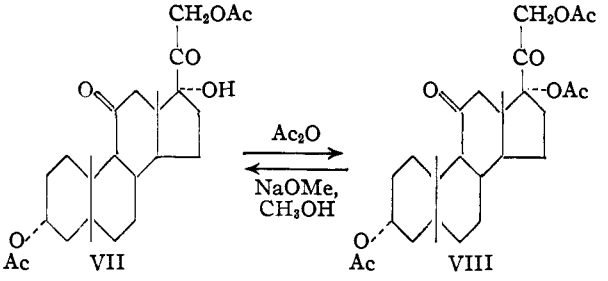
dride as described by Turner[5] and Huang-Minlon, *et al.*,[6] V was converted to the diacetate VI. That no fundamental alteration of the molecule occurred under these conditions was demonstrated by conversion of VI to the methyl ester IV, with one mole of sodium methoxide in methanol.

This selective removal of the 17α-acetoxy group is a general reaction as evidenced by the ready conversion of 3α,17α,21-triacetoxypregnane-11,20-dione (VII) to 3α,21-diacetoxy-17α-hydroxypregnane-11,20-dione (VIII) under the same conditions without any indication for the formation of either the 17α,21-dihydroxy or 3,17,21-trihydroxy compound.



Experimental[7]

3,5-Seco-17α-hydroxy-21-acetoxy-5,11,20-triketopregnane-3-oic Acid (II).—A stream of approximately 6% ozone was passed through a solution of 3.0 g. of cortisone acetate in 400 ml. of ethyl acetate cooled in a Dry Ice–methanol-bath. When the ozonolysis was complete, as evidenced by the oxidation of potassium iodide in a trap through which the effluent gases were passed, the reaction was discontinued and a solution of 4 ml. of 30% hydrogen peroxide and 4 ml. of methanol was added to the ethyl acetate solution. After standing at room temperature for 16 hours, the solvent was removed under reduced pressure. The oily solid was recrystallized from acetone–petroleum ether yielding 1.62 g. of rhombohedral crystals, m.p. 140–144°. Difficulty in recrystallization of the free acid prompted preparation of the hydrate which was obtained in long white needles from either aqueous methanol or aqueous acetone, m.p. 118–121°, $[\alpha]^{24}D$ +91.6° (ethanol). Both products had identical spectra in the region from 1150–850 cm.$^{-1}$.

Anal. Calcd. for $C_{22}H_{30}O_8 \cdot H_2O$: C, 59.98; H, 7.31. Found: C, 59.98; H, 7.17.

The methyl ester of the acid was prepared with an excess of ethereal diazomethane and was recrystallized with difficulty from methanol. After recrystallization, long white needles, m.p. 154.5–156.5°, $[\alpha]^{23}D$ +99.1° (chloroform) were obtained. The hydrate likewise yielded this same methyl ester.

Anal. Calcd. for $C_{23}H_{32}O_8 \cdot {}^1/_2CH_3OH$: C, 62.37; H, 7.57. Found: C, 62.34; H, 7.43.

4-Oxa-17α,21-diacetoxy-Δ5-pregnene-3,11,20-trione (VI).—A slurry of 209 mg. of II in 15 ml. of acetic anhydride containing 53 mg. of anhydrous sodium acetate was heated to boiling under reflux for 1 hour. The product went into solution during heating and at the end of the reaction the acetic anhydride was removed by distillation under diminished pressure. The oily solid was dissolved in ether–ethyl acetate and extracted several times with water followed by dilute sodium bicarbonate solution and again with water, dried over anhydrous sodium sulfate, and the solvent was removed. The residue, 214 mg. of yellow oil, was chromatographed upon silica gel, and 95 mg., m.p. 200–220°, was obtained. After successive recrystallizations from acetone–petroleum ether 4-oxa-17α,21-diacetoxy-Δ5-pregnene-3,11,20-trione (VI) melted at 231–234°, $[\alpha]^{25}D$ −24° (chloroform).

Anal. Calcd. for $C_{24}H_{30}O_8$: C, 64.57; H, 6.77. Found: C, 64.97; H, 6.73.

4-Oxa-17α-hydroxy-21-acetoxy-Δ5-pregnene-3,11,20-trione (V).—A slurry of 2.01 g. of the keto acid II in 80 ml. of acetic anhydride containing 240 mg. of anhydrous sodium acetate was heated under reflux for 15 to 20 minutes. The acetic anhydride was removed under diminished pressure and the product was isolated as in the preceding section. After crystallization from acetone–petroleum ether 986 mg. of product, m.p. 200–220°, was obtained; recrystallization from the same solvents afforded prisms of 4-oxa-17α-hydroxy-21-acetoxy-Δ5-pregnene-3,11,20-trione (V), m.p. 247–253°, $[\alpha]^{23}D$ +30° (chloroform).

Anal. Calcd. for $C_{22}H_{28}O_7$: C, 65.32; H, 6.73. Found: C, 64.95; H, 6.77.

4-Oxa-17α,21-diacetoxy-Δ5-pregnene-3,11,20-trione (VI) **from V.**—A solution of 107 mg. of 4-oxa-17α-hydroxy-21-acetoxy-Δ5-pregnene-3,11,20-trione (V) in 10 ml. of acetic anhydride was heated under reflux for 16 hours, and at the end of this time the acetic anhydride was removed under diminished pressure. The residual yellow oil after chromatography upon silica gel was recrystallized from acetone–petroleum ether to yield 99 mg. of VI, m.p. 231–234°, identical in all respects including infrared spectrum with the product prepared from the keto acid.

A solution of 25 mg. of the enol lactone V in 2 ml. of glacial acetic acid and 2 ml. of acetic anhydride containing 26 mg. of *p*-toluenesulfonic acid monohydrate was allowed to stand at room temperature for 60 hours. The solution was diluted with ethyl acetate, extracted with water, 10% sodium bicarbonate solution and again with water and dried over anhydrous sodium sulfate. The solvent was removed and the product crystallized from acetone–petroleum ether to yield 19 mg. of 4-oxa-17α,21-diacetoxy-Δ5-pregnene-3,11,20-trione (VI) identical in all respects including infrared spectrum with the product obtained in the preceding reaction.

Methyl 3,5-Seco-17α-hydroxy-21-acetoxy-5,11,20-triketopregnanoate (IV) **from VI.**—A stream of nitrogen was passed through a solution of 30 mg. of 4-oxa-17α,21-diacetoxy-Δ5-pregnene-3,11,20-trione in 3 ml. of methanol, and to this was added 1 ml. of 0.67 *M* sodium methoxide in methanol (1 equivalent). The solution was stirred for 5 minutes by means of the nitrogen stream and at that point, 1 ml. of water was added. After an additional 3 minutes, 1 ml. of glacial acetic acid was added and the solution was extracted with ether. The ether solution was washed with water, dilute sodium bicarbonate and water and dried over anhydrous sodium sulfate. The solvent was removed and after chromatography on silica gel followed by recrystallization from methanol, 21 mg. of methyl 3,5-seco-17α-hydroxy-21-acetoxy-5,11,20-triketopregnanoate (IV) was obtained, m.p. 154.5–156.5°. The product was identical in all respects with that obtained directly from the esterification of the keto acid II.

3α,21-Diacetoxy-17α-hydroxypregnane-11,20-dione (VII) **from 3α,17α,21-Triacetoxypregnane-11,20-dione** (VIII).—In the manner described in the preceding experiment, 49 mg. of 3α,17α,21-triacetoxypregnane-11,20-dione was converted with sodium methoxide to 16 mg. of 3α,21-diacetoxy-17α-hydroxypregnane-11,20-dione, m.p. 228–232°. The mother liquors contained the triacetate VIII as shown by the infrared spectra but there was no indication of any 3α-acetoxy-17α,21-dihydroxypregnane-11,20-dione.

Acknowledgments.—We wish to thank Dr. T. F. Gallagher for his valuable suggestions and discussion of this work. We are indebted to Friederike Herling for the determination and interpretation of infrared spectra.

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NEW YORK, NEW YORK

(5) R. B. Turner, THIS JOURNAL, **74**, 4220 (1952).

(6) Huang-Minlon, E. Wilson, N. L. Wendler and M. Tishler, *ibid.*, **74**, 5394 (1952).

(7) All melting points are corrected.

The Use of the Schlieren Optical System for Sampling after Preparative Angle Ultracentrifugation[1]

BY SAM SOROF

RECEIVED JUNE 22, 1953

The present communication demonstrates that

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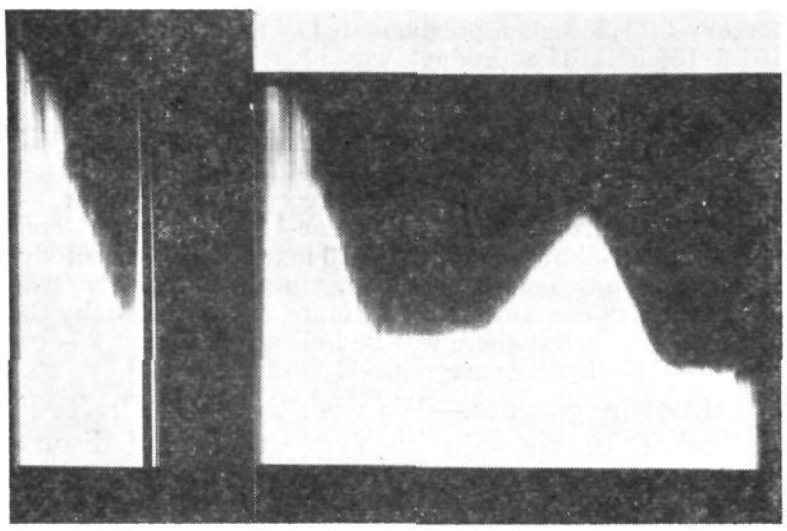
the schlieren optical system of Philpot[2] and Svensson[3] may be used successfully for the optically controlled sampling of partly sedimented proteins from lusteroid tubes after preparative angle ultracentrifugation. The method is of general applicability to proteins irrespective of their color or biological activity. It has yielded reproducible protein fractions of high ultracentrifugal purity when applied to the isolation of the slowest sedimenting constituent from a mixture of proteins.

Previous investigators have demonstrated that relatively sharp, partly sedimented boundaries of proteins can be obtained in the preparative tubes after angle ultracentrifugation. This fact has been demonstrated by subsequent chemical or biological analyses on samples arbitrarily removed from successive levels of solution in the preparative tubes (Hughes, Pickels and Horsfall[4]), and also by the absorption of light by the blue protein, hemocyanin (Pickels[5]). The position of the partly sedimented boundary in the angle ultracentrifuge tube may be used to calculate an approximate sedimentation rate.

The present study serves to demonstrate the general application of the schlieren optical system in sampling after preparative ultracentrifugation. The example chosen is the isolation in high ultracentrifugal purity of the A component,[6] consisting chiefly of albumin, from human plasma.

Experimental

Prior to angle ultracentrifugation in the Spinco Ultracentrifuge, Model E,[7] each lusteroid preparative tube was tested individually for the absence of optical imperfections and for the presence of a straight baseline in the schlieren optical system of Philpot and Svensson of the Perkin–Elmer electrophoresis apparatus, model 38.[8] The water-filled Spinco tube, $^5/_8$ inch diameter by 3 inches long, capped, and fastened in the preparative tube clamp, was placed in a preset vertical position in optical alignment with the vertical slit at the first schlieren lens. The slit width used was $^5/_8$ inch, *i.e.*, equal to the width of the preparative tube. The tube was examined in the schlieren optical system throughout a rotation of 90°, and the best orientation so determined was marked on the tube 90° to the pathway of light. Only tubes with good optical characteristics were used subsequently.



b a

Fig. 1.—(a) Cylindrical lens schlieren pattern of diluted human plasma in lusteroid tubes after preparative angle ultracentrifugation (see text). (b) Same as a, after removal of the trailing portion of the partly sedimented proteins of the A component.

Irradiated human plasma,[9] which had been stored at −17°, was diluted with two volumes of cold 0.15 *M* sodium chloride containing 0.02 *M* sodium phosphate at *p*H 7.40. Analytical ultracentrifugation of a dialyzed aliquot of the diluted plasma displayed the presence of the A, G and 20-components[6] (3.20 mg. nitrogen per ml. in 0.15 *M* sodium chloride containing 0.02 *M* sodium phosphate at *p*H 7.40). Subsequently, preparative lusteroid tubes were filled with more of the undialyzed diluted plasma, capped, and then oriented in a cold 26° angle preparative rotor A so that the above mentioned marks on the tubes were closest to the axis of the rotor. This was done in order to minimize any optical effect of deformity of the tubes resulting from ultracentrifugal stresses. The solution was then spun for 7.5 hours in an evacuated and refrigerated chamber at 50,740 r.p.m. with a relative centrifugal force in the center of the tubes equal to 152,000 × *g*. Subsequently, the rotor was slowly decelerated and the rotor and tubes carefully handled to minimize disturbance of the protein boundary. The tubes were placed one at a time into the pre-set position in the bath of the electrophoresis apparatus, examined, and recorded photographically as in Fig. 1a. A well-resolved, single peak, corresponding to the slowest sedimenting A component observed earlier in the ultracentrifugal analysis, is seen. The protein "pile-up" gradient in the lower part of the tube is shown at the left end of the pattern of Fig. 1a. A needle ($3^1/_2$" #18), attached to a syringe through a metallic three-way stopcock adapter,[10] was then entered slowly through the hole of the cap to a depth in the solution just above the "pile-up" gradient without any visible disturbance to the boundary. The syringe was mounted on a holder containing a rack and pinion for fine vertical motion of the needle. Removal of the solution above the needle tip was accomplished by means of the movement of a fine screw attached to the head of the plunger of the syringe. Figure 1b shows the resultant pattern after removal of all the solution above the tip of the needle. (Sufficient light to be photographed does not penetrate the tube above the level of the meniscus.) That extremely little, if any, protein was sucked up from below the needle tip is attested

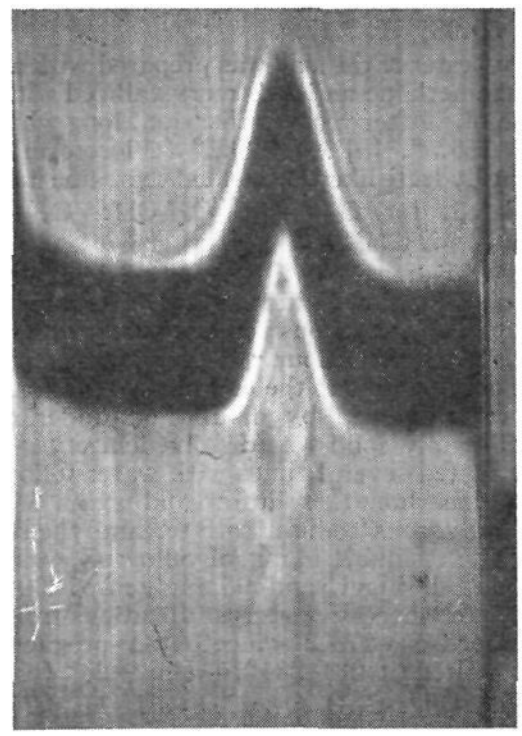


Fig. 2.—Analytical ultracentrifuge pattern of isolated A component: 0.95 mg. nitrogen per ml., 81 minutes at 59,780 r.p.m., 32° bar angle.

(2) J. S. L. Philpot, *Nature*, **141**, 283 (1938).

(3) H. Svensson, *Kolloid-Z.*, **87**, 181 (1939); **90**, 141 (1940).

(4) T. P. Hughes, E. G. Pickels and F. L. Horsfall, *J. Exp. Med.*, **67**, 941 (1938).

(5) E. G. Pickels, *J. Gen. Physiol.*, **26**, 341 (1943).

(6) K. O. Pedersen, "Ultracentrifugal Studies on Serum and Serum Fractions," Almqvist and Wiksells Boktryckeri AB, Uppsala, 1945.

(7) Manufactured by the Specialized Instruments Corp., Belmont, Calif.

(8) Manufactured by the Perkin-Elmer Corp., Norwalk, Conn.

(9) Obtained through the kindness of Sharp and Dohme, Philadelphia, Pa., Lot No. 32335.

(10) Manufactured by Becton, Dickinson and Co., Rutherford, N. J.

by the unchanged nature of the "pile-up" gradient after withdrawal.

In order to determine the purity of the isolated slow sedimenting fraction, a sample of the removed solution was dialyzed against 0.15 *M* NaCl containing 0.02 *M* sodium phosphate at *p*H 7.40, and then examined in the analytical rotor. The resultant pattern, shown in Fig. 2, displayed the A component and a barely perceptible trace of the faster G component. The former exhibited a corrected sedimentation constant, $s^{\circ}_{20} = 4.09\ S$, which agrees with the literature.[6,11]

We have found this technique to be reproducible with respect to the nature of the schlieren patterns of the protein solutions obtained in the preparative tubes, the sampling of the fractions, and the analytical ultracentrifugal purity of the proteins isolated. In addition, such use of the schlieren optical system permits the determination of the location of partly sedimented protein boundaries after preparative angle ultracentrifugation without resort to chemical or biological analysis of isolated fractions. In summary, sampling controlled by observation in the schlieren optical system has a general applicability to colorless as well as colored proteins, and when applied to the slowest sedimenting component of a mixture, is capable of reproducibly yielding isolated proteins of high ultracentrifugal purity by mild physical means.

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(11) See: G. Kegeles and F. J. Gutter, THIS JOURNAL, **73**, 3770 (1951); G. L. Miller and R. H. Golder, *Arch. Biochem. and Biophys.*, **36**, 249 (1952); J. F. Taylor, *ibid.*, **36**, 357 (1952).

COMMUNICATIONS TO THE EDITOR

THE ENZYMIC SYNTHESIS OF TREHALOSE PHOSPHATE[1]

Sir:

Uridine diphosphate glucose (UDPG)[2] has been found to disappear when incubated with a yeast extract and glucose monophosphate. This disappearance may be measured by estimating UDPG by its coenzymatic activity[3] and also as a decrease in acid-labile glucose. During the reaction UDP is formed and the reducing power of the mixture decreases. As shown in Table I, these changes are equivalent and do not take place when any one of the reactants is added at the end of the incubation period.

TABLE I

ANALYTICAL CHANGES PRODUCED BY THE ENZYME

Incubation of 0.4 μmole of glucose-6-phosphate, 0.6 μmole of UDPG and 0.02 ml. of enzyme in 0.14 *M* tris-(hydroxymethyl)-aminomethane buffer of *p*H 7 during 100 minutes at 37°; total volume, 0.1 ml.; results expressed in μmoles. The enzyme was obtained by disintegrating brewer's yeast cells with sand in a 50 cycles per second oscillator. After centrifuging the supernatant was made 0.5 saturated with ammonium sulfate and the precipitate was dialyzed.

Sample	Substance omitted during incubation[a]	Δ Reducing power[b]	Δ Labile glucose[c]	Δ UDP[d]
1	Glucose-6-phosphate	0	−0.04	+0.02
2	UDPG	0	0	0
3	None	−0.13	−0.14	+0.14

[a] The substance omitted was added at the end of the incubation period. The Δ values represent the difference with sample 2. [b] Calculated as glucose. [c] Hydrolyzed 10 minutes at pH 2 followed by precipitation with zinc sulfate and barium hydroxide. Practically all the glucose liberated under these conditions is that of UDPG. [d] Estimated by a method based on the reaction: phosphopyruvate + UDP → pyruvate + UTP (A. Kornberg, in "Phosphorus Metabolism," The Johns Hopkins Press, Baltimore, Md., 1951, Vol. I, p. 392). Pyruvate measured colorimetrically.

Samples equal to those shown in Table I were submitted to fractionation of the barium salts. The water-soluble, alcohol-insoluble fractions were used for paper electrophoresis with borate buffer[4] and the phosphate containing compounds were subsequently developed with a molybdate spray reagent.[5] The experiment showed that sample 3, but not samples 1 or 2, contained a phosphate compound which migrated at 60% the rate of glucose-6-phosphate. Dephosphorylation of this compound with kidney phosphatase produced a substance which gave the same R_f value as trehalose when chromatographed on paper.

In other experiments the reaction products were deproteinized by heating, treated with charcoal in order to remove the nucleotides and submitted to the action of phosphatase. When chromatographed on paper a substance migrating like trehalose was found to be present in sample 3 but not in the others. The substance extracted from the paper was hydrolyzed in 1 *N* acid during 3 hours at 100° and compared chromatographically with trehalose treated in the same manner. In both cases a glucose and a trehalose spot were obtained.

The solvent used for paper chromatography was pyridine–ethyl acetate–water[6] with which trehalose, saccharose, maltose and lactose can be separated and the developer was an alkaline silver reagent[7] which reacts slowly with non-reducing disaccharides. Furthermore, reducing from non-reducing sugars can be distinguished because only the latter give color with the aniline-phthalate spray reagent.[8] Thus the ester appears to be a phosphate of trehalose which is presumably identical to that iso-

(1) This investigation was supported in part by a research grant (G-3442) from the National Institutes of Health, U. S. Public Health Service, and by the Rockefeller Foundation.

(2) These abbreviations will be used: UDPG for uridine diphosphate glucose, UDP for uridine diphosphate, and UTP for uridine triphosphate.

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(4) R. Consden and W. M. Stanier, *Nature*, **169**, 783 (1952).

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(8) S. M. Partridge, *ibid.*, **164**, 443 (1949).