[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, DUKE UNIVERSITY SCHOOL OF MEDICINE]

The Precipitation of Proteins by Synthetic Detergents[1a]

BY FRANK W. PUTNAM AND HANS NEURATH

Synthetic detergents are capable of reacting with proteins to produce precipitation[1,2,3,4] and denaturation,[1,2,5,6] the inactivation of some viruses[7,8] and enzymes,[9] the fragmentation of certain natural pigment-protein complexes,[2,10] and the catalytic hydrolysis of protein amide and peptide bonds.[11] The inhibition of bacterial metabolism by synthetic detergents has also been observed.[12]

While this diversity of effects need not be governed by a common mechanism, two of the phenomena, *i. e.*, precipitation and denaturation, afford particularly suitable conditions for studying the mode of action of detergents on proteins: precipitation as compared to that produced by specific protein precipitants and salting-out agents commonly employed for the isolation of proteins; denaturation in analogy to that caused by concentrated solutions of urea and guanidine hydrochloride, previously studied in this Laboratory.[13]

The present communication deals primarily with the conditions requisite for the precipitation of proteins by anionic detergents, particularly, the precipitation of crystalline horse serum albumin by sodium dodecyl sulfate, and with the properties of the protein-detergent complex.[14] The effects of anionic detergents on the denaturation and regeneration of proteins will be the subject of subsequent papers.

(1a) Presented before the Division of Biological Chemistry at the 106th Meeting of the American Chemical Society which was held on September 6–10, 1943, in Pittsburgh, Pennsylvania. A preliminary communication has already been published, see ref. 1.

(1) F. W. Putnam and H. Neurath, *J. Biol. Chem.*, **150**, 263 (1943).

(2) R. Kuhn, H. J. Bielig and O. Dann, *Ber.*, **73B**, 1080 (1940).

(3) T. L. McMeekin, *Federation Proc.*, **1**, Pt. 2, 125 (1942).

(4) K. H. Schmidt, *Z. physiol. Chem.*, **277**, 117 (1943).

(5) M. L. Anson, *J. Gen. Physiol.*, **23**, 239 (1939).

(6) H. P. Lundgren, THIS JOURNAL, **63**, 2854 (1941).

(7) M. Sreenivasaya and N. W. Pirie, *Biochem. J.*, **32**, 1707 (1938).

(8) F. C. Bawden and N. W. Pirie, *ibid.*, **34**, 1278 (1940).

(9) D. Shoch and S. J. Fogelson, *Proc. Soc. Exp. Biol. Med.*, **50**, 304 (1942).

(10) E. L. Smith and E. G. Pickels, *J. Gen. Physiol.*, **24**, 753 (1941).

(11) J. Steinhardt and C. H. Fugitt, *J. Res. Natl. Bur. Stds.*, **29**, 315 (1942).

(12) Z. Baker, R. W. Harrison and B. F. Miller, *J. Exp. Med.*, **73**, 249 (1941).

(13) H. Neurath, G. R. Cooper and J. O. Erickson, *J. Biol. Chem.*, **142**, 249 (1942).

(14) Since the work reported in this paper was completed, electrophoretic[15] and X-ray studies[16] have been reported dealing with the action of alkylbenzene sulfonates on egg albumin. Those results together with unpublished observations of our own indicate the profound effect of anionic detergents on proteins under conditions unfavorable for precipitation.

(15) H. P. Lundgren, D. W. Elam and R. A. O'Connell, *J. Biol. Chem.*, **149**, 183 (1943).

(16) K. J. Palmer and J. A. Galvin, THIS JOURNAL, **65**, 2187 (1943).

Experimental

Materials and Methods

Serum Albumin.—Crystalline horse serum albumin was prepared by low temperature precipitation of the globulins with ammonium sulfate (2.1 *M*) at *p*H 6.4, followed by room temperature precipitation of the albumin at its isoelectric point (*p*H 4.8) by 2.3 *M* ammonium sulfate, the procedure being analogous to that already described for the preparation of bovine serum albumin.[17] The four-times crystallized albumin had a carbohydrate content of 0.10% and corresponded approximately to Kekwick's Fraction B. The preparation was electrophoretically homogeneous at *p*H 7.6.

Other proteins investigated are described in the text.

Detergents.—In preliminary experiments a number of commercial detergents of different type formulas were used. Quantitative investigation was restricted to purified sodium dodecyl sulfate[18] and Duponol P C, free of sodium sulfate.[19]

Apparatus.—The apparatus used in diffusion,[20] viscosity,[21] and electrophoresis studies[22] has already been described, as has the manner of calculation of the diffusion constant, molecular asymmetry and molecular weight.[20,21] Nitrogen determinations were made by the micro-Kjeldahl method.

Preliminary Observations

The action of a number of commercial detergents[19,23,24] on several purified proteins was investigated qualitatively in the following manner: to Wassermann tubes containing 2-cc. aliquots of 2% protein in phosphate buffer *p*H 7.7, or in acetate buffer *p*H 4.2, were added serially from another tube 2 cc. of tenfold dilutions (0.0002–2.0%) of an aqueous solution of the detergent. Thorough mixing was accomplished by repeated pouring back and forth.

It was observed that with anionic detergents precipitation was restricted to the acidic *p*H and that with all types of detergents maximum precipitation occurred at 0.1–1.0%, increasing detergent concentrations resulting in dispersion of the precipitate.

***p*H Region of Precipitation.**—In order to investigate further the effect of *p*H on precipitation, a series of buffers hydrochloric acid–sodium chloride, sodium acetate or phosphate) differing by 0.1 or 0.2 *p*H unit, was prepared.[25]

(17) F. W. Putnam, J. O. Erickson, E. Volkin and H. Neurath, *J. Gen. Physiol.*, **26**, 513 (1943).

(18) We are indebted to Dr. S. Lenher of the Fine Chemicals Division of E. I. du Pont de Nemours and Co. for a generous supply of purified sodium alkyl sulfates. Dr. Lenher's communication gives the analysis of sodium dodecyl sulfate as follows: Calcd. for $C_{12}H_{25}SO_4Na$: S, 11.1; Na, 7.98. Found: S, 10.57; Na, 7.63. Moisture content 1.37%. NaCl 0.31%. The alcohol from which the product was prepared was 99.7% pure.

(19) A mixture of the sodium salts of homologous alkyl sulfates of chain length C_8-C_{18}, obtained free of sodium sulfate, through the courtesy of E. I. du Pont de Nemours and Co.

(20) H. Neurath, *Chem. Rev.*, **30**, 357 (1942).

(21) H. Neurath, G. R. Cooper and J. O. Erickson, *J. Biol. Chem.*, **138**, 411 (1941).

(22) D. G. Sharp, G. R. Cooper and H. Neurath, *ibid.*, **142**, 203 (1942).

(23) Nacconol N. R. S. F., a sodium alkylbenzenesulfonate mixture, National Aniline Division, Allied Chemical and Dye Corporation.

(24) Aerosol OT, di-octyl sodium sulfosuccinate, American Cyanamid and Chemical Corporation.

(25) The relative insolubility of the potassium salts of certain detergents, *e. g.*, the homologous alkyl sulfates, precludes the use of this cation in the buffers employed. The high temperature coeffi-

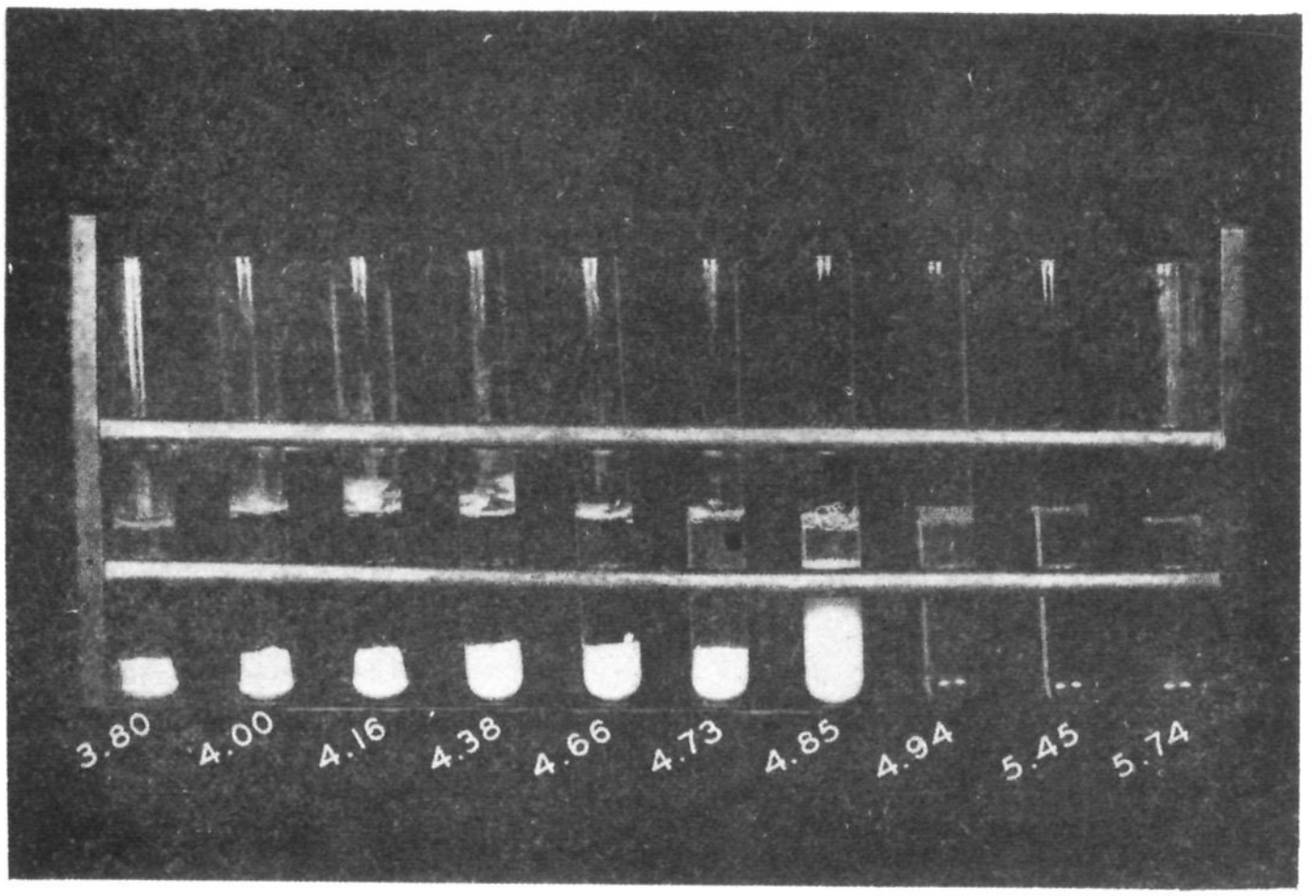

Fig. 1.—*p*H region of precipitation. The test-tubes contain 40 mg. of crystalline horse serum albumin and 10 mg. of purified sodium dodecyl sulfate in 4 cc. of 0.1 *N* sodium acetate buffer of the *p*H indicated. Photographed without centrifuging two hours after mixing. Since up to *p*H 4.85 the protein was completely precipitated, in all of the tubes, the heights of the precipitates are indicative only of the rate of sedimentation.

The protein was dissolved in the buffers and sufficient sodium dodecyl sulfate (SDS) was then added to ensure complete precipitation. The final *p*H was determined on the supernatant with the glass electrode since slight changes in *p*H occurred incidental to mixing.

Serum Albumin.—The test tubes depicted in Fig. 1 contain 40 mg. of crystalline horse serum albumin to which had been added 10 mg. of SDS, the solutions being 0.1 *N* with respect to sodium acetate, in a total volume of 4 cc. The isoelectric point of horse serum albumin in this buffer is reported to be *p*H 4.75[26] while precipitation ceases at *p*H 4.85.

Except in the region of the isoelectric point, settling is very rapid. Within less than one hour a compact pellet is formed from which the supernatant solution may be decanted readily without prior centrifugation. The photograph taken after standing for two hours after mixing is evidence of this fact. Since all the protein was precipitated in the *p*H range 3.80–4.85, the heights of the precipitates are indicative only of the rate of sedimentation.

Other Proteins.—Other proteins investigated in a similar manner were: pepsin, β-lactoglobulin, horse serum pseudoglobulin GI, and crystalline beef serum albumin, obtained as already described,[16,21] egg albumin, made available through the courtesy of Dr. A. R. Taylor of the Duke Medical School; and crystalline human carboxyhemoglobin prepared according to the method of Cannan and Redish.[27] All preparations were found to be homogeneous in diffusion or electrophoresis, except for hemoglobin which was not investigated.

As revealed by the data given in Table I, all these proteins are precipitated only in the cationic form, the maximum *p*H at which precipitation ensues closely approximating the isoelectric point except for hemoglobin. Agreement between the isoelectric point and the maximum *p*H of precipitation is most satisfactory for proteins which are isoelectric within the acetate buffer range. In the case of pseudoglobulin GI, a small amount of precipitate which formed above the isoelectric point redissolved spontaneously on standing overnight. Likewise, in the case of pepsin, a small amount of precipitate was observed at

TABLE I

COMPARISON OF ISOELECTRIC POINT, AND MAXIMUM *p*H FOR PRECIPITATION OF PROTEINS BY SDS

Protein	I. P.	Ref.	Maximum *p*H for precipitation
Pepsin	2.7	(28)	about 2.7
Egg albumin	4.6	(29)	4.6
Horse serum albumin	4.75	(26)	4.85
Beef serum albumin	4.8	(30)	4.8
β-Lactoglobulin	5.2	(31)	5.2
Horse pseudoglobulin GI	6.0	(22)	5.9
Human carboxyhemoglobin	7.1	(32)	6.4

cient of solubility also restricts the conditions of the experiment: for example, Duponol PC which may readily be kept at room temperature as a 10% stock solution crystallizes out at 4° above concentrations of 0.2%.

(26) H. A. Abramson, L. S. Moyer and M. H. Gorin, "Electrophoresis of Proteins," New York, 1942, p. 182.

(27) R. K. Cannan and J. Redish, in "Blood substitutes and blood transfusion" (S. Mudd and W. Thalhimer editors), Springfield, Illinois, 1942, p. 147.

(28) J. H. Northrop, "Crystalline Enzymes," New York, 1939, p. 49.

(29) A. Tiselius and H. Svensson, *Trans. Faraday Soc.*, **36**, 16 (1940).

(30) E. J. Cohn, *Chem. Rev.*, **28**, 395 (1941).

(31) K. O. Pedersen, *Biochem. J.*, **30**, 961 (1936).

(32) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford, 1940, p. 407.

reactions exceeding the isoelectric point; however, acidification of the supernatant solutions yielded additional precipitation only in tubes initially at *p*H 2.75 or higher.

The conclusion derived from these experiments is that *anionic detergents precipitate proteins only when the latter are in the cationic form*[33] and that the maximum *p*H at which precipitation occurs closely approximates the isoelectric point.

Analytical Investigations

Quantitative investigations of the influence of the following factors on the precipitation of crystalline horse serum albumin by purified SDS was undertaken: (1) the protein-detergent weight concentration ratio, (2) *p*H, and (3) temperature and salt concentration. SDS was chosen because (a) it is available in the pure anhydrous form, (b) it is nitrogen free, and (c) its presence may readily be detected and its removal effected by the addition of barium salts.

1. Protein-Detergent Weight Concentration Ratio.—The effect of varying detergent concentration on the precipitation of a given amount of serum albumin was studied at three different protein concentrations (4.80, 9.80 and 19.72 mg. per cc.), in the presence of 0.1 *N* sodium acetate, *p*H 4.5, at room temperature. One-half hour after mixing the tubes were centrifuged and aliquots of the filtered supernatant solutions were analyzed for nitrogen, the amount of protein precipitated being obtained by difference.

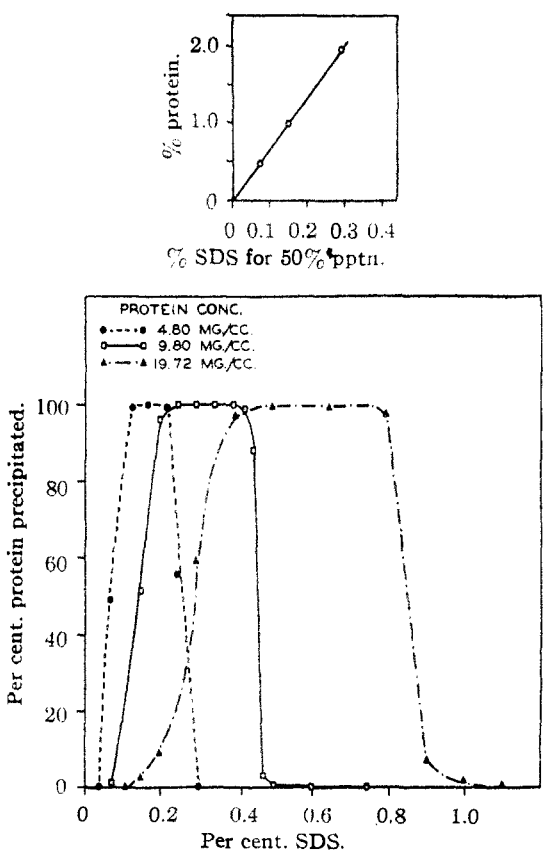

Fig. 2.—Per cent. serum albumin precipitated at *p*H 4.5 in 0.1 *N* sodium acetate plotted against concentration of sodium dodecyl sulfate. The initial protein concentration of the solution is denoted as follows: - - - - 4.80 mg. per cc., —— 9.80 mg. per cc., and ·—· 19.72 mg. per cc. The small graph represents the linear relation between the protein concentration and the per cent. SDS required for 50% precipitation.

In Fig. 2 the per cent. of total protein precipitated is plotted against the final concentration of SDS.

Taking the middle curve representing a protein concentration of 9.80 mg. per cc. as an example, three distinct regions may be discerned: (a) that of *protein excess* where the protein is incompletely precipitated; (b) the *equivalence zone* where complete precipitation is achieved; and (c) the region of *detergent excess*, in which the precipitate initially formed may be partly or completely dispersed by shaking. At a given protein concentration, the transition between these regions with increasing concentration of SDS is abrupt. Therefore, to ensure 100% precipitation an optimal protein-detergent concentration ratio must be observed. Since the equivalence zone is fixed within the limits of the protein-detergent weight ratio (5:1 to 2.5:1), its width varies directly with protein concentration.

If the data represented in Fig. 2 are reduced to a common basis by plotting the protein-detergent weight ratio as abscissa against the per cent. protein precipitated as ordinate, the three curves very closely superimpose, indicating that precipitation is governed by this ratio. This is also illustrated by the small inset graph of Fig. 2 in which the per cent. SDS required to precipitate 50% of the albumin is plotted against the protein concentration, the figures being interpolated from the larger graph in Fig. 2.

Qualitative tests for SDS made by the addition of barium chloride to the supernatant solutions indicated that below the region of detergent excess nearly all of the SDS was combined with the precipitate. The maximal concentration of SDS that will completely precipitate a given amount of horse serum albumin at room temperature corresponds approximately to the total acid-binding capacity of the protein. The average value obtained by interpolation from the curves in Fig. 2 is 145 moles of SDS per g. of protein $\times 10^5$. This correlation suggests that anionic detergents may act to precipitate proteins by combining stoichiometrically with the positively charged groups of the protein. However, since precipitation is already complete at a detergent concentration corresponding to about one-half of the total acid-binding capacity, this relation may be fortuitous and, therefore, did not warrant a detailed analysis of the composition of the precipitates in the region of protein excess.

2. *p*H Region of Precipitation.—The effect of *p*H on the precipitation of horse serum albumin by SDS was determined in the acetate buffer range (0.1 *N* sodium acetate) at a final protein concentration of 9.70 mg. per cc., at 31°. Two different concentrations of SDS were chosen; the one, 2.5 mg. per cc., was calculated as just sufficient to precipitate all the protein present, while the other, 1.5 mg. per cc., was estimated to yield about 50% precipitation (Fig. 3).

The lower curve, corresponding to partial precipitation, was established by Kjeldahl analysis. The upper curve was obtained by the observation that no precipitate was formed at *p*H 4.94 or higher, while at *p*H 4.73 and lower, no precipitate resulted on the addition of trichloroacetic acid to the clear supernatant solutions.[34]

When a solution of SDS or Duponol (adjusted to *p*H 4.85) is added to a salt-free, isoelectric solution of serum albumin (*p*H 4.85), no precipitation ensues, and the *p*H rises to a maximum value of about 6.4. On the addition of acid to the clear solution it becomes cloudy at the isoelectric point, and the protein is completely precipitated on further acidification. The *p*H increase undergone upon the titration of 1 g. of horse serum albumin in 100 cc. of water with 100 cc. of 0.25% Duponol is illustrated in Fig.

(33) A personal communication from Dr. T. L. McMeekin reveals that this point is in substantial agreement with his unpublished observations.

(34) We have verified the statement of Anson[8] that under specified conditions the presence of Duponol interferes with the precipitation of hemoglobin by trichloroacetic acid (see also (11)). Under similar conditions we have observed no interference with the trichloroacetic acid precipitation of serum albumin or serum globulin.

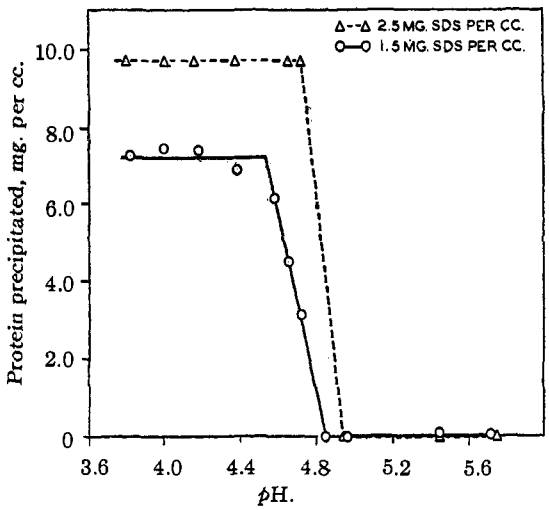

Fig. 3.—*p*H region of precipitation of crystalline horse serum albumin (9.70 mg. per cc.) by sodium dodecyl sulfate in 0.1 *N* sodium acetate. The upper curve (complete precipitation) refers to a detergent concentration of 2.5 mg. per cc., the lower curve (partial precipitation) to 1.5 mg. cc.

4. It is evident that in order to precipitate proteins with anionic detergents it is necessary to work in buffered solutions.

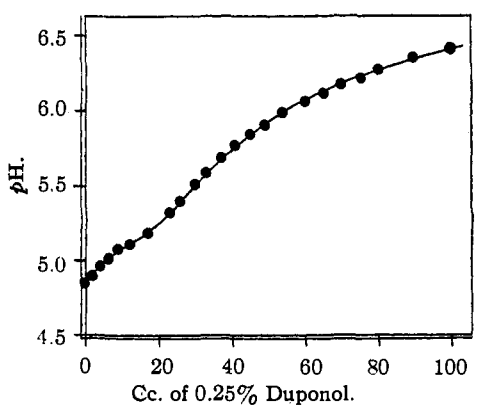

Fig. 4.—Change in *p*H of a salt-free 1% serum albumin solution (isoelectric point, *p*H 4.85) upon the addition of increments of Duponol (adjusted to *p*H 4.85).

3. Influence of Temperature and Salt Concentration.—The effect of temperature was first studied at a protein-detergent concentration ratio corresponding to the half-precipitation level. The reacting solutions were equilibrated at various temperatures, mixed, allowed to stand one-half hour, then centrifuged at the same temperature, and analyzed as already described. The protein concentration was 9.76 mg. per cc., the SDS concentration 1.5 mg. per cc., the solvent being 0.1 *N* sodium acetate, *p*H 4.5.

The large temperature effect observed under these conditions is shown in Fig. 5.

Although an albumin solution precipitated at 4° yields additional precipitate upon equilibration at 36°, to an extent corresponding to this point, the path going from 36 to 4° is only partially reversible.

Investigation of the influence of temperature on precipitation at various protein-detergent ratios revealed that this effect was confined to the protein or detergent excess regions, the width of the equivalence zone being but little affected by changing temperature.

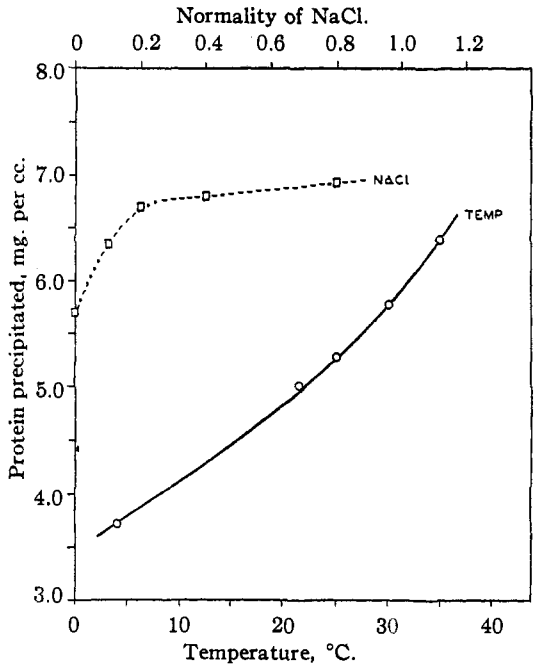

Fig. 5.—Influence of temperature and salt concentration on the partial precipitation of horse serum albumin (9.76 mg. per cc.) by sodium dodecyl sulfate (1.5 mg. per cc.). The bottom abscissa refers to temperature, the solvent being 0.1 *N* sodium acetate, *p*H 4.5. The top abscissa refers to sodium chloride concentration, the solvent likewise containing 0.1 *N* sodium acetate, *p*H 4.5 (t = 31°).

As shown in Fig. 5, increasing the salt concentration likewise enhances the precipitation of serum albumin at the half-precipitation level, the first increments of salt being most effective.

Properties of the Protein-Detergent Complex and of the Recovered Protein.—The complex precipitated in the equivalence zone is sparingly soluble in water, neutral salt solutions, or in weakly acidic buffers, but dissolves in alkaline solutions or on dialysis against water. Upon prolonged dialysis of an alkaline solution, some of the SDS passes through the membrane. However, complete separation of protein and detergent cannot be accomplished by this method, for the presence of undialyzable SDS may be demonstrated by the addition of barium chloride, or by the precipitation of the protein upon slight acidification of the solution. Moreover, considerably lower concentrations of ammonium sulfate are required for the precipitation of the protein under these conditions, than for the precipitation of native serum albumin.

Complete dissociation of the complex and removal of SDS may be accomplished by the addition of a small excess of barium chloride (1 mM. per 0.9 mM. of SDS). The highly insoluble barium dodecyl sulfate so formed is negative to qualitative protein tests after two washings. Excess barium remaining in the supernatant pro-

tein solution is removed by the addition of ammonium sulfate incidental to the reprecipitation of the protein.

The recovered albumin resembles the native protein in solubility in ammonium sulfate but is not readily crystallizable. It is electrophoretically homogeneous but exhibits a higher degree of boundary spread than the native material. Moreover, in a veronal buffer *p*H 7.6, $\mu = 0.1$, the mobility is 7% greater than the observed for native horse serum albumin (Table II). Viscosity measurements indicate that the specific hydrodynamic volume of the recovered protein is about 25% less than that of the native, whereas the diffusion constant of the former undergoes a corresponding increase with the result that the molecular weight remains essentially unchanged. Evaluation of the diffusion curves by methods already described[20] indicates that the recovered protein is molecularly homogeneous. A summary of the molecular properties is given in Table II.

TABLE II

MOLECULAR CONSTANTS OF NATIVE HORSE SERUM ALBUMIN AND OF ALBUMIN RECOVERED FROM SODIUM DODECYL SULFATE PRECIPITATE

U = the electrophoretic mobility in sq. cm. per second per volt $\times 10^{-5}$ in 0.03 N sodium veronal − 0.07 N sodium chloride buffer, *p*H 7.60, at 1.0° after four hours migration. For the explanation of other symbols see ref. (21).

Protein	η_{sp}	$(b/a)_k$	$(f/f_0)_\eta$	$d_{20,w}$, cm.^{2} sec.$^{-1}$ $\times 10^{-7}$	M	U, cm.^{2} sec.$^{-1}$ volt^{-1} $\times 10^{-5}$
Native	4.1	3.2	1.23	6.99	75,700	6.25
Recov.	3.1	2.0	1.15	7.44	77,000	6.70

Discussion

The precipitating action of detergents on proteins has already been observed in previous work. Following an observation by Bull and Neurath on the precipitating and dispersing effects of sodium dodecyl sulfate on egg albumin,[35] similar findings were reported by Miller and Andersson for insulin.[36] McMeekin[3] has investigated quantitatively the relative effectiveness of a number of homologous anionic detergents in precipitating crystalline egg albumin and β-lactoglobulin, while Schmidt[4] has reported analogous experiments involving cationic detergents.

Precipitation of proteins by synthetic detergents appears to be caused by electrostatic forces. This is suggested by the finding reported herein that with anionic detergents, precipitation is restricted to protein cations, while with cationic detergents it is limited to protein anions.[2,37] Ultracentrifugal and diffusion measurements indicate that the sodium alkyl sulfates exist in solution in micellar form, with a molecular weight of about 12,500.[36] Accordingly, it may be calculated that in the region of complete precipitation each protein molecule combines with 1 to 2 detergent micelles. Since the micelles are negatively charged, precipitation may be ascribed to the mutual coagulation of oppositely charged micelles, while the redispersion of the precipitate may be explained as resulting from a reversal of charge arising under conditions of detergent excess. This explanation is further strengthened by the observation that anionic and cationic detergents themselves are capable of similar interaction, *i. e.*, precipitation and redispersion.

Although the interpretation of the mutual precipitation of proteins and detergents as a colloidal phenomenon has much to recommend itself, it may be significant that the reactivity of *detergent acids*, as well as of other acidic protein precipitants such as flavianic, trichloroacetic, metaphosphoric and picric acids, arises from an apparently stoichiometric anion association with the positively charged groups of the protein.[38,39] The precipitating efficiency of these reagents, as well as their catalytic effectiveness in the hydrolysis of protein amide and peptide bonds, parallels their anion affinities, the affinities of dodecyl sulfate and dodecyl sulfonate being among the highest so far measured.[11] However, not all proteins are precipitated by detergents at a given acidity but each at a *p*H determined by the respective isoelectric point.[40] Moreover, in contradistinction to the action of other specific protein precipitants, detergents when present in excess redisperse the precipitate initially formed, in a manner typical of colloidal reactions.

Proteins and detergents are subject to interaction even at conditions unfavorable for precipitation, *i. e.*, when they carry charges of the same sign. This is evidenced by ultracentrifugal and diffusion studies on mixtures of Duponol and insulin,[36] as well as by electrophoresis. In agreement with a recent report by Lundgren, *et al.*,[15] we have found that a solution of the dialyzed protein-detergent complex, obtained after dissolution of the precipitate, exhibited anomalous behavior, an unequal number of boundaries arising on the ascending and descending sides of the cell. This phenomenon is similar in kind to that observed in the interaction between thymonucleic acid and serum albumin in *p*H regions alkaline to the isoelectric point of the protein.[42] In this case, too, precipitation ensues when the *p*H is adjusted to the acid side of the isoelectric point.

(35) H. B. Bull and H. Neurath, *J. Biol. Chem.*, **118**, 163 (1937).

(36) G. L. Miller and K. J. I. Andersson, *ibid.*, **144**, 475 (1942).

(37) W. J. Jaffé, *J. Biol. Chem.*, **148**, 185 (1943).

(38) J. Steinhardt, C. H. Fugitt and M. Harris, *J. Res. Natl. Bur. Stds.*, **26**, 293 (1941).

(39) J. Steinhardt, C. H. Fugitt and M. Harris, *ibid.*, **28**, 201 (1942).

(40) Polymerized sodium metaphosphate offers an interesting analogy to the action of SDS. Briggs[41] has observed that on mixing sodium metaphosphate (*p*H 5.0) with isoelectric salt-free horse serum albumin, the solution remains clear and the *p*H rises abruptly in a manner somewhat similar to that observed herein for the addition of SDS (Fig. 4). On acidification, the metaphosphate protein solution becomes cloudy just above the isoelectric point and precipitates as the pH is decreased.

(41) D. R. Briggs, *J. Biol. Chem.*, **134**, 261 (1940).

(42) E. Stenhagen and T. Teorell, *Trans. Faraday Soc.*, **35**, 743 (1939).

While the very structural features which render detergents protein precipitants may likewise be involved in their denaturing action, the two phenomena may be discerned under certain conditions. Thus, while for anionic detergents precipitation is confined to the acid side of the isoelectric point, denaturation, as revealed by viscosity measurements,[43] occurs to about the same extent in both acid and alkaline regions, with detergents of both the anionic and cationic types. Moreover, while relatively low concentrations of detergents exert a high denaturing action on proteins,[1,5] this effect increases with detergent concentration in the region of detergent excess.[43]

The properties of serum albumin recovered from the precipitated complex approximate more closely those of the protein regenerated from concentrated urea or guanidine hydrochloride solutions[13,44] than they do those of the native material. Since precipitation by SDS leads to changes in properties which may not be wholly reversed even upon subsequent dissociation of the protein-detergent complex, the recovered protein must be denatured according to the general definition proposed for denaturation.[45] However, it is the degree of denaturation that varies with detergent concentration, regardless of whether the combination between protein and detergent leads to precipitation.

While the application of detergents to the isolation of proteins is restricted by the degree of denaturation incurred, nevertheless, their use may be warranted in specific instances, even in the preparation of biologically *active* materials. For example, the serological activity of antibodies[46] or the hormonal activity of insulin[47] have been shown to be rather insensitive to even high degrees of denaturation. Also, under suitable conditions detergents may be employed in the preparation of protein-free filtrates or of regenerated proteins. Since precipitation of a given protein ceases at the isoelectric point, detergents may conceivably be adapted to the separation of protein mixtures under conditions at which the components carry opposite charges, *e. g.*, serum proteins. This aspect of the problem is now being investigated in this Laboratory.

The authors are indebted to the Rockefeller Foundation, to the Lederle Laboratories, Inc., and to the Duke University Research Council for support of this work.

Summary

Investigation of the effects of synthetic detergents on proteins has revealed that anionic detergents precipitate proteins only in the cationic form, precipitation ceasing above the isoelectric point of the protein.

Quantitative study of the system crystalline horse serum albumin-sodium dodecyl sulfate has shown that precipitation is governed by the following factors: protein-detergent weight concentration ratio, *p*H, temperature and ionic strength. At low detergent concentrations the protein may be completely precipitated while in regions of detergent excess redispersion of the precipitate occurs.

Dissociation of the protein-detergent complex by means of barium salts yields a protein which, as indicated by diffusion, viscosity and electrophoresis, is in a regenerated rather than in the native state.

The mechanism of precipitation and the potential application of detergents to the preparation and separation of proteins have been considered.

(43) J. O. Erickson, F. W. Putnam and H. Neurath, unpublished observations; see also ref. 1.

(44) D. G. Sharp, G. R. Cooper, J. O. Erickson and H. Neurath, *J. Biol. Chem.*, **144**, 139 (1942).

(45) H. Neurath, J. P. Greenstein, F. W. Putnam and J. O. Erickson, *Chem. Rev.*, in press.

(46) J. O. Erickson and H. Neurath, *Science*, **98**, 284 (1943).

(47) A. Rothen, B. F. Chow, R. O. Greep and H. B. van Dyke, *Cold Spring Harbor Symposia Quant. Biol.*, **9**, 272 (1941).

DURHAM, N. C. RECEIVED DECEMBER 31, 1943

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF DELAWARE, AND THE AMMONIA DEPARTMENT OF E. I. DU PONT DE NEMOURS AND COMPANY, INC., WILMINGTON, DELAWARE]

Polarographic Examination of Carbonyl Compounds

BY JOHN M. LUPTON AND CECIL C. LYNCH

Although the polarographic reduction of many carbonyl compounds has been reported,[1] it is not possible to obtain reduction waves with saturated, unsubstituted ketones. This fact led us to the study of methods by which carbonyl compounds may be examined polarographically.

In searching for an environment in which carbonyl reaction products would give polarographic waves, several reagents were tested. In acid hydrazine solution,[2] ketones give double waves as shown in Fig. 1. Aldehydes give similar waves. The half-wave potentials vary somewhat with concentration, but for ketones the first wave occurs near -1.1 v. (*vs.* S. C. E.); for aldehydes, near -0.9 v. For both aldehydes and ketones the half-wave potential of the second wave is in the

(1) I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience Publishers, Inc., New York, N. Y., 1941.

(2) The polarographic reduction of the hydrazonium and phenylhydrazonium ions has not been investigated. Since they do not appreciably alter the hydrogen discharge in hydrochloric and sulfuric acid solutions (0.1 *N*) their half-wave potentials must be more negative than -1.5 v.