esses[26,27] is interesting. In autoxidation reactions, certain substances will cause inhibition of oxidation by processes which involve their own oxidation. This type of system has been described lately in detail by Kolthoff and Medalia.[28] In the present work, inhibition by ascorbic acid is indicated in the direct analysis experiments, while the concurrent induced oxidation of the ascorbic acid is indicated by the increased rate of oxygen uptake as demonstrated in Table I and in the experiments with the *o*-dihydroxy compounds.

Addendum.—While this manuscript was in preparation, Kendal[29] published some results which are closely related to the present work. It was shown that in the presence of ascorbic acid certain monophenols, including tyrosine, were oxidized by tyrosinase without an induction period. The view was presented that the ascorbic acid effect was not "dependent on its favoring *o*-dihydroxyphenol accumulation." No explanation can be offered, however, to account for Kendal not finding retardation of tyrosine oxidation during the later stages of the reaction.

(26) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 370.

(27) W. A. Waters, "The Chemistry of Free Radicals," The Claredon Press, Oxford, 1946, p. 232.

(28) I. M. Kolthoff and A. I. Medalia, THIS JOURNAL, **71**, 3777 (1949).

(29) L. Kendal, *Biochem. J.*, **44**, 442 (1949).

Acknowledgment.—This investigation was conducted under the guidance of Professor J. M. Nelson to whom the author is indebted for his interest and coöperation. The author appreciates the friendly council of Professor C. R. Dawson and also wishes to express his gratitude to the Trustees of Columbia University for their award of a University Fellowship granted during the course of this work.

Summary

The effect of ascorbic acid on the enzymatic oxidation of *l*-tyrosine, 3,4-xylenol, β-(3,4-dihydroxyphenyl)-alanine (dopa) and protocatechuic acid has been described. The rate of oxygen uptake in the oxidation of *l*-tyrosine, dopa and protocatechuic acid is increased by the addition of ascorbic acid. In the case of the tyrosine oxidation, it has been shown that the increase in the rate of oxygen uptake could not be completely attributed to the oxidation of the dopa formed in the reaction. Both an increase and a retardation in the rate of disappearance of tyrosine during the course of its oxidation in the presence of ascorbic acid has been found. The results indicate a parallel between the effect of ascorbic acid on the systems studied and the effect of inhibitors on autoxidation reactions.

NEW YORK, N. Y. RECEIVED SEPTEMBER 15, 1949

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE UNIVERSITY OF CALIFORNIA]

The Physical Properties of Elinin, a Lipoprotein from Human Erythrocytes[1]

BY WALTER B. DANDLIKER,[2a] MERWIN MOSKOWITZ,[2b] BRUNO H. ZIMM AND MELVIN CALVIN[2c]

Introduction

The structure and chemical composition of red blood cells have been the subject of repeated investigations. The results of this work have been adequately summarized in several reviews.[3a,b,4,5]

Some time ago, work was begun in these laboratories for the purpose of concentrating or isolating the Rh factors present in human blood. It was thought that a concentrated or pure Rh preparation might offer an avenue of approach for therapy in cases of *erythroblastosis fetalis*. A preliminary communication[6] reported the initial results of this project. The present work describes subsequent investigations of elinin, a lipoprotein preparation derived from the red cell membrane, which when prepared from Rh positive or type A or type B blood is capable of reacting specifically (in a serological sense) with antibodies to these factors. The method of preparation of elinin and its serological properties are described elsewhere.[7]

General Properties of Elinin Solutions

An elinin solution is a transparent, viscous, yellow-brown liquid having easily visible Tyndall scattering and double refraction of flow. A concentration of about 1% or greater is needed to demonstrate the latter property readily. At elinin concentrations of about 5% or more, gels result. These can easily be prepared from a

(1) A preliminary report of the present work was presented before the American Association for the Advancement of Science, *Chem. Eng. News*, **26**, 2118 (1948).

(2) (a) University Laboratory of Physical Chemistry Related to Medicine and Public Health, Harvard University, Boston, Mass.; (b) Department of Bacteriology, Yale University Medical School, New Haven, Conn. (c) The authors wish to express their gratitude to the Rockefeller Foundation and to the United States Public Health Service for financial support which made this work possible.

(3) (a) Ponder, *Ann. N. Y. Acad. Sci.*, **48**, 577 (1947); (b) Parpart and Dziemian, *Cold Spring Harbor Symposia*, **8**, 17 (1940).

(4) Morgan, *Experientia*, **3**, No. 7, 257 (1947).

(5) Kabat, *Bact. Rev.*, **13**, 189 (1949).

(6) Calvin, Evans, Behrendt and Calvin, *Proc. Soc. Exp. Biol. Med.*, **61**, 416 (1946).

(7) Moskowitz, Dandliker, Calvin and Evans, *J. Immunol.*, **65**, 383 (1950); Evans, Moskowitz and Calvin, *Proc. Soc. Exp. Biol. Med.*, in press.

water solution of elinin by centrifugation at high speeds and as deposited inside a centrifuge tube show spontaneous double refraction. We have never detected spontaneous separation of an elinin solution into two phases merely on standing, which phenomenon has been observed with tobacco mosaic virus.[8]

Water solutions at *p*H 8 to 9 containing up to about 1.5% elinin may be passed through a sterilizing sintered glass filter (Corning ultra-fine).

The addition of sodium chloride to water solutions of elinin results in an immediate increase in turbidity and a loss of double refraction of flow. This effect is achieved at about 0.05 *M* sodium chloride at *p*H 9; further addition of salt does not change the appearance of the solution up to at least 1 *M* and does not cause precipitation. Similar effects are obtained by lowering the *p*H of the solution in water. However, if the *p*H be brought as low as 6, even at 0°, the elinin will be found to have irreversibly flocculated. Freezing and thawing or drying from the frozen state also gives rise to aggregation which does not reverse on standing.

Inasmuch as the method of preparation would remove all low molecular weight materials not rather firmly combined with the protein, we have determined protein concentrations by drying a portion of solution in an oven at 90° to constant weight. This dried residue does not show significant further loss in weight when left *in vacuo* at 56° over phosphorus pentoxide. Such dried preparations contain about 8% N by micro-Dumas, 54% C, 8% H and 1.7% P.

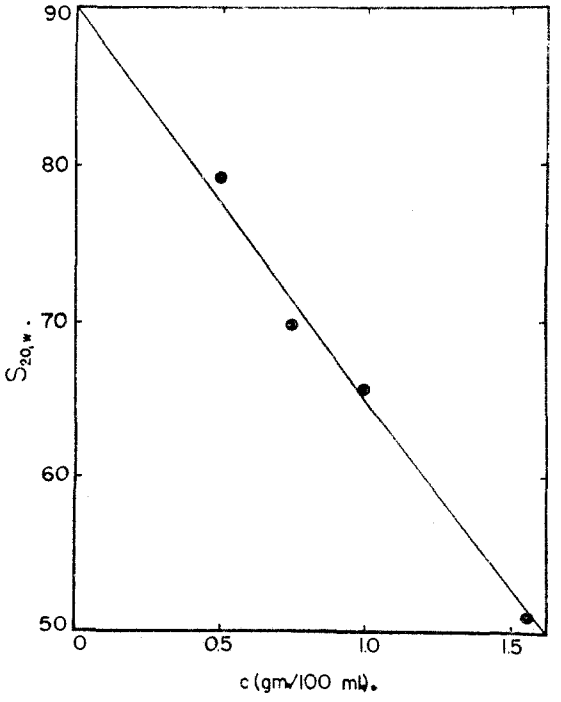


Fig. 1.—Sedimentation constants of elinin as a function of concentration.

(8) Bawden and Pirie, *Proc. Roy. Soc.* (*London*), **B123**, 274 (1937).

Sedimentation

Sedimentation measurements were carried out on elinin using a Spinco electrically driven ultracentrifuge.[9] The optical system of this instrument employs an inclined bar and cylindrical lens. The radial depth of the cell is 1.5 cm. and the cell center is 6.5 cm. from the axis of rotation. The speed was 25,500 ± 100 r.p.m. and the temperature 20–25°. The solvent was a mixture of sodium carbonate and bicarbonate solutions at *p*H 8.9 and ionic strength 0.15.

The photographs of the sedimentation diagrams were measured in a projection comparator magnifying twenty times; the maximum ordinate of the diagram was taken as the position of the boundary. Inasmuch as the sedimentation diagrams of elinin are somewhat skewed (skewness about 0.3) this measurement results in a statistically undefined average value[10] for the sedimentation constant. This average lies below the weight average if the skewness is positive.[10] Sedimentation constants, *s*, were calculated from the equation.[11]

$$s = (\Delta x/\Delta t)(1/\omega^2 x_m)$$

where Δx is the distance traversed by the boundary in time Δt; x_m is the mean position of the boundary during the time interval being considered and ω is the angular velocity of the rotor. The results have been corrected to sedimentation in a solvent having the viscosity and density of water at 20° (s^{w}_{20}).[11] The change of partial specific volume of the protein between 20 and 25° has been neglected. Results are expressed in Svedberg units.

Figure 1 shows the concentration function of s^{w}_{20} which extrapolates to a sedimentation constant of 90 at $c = 0$. Figure 2[11a] is the sedimentation diagram for elinin (1 g./100 ml.); the time intervals were four minutes with the first exposure taken three minutes after the centrifuge had reached 25,500 r.p.m. The temperature range was 21.7 to 21.9°.

The boundary spreading observed in Fig. 2[11a] was shown to be due mainly to heterogeneity by comparison of the sedimentation diagrams of elinin and human hemoglobin (Fig. 3)[11a] in the following manner. The diffusion constant, *D* of a homogeneous, normally diffusing solute is related to the area and the maximum height of the diffusion curve and the time. For hemoglobin, D^{w}_{20} was found to be 6.4×10^{-7} cm.^{2} sec.$^{-1}$.

(9) Available from the Specialized Instruments Corp., Belmont, California.

(10) Jullander, *Arkiv. Kemi, Mineral. Geol.*, **21A**, No. 8 (1945).

(11) Svedberg and Pedersen, "The Ultracentrifuge," Oxford University Press, 1940.

(11a) Figures 2, 3, 6 and 7 can be obtained from the American Documentation Institute, 1719 N Street, N. W., Washington 6, D. C., by ordering Document 2864, remitting $0.50 for microfilm (images 1 inch high on standard 35 mm. motion picture film) or $0.50 for photocopies (6 × 8 inches) readable without optical aid.

The value given by Svedberg (ref. 11, Table 48) for the diffusion constant of hemoglobin is 6.9×10^{-7}. The sedimentation constant for hemoglobin was found to be 4.3 as compared with the value of 4.48 taken from Svedberg's table.[11] The speed of the centrifuge was 49,300 r.p.m. and the photographic intervals 32 minutes. These data indicate that the boundary spreading observed for hemoglobin in this centrifuge can be accounted for by diffusion alone.

A similar treatment of the sedimentation data for elinin results in an apparent diffusion coefficient varying from 39×10^{-7} in the second exposure to 56×10^{-7} cm.^{2} sec.$^{-1}$ in the last exposure. The diffusion constant of an unsolvated sphere with a partial specific volume of 0.866 cc./g. and a sedimentation constant of 90 may be calculated to be 1.34×10^{-7} cm.^{2} sec.$^{-1}$ in water at 20°. It is evident from these results that heterogeneity must account for practically all of the boundary spreading observed with elinin. At lower concentrations of elinin the boundary spreading proceeds even faster than at 1% and that this should be qualitatively so can be seen from Fig. 1, since the concentration dependence will result in an artificial sharpening of the boundary at higher concentrations.

It must be noted also that considerable variability may be found between different preparations of elinin if the preparative technique is altered. The data discussed in this section were obtained on a preparation essentially identical to that studied in the light scattering section. The solution of human hemoglobin used in the foregoing experiment was prepared by washing fresh human erythrocytes with 0.15 *M* sodium chloride, hemolyzing in distilled water and adding 5 *M* sodium chloride to give an ionic strength of 0.17. The *p*H was 7.0.

The sedimentation data alone do not afford a means of deducing the nature of the change which occurs when electrolytes are added to an elinin solution. The sedimentation constant in water at 1% protein (*p*H~8.5–9) is found to be about 40, *i. e.*, about 0.6 times the value in bicarbonate buffer. The addition of salts is accompanied by the disappearance of the primary charge effect (ref. 11, p. 23) and gives rise to more rapid sedimentation in buffer. It is evident then that the change in sedimentation constant might include contributions from both the charge effect and aggregation.

No systematic drift was found for sedimentation constants calculated at different levels in the cell. This indicates that under the conditions studied elinin solutions are not noticeably gel-like.

The apparent partial specific volume (v_2) of elinin was obtained at about 1% protein. The formula

$$v_2 = \frac{1}{d_1} - \frac{\Delta m}{cm}$$

gave a result of 0.866 ± 0.002 cc./g. at 26° where d_1 is the density of solvent, m is the weight solvent held by the pycnometer, Δm is the weight of solution held by the pycnometer minus m and c is the weight of solute/cc. of solution.

Light Scattering Measurements

Theoretical.—It is now well-known that light scattering can be used to investigate the shape and size of suspended particles.[12] However, since the method has not yet achieved wide use, a brief summary of the pertinent theory will be given before the experiments are described in the following section.

The light scattered from a dilute suspension of particles may be discussed from several points of view. To fix our ideas, let us consider a cc. of solution illuminated by a parallel horizontal beam of polarized light with the electric vector vertical. Let the intensity of this beam be I^0. Let the scattering be measured in the horizontal plane at a point whose distance from the solution is r, with the direction of the ray to the observation point making an angle ϑ with the incident light. Let the scattered intensity at the observation point be i. We then define the *reduced intensity with vertically polarized incident light* as

$$\mathrm{I}_{\vartheta,v} = ir^2/I^0$$

The reduced intensity is convenient since it is independent of r and I^0; it has the dimensions of reciprocal length.

If the solution contains rod-like particles of length L, molecular weight M and at concentration c, and if the refractive index of the solution is n and the wave length of the light *in vacuo* is λ_0 while in the solution it is λ, the following equations may be obtained for the scattering at infinite dilution.[12,13]

$$\mathrm{I}_{\vartheta,v} = \frac{4\pi^2 n^2 (\partial n/\partial c)^2 Mc}{\lambda_0^4 N_0} P(\vartheta, L/\lambda) \quad (1)$$

$$P(\vartheta, L/\lambda) = (1/x)Si(2x) - (\sin x/x)^2 \quad (2)$$

$$x = \frac{2\pi L}{\lambda} \sin \vartheta/2$$

The function Si is the integral sine function which is tabulated, for example, in Jahnke and Emde.[14] N_0 is Avogadro's number.

The angular dependence of the scattering and the length L of the rods are involved in only the function $P(\vartheta, L/\lambda)$. The length may therefore be determined from the angular dependence alone while the evaluation of the molecular weight M requires determination of absolute values of reduced intensity.

If the suspended particles are spherical instead of rod-like, nothing in the above formulas is altered except the form of the function P. If

(12) P. Debye, *J. App. Phys.*, **15**, 338 (1944); *J. Phys. Colloid Chem.*, **51**, 18 (1947).

(13) Zimm, Stein and Doty, *Polymer Bulletin*, 1, 90 (1945).

(14) Jahnke and Emde, "Tables of Functions," Dover Publications, New York, N. Y., 1945.

the diameter of the spheres is D then $P(\vartheta,L/\lambda)$ is simply replaced by

$$P(\vartheta, D/\lambda) = \frac{9}{y^6}(\sin y - y\cos y)^2 \qquad (3)$$

$$y = \frac{2\pi D}{\lambda}\sin\vartheta/2$$

The angular variation of the function P is more pronounced for spheres than for rods of equal size ($L = D$). Presumably the angular function for particles of intermediate shape would be similar to an average of the P functions of the two extremes. Figure 4 gives the reciprocals of the P functions for rods and spheres together with the asymptotes and limiting tangents.

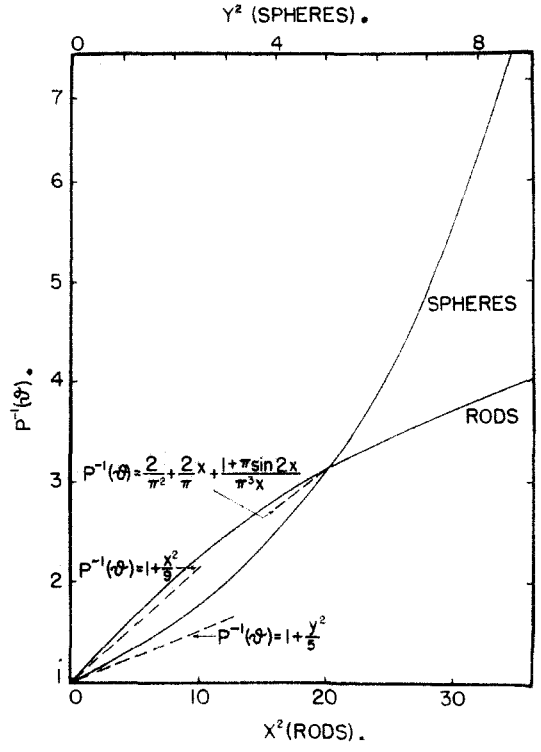


Fig. 4.—Reciprocal P functions for rods and spheres.

In any case, $P(\vartheta)$ becomes unity as ϑ approaches zero, so that the molecular weight M may be obtained from $\mathbf{I}_{\vartheta v}$ using Equation (1).

To obtain M the turbidimeter is calibrated with a material of known reduced intensity as a standard. Carr[15] has recently carried out reduced intensity measurements on benzene and obtained a value of 16.95×10^{-6} for the total scattered intensity at 90° in unpolarized incident light of wave length λ_0 5460 Å. This type of calibration permits one to calculate M directly from Equation (1).

Apparatus.—The turbidimeter has been described previously.[16] Essentially it consists of a small glass bulb containing the material under investigation and surrounded by a larger vessel of clean solvent. The cell is illuminated by the condensed beam of a mercury arc and the scattering is measured by a multiplier phototube which can be swung around the cell. In this way relative values of $\mathbf{I}_{\vartheta,v}$ at angles from 20 to 145° are obtained.

Procedure and Results.—Measurements of angular dependence were made on elinin in water at pH 8.5-9 and in borate buffer at pH 8.8 (0.1 mole B/l.) each at three protein concentrations, *viz.*, 1.17×10^{-3}, 1.94×10^{-4} and 3.24×10^{-5} g./ml. Dust, lint and denatured protein particles were removed from the solutions by centrifugation at 20,000 times gravity for 20 minutes and the clear supernatant liquids introduced directly into the turbidimeter cell by means of a pipet. The weight of material thrown down in this way was negligible. The incident light was plane polarized (electric vector vertical), *i. e.*, perpendicular to the plane of revolution of the photometer and of wave length 5460 Å. *in vacuo*.

The index of refraction of an elinin solution was measured in a Zeiss interferometer at room temperature using Na$_D$ lines. A linear concentration dependence of the refractive index was assumed and $(\partial n/\partial c)$ calculated to be 0.169 with c in g./ml. of solution.

Reduced intensities were computed from Carr's absolute calibration[15] and the results plotted in Fig. 5 in the reciprocal form used by Zimm[16] to facilitate extrapolation to infinite dilution. The limiting value of $c/\mathbf{I}_{0,v}$ at $c = 0$ may be found by extrapolation in Fig. 5 to be 0.072. This gives a value of M (from Equation (1)) equal to 37×10^6. If we assume the particles to be thin rods we can apply Equation (2) to find the length of the rods. Equation (2) may be expanded in terms of x^2 to give

$$P_{(\vartheta)}^{-1} = 1 + x^2/9 + 7x^4/2025 + \ldots \qquad (4)$$

Thus, the ratio of the limiting slope to the intercept in Fig. 5 ($c = 0$) gives the quantity $4\pi^2L^2/9\lambda^2$. This ratio is found to be 2.8 and $L = 3300$ Å. It can be seen by com-

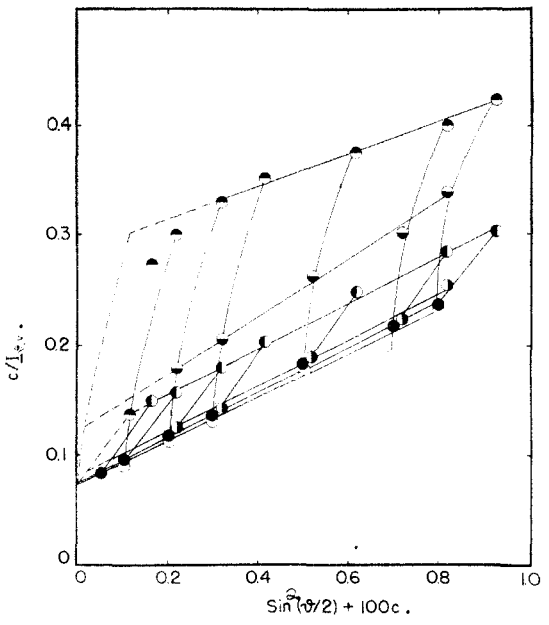


Fig. 5.—Light scattering data for elinin in water (pH 8.5-9) and in borate buffer (pH 8.8, 0.1 mole B/l.): ◓, water; ◐, buffer; $c = 1.17 \times 10^{-3}$ g./ml. ◒, water; ◑, buffer; $c = 1.94 \times 10^{-4}$ g./ml. ○, water; ●, buffer; $c = 3.24 \times 10^{-5}$ g./ml.

(15) C. I. Carr, Dissertation, University of California, 1949.
(16) Zimm, *J. Chem. Phys.*, **16**, 1099 (1948).

paring Figs. 4 and 5 that the sphere formula does not fit the data.

From Fig. 5 we can obtain some information concerning the interaction of elinin particles in solution. The concentration dependence in borate buffer is much less pronounced than that in water. This is just the result to be expected if electrostatic repulsive forces were tending to arrange the elinin particles in a somewhat orderly array in the water solutions. Such forces would be especially effective for the orientation of rod-like particles. At sufficiently low protein concentrations the behavior of elinin becomes the same in either solvent. The effect of electrostatic charge on turbidity has been observed previously[17] with tobacco mosaic virus solutions. An extensive study of related phenomena in serum albumin solutions recently has been made by Edsall and coworkers.[18] The data in borate buffer argue against an aggregation of elinin in the presence of salts at least at these rather low concentrations since the scattered intensity per unit concentration rises with decreasing concentration.

If we assume the elinin particle to be an unhydrated, elongated ellipsoid, we can estimate the axial ratio to be 33 and the sedimentation constant to be 78. The measured value of s is 90.

Light Absorption.—Measurements were made on elinin solutions in water at pH 9 using a Cary recording spectrophotometer in the region from 6000 to 3500 Å. and a protein concentration of 0.17%. At lower wave lengths, a Beckman spectrophotometer and a protein concentration of 0.03% was used. No absorption bands were found in the visible region showing that the yellow-brown color of elinin solutions is due to preferential scattering of the shorter wave lengths and not due to residual blood pigments in the protein. Occasionally preparations were obtained from which such pigments had not been completely removed, and in these cases a low, rather broad maximum was obtained in the region of 4000 Å. In the ultraviolet region true absorption is present and shows a maximum at about 2740 Å. As in the visible region, a large portion of this extinction is attributable to scattering. The absorption at 2740 Å. is probably due to the presence of aromatic amino acids, the shape of the curve resembling that of pepsin[19] rather than that of tobacco mosaic virus[19] which has a maximum at 2650 Å. and contains nucleic acid.

Electron Microscope Studies

Cellulose nitrate films were made by evaporating on water one to two drops of a solution (2 g./100 ml.) of Parlodion (Mallinckrodt) in amyl acetate or a solution of Merck Collodion diluted to the above concentration with amyl acetate. After mounting on electron microscope screens, the films were treated with sodium hydroxide solution to facilitate wetting.[20] The films were then washed thoroughly in water and a solution of elinin (0.02%) in water at pH 9 applied with a small dropper. Drying was done in air at room temperature.

The dry specimens were then examined[21] in an R. C. A. electron microscope at a magnification of 7500. Figure 6[11a] shows some of the first pictures made using a material produced in a way slightly different from the final preparative method.[7] Relatively few of the fields showed structures of noticeable regularity. In most cases the protein material was present in large lumps usually near a wire in the specimen screen. However, fields were occasionally observed which showed well formed rod-like or ribbon-like particles varying in size. Also present were rod-like structures in varying stages of aggregation and decomposition. The lengths of the rods in Fig. 6 vary from about 2500 to 10,000 A. The occurrence of aggregated and apparently denatured particles in an electron microscope preparation might easily be due to surface denaturation during the time required for drying.

Some of the rods in Fig. 6 appear to be composed of smaller parallel rods lying close together; the smallest of these rods is about 125 A. in width. In addition, the original negatives show that these smallest rods frequently have varying structural density along the long axis of the rod with a period of about 125 Å.

Omission of the sodium hydroxide treatment or lowering the concentration of elinin to 0.002% did not result in better preparations. Preliminary treatment[22] of the films with cinchonine hydrochloride did not noticeably facilitate even distribution of the protein over the film. Better spreading of the protein was obtained by casting the collodion films on 10^{-4} M sodium hydroxide and subsequently washing the film with water from a wash bottle. This procedure resulted, however, in the appearance of many partially decomposed particles as shown in Fig. 7.[11a] It is possible that films prepared in this manner still contained appreciable amounts of sodium hydroxide when the protein solution was applied. The particles in Figs. 7a and b were gold shadow cast and those in Fig. 7c uncoated. The source of gold atoms was about 20° above the plane of the sample. The larger particles in Fig. 7a are about 4000 Å. in length. It is highly probable that the very large aggregates observed in Fig. 7b were formed during the preparation of the electron microscope specimen since particles of this size would have been removed in the clarifying centrifugations during the elinin preparation. Figures 7a, b and c were made from an elinin preparation comparable to those studied by light scattering and sedimentation methods in the previous sections and showed slower sedimentation than the material in Fig. 6. A plausible interpretation of this fact is that the larger structures in Fig. 6 can be broken down further to yield much smaller particles such as those seen in the later photographs.

Since the type of field shown in Fig. 6 was quite rare, the possibility must be considered that the structures observed were merely some extraneous impurity unrelated to red cell proteins. The following considerations make us think that this possibility is rather unlikely. First, there was no suggestion of a more or less continuous background of protein material on the films. Instead, the films were either empty, *i. e.*, the same as a collodion film with no applied protein, or showed very large clumps of material of no regular form or showed the rod-like structures of Fig. 6. It appears that the films were wet very poorly by the applied solutions so that in only rare cases were particles deposited in positions where they would not be drawn together as the droplet of liquid evaporated. Second, the particles do, in many cases, appear to undergo the surface denaturation which would be expected of a protein. Third, the protein solutions had been subjected to repeated clarifying centrifugations during the preparation so that dense foreign material would be removed.

However, to establish with certainty that the structures observed in the electron microscope were indeed derived from the protein particles in the solutions would require further work to find conditions under which the solution would spread readily without producing too extensive surface denaturation. In this way one might be able to account for a large percentage of the total material in the form of observable isolated particles.

Discussion

Two important considerations must be taken into account in a discussion of the properties of elinin. First of all, it must be recognized that the material which we have called elinin is de-

(17) Oster, Doty and Zimm, THIS JOURNAL, **69**, 1193 (1947).

(18) Edsall, Edelhoch, Lontie and Morrison, *ibid.*, **72**, 4641 (1950).

(19) Lavin and Stanley, *J. Biol. Chem.*, **118**, 269 (1937).

(20) Cravath, Smith, Vinograd and Wilson, *J. App. Phys.*, **17**, 309 (1946).

(21) The aid of R. S. McKay who operated the electron microscope is gratefully acknowledged.

(22) Zbinder and Huber, *Experientia*, **3**, No. 11, 452 (1947).

fined essentially by a method of preparation[7] rather than by a set of invariant chemical and physical properties which might be assigned to a single compound. Both the method of preparation and the close association of the Rh, A and B factors with elinin indicate that elinin is derived from the red cell wall. Evidently, then, we have degraded a tissue into relatively small particles by a series of chemical steps in such a way that the final product still has certain desired serological properties of the original tissue. The second consideration is the great heterogeneity in the particle size of elinin. This is really a consequence of the first. That elinin is very polydisperse even in solution is evident from the ultracentrifuge results and it is with these limitations in mind that we make some estimate of the size and shape of elinin particles.

We should remark that no physico-chemical differences have been observed among elinin samples prepared from the various blood types.

As noted before, the sedimentation studies, the light scattering studies and the electron micrographs in Fig. 7[11a] were all made on essentially identical preparations. The average particle weight from light scattering is in the region of forty million and the length about 3000 Å. The sedimentation data are in fair agreement with this picture. All of the data are certainly in qualitative accord in indicating the presence of very large asymmetric particles, all of which are, however, too small to be resolved in the light microscope.

Summary

Some of the physical characteristics of elinin, a lipoprotein preparation from red blood cells, have been studied, and an estimate made of its particle size and shape. It appears to consist of large asymmetric particles. The preparation of elinin is described elsewhere.[7] The chief practical interest of elinin resides in its content of Rh factor and A and B substances.

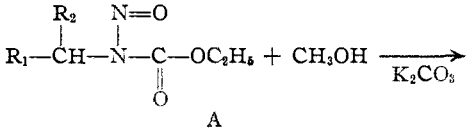
BERKELEY, CALIFORNIA RECEIVED APRIL 25, 1950

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ILLINOIS INSTITUTE OF TECHNOLOGY]

The Base-Catalyzed Decomposition of N-Nitroso-N-cyclohexylurethan[1,2]

BY FREDERICK W. BOLLINGER, F. N. HAYES AND SAMUEL SIEGEL

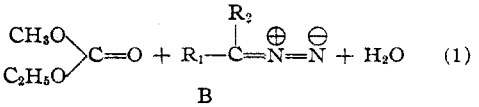
Meerwein's[3] process for the preparation of nascent diazomethane (IB) consists of the decomposition of N-nitroso-N-methylurethan (IA) in methanol containing a catalytic amount of finely-powdered potassium carbonate. The reaction is believed to take the course



A

A = Urethan

I, $R_1 = R_2 = -H$
II, $R_1 = -CH_3$, $R_2 = -H$
III, $R_1 = -n\text{-}C_7H_{15}$, $R_2 = -H$



(1)

B

B = Diazoalkane

IV, $R_1 = -(CH_2)_4-C(=O)-OC_2H_5$, $R_2 = -H$
V, $R_1 = -C_6H_5$, $R_2 = -H$
VI, $R_1R_2 = -(CH_2)_5-$

If it is desired that diazomethane react with an aldehyde or ketone, neither carbonyl compound interferes with the above preparation and may, therefore, be present in the methanol solution.

Other bases such as sodium carbonate or magnesium ethoxide may be substituted for potassium carbonate.[3,4] Because these bases exhibit an exceedingly small solubility in methanol, a solid phase is present throughout the reaction.

The preparation and use of diazoalkanes other than diazomethane by the Meerwein process, which have been reported in the literature, include diazoethane[5] (IIB), 1-diazoöctane[5] (IIIB), 5-carbethoxy-1-diazopentane[5] (IVB) and phenyldiazomethane[6] (VB). No example in which an N-nitroso-N-alkylurethan (R_1 and R_2 = alkyl groups) was the reactant has been reported. Yields of diazoalkanes based on reaction products, range from practically quantitative downward to 10%. By-products of Meerwein's process for the preparation of diazoalkanes have not been reported.

This investigation reports the products of the base-catalyzed decomposition of N-nitroso-N-cyclohexylurethan (VIA). N-Cyclohexylurethan[7] prepared from cyclohexylamine and ethyl chloroformate, was treated with excess nitrous acid to

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(2) Abstracted in part from a dissertation to be submitted by Frederick W. Bollinger to the Graduate School of Illinois Institute of Technology in partial fulfillment of the requirements for the degree Doctor of Philosophy.

(3) Meerwein, German Patent 579,309; *Chem. Zentr.*, **104**, II, 1758 (1933).

(4) Kohler, Tishler, Potter and Thompson, THIS JOURNAL, **61**, 1057 (1939).

(5) Adamson and Kenner, *J. Chem. Soc.*, 181 (1939).

(6) Gutsche, THIS JOURNAL, **71**, 3513 (1949).

(7) Skita and Rolfes, *Ber.*, **53**, 1249 (1920).