the rapid separation of sodium chloride. The salt was filtered and the filtrate was diluted with 800 cc. of water. The resulting partially cloudy mixture was acidified with hydrochloric acid and the yellow precipitate of 5-chloro-2-pyrimidinethiol collected and dried: 37.5 g. (76%), m. p. 218–223°. After crystallization from methanol the melting point was constant at 221–222°.

Anal.[2] Calcd. for $C_3H_2ClN_2S$: C, 32.8; H, 2.0; S, 21.8. Found: C, 33.0, 33.0; H, 2.3, 2.4; S, 22.1, 22.0.

The yellow color of the compound could not be removed by treatment in alkaline solution with hydrosulfite, zinc dust, charcoal, or a combination of the last two agents.

(2) Carried out under the direction of Dr. J. A. Kuck.

CHEMOTHERAPY DIVISION
STAMFORD RESEARCH LABORATORIES
AMERICAN CYANAMID COMPANY JACKSON P. ENGLISH
STAMFORD, CONNECTICUT ESTHER B. LEFFLER

RECEIVED MARCH 31, 1950

COMMUNICATIONS TO THE EDITOR

BOUNDARY SPREADING IN SEDIMENTATION VELOCITY EXPERIMENTS

Sir:

The only case in which sedimentation velocity experiments have been successfully used for size distribution analysis is the one in which no significant amount of diffusion occurs during the course of the experiment.[1] This situation does not ordinarily obtain, and in this Communication we present in outline a method to sort out the contributions of heterogeneity and diffusion to the spreading of the sedimentation boundary, thus providing means for the computation of each.

Since the second moments about the mean are additive in a combined distribution composed of independent distributions[2] we may derive the expression

$$\sigma^2 = \sigma_0^2 + 2Dt + \bar{x}^2\left[p\omega^2t + \frac{(p\omega^2t)^3}{3!} + \frac{(p\omega^2t)^5}{5!} + \ldots\right]^2 \quad (1)$$

Thus, and to a good approximation, we have

$$\frac{\sigma^2 - \sigma_0^2}{2t} = D + \frac{p^2\omega^4}{2}\bar{x}^2t$$

In these equations, σ^2 and σ_0^2 are the second moments of the curve which defines the sedimenting boundary at times $t = t$ and $t = 0$, D is the weight-average diffusion constant, ω is the angular velocity of the ultracentrifuge rotor, p is the standard deviation of the sedimentation constant distribution and $\bar{x}$ may be taken as the distance from the center of rotation to the centroidal ordinate of the boundary. This equation shows that when the apparent diffusion coefficient $(\sigma^2 - \sigma_0^2)/2t$, is plotted against $\bar{x}^2t$, a straight line is obtained with intercept D and slope $p^2\omega^4/2$. A drift of "diffusion coefficient" with time has long been recognized as a test of homogeneity with respect to sedimentation behavior.[3]

(1) *Cf.* for example, W. B. Bridgman, THIS JOURNAL, **64**, 2349 (1942).

(2) C. E. Weatherburn, "A First Course in Mathematical Statistics," Cambridge University Press, Cambridge, 1946, p. 82.

(3) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, Oxford, 1940, p. 287.

The distribution function, $g(s)$ which gives the relative amount of the molecular species with s_{20} of s is given by

$$g(s) = \frac{dn}{dx}\left(\frac{x}{x_0}\right)^2\frac{x\omega^2t}{n_1 - n_2}\frac{\eta_{20}}{\eta_t} \quad (2)$$

when diffusion is negligible.[4] When diffusion is not negligible, an "apparent distribution" defined in this manner may be extrapolated to infinite time to give the actual distribution of sedimentation constants, since the spreading of the boundary due to differences in s is proportional to $\bar{x}t$, while the spreading due to diffusion is proportional to $t^{1/2}$, as is shown by equation (1).

The method has been applied in the analysis of sedimentation velocity diagrams for pepsin-digested γ-globulin systems from horse anti-diphtheric serum. Additional information has been obtained, not only as regards the actual size distribution in these systems, but also with respect to the mechanism of the enzymatic degradation. A definitive account of these studies will be submitted at a later date.

Grateful acknowledgment is made to the U. S. Public Health Service and to the Wisconsin Alumni Research Foundation.

(4) R. Signer and H. Gross, *Helv. Chim. Acta*, **17**, 726 (1934).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WISCONSIN ROBERT L. BALDWIN
MADISON, WISCONSIN J. W. WILLIAMS

RECEIVED AUGUST 7, 1950

SYNTHESIS AND ISOLATION OF A CRYSTALLINE SUBSTANCE WITH THE PROPERTIES OF A NEW B VITAMIN

Sir:

A naturally occurring factor active for *Leuconostoc citrovorum* 8081 has been described,[1,2] and a synthetic reaction mixture derived from folic acid has been reported to be active for this organism.[3]

We wish to report the synthesis and isolation

(1) Sauberlich and Baumann, *J. Biol. Chem.*, **176**, 165 (1948).

(2) Broquist, *et al.*, *Proc. Soc. Exp. Biol. Med.*, **71**, 549 (1949).

(3) Shive, *et al.*, THIS JOURNAL, **72**, 2818 (1950).