in dry benzene (20 ml.) were treated when refluxing with phosphorus pentoxide (0.3 g., 0.0021 mole) and then refluxed for 1 hr. A further portion of phosphorus pentoxide (0.2 g., 0.0014 mole) then was added and the mixture refluxed for another 2 hr. The solvent was removed *in vacuo* and the residue solidified, washed with petroleum ether (b.p. 40–60°) and then dissolved in benzene and chromatographed over activated alumina (using benzene as the eluting solvent). The product, after removing the benzene, was recrystallized from benzene and petroleum ether (b.p. 60–80°) to yield the required 1-benzoyl-2-cyano-1,2,-3,4-tetrahydroquinoline (0.2 g., m.p. 149°). The m.p. was not depressed upon admixture with product C (m.p. 148–149°) obtained from the partial hydrogenation of the Reissert compound (I).

Anal. Calcd. for $C_{17}H_{14}ON_2$: C, 77.84; H, 5.34; N, 10.68. Found: C, 77.90; H, 5.35; N, 10.50.

Acid Hydrolysis of Pure 1-Benzoyl-2-cyano-1,2,3,4-tetrahydroquinoline.—1-Benzoyl-2-cyano-1,2,3,4-tetrahydroquinoline (0.1 g., 0.0004 mole), which had been synthesized from the amide, was refluxed for 1 hr. with concentrated hydrochloric acid (2.5 ml.) and water (2.5 ml.) containing 2,4-dinitrophenylhydrazine (0.1 g., 0.00055 mole). The solution remained completely clear and was treated with water (10 ml.) before cooling. The clear solution was poured into sodium bicarbonate (2.5 g.) in water (20 ml.) and filtered to remove the 2,4-dinitrophenylhydrazine. The alkaline solution was concentrated *in vacuo* to a small bulk (5 ml.) and acidified with concentrated hydrochloric acid. The precipitate was filtered off, after cooling, and recrystallized from water to yield benzoic acid (0.015 g., m.p. 122–123°).

The addition of excess sodium nitrite to the mother liquors gave an oily precipitate which was extracted with chloroform and the extract dried and concentrated. The solidified residue was recrystallized from benzene and petroleum ether (b.p. 40–60°) to yield 1-nitroso-1,2,3,4-tetrahydroquinoline-2-carboxylic acid (0.03 g., m.p. 119–123° dec.).

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The Evaluation of the Kinetic Constants of Enzyme-Catalyzed Reactions by the Method of Foster and Niemann[1]

BY THOMAS H. APPLEWHITE AND CARL NIEMANN[2]

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In a recent communication from these laboratories Foster and Niemann,[3,4] in extending an earlier treatment of Walker and Schmidt,[5] described a procedure for the graphical evaluation of the kinetic constants of enzyme catalyzed reactions whose rates, in so far as they are dependent upon the concentration of enzyme and specific substrate, are described by equation 1

$$k_3[E]t = K_S\left(1 + [S]_0\sum_{j=1}^{n} 1/K_{P_j}\right)\ln [S]_0/[S]_t + \left(1 - K_S\sum_{j=1}^{n} 1/K_{P_j}\right)([S]_0 - [S]_t) \quad (1)$$

This procedure, which was developed for the case where $\sum_{j=1}^{n} 1/K_{P_j} = 1/K_P$, is based upon the fact that in a plot of $([S]_0 - [S]_t)/t$ *vs.* $(\ln[S]_0/[S]_t)/t$ lines of slope $[S]_0$ drawn through the origin will intersect those of slope $-K_S(K_P + [S]_0)/(K_P - K_S)$ at points which define, in terms of the ordinate $([S]_0 - [S]_t)/t$, the corresponding initial velocities. As this fact was not clearly established, nor clearly stated, in the earlier communication[3] we shall in this communication offer proof of its validity.

If equation 1 for the case where $\sum_{j=1}^{n} 1/K_{P_j} = 1/K_P$ is transformed into the usual slope-intercept form one obtains equation 2

$$([S]_0 - [S]_t)/t = k_3[E]K_P/(K_P - K_S) - K_S(K_P + [S]_0)(\ln[S]_0/[S]_t)/t(K_P - K_S) \quad (2)$$

It will be seen from equation 2 that with a plot of $([S]_0 - [S]_t)/t$ *vs.* $(\ln[S]_0/[S]_t)/t$ one will obtain, for various values of $[S]_0$ and t, a series of lines of slope $-K_S(K_P + [S]_0)/(K_P - K_S)$. For each of these lines there is a point corresponding to $t = 0$ and this point may be located by examination of the limits of the two parameters $([S]_0 - [S]_t)/t$ and $(\ln[S]_0/[S]_t)/t$ as $t \rightarrow 0$. The limit of $([S]_0 - [S]_t)/t$ as $t \rightarrow 0$ is clearly $-d[S]/dt$ and that of $(\ln[S]_0/[S]_t)/t$ as $t \rightarrow 0$ is $(-d[S]/dt)\ (1/[S]_0)$. Therefore, for the condition that $t = 0$ lines of slope $[S]_0$ drawn through the origin of a $([S]_0 - [S]_t)/t$ *vs.* $(\ln[S]_0/[S]_t)/t$ plot will intersect the lines of slope $-K_S(K_P + [S]_0)/(K_P - K_S)$ at points corresponding to $t = 0$. It is emphasized that the relation $([S]_0 - [S]_t)/t = [S]_0(\ln[S]_0/[S]_t)/t$ defines a point for the condition that $t = 0$ and does not describe the relation between $([S]_0 - [S]_t)/t$ and $(\ln[S]_0/[S]_t)/t$ for other values of t.

It has been noted above that the parameters of the points corresponding to $t = 0$, *i.e.*, the points of intersection of the lines of slope $[S]_0$ with those of slope $-K_S(K_P + [S]_0)/(K_P - K_S)$, are, respectively, $-d[S]/dt$ for the ordinate and $(-d[S]/dt)\cdot(1/[S]_0)$ for the abscissa. Since by definition $-d[S]/dt = v_0$ and $(-d[S]/dt)(1/[S]_0) = v_0/[S]_0$ it follows that a line drawn through the points of intersection corresponding to $t = 0$ for various values of $[S]_0$ will possess the same characteristics as one obtained by a plot of v_0 *vs.* $v_0/[S]_0$,[6] *i.e.*, will have a slope of $-K_S$, an ordinate intercept of $k_3[E]$ and an abscissa intercept of $k_3[E]/K_S$. That the initial velocities, *i.e.*, the values of v_0, are defined in terms of the ordinate, *i.e.*, $([S]_0 - [S]_t)/t$, is evident when the terms in K_P in equation 2 are eliminated through the use of the relation $([S]_0 - [S]_t)/t = [S]_0(\ln[S]_0/[S]_t)/t$ to give equation 3 which is singular for the condition that $t = 0$.

$$([S]_0 - [S]_t)/t = k_3[E][S_0]/(K_S + [S]_0) = v_0 \quad (3)$$

(1) Supported in part by a grant from Eli Lilly and Co.

(2) To whom inquiries regarding this article should be sent.

(3) R. J. Foster and C. Niemann, *Proc. Natl. Acad. Sci.*, **39**, 999 (1953).

(4) Attention is called to three typographical errors in ref. 3: on p. 1000 the left hand member of equation 4 should read "$k_3[E]t$" instead of "$k_3[E]_t$," on pp. 1000 and 1002 the equation describing the slopes of the $([S]_0 - [S]_t)/t$ *vs.* $(\ln[S]_0/[S]_t)/t$ plots should read "$-K_S(K_P + [S]_0)/(K_P - K_S)$" instead of "$-K_S(K_P + [S]_0)/(K_S - K_P)$" and in Fig. 1, the slopes of the lines drawn through the hypothetical experimental points should read "$-K_S(K_P + [S]_0)/(K_P - K_S)$" instead of "$K_S(K_P + [S]_0)/(K_P - K_S)$."

(5) A. C. Walker and C. L. A. Schmidt, *Arch. Biochem.*, **5**, 445 (1944).

(6) G. S. Eadie, *J. Biol. Chem.*, **146**, 85 (1942).

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The Sedimentation Behavior of Bovine Serum Albumin in Acid Solutions[1]

BY P. BRO,[2] S. J. SINGER AND J. M. STURTEVANT

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An extension of the calorimetric studies reported by Gutfreund and Sturtevant[3] has revealed that at low *p*H bovine serum albumin (BSA) and bovine serum mercaptalbumin (BSMA) may undergo an exothermic reaction in addition to the previously reported endothermic reaction. In an effort to find the cause of the exothermic reaction, the calorimetric work was complemented by sedimentation studies of BSA and BSMA in 0.1 *M* ionic strength HCl–NaCl solutions.

In agreement with published results, we have found that aggregates are formed[4,5] at low *p*H and that the sedimentation constants of the unaggregated[6] and aggregated species decrease with *p*H. The nature of the aggregation phenomenon is under investigation and will not be discussed further here. A detailed study has been made, however, of the sedimentation constants as a function of *p*H in solutions approximately 1% in protein (Fig. 1). Several features may be noted. In the range of *p*H 3.8 to 2.8 the sedimentation constants show an inflection; at higher *p*H, the slopes of the sedimentation curves do not appear to decrease to zero near the isoelectric point; the changes in the sedimentation constants are reversible; and the behavior of BSA and BSMA are indistinguishable.

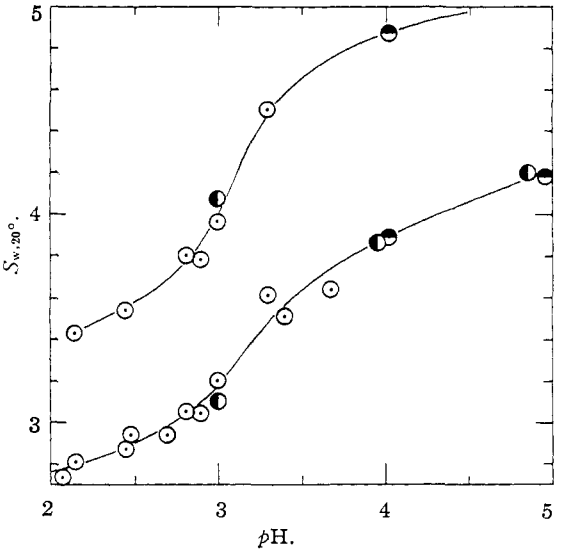

Fig. 1.—Sedimentation curves of BSA and aggregate; 0.1 *M* ionic strength HCl–NaCl solutions: ⊙, Armour and Pentex bovine serum albumin; ◐, bovine serum mercaptalbumin: ◓, B.S.A. after exposure to *p*H 3.1.

There are at least three factors which might be involved in the decrease in sedimentation constants: a decrease in molecular weight; the presence of electric potentials during sedimentation of charged particles; and configurational changes in the molecule which produce an increased frictional coefficient. The first of these is unlikely since the unaggregated and aggregated species behave in a parallel manner. The contribution of the second factor may be evaluated, as shown by Pedersen.[7] At *p*H 3.0 the charge correction amounts to less than 1% of the isoelectric sedimentation constant. The difference between the isoelectric sedimentation constant (corrected for the charge effects at various *p*H levels) and the observed values at lower *p*H, together with the pronounced inflection in the curve of Fig. 1, therefore provide additional evidence for the occurrence of a reversible configurational change in the BSA molecule in acid solution.[8,15] It is interesting that the aggregate appears to undergo a similar change.

In order to investigate the nature and magnitude of this configurational change, sedimentation constants extrapolated to infinite dilution should properly be determined. If the observed and isoelectric sedimentation constants in 1% solutions are compared, the magnitudes of the average changes so calculated will most likely be too large. Assuming that the molecule "swells" isotropically in acid solution, and using the model of the hydrodynamically equivalent sphere for the BSA molecule, in conjunction with Stokes' law, we find[16]

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(2) General Electric Company Fellow, 1954–1955.

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(8) C. Tanford, paper presented at the meeting of the Electrochemical Society in New York, April, 1953.

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