be more stable in the presence of less reactive groups, such as hydroxyl.

Experimental

The dimer of 2,4-tolylene diisocyanate was prepared by mixing 1350 g. of dry pyridine and 1590 g. of the diisocyanate with stirring.[2] Some cooling was used to keep the temperature below 40°. After standing overnight the solid reaction mass was diluted with 3.5 liters of dry carbon tetrachloride, pulverized, filtered and dried in a vacuum desiccator. The dimer was recrystallized from chloroform, m.p. 155.4–155.7°, corrected.[4]

Anal. Calcd. for $C_{18}H_{12}O_4N_4$: C, 62.06; H, 3.47; N, 16.09. Found: C, 62.50; H, 3.75; N, 16.16.

The dimer was fairly stable toward atmospheric moisture.[2] After 48 hours exposure to the air the melting point was 4° lower than initially.

The reactions between the dimer and dibutylamine were performed in a dry 1-liter, 3-necked flask equipped with a sealed stirrer, thermometer and reflux condenser which was protected by a calcium chloride tube. The flask was immersed in a constant-temperature oil-bath. Redistilled technical *o*-dichlorobenzene which had been dried over calcium hydride was used as the solvent. The solvent (500 g). was added to the flask and was permitted to come to the bath temperature, when 25.0 g. (0.072 mole) of dimer was added. At zero time 37.6 g. (0.287 mole) of redistilled di-*n*-butylamine, neutralization equivalent 131, was added from a pipet. At intervals a sample of the reaction mixture was withdrawn and added to 25 ml. of 1.06 *N* hydrochloric acid, the mixture shaken, and the sample weight determined. Titration of the excess acid with 0.50 *N* sodium hydroxide solution to a brom phenol blue end-point permitted calculation of unreacted amine, and hence of unreacted dimer.

In the experiments at 50° 850 g. of solvent was required to dissolve the dimer. One experiment was performed in refluxing solvent (170–180°) with amine:dimer molar ratio of 8:1 to find the time required for complete reaction.

A blank experiment showed that dibutylamine did not react appreciably with the solvent during 2.25 hours refluxing. It was also found that no hydrolysis of the dimer occurred during analysis. A mixture of 50 ml. of solvent and 1 g. of dimer was refluxed and added quickly to 25 ml. of acid. Titration after 25 minutes showed that no amine had been liberated by hydrolysis of the dimer.

The results at 0–240 min. and at 1440 min. are summarized in Fig. 1.

The product from a reaction between the dimer and dibutylamine in refluxing dichlorobenzene was obtained by vacuum distillation of the solvent. Repeated recrystallizations from hexane gave a white solid, m.p. 111.1–111.7°, corrected.

Anal. Calcd. for $C_{25}H_{44}O_2N_4$: C, 69.40; H, 10.25; N, 12.95. Found: C, 69.70; H, 10.37; N, 12.74.

From a reaction between monomeric tolylene diisocyanate and dibutylamine in benzene there was obtained a white solid, m.p. 111.1–111.6°, corrected. This solid showed no depression of melting point when mixed with the reaction product from the dimer and the amine, thus confirming structure II.

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

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The ultracentrifuge studies of the Santomerse D–calf thymus nucleohistone system[2] have been extended to provide more information on the nature of complex formation in this system. The behavior of the components of the nucleohistone molecule, histone and nucleic acid, in Santomerse D solution, in the ultracentrifuge is reported here.

Experimental and Results

The nucleohistone, histone hydrochloride and tetrasodium nucleate were prepared as previously described.[2] All solutions containing Santomerse D were used for ultracentrifuge, viscosity and diffusion measurements immediately after dialysis was completed. All solvents contained 0.02 ionic strength phosphate buffer.

Ultracentrifuge Studies.—The experimental results with the customary optical rotor are summarized in Table I. "Schlieren" patterns of tetrasodium nucleate and histone hydrochloride sedimenting in buffer and in buffered Santomerse D solution are shown in Fig. 1.

TABLE I

SEDIMENTATION VELOCITY OF TETRASODIUM NUCLEATE AND HISTONE HYDROCHLORIDE

Solute	Concn., %	Solvent	s_{20}[a]
Tetrasodium nucleate	0.31	Water	4.23
	.155	Water	5.29
	.103	Water	6.34
	.000	Water	8.33[c]
	.31	0.35% Santomerse D	4.36
	.155	.35% Santomerse D	5.53
	.103	.35% Santomerse D	6.24
	.000	.35% Santomerse D	8.00[c]
Histone hydrochloride	0.22	Water	21.2,[b] 12.8[b]
	.32	0.35% Santomerse D	3.38
	.157	.35% Santomerse D	2.45
	.078	.35% Santomerse D	2.13
	.000	.35% Santomerse D	1.90[c]
Santomerse D	0.35	Water	0.89[d]

[a] Expressed in Svedberg units. [b] Fast moving components. The sedimentation constant of the slow moving component was too low for measurement. [c] Extrapolated value. [d] Obtained with Spinco Model E ultracentrifuge at 59,780 r.p.m.

To measure the amount of Santomerse D actually involved in complex formation, the experiments with the optical rotor were parallelled with a quantity type rotor[3]. The rotor was fitted with two plastic tube liners, each containing a stainless steel thimble which extended about half way to the bottom of the tube. The perforated bottom of the

TABLE II

BINDING OF SANTOMERSE D BY NUCLEOHISTONE, TETRASODIUM NUCLEATE AND HISTONE HYDROCHLORIDE FROM QUANTITY TYPE ROTOR EXPERIMENTS

Run	System[a]	Time, hr.	RCF, g's	Santomerse D in supernatant, %	Wt. fraction Santomerse D in complex
1	Santomerse D–nucleohistone	6	104,000	0.25	0.45[b]
2	Santomerse D–tetrasodium nucleate	6	104,000	.35	.00
3	Santomerse D–histone hydrochloride	12	129,000	.07	.64
4	Santomerse D	12	100,000	.16	

[a] Contains 0.35% Santomerse D with 0.02 ionic strength phosphate buffer, and 0.16% of second component listed. [b] Corrected for 0.03% nucleohistone remaining in the supernatant, as determined by micro-Kjeldahl nitrogen analysis. This result is in agreement with the previously reported[2] value obtained from area measurements.

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

(2) M. W. Renoll and Q. Van Winkle, THIS JOURNAL, **73**, 2504 (1951).

(3) R. W. G. Wyckoff and J. B. Lagsdin, *Rev. Sci. Instr.*, **8**, 427 (1937).

thimble was covered by a disc of hardened filter paper. The tubes were filled with solution to within about an inch from the top of the thimble (7–10 ml.) and that remaining above the disc removed for analysis at the completion of the run. Sedimentation was allowed to continue for 6–12 hours at 100,000–129,000 times gravity (40,000–50,000 r.p.m.). The amount of Santomerse D present in the supernatant liquid removed was determined by comparison of the surface tension of a water dilution with a previously prepared curve relating surface tension to per cent. Santomerse D. A du Noüy tensiometer was used. The results are summarized in Table II.

Run 2 above was repeated at 100,000 times gravity for 9 hours and similar results obtained. The nitrogen content of the supernatant was measured by the micro-Kjeldahl method and essentially complete sedimentation of nucleic acid was found.

The concentration of Santomerse D sedimenting in micelle form in the ultracentrifuge with the optical rotor (see Table I) was calculated from area measurements of the "schlieren" patterns as 0.16%

Viscosity Measurements.—The results of viscosity measurements[2] on tetrasodium nucleate are summarized in Table III. Since the change in viscosity with dilution for histone hydrochloride in water solution and in Santomerse D is very small, no calculation of $[\eta]$ was made.

TABLE III

VISCOSITY OF TETRASODIUM NUCLEATE AT 20°

Concn. of tetrasodium nuclate, %	Solvent	η, cp.	η_{sp}/c	$[\eta]$
0.31	Water	15.353	45.87	
.232	Water	8.338	31.32	
.155	Water	4.163	20.17	
.103	Water	2.702	16.30	
.000	Water			12.5[a]
.31	0.35% Santomerse D	15.082	44.63	
.155	.35% Santomerse D	4.256	20.55	
.103	.35% Santomerse D	2.676	15.84	
.000	.35% Santomerse D			12.5[a]

[a] From $\eta_{sp}/c/c^2$.

Diffusion Measurements.—The diffusion constants[2] of histone hydrochloride in 0.35% Santomerse D diffusing into Santomerse D solution were essentially constant in the range of 0.32, 0.24 and 0.16%, respectively, histone hydrochloride concentration and the average values of $D_{0.6}$, 2.51×10^{-7} and D_{20}, 4.68×10^{-7} were obtained.

Molecular Weights.—The molecular weight of tetrasodium nucleate in water and in Santomerse D solution was calculated[2] from s_{20} and η. The results were 6.4×10^5 and 6.2×10^5, respectively, which are considered significant on a relative basis only. Since the slow moving component, which comprised the larger part of the histone hydrochloride sample in water solution, had a sedimentation rate too low for measurement in the ultracentrifuge its molecular weight of 18,000 was obtained for measurement of the osmotic pressure in 0.02 ionic strength phosphate buffer, pH 6.87, by the capillary rise method in an osmometer with stainless steel cell.[4] The histone hydrochloride concentrations used in the osmotic pressure measurements were 0.073, 0.11 and 0.22%. The molecular weight of histone hydrochloride in Santomerse D solution was calculated[2] from s_{20} and D_{20}. The value of 0.729 for the partial specific volume of histone hydrochloride[5] was used. The density of 0.35% Santomerse D solution at 20° was measured with a pycnometer of approximately 20-ml. capacity[6] and was found to be 1.00018 g./ml. The apparent specific volume of Santomerse D was calculated from the equation[7]

$$V_1^* = V - w_2 V_0 / w_1$$

where V and V_0 are the specific volumes (*i.e.*, $1/\rho$, where ρ is the density) of solution and solvent, respectively, and w_1 and w_2 are the concentrations of solute and solvent, respectively, in g./g. of solution. It was found to be 0.77. The apparent specific volume of histone hydrochloride in Santomerse D solution was calculated as 0.75 from weight fractions of 0.64 for Santomerse D and 0.36 for histone hydrochloride obtained in the quantity type rotor experiment of Table II. Because the dissociation tendency of the complex caused a decrease of s_{20} with decreasing histone hydrochloride concentration, the value of s_{20} for 0.157% protein concentration was used to calculate the molecular weight of 5.1×10^4. On the basis of a complex containing one histone molecule per molecule of complex, this corresponds to a weight fraction of 0.65 for Santomerse D. Area measurements of the "schlieren" pattern, Fig. 1, I, for the system containing 0.157% histone hydrochloride gave weight frac-

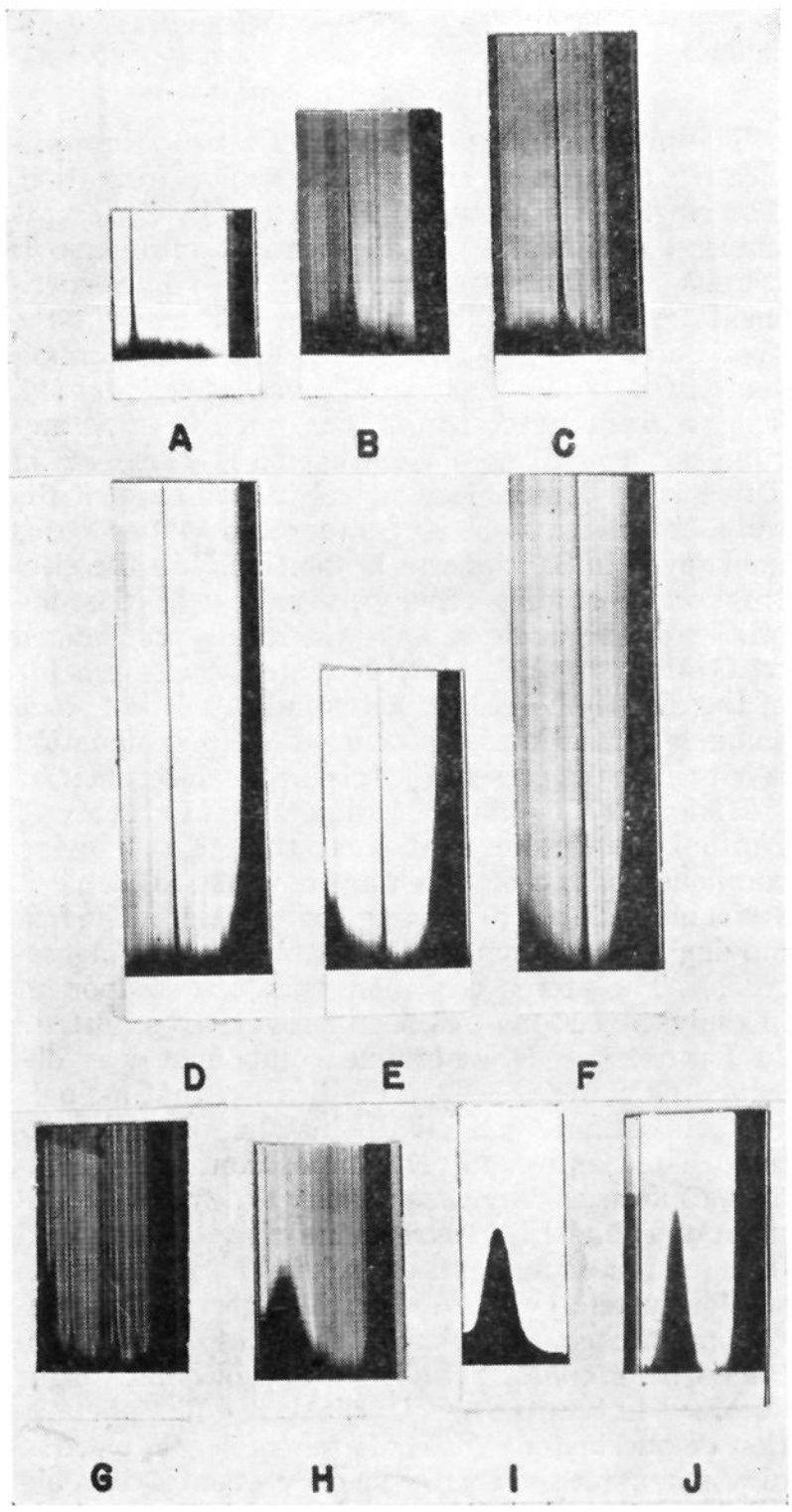

Fig. 1.—Ultracentrifuge "schlieren" scanning patterns of the sedimenting components in the tetrasodium nucleate and histone hydrochloride systems. A, B and C represent tetrasodium nucleate at percentages of 0.103, 0.155 and 0.31, respectively; D, E and F tetrasodium nucleate with 0.35% Santomerse D at nucleate percentages of 0.103, 0.155 and 0.31, respectively; G, 0.22% histone hydrochloride; H, I, J histone hydrochloride with 0.35% Santomerse D at histone hydrochloride percentages of 0.078, 0.157 and 0.32, respectively; direction of sedimentation, left to right.

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tions of 0.65 and 0.35 for Santomerse D and histone hydrochloride, respectively.

Discussion of Results

The ultracentrifuge behavior of tetrasodium nucleate shows no evidence of complex formation. The single peak shown in Fig. 1 (A, B, C) for the nucleate sedimenting in absence of Santomerse D is unchanged in its presence (D, E, F). The experiments with the quantity type rotor confirm the absence of complex formation. Under the conditions used no Santomerse D micelles sedimented. The intrinsic viscosity of the nucleate was unchanged in presence of Santomerse D, as shown in Table III. The molecular weight, which is of the same order of magnitude as reported values[8,9] was unchanged in Santomerse D solution. In the electrophoretic studies previously reported,[2] the mobilities of the nucleate were essentially unchanged by Santomerse D. However, area measurements of the electrophoretic patterns indicated that some complex formation had occurred. No explanation is offered at the present for this lack of agreement.

Histone hydrochloride sedimenting in absence of Santomerse D shows at least three fast moving components, two of which are reported in Table I, and a main slow sedimenting component. The fast moving components are probably histone aggregates. The presence of more than one component in dialyzed histone has been previously reported.[5] In Santomerse D, only one component was observed (Fig. 1, H, I, J), an indication that a single complex of Santomerse D and histone was sedimenting. The Santomerse D composition of the complex, as measured by the quantity type rotor experiment of Table II is in agreement with that obtained from area measurements of "schlieren" patterns of a similar system (Fig. 1). Further information concerning the composition of the complex was obtained from consideration of the molecular weight obtained in Santomerse D solution. The assumption of one histone molecule per molecule of complex is in agreement with the experimental data obtained in the present work.

A proposed structure for the histone hydrochloride–Santomerse D complex[10] assumes the binding of a monomolecular layer of detergent to the histone molecule by means of a primary ionic interaction, followed by binding of additional detergent molecules to the complex through attractive forces between the hydrocarbon chains of the detergent. The existence of a double layer of Santomerse D on the histone molecule would be consistent with the reversal of the charge by Santomerse D in electrophoresis,[2] the quantity type rotor experiments, and the results of area measurements of ultracentrifuge "schlieren" patterns.

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The Preparation and Properties of 4-Oxa-17α-hydroxy-21-acetoxy-Δ⁵-pregnene-3,11,20-trione[1]

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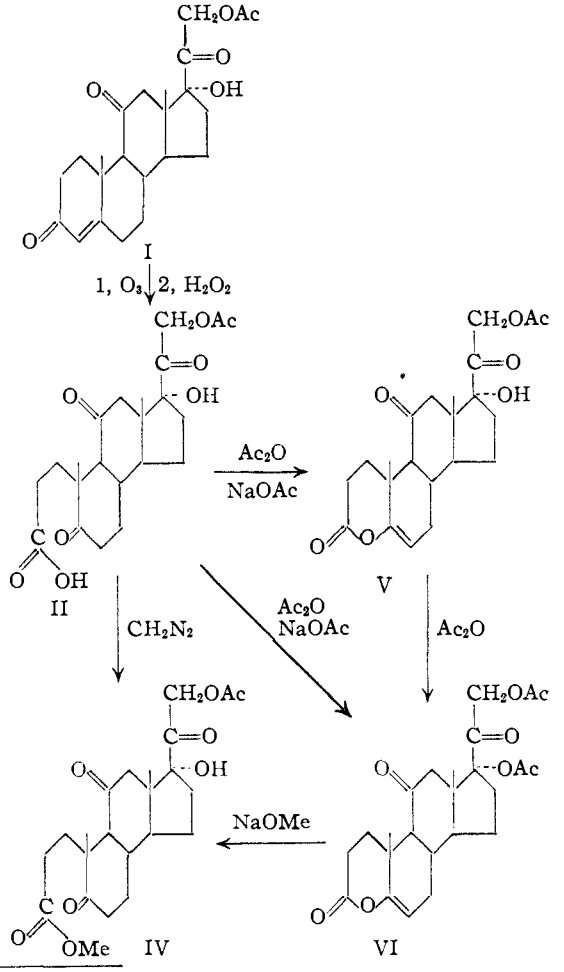
In the course of investigations designed to introduce carbon-14 into the ring system of cortisone, the preparation of 4-oxa-17α-hydroxy-21-acetoxy-Δ⁵-pregnene-3,11,20-trione (V) was undertaken. Ozonolysis of cortisone acetate (I) followed by oxidation with hydrogen peroxide[3] yielded an acid, 3,5-seco-17α-hydroxy-21-acetoxy-5,11,20-triketopregnane-3-oic acid (II). The acid II was converted to an enol lactone in refluxing acetic anhydride in the presence of a small amount of sodium acetate. The product obtained proved to be 4-oxa-17α,21-diacetoxy-Δ⁵-pregnene-3,11,20-trione (VI).[4] With less prolonged heating, the 17α-hydroxyl group was not acetylated and 4-oxa-17α-hydroxy-21-acetoxy-Δ⁵-pregnene-3,11,20-trione (V) was produced. By heating with acetic anhy-



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