suspensions is unaffected by incubation with lysozyme and ethylenediaminetetraacetate[9]. Viability of strain V5 is well preserved in 0.4 *M* sucrose, buffered to pH 7.2 with 0.01 *M* phosphate[9], but the cells are easily killed by exposure to distilled water. When grown under the conditions used here they may be washed in distilled water with the loss of only a small amount of acid-precipitable nucleic acid (less than 2 %), although acid-soluble nucleotides are lost and at least 99.9 % of the cells are killed. Moreover, suspensions in 0.4 *M* sucrose —0.01 *M* phosphate may be diluted 10-fold into distilled water or 1 % (w/v) aqueous ethylene diaminetetraacetic acid without undergoing visible lysis. It may be noted that a small organism such as *M. mycoides* (diameter 0.2 μ or less) immersed in a hypotonic medium will be subject to a much smaller disruptive force than will the protoplast of a typical bacterium under similar conditions. Other factors which may contribute to the osmotic stability of the pleuropneumonia-like organisms are increased permeability to small molecules, and pliability of the surface membrane, as suggested by SMITH AND SASAKI[10], who found that several strains of human and avian origin were not killed by osmotic shock.

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Optical-rotatory changes associated with the dimerization of bovine mercaptalbumin with mercurials

The dimerization of both human and bovine mercaptalbumin with mercurials is characterized by a large positive entropy of activation, $\Delta S^{\neq}$ (EDELHOCH *et al.*[1]; KAY AND EDSALL[2]). This is in contrast to the values of the order of —50 cal/deg./mole which are commonly found in reactions between simpler substances when two molecules unite to form one. EDSALL *et al.*[3] have previously suggested that the positive terms in the entropy of activation may be due to a preliminary unfolding of the albumin molecule in the vicinity of the sulfhydryl group which is necessary if dimerization is to occur. This hypothesis received experimental support when it was observed that $\Delta S^{\neq}$ for bovine mercaptalbumin in 8 *M* urea (—18 cal/deg./mole) was much lower than any of the values for the dimerization reaction in water[2]. Presumably, when the albumin molecule is dissolved in urea, the requisite folding has, to a large extent, already occurred and the way has thus been prepared to make the dimerization reaction occur more easily.

The possibility that this molecular unfolding might be detected by the technique of optical rotation was first explored by SAROFF AND SIMPSON[4] who found essentially no change in $[\alpha]_D$ accompanying the dissociation of human mercury dimer. In view of the more recent advances in polarimetry instrumentation and the theory of optical rotatory dispersion[5,6] it was felt desirable to repeat this work with bovine mercaptalbumin and to extend the polarimetric measurements over a considerable wavelength range.

The optical rotations were measured with a Rudolph high-precision photoelectric polarimeter Model 200 equipped with quartz optics. The monochromator was a Bausch and Lomb grating instrument, Model 33-86-45 and the light source was a GE lamp (H 100 – A 4) from which the glass envelope had been removed in order to utilize the shorter-wavelength lines of the mercury spectrum. The concentration of the protein solutions, contained in 1-dm tubes, was of the order of 1–2 % and the degree of precision of the specific rotation values was ± 0.5° or better. Measurements were made at room temperature, 25 ± 2°.

Two preparations of bovine mercaptalbumin were used: a 5-times recrystallized dimer (ASHgSA) sample obtained from Pentex Inc., lot D8504, and a monomer (ASH) preparation made by Dr. JOEL FEIGON and kindly supplied by Professor J. T. EDSALL of Harvard University. Solutions of the monomer and dimer were made up by the method of direct weighing, a correction being applied for moisture content, which was found, in both cases, to average 5 % by drying samples to constant weight at 70° *in vacuo*. Dr. R. BRUCE MARTIN determined the sulfhydryl content of the monomer, using the amperometric titration technique of BENESCH, LARDY AND BENESCH[7], as 0.99 ± 0.02 moles/molecular weight of 69,000. The solvent in all the experiments was acetate buffer, pH 4.75, $I = 0.05$. It should also be mentioned that solutions of the Pentex dimer and solutions of monomer derived from this dimer were all slightly colored, so that in these cases it was not possible to measure the optical rotatory dispersion below 405 mμ. This coloring suggests the presence of a small degree of impurity in the Pentex preparation and probably accounts for the differences observed in the optical-rotatory dispersions of monomer derived from Pentex dimer and the Harvard monomer (Expt. 7 as compared with Expt. 1 in Table I).

When the association reaction was studied polarimetrically a solution of previously clarified monomer of known concentration was added to a 1-dm tube. A solution of mercuric acetate in acetate buffer was then introduced, the final concentration of mercury being 1 mole/2 moles ASH. The top of the 1-dm tube was covered with a polyethylene cap and the tube was rapidly inverted several times to insure mixing of the reactants and then set in position for reading. A similar procedure was followed when dimer dissociation was studied polarimetrically; a solution of NaBr in acetate buffer was added to the dimer solution in the 1-dm tube such that the final concentration of bromide was 2 moles/mole dimer mercury. Optical-rotatory dispersions were determined continuously for the two processes for the first 3 h and then at longer time intervals for an additional 1–2 days.

When the dimerization reaction was effected an increase in laevoratation, over the entire wavelength range studied, was observed to take place during the first 30 min of the reaction (Expt. 2 as compared with Expt. 1); thereafter no further

TABLE I

ROTATORY DISPERSIONS OF MERCAPTALBUMIN SOLUTIONS

	Expt. 1 *ASH* *(Harvard Prep.)*	*Expt. 2* *ASH + Hg++* *(Hg++/ASH = 0.5)*	*Expt. 3* *ASH + Br−* *(Br−/ASH = 1)*	*Expt. 4* *ASHgSA* *(Pentex)*	*Expt. 5* *ASHgSA + Br−* *(Br−/dimer Hg++ = 2)*	*Expt. 6* *ASHgBr****	*Expt. 7* *ASH§*
λ *(mμ)*	$-[\alpha]$	$-[\alpha]$*	$-[\alpha]$	$-[\alpha]$	$-[\alpha]$**	$-[\alpha]$	$-[\alpha]$
577	63.0	64.2	63.2	61.1	59.7	57.0	60.2
546	72.3	73.8	72.5	70.6	68.1	61.8	69.3
436	140.4	142.0	139.4	135.0	130.8	124.4	133.7
405	180.7	184.1	180.9	170.3	165.4	156.8	168.1
365	264.4	269.0	264.0				
334	391.9	398.5	390.9	Measurements could not be made below 405 mμ because of intense coloring of solution.			
313	545.9	554.1	544.5				

* Rotations listed here are the final rotations observed some 30 min after addition of reactants to ASH solution. There was no further significant change after 36 h.

** Rotations listed here are the final rotations observed some 25 min after addition of NaBr to ASHgSA solution. No further significant change occurred during the ensuing 36 h.

*** Formed by the addition of 1 mole $HgBr_2$/mole ASHgSA.

§ Formed from ASHgSA (Pentex) by removing mercury of the dimer with thioglycollate adsorbed on Amberlite resin (IRA-400, 20–50 mesh) according to DINTZIS[8].

change occurred. On the other hand, when dimer dissociation was followed polarimetrically a decrease in laevorotation was found to accompany the first 25 min of the reaction (Expt. 5 as compared with Expt. 4); here too, no further significant change occurred with time. In order to test the possibility that the rotational change observed in Expt. 5 might simply be due to the presence of bromide in the solution, either as protein-bound bromide or free bromide, rather than to an actual dimer dissociation, Expt. 3 was carried out in which bromide was added to monomer. Since no significant difference was observed between the rotations of Expts. 1 and 3, it may be concluded that the results of Expt. 5 are not due to a specific bromide effect.

The increase in laevorotation observed during association is consistent with the view that the serum albumin molecule may have to undergo a preliminary unfolding in the neighborhood of the reactive centers as a necessary prelude to dimerization. Such a process would be analogous to a partial denaturation, and denaturation of proteins, in general, is accompanied by an increase in laevorotation[9]. The decrease in laevorotation accompanying dimer dissociation may reflect a refolding of the albumin molecule. This view agrees well with all the light-scattering evidence which indicates that the dimerization process is entirely reversible and that the albumin molecule can, therefore, return from the unfolded to the original folded configuration when the dimer dissociates. The smallness of the rotational change observed might be due to the fact that only part of the polypeptide chain containing the amino acids in the immediate vicinity of the –SH group is involved in any changes in configurational folding. This may be a segment of the chain containing a small number of amino acids as compared with the 580 present in the bovine serum albumin molecule.

The major drawback to this explanation is that the changes in rotation are not consistent with the light-scattering kinetic data since at the end of 25–30 min monomer association is only 10 % complete and dimer dissociation 15 % complete[2]. It therefore seems likely that if the rotatory-dispersion changes are reflecting configurational changes in the albumin molecule, these must be a necessary preliminary

TABLE II

A COMPARISON OF THE OBSERVED AND CALCULATED ROTATIONS FOR DIMER DISSOCIATION

λ (mμ)	*Observed rotation*	*Calculated rotation*
577	59.7	59.8
546	68.1	68.1
436	130.8	132.0
405	165.4	166.4

to either association or dissociation. Perhaps the folding or unfolding takes place at the activated complex level and persists in the final products when these are formed. Some experimental support for this idea was obtained by determining the dispersion curves for mercaptide (Expt. 6) and monomer derived from Pentex dimer (Expt. 7). The results of these experiments were utilized in the following calculation. When dimer dissociation is effected by the addition of 2 moles bromide/mole ASHgSA, one ends up with an equilibrium mixture of 50 % dimer, 25 % mercaptide and 25 % monomer. Upon weighting the rotational data for each of the molecular species present at equilibrium throughout the wavelength range employed, the resultant calculated rotations agreed very well with the observed rotations of Expt. 5 (see Table II).

It should also be noted that although there is a difference in the values of [α] between the ASH forms of the Harvard and Pentex preparations, the change on dimerization is essentially the same in both cases, so that the reality of the effects observed seems quite clear.

In summary, the observed changes in optical-rotatory dispersion accompanying the dimerization reaction are not sufficiently large to state unequivocally that they represent changes in configurational folding but the evidence is strongly suggestive that they in fact do.

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