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Physical-chemical Studies on the Interaction of Surface-active Agents with Nucleoproteins. I[1]

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The solubilizing action of thirty-one surface-active agents on calf thymus nucleohistone has been investigated in a qualitative way. The electrophoretic behavior of nucleohistone with three of these agents, Santomerse D, sodium lignin sulfonate and Igepon-T has been studied and evidence for complex formation shown. Further investigation of the Santomerse D–nucleohistone system containing 0.02 ionic strength phosphate buffer in the ultracentrifuge has shown that the nucleic acid–protein bond is not broken in complex formation. The molecular weight of nucleohistone in 0.35% Santomerse D solution is considerably lower than that in 0.02 ionic strength phosphate buffer.

Introduction

The limited solubility of many nucleoproteins in water and in 1 *M* sodium chloride, and the tendency of the latter solvent to cause dissociation of the nucleoprotein into nucleic acid and protein[2] make desirable a different method of isolation from animal tissues. The increase of solubility through the formation of a soluble complex between nucleoprotein and surface-active agents presents one method of attack on this problem. Although the action of surface-active agents on the simple proteins has been well studied,[3] little attention has been given to their behavior with nucleoproteins. Sodium dodecyl sulfate and sodium desoxycholate have been found to dissolve nucleoproteins[4] and the former to split nucleic acid from plant viruses,[3] but physical-chemical study of the interaction of surface-active agents with nucleoproteins has not been reported.

Therefore, thirty-one compounds have been tested for their ability to form saline-soluble complexes with calf thymus nucleohistone. Physical-chemical studies involving electrophoretic, ultracentrifugal, diffusion and viscosity measurements are presented here for the system nucleohistone–Santomerse D, as well as electrophoretic studies on sodium lignin sulfonate and Igepon-T.

Experimental and Results

Isolation of Nucleohistone, Histone Hydrochloride and Tetrasodium Nucleate.—The nucleohistone of calf thymus was isolated by the procedure of Carter and Hall.[5] All operations were carried out in a cold room at 4°. The macerated glands of freshly slaughtered calves were extracted with 0.02 ionic strength phosphate buffer, *p*H 6.98, which was discarded. The crude undissolved nucleohistone was dissolved in water and purified by reprecipitation twice from water by addition of 1 *M* sodium chloride containing 0.02 ionic strength phosphate buffer, *p*H 6.72, to 1% sodium chloride concentration. The final water solution, after centrifugation, contained 0.81% nucleoprotein.[6] It was stored in a deep-freeze at −30°. The ultraviolet absorption curve[7] of an 0.007% solution[6] of the nucleohistone was in agreement with that of Mirsky and Pollister[4] for chromosin. Although the final solution of nucleohistone appeared to be homogeneous, freezing caused agglomeration of nucleohistone to form a thick suspension. Histone hydrochloride was isolated from thymus nucleohistone by 0.2 *N* hydrochloric acid.[4] After dialysis in cellophane against distilled water until free of chloride ion, the slightly opalescent solution had a *p*H of 4.45 and a protein content of 0.26%.[6] The ultraviolet absorption curve[7] of an 0.026% solution showed a maximum at 275 mμ, which checks the reported value.[4] Nucleic acid was isolated as the tetrasodium salt from crude thymus nucleohistone by a modified[8] Hammarsten[9] procedure using saturated sodium chloride. Washing of the precipitated nucleate was facilitated by use of a Waring blendor, followed by centrifugation. After drying over concentrated sulfuric acid at 20° *in vacuo*, the white, fibrous product contained 4.41% moisture (determined by loss of water at 110° *in vacuo*). It contained 14.04% nitrogen,[6] 7.44% phosphorus and 6.23% sodium, calculated on the dry basis. The content of desoxyribose nucleic acid by the diphenylamine reaction[10] was 2.26 mg./ml. in an 0.2% solution. The ultraviolet absorption curve[7] showed $\epsilon = 18$ cc./mg., compared to the value of 16.5 cc./mg. calculated from Chargaff's[11] data. An 0.41% solution of the nucleate (dried over sulfuric acid) was used for electrophoretic studies.

Surface-Active Agents.—A qualitative study of thirty-one surface-active agents was made to evaluate their solubilizing action on thymus nucleohistone. The compounds investigated were Santomerse D, sodium decylbenzenesulfonate; sodium lignin sulfonate; Igepon-T, sodium N-methyl-N-oleoyltaurate; sodium cellulose phthalate; Vel, neutral sulfated monoglyceride; sodium desoxycholate; Santomerse No. 3, sodium dodecylbenzenesulfonate; Aerosol OT, bis-(2-ethylhexyl) sodium sulfosuccinate; alkyl naphthalenesulfonate, mol. wt. 470; sodium glycerophosphate; Sterox CD, SE and SK, non-ionic wetting agents; potassium cellulose sulfate; deacetylated chitin acetate; Asolectin, a mixture of about 30% lecithin with equal amounts of cephalin and lipositol; sodium glycocholate; sodium cholate; Zephiran chloride, a mixture of alkylbenzyldimethylammonium chlorides; chlorophyll; Triton A-20, alkyl aryl polyether alcohol; Triton 720, alkylated aryl polyether sodium sulfonate; gelatin sulfate; ethylene oxide condensation products with mercaptan (mol. wt. 685), with sorbitan monolaurate (mol. wt. 1240), with fatty alcohols (mol. wts. 520 and 880), with C_{18} amide (mol. wt. 944); the lauric ester of polyethylene glycol (mol. wt. 769); sodium dodecylsulfate; sodium carboxymethyl cellulose.

The compounds were purified if necessary. The purification of the three compounds studied further illustrates the procedure used in numerous cases. **Santomerse D**, obtained from Monsanto Chemical Co., was purified by solution in absolute ethanol, filtration and evaporation of the alcohol. The residue was dried at 70° *in vacuo*. **Sodium lignin sulfonate** was prepared from crude calcium lignin sulfonate (No. 90, Master Builders Co.). The calcium salt was dialyzed in cellophane against running distilled water for 48 hours. Calcium and acid impurities were removed by passage over ion-exchange resins. The purified lignin sulfonic acid was neutralized to *p*H 7 with sodium hydroxide. **Igepon-T** was purified by dialysis and by solution in absolute ethanol (*cf.* Santomerse D). The latter method gave a product dissolving completely in water and remaining

(1) Based on research carried out under contract between the Office of Naval Research and The Ohio State University Research Foundation.

(2) A. E. Mirsky and A. W. Pollister, *Proc. Nat. Acad. Sci. U. S.*, **28**, 344 (1942).

(3) F. W. Putnam, "Advances in Protein Chemistry," Academic Press, Inc., New York, N. Y., 1948, Vol. 4, p. 79.

(4) A. E. Mirsky and A. W. Pollister, *J. Gen. Physiol.*, **30**, 117 (1946).

(5) R. O. Carter and J. L. Hall, THIS JOURNAL, **62**, 1194 (1940).

(6) Determined by micro-Kjeldahl analysis for nitrogen.

(7) Ultraviolet absorption measurements were carried out with a Beckman quartz spectrophotometer, model DU.

(8) L. Ahlström, H. v. Euler and L. Hahn, *Arkiv Kemi, Mineral. Geol.*, **A22**, No. 13 (1946); B. Taylor, J. P. Greenstein and A. Hollaender, *Arch. Biochem.*, **16**, 19 (1948).

(9) E. Hammarsten, *Biochem. Z.*, **144**, 383 (1924).

(10) Z. Dische, *Mikrochemie*, **8**, 4 (1930).

(11) E. Chargaff, *et al.*, *J. Biol. Chem.*, **177**, 405 (1949).

clear at 4° for a much longer time than the former. The ethanol-purified material was used for all work reported.

The solubilizing action of each agent on a water suspension of freshly precipitated nucleohistone, and the protective action of the agent against precipitation of the dissolved nucleohistone by sodium chloride present in 1% concentration were the criteria used in evaluating the agents. For the test, a nucleohistone solution containing 0.53% nucleoprotein[6] was used. It was prepared from a representative sample of the nucleohistone suspension mentioned above by dialysis in cellophane against distilled water for 16 hours, followed by centrifugation. The surface-active agents were made up in water to a concentration of about 1% and the *p*H adjusted with acid or alkali to as close to 7 as possible. Five ml. of nucleohistone solution was shaken with 1 ml. of 1 *M* sodium chloride containing 0.02 ionic strength phosphate buffer, *p*H 6.72, in a 15-ml. graduated centrifuge tube. After centrifuging for 5 minutes in a clinical centrifuge, the residue of white precipitated nucleohistone, 0.35 to 0.45 ml. in volume, was diluted to 5 ml. with water, well shaken to disperse the precipitate, and 5 ml. of the surface-active agent added. If a clear, viscous solution resulted, 2 ml. of 1 *M* sodium chloride was added and the tube well shaken. A surface-active agent which prevented the formation of turbidity or a precipitate in presence of sodium chloride was considered to have positive solubilizing action.

Positive solubilizing action was shown by the first five compounds listed; the next four had partial action and the remainder negative action. The surface-active agents which had positive action—Santomerse D, sodium lignin sulfonate, Igepon-T, sodium cellulose phthalate[12] and Vel[13]—are all of the anionic type. The agents of the non-ionic type studied had no action and the single example of the cationic type precipitated the nucleohistone.

Stock solutions of Santomerse D (0.88%[14]), sodium lignin sulfonate (1.56%[15]) and Igepon-T (0.89%[16]) were prepared for the measurements which follow.

Extraction of Nucleohistone from Calf Thymus by Surface-active Agents.—In order to evaluate Santomerse D as an extracting agent for nucleohistone from calf thymus, the fresh tissue, which had been macerated and washed with 0.85% sodium chloride, was treated at 4° with 0.43 and 0.18%, resp., Santomerse D solutions. The nucleohistone, isolated by alcohol precipitation from the clarified extract, was insoluble in water. The clear, viscous extract, obtained from 250 ml. of 0.18% Santomerse D solution and 50 g. of prepared tissue, after filtration through cheesecloth, dialysis for 94 hours in cellophane against water to a negative barium chloride reaction and centrifugation, contained only 0.15% nucleoprotein.[6] The presence of Santomerse D in this material was indicated by a positive test for sulfur and its electrophoretic behavior (Run 20). The incomplete removal of Santomerse D is to be expected since it was found that Santomerse D does not dialyze completely from solution in water, through cellophane, into water. Attempts to remove Santomerse D completely by barium chloride precipitation from nucleohistone solution were likewise unsuccessful.

Preliminary experiments on the extraction of tissue with sodium lignin sulfonate, which is non-dialyzable, showed that an extract of lower viscosity than with Santomerse D was obtained. However, attempts to remove the surface-active agent from the nucleohistone solution by chromatographic adsorption were unsuccessful.

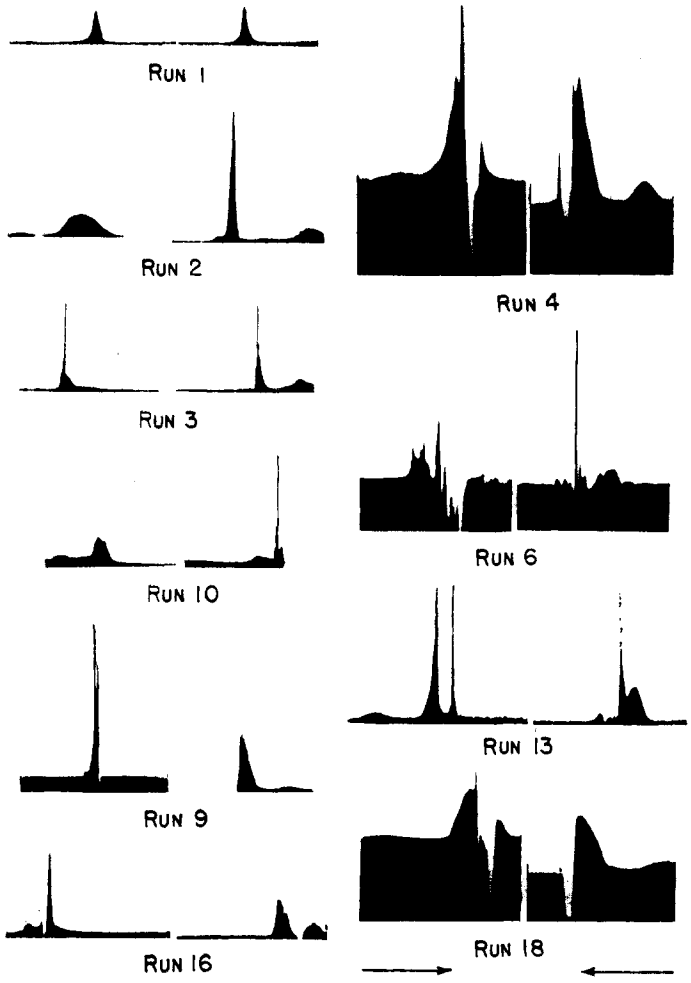

Fig. 1.—Electrophoretic patterns of thymus nucleohistone, histone hydrochloride, tetrasodium nucleate and surface- active agents. For each run, the pattern of the ascending boundary is shown on the right, the descending on the left. The direction of movement of the boundaries in all cases is as indicated for Run 18.

Electrophoretic Studies.—The electrophoretic behavior of the selected surface-active agents with thymus nucleohistone, histone hydrochloride and tetrasodium nucleate was investigated using the Tiselius–Klett electrophoresis apparatus with schlieren optical system.[17] The nucleohistone used was the 0.81% solution previously described. The water solution of the sample (thoroughly mixed with surface-active agent, if used, and made up to 25 ml. with water) was dialyzed in cellophane against 200 ml. of buffer overnight, followed by a similar dialysis against 1500 ml. of buffer which was reserved for filling the electrode vessels. The dialyzed sample was centrifuged if not entirely clear.

The mobilities summarized in Table I were calculated from the observed rate of movement of the boundary corresponding to the main component. The heat dissipation was less than 0.1 watt/cc. No corrections were made for δ and ϵ effects in calculating the mobilities of the ascending boundaries. Some representative electrophoretic patterns are shown in Fig. 1.

The average composition of all Santomerse D complexes with nucleohistone, histone or nucleic acid present in a given system was calculated from (1) the sum of the areas for all components in the "schlieren" scanning pattern for the system, (2) the known concentration of nucleohistone, histone hydrochloride or tetrasodium nucleate and (3) the refractive index increments for Santomerse D, nucleohistone, histone hydrochloride and tetrasodium nucleate. The refractive index increment for Santomerse D was determined in this

(12) Not studied further because of precipitation of nucleohistone during dialysis prior to electrophoresis.

(13) Not studied further because of insufficient solubility at 4°.

(14) Analysis by the method of T. U. Marron and J. Schifferli, *Ind. Eng. Chem., Anal. Ed.*, **18**, 49 (1946), adapted to quantities of 0.05 to 0.10 g. of Santomerse D.

(15) Determined from weight of residue after drying to constant weight *in vacuo* at 70°.

(16) Analysis by the method of D. A. Shiraeff, *Am. Dyestuff Reptr.*, **36**, 313 (1947).

(17) L. G. Longsworth, *Ind. Eng. Chem. Anal. Ed.*, **18**, 219 (1946).

TABLE I

MOBILITIES OF THYMUS NUCLEOHISTONE, HISTONE HYDROCHLORIDE, TETRASODIUM NUCLEATE AND SURFACE-ACTIVE AGENT COMPONENTS AT 0.5°

	Sample			Buffer				Dialysis time, days	pH 20°	Mobility cm.^{2} sec.$^{-1}$ volt^{-1} × 10^5	
Run	Solute	Concn., %	Surface active agent	Concn., %	Inorg. salt[a]	Surface-active agent	Concn., %			Asc.	Desc.
1	Nucleohistone	0.068			Phosphate			2	6.81	−11.9	−11.4
2	Histone HCl	.26			Phosphate			2	6.64	+ 6.2	+ 6.4
3	Na_4 nucleate	.16			Phosphate			3	6.75	−16.0	−18.0
4	Nucleohistone	.32	Santomerse D	0.35	Phosphate	Santomerse D	0.35	2	6.75	−15.2	−15.0
5	Nucleohistone	.32	Santomerse D	.35	Acetate	Santomerse D	.35	2	5.15	− 8.3	−11.6
6	Nucleohistone	.48	Santomerse D	.35	Phosphate, 1% NaCl	Santomerse D	.35	2	6.72	[b]	[b]
7	Nucleohistone	.32	Santomerse D	.53	Phosphate			1	6.93	−16.6	−15.2
8	Nucleohistone	.32	Santomerse D	.69	Phosphate	Santomerse D	.69	2	6.81	−16.9	−15.5
9	Histone HCl	.16	Santomerse D	.35	Phosphate	Santomerse D	.35	2	6.72	−16.7	−15.7
10	Na_4 nucleate	.16	Santomerse D	.35	Phosphate	Santomerse D	.35	2	6.77	−16.0	−18.7
11	Na_4 nucleate	.16	Santomerse D	.35	Acetate	Santomerse D	.35	2	5.14	−18.6	−18.9
12			Santomerse D	.35	Phosphate			0	6.85	−21.4	−20.8
13	Nucleohistone	.32	Na lignin sulfonate	.35	Phosphate	Na lignin sulfonate	.35	3	6.77	−16.7	−13.5
14	Nucleohistone	.32	Na lignin sulfonate[c]	.35	Phosphate	Na lignin sulfonate[c]	.35	2	6.83	−12.8	−13.5
15	Nucleohistone	.32	Na lignin sulfonate	.54	Phosphate			1	6.83	−16.1	−14.7
16	Na_4 nucleate	.16	Na lignin sulfonate	.35	Phosphate	Na lignin sulfonate	.35	3	6.70	−22.5	−21.7
17			Na lignin sulfonate	.35	Phosphate			2	6.82	−20.3	−18.0
18	Nucleohistone	.32	Igepon-T	.35	Phosphate	Igepon-T	.35	2	6.85	−15.20[d]	−15.30[d]
19	Nucleohistone	.32	Igepon-T	.35	Borate	Igepon-T	.35	2	8.34	−10.79	−13.35
20	Nucleohistone extracted by Santomerse D	.15			Phosphate			2	6.87	−12.8	−12.8
21	Ovalbumin[e]	.45	Santomerse D	.35	Phosphate	Santomerse D	.35	2	6.78	−15.3	−15.5
22	Ovalbumin[e]	1.12			Phosphate[e] 0.08 *M* NaCl			2	7.1	− 6.07	− 6.07

[a] Ionic strength 0.02. [b] Measurement of main boundary impossible. [c] Prepared from a non-dialyzable calcium lignin sulfonate, courtesy of Dr. J. L. McCarthy, Pulp Mills Research, University of Washington. [d] Turbidity developed in sample during run. [e] Four times recrystallized, prepared by Mrs. Justine S. Garvey, to whom we are indebted for this material and for its mobility. Ionic strength of buffer, 0.2.

Laboratory and was found to be 2.2 × 10^{-4} cc./mg. The increments used for nucleohistone,[5] and tetrasodium nucleate[18] were 1.76 × 10^{-4} and 1.60 × 10^{-4} cc./mg., respectively. For histone hydrochloride a calculated value of 1.92 × 10^{-4} cc./mg. was used. The results are listed in Table II.

TABLE II

BINDING OF SANTOMERSE D BY HISTONE HYDROCHLORIDE, TETRASODIUM NUCLEATE AND NUCLEOHISTONE FROM ELECTROPHORETIC PATTERNS

Material	Material, %	Santomerse D, %	Total area, sq. cm.	Calcd. area, material, sq. cm.	Area due to Santomerse D, sq. cm.	Wt. reaction Santomerse D in complex
Histone hydrochloride	0.16	0.35	0.69	0.43	0.26	0.34
Tetrasodium nucleate	.16	.35	.54	.35	.19	.28
Nucleohistone	.32	.35	0.88, 1.02[a]	.78	0.10, 0.24[a]	0.09, 0.20[a]

[a] Area due to peak below base line not deducted.

Ultracentrifuge Studies.—Sedimentation velocity measurements were made with an air-driven ultracentrifuge of the Beams type[19] at speeds of 30,000–47,000 r.p.m., corresponding to an average centrifugal acceleration of 100,000 times gravity. The customary lens system was replaced by parabolic mirrors[20] to permit the investigation of extremely low concentrations of nucleoprotein by means of ultraviolet light. Light of wave length mainly 2600 Å. was isolated from the mercury vapor source (G.E. Type A-H 6 lamp) by series of filters, cobalt-nickel sulfate, gaseous chlorine[21] and Corning No. 5893 cobalt glass. Centrifuge cell windows of quartz were used throughout. Sedimentation pictures were obtained by the "schlieren" method[17] when the filters were not used and boundary positions measured with a Gaertner comparator. The "light absorption"[22] patterns obtained with light of 2600 Å. were scanned with a Leeds and Northrup microphotodensitometer equipped with Speedomax recorder. The concentration scale was obtained by similar measurement of patterns from a series of uracil solutions in 2 *N* hydrochloric acid—E_{260} = 0.133 to 0.043 (1 cm. cell)[7] contained in a 5 cm. cell with quartz windows. The same cell, filled with the solvent, remained in the light path during the entire sedimentation process.

The sedimentation constants were calculated from the equation[23]

$$s_{20} = \frac{\Delta x/\Delta t}{\omega^2 X_m} \cdot \frac{\eta_t}{\eta_{20}}$$

Where η_t is the viscosity of the solvent at the average temperature t, and η_{20} is the viscosity of water at 20°. Since the buoyancy correction $(1 - V\rho_{20})/(1 - V\rho_t)$ is very small, due to variation in density of only 0.3% over a 10° range, it has been omitted from the calculations.

All solutions contained 0.02 ionic strength phosphate buffer and were prepared as described for the electrophoretic studies. The solutions of Santomerse D with 0.35 and 0.48% nucleohistone were used in the ultracentrifuge immediately after completion of dialysis since precipitation occurred within a few days. This behavior was not observed with the diluted samples. The experimental results have been summarized in Table III. The sedimentation constant of Santomerse D in 0.35% solution was too low to be measurable under the experimental conditions used. The components of the sedimenting sample in the nucleohistone–Santomerse D system, using freshly prepared nucleohistone, are shown in the "schlieren" scanning patterns of Fig. 2.

The composition of the nucleohistone–Santomerse D complex, as shown in "schlieren" patterns of the sedimenting samples (Fig. 2), was determined by calculations similar to those described previously for the electrophoretic patterns. The results are listed in Table IV.

(18) H. G. Tennent and C. F. Vilbrandt, THIS JOURNAL, **65**, 424 (1943).

(19) E. R. Lang, Q. Van Winkle and W. G. France, *J. Colloid Sci.*, **2**, 315 (1947).

(20) A description of this optical system will be published elsewhere.

(21) H. L. J. Bäckström, *Arkiv Kemi, Mineral. Geol.*, **A13**, No. 24 (1940).

(22) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Clarendon Press, Oxford, 1940, p. 240.

(23) T. Svedberg and K. O. Pedersen, *ibid.*, p. 274.

TABLE III

SEDIMENTATION VELOCITY DETERMINATIONS ON THYMUS NUCLEOHISTONE

Concn. of nucleohistone, %	Solvent	s_{20}/
0.029[b]	Water	15.7[a]
.32[c]	0.35% Santomerse D	4.14, 2.36[d]
.24	.35% Santomerse D	5.31
.16	.35% Santomerse D	5.95
.16[c]	.35% Santomerse D	6.20, 1.70[d]
.128	.35% Santomerse D	7.08
.107	.35% Santomerse D	7.01
.107[c]	.35% Santomerse D	6.91
.000	.35% Santomerse D	11.36,[e] 1.32[d,e]
.48	.35% Santomerse D + 1% NaCl	5.33

[a] Ultraviolet light used. [b] $E_{260} = 3.88$, calculated from measurements on diluted samples. [c] Freshly prepared nucleohistone used. [d] Diffuse boundary (slow moving component). [e] Extrapolated from "schlieren" results. [f] Expressed in Svedberg units.

TABLE IV

BINDING OF SANTOMERSE D BY NUCLEOHISTONE FROM SEDIMENTATION PATTERNS

Nucleohistone, %	Santomerse D, %	Total area, sq. cm.	Calcd. area, nucleohistone, sq. cm.	Area due to Santomerse D, sq. cm.	Wt. fraction Santomerse D in complex
0.32	0.35	0.70	0.38	0.32	0.40
.16	.35	.46	.19	.27	.53
.107	.35	.43	.13	.30	.65

Diffusion Studies.—Diffusion measurements were made with a Claesson type diffusion cell[24] and the electrophoresis apparatus mentioned previously. The diffusion constants were calculated by the maximum ordinate–area method[25] using the equation

$$D = \left(\frac{A}{H}\right)^2 \frac{1}{m^2 4\pi t}$$

Since the measurements were made at 0.5°, the diffusion constants were converted to D_{20} by the equation

$$D_{20} = D_{0.5} \frac{\eta_{0.5}}{\eta_{20}} \frac{293.16}{273.66}$$

The solutions used for the diffusion studies were those described for the ultracentrifuge studies, with dilutions as indicated in Table V. Because precipitation occurred within several days in Santomerse D solution containing 0.32% nucleohistone, the value for the diffusion constant at this concentration is not considered reliable. No precipitation was noticed in the diluted samples.

TABLE V

DIFFUSION MEASUREMENTS ON THYMUS NUCLEOHISTONE

Concn. of nucleohistone, %	Solvent	D(sq. cm./sec.) × 10^6 (av.) 0.5°	20°
0.03	Water	3.02	5.71
.01	Water	3.10	5.86
.32	Santomerse D	4.03	7.52
.16	Santomerse D	4.10	7.65
.11	Santomerse D	3.17	5.91

Viscosity Measurements.—The viscosity of nucleohistone in 0.35% Santomerse D solution was determined in the usual way with an Ostwald viscosimeter. The solutions used were those described for the ultracentrifuge studies, with dilutions as indicated in Table VI. The intrinsic viscosity, $[\eta]$, for nucleohistone in Santomerse D solution was 5-10 (100 ml./g.).

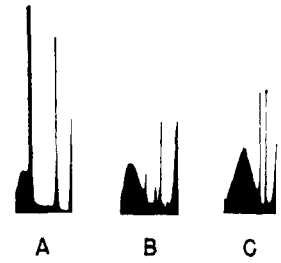

Fig. 2.—Ultracentrifuge "schlieren" scanning patterns of the sedimenting components in the nucleohistone–Santomerse D system: A, 0.107% nucleohistone; B, 0.16%; C, 0.32%. Direction of sedimentation, left to right.

TABLE VI

VISCOSITY OF THYMUS NUCLEOHISTONE IN 0.35% SANTOMERSE D AT 20°

Concn. of nucleohistone, %	Solvent[a]	η, cp.	η, sp/c
	Santomerse D	1.0167	
0.32[b]	Santomerse D	10.704	29.77
.24	Santomerse D	4.2022	13.05
.16[b]	Santomerse D	3.0074	12.24
.16	Santomerse D	2.7481	10.64
.128	Santomerse D	2.1551	8.75
.107[b]	Santomerse D	2.2865	11.67
.107	Santomerse D	2.0079	9.11

[a] Contains 0.02 ionic strength phosphate buffer. Freshly prepared nucleohistone used.

Molecular Weights.—The molecular weight of nucleohistone in water and in Santomerse D solutions was calculated in the customary way from the equation

$$M = RTs/D(1 - V\rho)$$

using sedimentation velocity and diffusion data. The value of 0.658 for the partial specific volume V of nucleohistone[26] was used. For the solution of nucleohistone in water containing 0.02 ionic strength phosphate buffer, the values of s_{20}, 15.7, and D_{20}, 5.71×10^{-8}, for the 0.03% solution were used. The molecular weight obtained, 1.9×10^6, agrees reasonably well with the values of 2.3 and 2.0×10^6 reported by Carter.[26] For the solution in Santomerse D, the value of s_{20}, 1.70, for the slow sedimenting component for 0.16% nucleohistone was used, together with the value of D_{20}, 7.65×10^{-8}, for the same concentration. The density of the solvent was 1.0001 at 20°. Since the slow sedimenting component comprises the larger part of the sedimenting sample, its molecular weight is believed to be representative of nucleohistone in Santomerse D. The molecular weight for the slow component was 1.6×10^5.

The molecular weight of nucleohistone in Santomerse D was also calculated from s_{20} and η using an equation combining Perrin's law[27] for rod-like ellipsoids and the Simha equation[28]

$$M = \left[\left(\frac{s}{1 - V\rho}\right)(f/f_0)\, 6\eta\right]^{3/2} \pi N (3V/4)^{1/2}$$

The value of $6.3 - 8.5 \times 10^4$ was obtained.

Discussion of Results

The general effect of solutions of surface-active agents, particularly detergents, on simple proteins has been found to be mainly one of denaturation accompanied by solution of the protein, with the formation of complexes of varying composition

(24) H. Walker, Jr., Ph.D. Dissertation, The Ohio State University, 1949.

(25) H. Neurath, *Chem. Revs.*, **30**, 357 (1942).

(26) R. O. Carter, THIS JOURNAL, **63**, 1960 (1941).

(27) F. Perrin, *J. phys. radium*, [7] **7**, 1 (1936).

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between detergent and protein.[3] Under certain conditions, the protein may be precipitated.[3]

Evidence for complex formation between nucleoproteins and surface-active agents consists in an increase in the electrophoretic mobility together with the difference in the electrophoretic patterns obtained from that of the original nucleoprotein, also in changes in molecular size and shape as shown by ultracentrifuge measurements. The increase in the electrophoretic mobilities of nucleohistone caused by Santomerse D, sodium lignin sulfonate and Igepon-T is evident from Table I. The multiplicity of peaks found in Runs 4, 6, 7, 13, 14, 18, 19 may indicate a series of different types of complexes with the intact nucleohistone molecule or a splitting of the molecule into fragments with which the surface-active agent combines. In order to determine the part of the molecule with which reaction takes place, electrophoretic measurements have been made using thymus tetrasodium nucleate and histone hydrochloride. With the nucleate the mobility of the main component was unchanged by Santomerse D, but from area measurements (Tables II and IV) complex formation is evident. The increase in mobility of histone hydrochloride in presence of Santomerse D is evidence that complex formation takes place with histone hydrochloride. The increase in mobility of the nucleate with sodium lignin sulfonate is an evidence of complex formation. However, the picture could not be completed for histone hydrochloride because it was almost completely precipitated by the sodium lignin sulfonate during dialysis.

Unusual electrophoretic patterns have been obtained with nucleohistone and surface-active agents (Fig. 1, Runs 4, 6, 18). These patterns show inverted peaks extending below the base line. Although this effect was not observed with sodium lignin sulfonate from one source (Run 13), it was seen in the pattern of Run 14. This is believed to be due to difference in composition of the materials from the two sources. The inverted peaks are caused by a change in the nature of the ionic species across the boundary, where the trailing species has a lower refractive index increment than the fast moving species. The nature of the species in the present systems is not known. Inverted peaks have been observed in solutions containing monovalent succinate and oxalate ions[29] and were attributed to the fact that the refractive index gradient does not parallel the density gradient at all of the boundaries.

The pronounced effect of sodium chloride in presence of Santomerse D, causing splitting of nucleohistone into numerous components, is shown in Fig. 1, Run 6. Its effect in presence of sodium lignin sulfonate was precipitation of the nucleohistone by formation of saline-insoluble complexes, leaving in solution an amount of nucleohistone insufficient for electrophoretic measurement. Because of these effects, no further work has been done on buffer systems containing sodium chloride.

In electrophoretic studies, the electric field itself can dissociate complexes formed by surface-active agents and proteins. The usual technique employed is that in which the surface-active agent is present with the protein but not in the buffer which is used in the remainder of the cell and in the electrode vessels. This generally results in a dissociation at the moving boundaries in the cell. That this occurs also with nucleoproteins was evident when Santomerse D was used (Run 7) and to a lesser extent with sodium lignin sulfonate (Run 15). This condition was avoided in all of the experiments reported here by using surface-active agent in both nucleoprotein and buffer solutions.

That the type of complex formed between nucleohistone and the three surface-active agents is of the dissociable type is evident from the electrophoretic patterns and ultracentrifuge data. It differs from the "all or none" type obtained with serum albumin and sodium dodecyl sulfate.[30] However, in the present work enough surface-active agent was available to combine with all the nucleohistone, therefore the peaks corresponding to uncombined protein are absent. The action of Santomerse D on ovalbumin (Run 21) illustrates the latter type of complex.

The ultracentrifuge patterns shown in Fig. 2 and the data of Table IV indicate that the composition of the nucleohistone–Santomerse D complex varies over a wide range of concentration, dependent on the ratio of nucleohistone to Santomerse D present in the solution. It is also evident from the areas measured in the patterns of Fig. 2 that Santomerse D does not break the nucleic acid–protein bond in water solutions containing 0.02 ionic strength phosphate buffer. If this bond were broken the protein would not sediment under the experimental conditions used and the peak areas would be at least 50% less than those obtained.

There is a difference between the values for per cent. Santomerse D in the nucleohistone–Santomerse D complex as measured by electrophoresis (Table II) and sedimentation (Table IV). This is brought about by the dialysis behavior of Santomerse D mentioned previously which causes the free Santomerse D in the nucleohistone–Santomerse D solution to be less than the Santomerse D concentration of the buffer solution against which it was dialyzed. This does not affect the areas of the sedimentation "schlieren" scanning patterns but does bring about a condition in which the areas of the corresponding electrophoresis patterns are too low by an amount which is proportional to the concentration difference in free Santomerse D across the moving boundaries.

Since the breaking of the nucleic acid–protein bond by Santomerse D was not observed in dilute salt solution the sedimentation behavior of nucleohistone in Santomerse D containing 1% sodium chloride (Table III) is of particular interest. From ultracentrifuge "schlieren" scanning patterns it was found that the sedimentable component accounted for only 50% of the nucleohistone present. A plausible explanation is that nucleic acid has been split from protein and sedimented either alone or in combination with Santomerse D.

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From the molecular weights obtained, it is evident that there is a decrease in molecular weight of nucleohistone from that in water to Santomerse D solution. The lack of agreement between the molecular weight in Santomerse D calculated from sedimentation and diffusion data as compared with that obtained from sedimentation and viscosity measurements is characteristic of polydisperse systems.

Although it has not been found possible to use the surface-active agents studied as agents for the extraction of tissue, information as to the mode of their combination with nucleohistone has been obtained.

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Small-Ring Compounds. IV. Interconversion Reactions of Cyclobutyl, Cyclopropylcarbinyl and Allylcarbinyl Derivatives

BY JOHN D. ROBERTS AND ROBERT H. MAZUR

A number of reactions have been investigated which lead to interconversion of cyclobutyl, cyclopropylcarbinyl and allylcarbinyl derivatives. Some degree of interconversion was observed in the following: vapor-phase light-catalyzed chlorination of methylcyclopropane; reaction of cyclopropylcarbinol with thionyl chloride, Lucas reagent, phosphorus tribromide and hydrobromic acid; cleavage of cyclopropylcarbinyl triethylsilyl ether with thionyl chloride; reaction of cyclobutanol with thionyl chloride and Lucas reagent; reaction of cyclopropylcarbinyl-, cyclobutyl- and allylcarbinylamines with nitrous acid; hydrolysis and acetolysis of cyclopropylcarbinyl and cyclobutyl chlorides; reaction of N-cyclopropylcarbinyl- and N-cyclobutylbenzamides with phosphorus pentabromide; and Grignard reactions of cyclopropylcarbinyl halides. No interconversion was observed in the following: reaction of allylcarbinol with thionyl chloride and phosphorus tribromide; vapor-phase light-catalyzed chlorination of cyclobutane; and Grignard reactions of allylcarbinyl and cyclobutyl halides. The products of those interconversion reactions which may reasonably be expected to involve carbonium ion intermediates have been correlated by consideration of carbonium ion stability and ease of interconversion, as well as the degree of reversibility of the reactions involved and the stabilities of the possible products. Cyclopropylcarbinyl halides have been shown to be unusually reactive in solvolysis reactions and, in fact, considerably more reactive than analogously constituted allylic halides.

Introduction

One of the principal limitations of carbonium-ion theory[1] as applied to rearrangement reactions occurring under the influence of polar catalysts or in polar media is the difficulty in deciding in advance which of several possible reaction paths should be most favorable. In no instance is this difficulty better exemplified than in the interconversion reactions of cyclobutyl, cyclopropylcarbinyl and allylcarbinyl derivatives where it has been reported that cyclobutyl- and cyclopropylcarbinylamines with nitrous acid give mixtures of cyclobutanol and cyclopropylcarbinol,[2] that cyclopropylcarbinol with phosphorus tribromide yields a bromide which on successive treatments with magnesium and carbon dioxide gives allylacetic acid,[3] and that cyclopropyldimethylcarbinol with hydrochloric acid forms (γ,γ-dimethylallyl)-carbinyl chloride which on hydrolysis with water regenerates cyclopropyldimethylcarbinol.[4] While simple *a posteriori* mechanistic interpretations of the above results may be made following the carbonium-ion theory of rearrangements, *prediction* of the product to be obtained from a given new carbonium-ion reaction must depend on a knowledge of some or all of several important factors such as: (1) the relative carbonium-ion stabilities, (2) the energy barriers to interconversion of the carbonium ions, (3) the relative reactivities of the carbonium ions toward nucleophilic substances (4) the reversibility of the reaction in question and (5) the thermodynamic stabilities of the possible products. It was of interest, therefore, to determine, insofar as possible, the importance of these factors in directing rearrangements under a variety of conditions in reactions of cyclobutyl, cyclopropylcarbinyl and allylcarbinyl derivatives.

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Preparation and Characterization of Allylcarbinyl, Cyclobutyl and Cyclopropylcarbinyl Halides

In order to determine qualitatively the relative stabilities of the cyclobutyl, cyclopropylcarbinyl and allylcarbinyl cations, one of the first objectives of the work was to prepare the corresponding halides and study their solvolytic reactivities. Allylcarbinyl chloride was readily obtained by the reaction of thionyl chloride with allylcarbinol prepared by the partial hydrogenation of 3-butyn-1-ol over palladium on calcium carbonate catalyst. Allylcarbinyl bromide was similarly obtained from the reaction of allylcarbinol with phosphorus tribromide. No rearrangements were noted in these reactions. The infrared spectra of the allylcarbinyl halides are shown in Figs. 1 and 2.

Cyclobutyl chloride was obtained by a vapor-phase light-catalyzed chlorination of cyclobutane. No other monochloride appeared to be formed in this preparation. Pure cyclobutyl bromide was obtained from the reaction of silver cyclobutane-