

# I. 7. THE SUPERCONTRACTION OF KERATIN FIBERS BY LITHIUM BROMIDE

Alfred E. Brown and Lawrence G. Beauregard

Harris Research Laboratories, Inc., Washington, D. C.

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## I. Introduction

Scientists in the keratin fiber field use measurements of the changes in mechanical properties of fibers to explain chemical effects which occur as a result of treatments with acids, alkalis, reducing agents, and oxidizing agents. Particularly useful are measurements of permanent set, supercontraction, stress-strain, and stress-relaxation behavior (1, 2). Although setting reactions are important in both the wool and hair-waving industries, scientific investigation of the problem began only a generation ago (3, 4). "Set" is the phenomenon whereby a stretched keratin fiber maintains a length greater than its original length after a given treatment. It is also

possible for a hair to shorten and be fixed at a length less than its original length, and such set is referred to as "supercontraction." \*

Keratin is a stable structure because of the large number of disulfide ( $-S-S-$ ) cross-links between polypeptide chains, and naturally much attention has been focused on the chemistry of the  $-S-S-$  bond (5). In our Laboratories industrial applications such as in permanent waving and wool textile treatments have been made through a better understanding of  $-S-S-$  reactions in keratin (6-10).

Historically, supercontraction was first noted in reactions which rupture  $-S-S-$  linkages (11) and for years it was thought that  $-S-S-$  breakdown is essential for contraction. Recently, it has become evident that secondary bonds also play an important part in contraction (12-15). Thus, treatments which produce contraction may be divided into two broad classes—those which predominantly cause cleavage of the disulfide bond (reducing agents) and those which predominantly rupture hydrogen and other secondary bonds (secondary-bond-breaking agents).

We selected contraction studies with a strong hydrogen-bond-breaking agent, lithium bromide, to isolate the contraction effect produced by secondary bond rupture from that produced by  $-S-S-$  breakdown.

## II. Experimental

### 1. MATERIALS

Human hair fibers three inches long were used for the contraction measurements. All fibers were extracted with water, alcohol, and ether prior to use.

### 2. PURIFICATION OF LiBr

The chemicals were reagent grade except for LiBr. This reagent was purified by filtering a saturated solution, heating it to 100°, and immersing wool in it. This treatment exhausted any reactive extraneous materials which might interfere with the contraction reactions (16). The LiBr concentration was determined at 25° with standard  $AgNO_3$ . This stock solution was used to prepare all solutions.

### 3. METHOD FOR SUPERCONTRACTION

The apparatus consisted of a large (175 ml. capacity) test tube, fitted with a two-hole rubber stopper (No. 9) through which a Bunsen valve and

\*Originally, *supercontraction* was used to denote the decrease in length of a stretched fiber on treatment in a given system, but today the term is used to denote decrease in length of either stretched or unstretched fibers. The terms *contraction* and *supercontraction* are used interchangeably in this paper.

a calibrated, centigrade scale thermometer were placed. Three brass cup hooks were screwed into the bottom of the stopper. The mounted fibers were attached to the hooks, and allowed to hang vertically in the solution. Three fibers were always treated simultaneously in each solution. Reproducibility of the contraction measurements was good; the individual values were within 5% of the mean value.

#### 4. ACETYLATION OF HAIR

Hair was acetylated in a 16% volume/volume solution of acetic anhydride in glacial acetic acid for 3 hours at 80°, then rinsed and dried. A white wool control, acetylated in the same bath, gave a negative ninhydrin test showing complete blocking of the amino groups.

#### 5. ESTERIFICATION OF HAIR

The esterified hair was prepared as described by Burton *et al.* (17) for leather, as adapted by Zahn and Wilhelm for wool (18). The hair was treated in anhydrous acidic methanol 0.1 *N* with respect to HCl. The treatment was for 7 days at room temperature with periodic shaking, after which the fibers were thoroughly rinsed and dried. The amount of methylation, determined by Alexander's method (19), showed 67% of the carboxyl groups were esterified.

### III. Results

#### 1. EFFECT OF LiBr CONCENTRATION

The effect of concentration of LiBr on the contraction of hair fibers at 80° is shown in Fig. 1. Maximum contraction is obtained in 7 to 8 *M* solution. Contraction of individual fibers at 80° is shown in Fig. 2. When the concentration of LiBr is 7.4 *M* or higher there is a considerable induction period. The fiber then rapidly contracts to about 6%. When the LiBr concentration is less than 7.4 *M*, the reaction occurs in a different manner. After the induction period the rate of contraction is fairly rapid until about 10%, after which similar behavior to that found in the 7.4 *M* solution is observed. Experiments at 100° also showed that an initial contraction of approximately 6% occurs rapidly in solutions 7 *M* or higher in concentration.

#### 2. EFFECT OF ACID AND ALKALI ON SUPERCONTRACTION

##### *a. Addition of Acid*

Contraction of hair fibers at 80° in acidic and alkaline 7 *M* LiBr solutions is shown in Fig. 3. The rate of contraction is markedly inhibited by

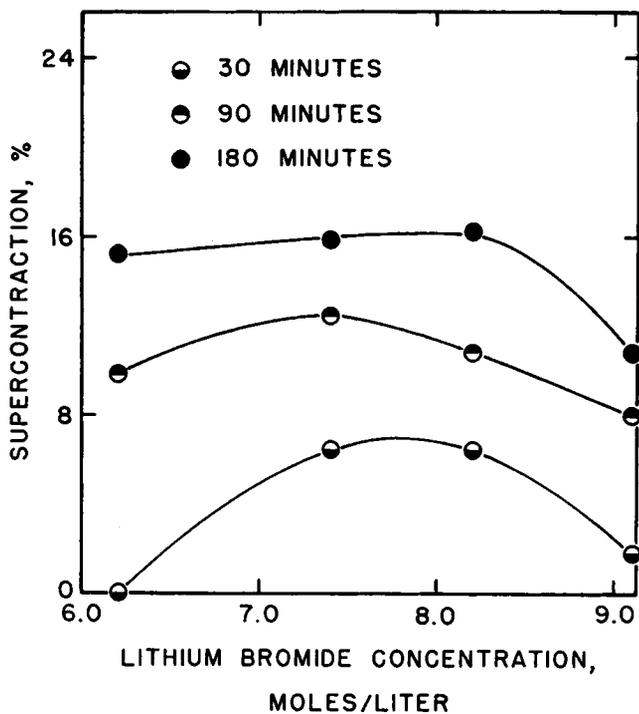


FIG. 1. The effect of concentration of LiBr on the supercontraction of human hair fibers at 80°.

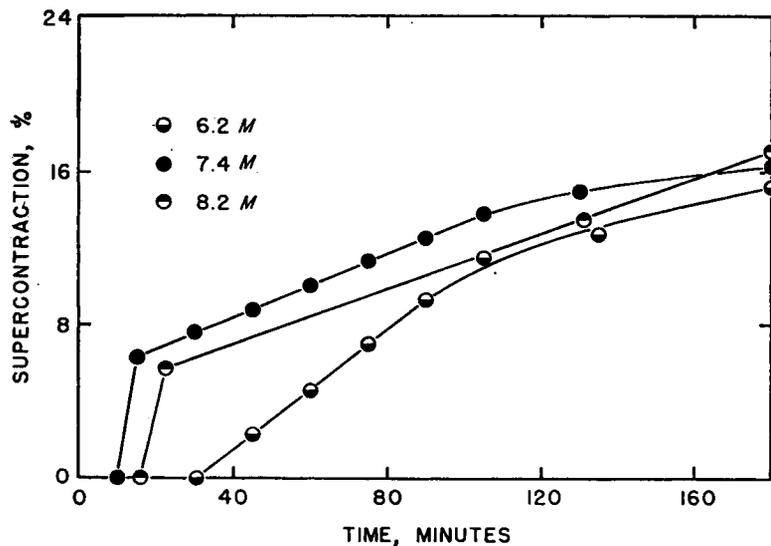


FIG. 2. The rate of supercontraction of individual human hair fibers in 6.2, 7.4, and 8.2 M LiBr at 80°.

0.001 *M* acid, and greatly enhanced by 0.001 *M* alkali. When the LiBr solution contained either 0.01 or 0.1 *M* HBr, the hairs did not contract.

Additional measurements were made using acid and alkali additions to LiBr solutions of various concentrations at 80° and 100°. The 0.01 *M* acid has two effects: (1) it prevents contraction in LiBr solutions below

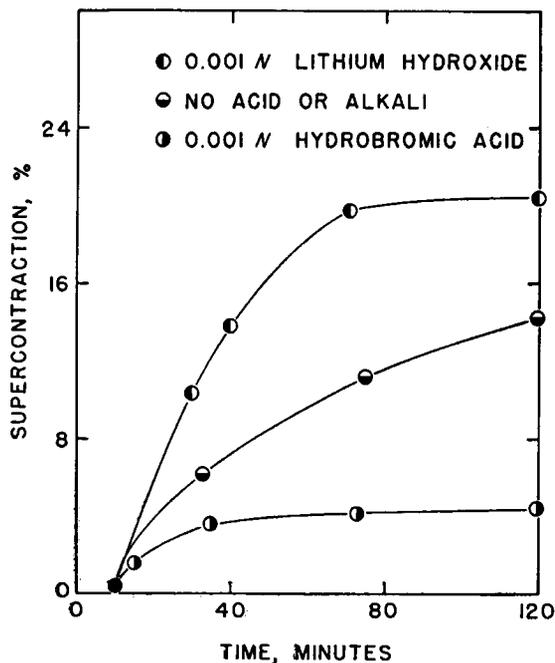


FIG. 3. The rate of supercontraction of human hair fibers at 80° in 7 *M* LiBr solutions containing acid or alkali as indicated.

7.4 *M* in concentration, and (2) at concentrations above 7.4 *M*, contraction is limited to 6% after a 3-hour treatment. The LiBr solutions effective in producing contraction in the presence of acid are those in which an initial rapid rate of reaction is observed in the absence of acid.

#### b. Addition of Alkali

Addition of 0.01 *M* LiOH to 5.3, 6.4, 7.2, and 8.7 *M* LiBr solutions produced rapid contraction at 80°. The rate is greatly accelerated in alkali, so that the former second stage of contraction cannot be differentiated from the initial rapid stage. In these solutions, 18 to 20% contraction was observed in 1 hour.

## 3. BEHAVIOR OF CHEMICALLY MODIFIED FIBERS

*a. Acetylated Hair*

Acetylated hair was treated in various concentrations of LiBr at 80°. The results are given in Table I. The marked inhibition of contraction produced by acetylation is evident. Addition of acid to the 6.2 *M* solution (which alone contracts acetylated hair) completely inhibits contraction

TABLE I  
SUPERCONTRACTION OF CHEMICALLY MODIFIED HAIR IN LiBr SOLUTIONS

Concentration	Per cent supercontraction at 80°					
	Untreated		Acetylated		Esterified	
	1 hour	3 hours	1 hour	3 hours	1 hour	3 hours
6.2 <i>M</i>	4.2	15.2	0	3.5	—	—
7.4 <i>M</i>	10.0	15.8	2.5	2.5	4.5	8.1
8.2 <i>M</i>	8.8	16.2	1.5	2.1	4.9	7.0
9.1 <i>M</i>	6.4	10.8	—	—	2.3	5.5

of untreated hair. Thus, the inhibitory effect of acetylation appears to be different from that produced by acid addition.

Untreated and acetylated fibers were treated for 3 hours in 7 *M* LiBr at 80°. Analyses before and after the treatment in the salt solution showed no significant change in cystine content. The contracted fibers also gave negative nitroprusside tests. These results indicate that —S—S— breakdown is not involved in LiBr contraction.

(1) *Acid Addition to LiBr.* At 80°, 0.01 *M* acid produced an added inhibitory effect in that no contraction was observed in 5.3, 6.2, or 7.4 *M* LiBr solutions. Approximately 3% contraction was noted after 3 hours in acidic 8.2 and 9.1 *M* solutions.

(2) *Alkali Addition to LiBr.* Addition of 0.01 *M* alkali to the LiBr increased contraction of acetylated hair after 1 hour at 80°. In the 6.4 *M* LiBr solution, 0.01 *M* alkali increased the contraction of acetylated hair from 3 to 12%. Despite the greatly increased amount of contraction of acetylated hair in these alkaline systems, the fibers still contracted much less than untreated fibers.

*b. Esterified Hair*

Esterification which blocks two-thirds of the free carboxyl groups in hair results in considerable inhibition of contraction in LiBr at 80°, as shown in Table 1.

(1) *Acid Addition to LiBr.* The behavior of esterified hair in acidic LiBr (7.4–9.2 *M* LiBr, 0.1 *M* HBr, 80°) is similar to that of untreated hair. Fibers contract approximately 6% in 1 hour and remain at that level after 3 hours.

(2) *Alkali Addition to LiBr.* Esterification inhibits contraction of hairs in 6.4–8.7 *M* LiBr solutions containing 0.01 *M* LiOH at 80°. The esterified hair contracts 12% compared to 20% for untreated hair.

#### 4. REVERSIBLE AND IRREVERSIBLE SUPERCONTRACTION

Contraction is considered reversible when the fiber recovers its original length after removal from the solution and immersion in water. Since reversibility is important for understanding mechanisms, it was determined for all fibers. After removal from the salt solutions, the fibers were immersed in distilled water maintained at the same temperature as the salt solution. Upon immersion in water, the fibers elongated and schlieren lines could be seen. Elongation occurred in a matter of seconds, and then the fiber either continued to elongate or contracted again.

It was found that the determining factor in reversibility is *not* the actual treatment to which the fiber is subjected but the *level* to which the fiber had originally contracted. When the contraction had not exceeded 6%, complete reversibility was obtained. When the contraction was in the range of 6 to 10%, only partial reversibility could be observed. When the contraction had exceeded 10%, the contraction was irreversible.

Only two exceptions to this generalization were found: hairs treated in LiBr solutions which are 0.1 *M* in acid were irreversibly contracted, regardless of the amount of contraction. Conceivably, peptide hydrolysis may be involved here. The contraction produced in LiBr solutions more dilute than 7.4 *M* was only partially reversible (about half) when contraction had not exceeded 6%. The different nature of the initial contraction reaction, which occurs in dilute versus concentrated LiBr solutions, is again indicated.

Since 6% contraction was the maximum level for reversibility and this level coincided with the previously observed break in the rate curve in concentrated solutions, a relationship between the initial phase of contraction and reversibility is indicated.

##### *a. Reversibility and Restoration of Hair Properties*

An important question which arises in terms of mechanisms is the following: does a fiber which contracted in LiBr and then recovered its original length on immersion in water behave like an untreated fiber on subsequent treatment in LiBr? A set of fibers was contracted 6% in 7.4 *M* LiBr at 80°. The fibers were immersed for 5 minutes in distilled water,

dried, and the cycle was repeated twice again. Under these conditions of treatment the hair contracts exactly the same way the second and third times it is treated in LiBr as it behaved initially.

#### IV. Discussion

In preliminary experiments, wool, modified by converting —S—S— bonds to bis-thioether linkages, was treated with hot sodium bisulfite and LiBr solutions. The reducing agent did not contract the fibers, whereas LiBr did, indicating that contraction with LiBr *did not* involve —S—S— breakdown (20). All of the results of this study support that conclusion.

Alexander (12) first showed that LiBr contracts wool fibers, and that secondary bond breakdown was involved. LiBr is capable of dissolving silk and cellulose, and it is generally accepted that hydrogen and other secondary bonds are responsible for cross-linking these molecules to provide strong fibers. The behavior of the LiBr solutions with human hair as noted here is similar to that observed in studies with wool under different conditions (12, 16, 21, 22), and may be explained as follows. The Li<sup>+</sup> in solution is surrounded by a hydration shell. Where there is insufficient water present to form a complete hydration shell, the Li<sup>+</sup> draws within its shell polarized groups containing hydrogen, such as NH and OH, which otherwise participate in hydrogen bonding in the protein. Thus the secondary valences in the lithium ion shell can be satisfied with either water or OH or NH groups. Above 6–7 M concentrations, the hydration shell is incomplete due to insufficient water, protein sites are involved, secondary bond breakdown occurs, and contraction follows. Maximal bond breakdown is achieved at 80° in 7–8 M LiBr solutions. On the other hand, the rate of diffusion of LiBr into hair is very slow (21) in solutions above 8 M, and maximal contraction is not attained in 3 hours. The lower reactivity of dilute LiBr solutions is related to less binding with polar sites; with concentrated solutions, slow transport to the protein sites is the problem. Thus, a 6.2 M LiBr solution, because of lower reactivity with protein sites, does not contract hair in 5 days at room temperature. On the other hand, a 7.4 M solution contracts hair 6% after 16 hours at room temperature.

At elevated temperatures, more bond breakdown occurs, peptide chains coil like rubber, and shorten, the driving force being an increase in entropy. Along with rubberlike properties, there is weakening of the fibers and disappearance of birefringence and the  $\alpha$ -keratin X-ray pattern (22). Complete reversibility of contraction is obtained by immersing the fibers in water because the water penetrates the fiber and rapidly replaces the

protein sites in the shell. The salt is removed, the protein groups reform hydrogen bonds in the hair, and the original hair properties are restored. There are analogies between this mechanism and the solubilizing activity of salts on proteins. It is known that ions have a salting-in or salting-out effect, dependent on whether the affinity of individual ions for particular groups in proteins is greater or less than their affinity for water (23). For example, the affinity of  $\text{Li}^+$  for amino, aliphatic hydroxyl, ether, and peptide groups is greater than its affinity for water, and  $\text{Li}^+$  increases solubility of materials with such groups. On the other hand, the affinity of  $\text{Li}^+$  for phenolic hydroxyl and for carboxyl groups is less than that for water, and a salting-out effect would be noted.

Silk, the peptide chains of which are held together by secondary bonds, is therefore solubilized by lithium salts. Hair would also dissolve in such salts were it not for the large number of  $-\text{S}-\text{S}-$  bonds present which cross-link the protein chains. Indeed, highly reduced hair can be solubilized in  $\text{LiBr}$  solutions. However, the contraction of untreated human hair as described in Figs. 1 and 2 does not involve  $-\text{S}-\text{S}-$  bonds, so that only a partial separation of the peptide chains is permitted, and contraction is due to the coiling of such chains.

#### 1. EFFECTS OF ACID, ALKALI, AND HAIR MODIFICATION

The discovery of the initial rapid phase of contraction, which levels off at approximately 6% when the  $\text{LiBr}$  concentration is approximately 7 *M* or higher, is most significant. No such phase is found in the more dilute solutions, where the mechanism may be similar to that which operates in concentrated solutions when contraction is greater than 6%. Addition of acid to the salt bath completely inhibits contraction in the dilute solutions, and limits contraction to about 6% in the concentrated solutions. This contraction is reversible. Tensile measurements showed that approximately 50% less work was required to stretch the contracted fibers. The original tensile properties were restored after the contraction had been reversed, indicating that the initial reversible contraction in  $\text{LiBr}$  is clearly independent of any disulfide bond breakdown, otherwise the original properties of the hair would not be restored (5).

Only secondary bonds are involved in the initial contraction in concentrated  $\text{LiBr}$  and since it is not affected by acid, it is suggested that the protein sites involved are not those which bind acid. It is probable that this initial phase is not affected by alkali, although this cannot be determined precisely. These results suggest that carboxyl and amino groups are not involved. The two types of groups in hair which have a strong affinity for  $\text{LiBr}$  and which are unaffected by acid and alkali are

peptide and aliphatic hydroxyl groups. Peptide groups form hydrogen bonds in considerable numbers and it seems likely that rupture of these bonds is involved in the initial phase of contraction with LiBr.

Contraction which occurs after the initial phase is another matter. Here the results obtained with the modified hair are of interest, since modification completely eliminates the second phase of contraction beyond 6%. Therefore, the amino (and possibly phenolic hydroxyl) groups, which would be masked by acetylation, and the carboxyl groups which would be masked by esterification, probably play the major role in this phase. Australian workers have mentioned the possible influence of basic side chains in contraction with LiBr based on the behavior of wool treated with anionic detergents (16).

In confirmation of previous work (20), no change in cystine content of the hairs, as measured before and after the supercontraction reactions, was noted. Consequently, LiBr produced contraction by secondary bond breakdown.

The effect of alkali in enhancing contraction is best explained by secondary bond breakdown plus disulfide bond breakdown. Weakly, alkaline phenol, another secondary bond breaker, contracts horsehair with rupture of —S—S— bonds (15). Thus, greater *total* bond breakdown is the key to more contraction. It has been shown that —SH groups in proteins can promote intra- and interchain sulfhydryl-disulfide interchange reactions which can produce marked structural changes (24, 25). It is also known that small amounts of —S—S— breakdown may occur on prolonged heating of hair in aqueous solutions. Since it cannot be positively stated that these reactions are not possible at the longer times used in these studies, it cannot be unequivocally stated that a small number of —S—S— groups may not be involved in the *second* stage of supercontraction with LiBr.

## 2. REVERSIBLE SUPERCONTRACTION

It can be stated for the first time that reversibility of contracted hair, with the exceptions noted, is related to *extent* of contraction and not to the reaction conditions used with the LiBr solution. This would indicate that the secondary bonds involved can be broken without large movements of the peptide chains, since otherwise restoration would be difficult. It also appears that the arrangement of the secondary bonds involved in the initial phase of contraction are important in maintaining the crystalline region of the keratin, since the X-ray pattern is reversibly changed by the treatment. On the other hand, after 15–25% contraction, massive disorder of keratin structure is obtained, and all properties, including X-ray pattern, are irreversibly altered.

From a mechanistic standpoint, the initial phase of reaction with LiBr breaks either weak or more accessible hydrogen bonds, and no supercontraction occurs until a considerable number of the bonds are broken. These are the bonds that are concerned in reversible supercontraction. In the second phase of reaction, stronger or less accessible hydrogen bonds are broken. At elevated temperatures, the peptide chains thus liberated coil due to entropy forces, the entire structure collapses, and a highly disorganized keratin is produced.

### V. Summary

Supercontraction of human hair in LiBr solutions does not involve —S—S— bonds and can be explained in terms of secondary bond breakdown. The initial phase of contraction in concentrated solutions is reversible and probably involves primarily cleavage of hydrogen bonds between peptide groups. In the later stages of contraction with such solutions, secondary bonds involving carboxyl and amino groups are involved. A mechanism is discussed which accounts for all of the changes in hair properties produced by contraction in LiBr solutions.

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### Discussion

MIDDLEBROOK: You mentioned the supercontraction of nylon. Is this a supercontraction in the sense that you only get it when you first extend the fiber, then steam it and allow it to relax, or do you actually get a contraction without this initial extension? I am referring to the original definition of supercontraction.

BROWN: I am glad you brought that point up. I might say that I have used supercontraction in the modern sense, which was not the sense used by Astbury. Originally supercontraction referred to the contraction of a stretched hair after a given treatment. It had to be stretched first. Astbury obtained the effect by stretching hair, steaming it for a few seconds, and it would shorten. In the last ten years supercontraction has been used broadly to denote shortening of either stretched or unstretched hair. The nylon supercontraction work was done on unstretched nylon, and that supercontracts about 5 or 6%.

I might mention from the standpoint of disulfide chemistry that nylon does not supercontract more in alkaline solutions than in acid solutions, whereas human hair does contract more in alkali. This would indicate that disulfide breakdown may also be involved with hair on the alkaline side.

SWAN: In regard to your statement that the disulfide bonds will be hydrolyzed in strong salt solutions, we have work in progress that points rather to a very marked stabilization of disulfide bonds in the presence of concentrated salt. For example, insulin was heated for various times in 0.5 M K<sub>2</sub>CO<sub>3</sub>, pH 11.7, in the presence and absence of added salts, and after first acidifying to remove any hydrogen sulfide, the disulfide remaining was measured both by the phosphotungstic acid method and by mercuric chloride titration in the presence of sulfite. The following results were obtained:

- 0.5 M K<sub>2</sub>CO<sub>3</sub> at 20°—75% loss of —S—S— in 120 hours
- 0.5 M K<sub>2</sub>CO<sub>3</sub> at 50°—75% loss of —S—S— in 4 hours
- 0.5 M K<sub>2</sub>CO<sub>3</sub> containing 4 M guanidine hydrochloride at 20°—no change in —S—S— up to 40 days
- 0.5 M K<sub>2</sub>CO<sub>3</sub> containing 4 M guanidine hydrochloride at 50°—no change in —S—S— up to 3½ days
- 0.5 M K<sub>2</sub>CO<sub>3</sub> containing 2 M Na<sub>2</sub>SO<sub>4</sub> at 50°—no change in —S—S— up to 48 days (insulin in suspension throughout)
- 0.5 M K<sub>2</sub>CO<sub>3</sub> containing 2 M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> at 20°—no change in —S—S— up to 36 hours
- 0.5 M K<sub>2</sub>CO<sub>3</sub> containing 2 M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> at 50°—no change in —S—S— up to 3 hours (insulin in suspension)

Dr. J. R. McPhee, working in the Wool Textile Research Laboratory at Geelong, has shown that the alkali-combining capacity of wool can be determined accurately in sodium hydroxide solutions saturated with various salts (*Textile Research J.*, 1958, in press). This result is due to the high salt concentration protecting —S—S— against alkali attack.

BROWN: The answer to your question is that we do not have any experimental evidence on —SH figures, but I don't think you are talking about a comparable situation. Here we have 0.001 *M* alkali, and whatever pH means in lithium bromide solutions, you would be talking about a pH of 10 or 11.

SWAN: pH 11.7.

KAUZMANN: I would like to call your attention to a paper by W. F. Harrington and J. A. Schellman (*Compt. rend. trav. lab. Carlsberg* **30**, 167, 1957) if you are not aware of it already, on the effect of concentrated lithium bromide on globular proteins. It is quite amazing. In the presence of lithium bromide the optical rotatory properties of typical proteins change in the opposite direction to that invariably observed during denaturation. Harrington and Schellman conclude that lithium bromide promotes the intramolecular hydrogen bonding of proteins. This raises all kinds of questions in my mind as to what the lithium ion actually does when it comes in contact with a protein. There must be something more complex than simply some kind of an "attack" on the wool of the kind suggested by you.

BROWN: Thank you for calling that reference to our attention. I want to make clear that the particular reversibility I am speaking of is in the very concentrated salt solutions. In dilute solutions something else must be happening. We believe that the amino group is concerned. So conceivably in the types of systems you are speaking of, amino and carboxyl groups, and many others may be involved in the lithium interactions. I don't know.