving sulfhydryl enzymes, especially in systems which appear to transfer energy over a distance. Other processes in which the possibility of disulfide interchange should be considered include the production of keratin and the formation of such physiological protein gels as the mitachondrial framework or the lens of the eye. Disulfide linkages are relatively abundant in most protein molecules, whereas sulfhydryl and disulfide are potentially among the most reactive of the protein functional groupings, although, as was pointed out above, their reactivity ordinarily is more or less restricted by the characteristic structure of the protein molecule. It is not unreasonable to consider that the physiological initiation and control of many important processes in living organisms may depend on factors which establish and regulate conditions under which interaction between protein sulfhydryl and disulfide groups can take place.

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