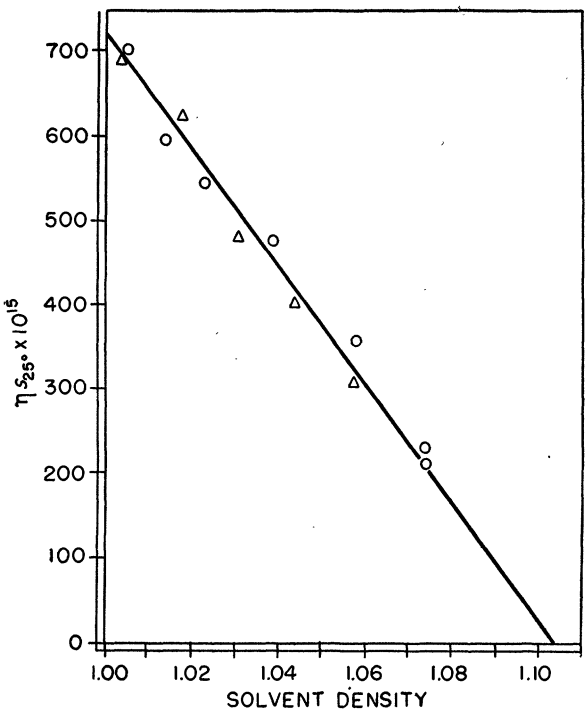

FIG. 2. Data from 2 different specimens of influenza virus A (PR8 strain) (triangles and circles, respectively) showing the linear dependence of $\eta S_{25°}$ on solvent density as the latter is varied with bovine albumin in concentrations of 0 to 25 per cent. The value of the density at the intercept at the base is 1.104.

virus A (PR8 strain) to consist of rounded or ovoid particles[6] which would be expected to sediment as spheres subject to retardation according to Stokes's law. The particle radius "r" can then be derived from the particle density ($\rho = 1.104$ from the intercept of the line of Fig. 2) and the sedimentation constant, $S_{20°}$, at infinite virus dilution.[9]

$$\tfrac{4}{3}\pi r^3(\rho_v - \rho_s)\,\omega^2\,R = 6\pi\eta r\frac{dR}{dt} \qquad (1)$$

where ρ_s = solvent density, ω = angular velocity, and R = distance from the particle to the axis of rotation. Inasmuch as $S = \frac{dR}{dt}\frac{1}{\omega^2 R}$, equation (1) for particle radius in solution then becomes

$$r = \sqrt{\frac{9}{2}\frac{\eta_{20°}\,S_{20°}}{(\rho_v - \rho_s)}} \qquad (2)$$

where the viscosity of water at 20° C. is $\eta_{20°}$. For influenza virus A (PR8 strain) $S_{20°} = 742 \times 10^{-13}$ [9], which gives for the radius of the sedimenting particle the value 57.7 m μ.

In previous reports[6,9] of estimates of the particle size of influenza virus A (PR8 strain) from sedimentation data, the density value employed in the absence of other data was the reciprocal of the partial specific volume ($\frac{1}{v}$) of the dry virus determined in the pyknometer. The diameter found in this way was 80 m μ. The density of the virus particle measured in solution in the present work, 1.104, was much smaller than $\frac{1}{v}$, 1.215, and the average diameter of the particle calculated with the present value was 115 m μ. This diameter is slightly larger, as might be expected, than that recently reported, 101 m μ, from electron micrographs[9] after direct calibration of the electron microscope by a method other than standardization on the basis of the width of tobacco mosaic rods.

It would appear that the procedures employed here provide a relatively simple method for the direct determination of the density of virus particles in solution. Further, the difference in the density of the dry virus and that of the virus in solution gives an estimate of the quantity of water associated with the particle of influenza virus A, namely, about 66 per cent. by volume. It is of interest to note that this amount of water is greatly in excess of that considered to be associated with protein molecules as water of hydration but is similar to the quantity found in organisms of complicated biological structure.

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THE POSSIBLE EXISTENCE OF A MICROBIOLOGICALLY INACTIVE "FOLIC ACID"-LIKE MATERIAL POSSESSING VITAMIN ACTIVITY IN THE RAT

IT is well established that certain nutritional deficiencies can be produced in the rat by incorporating various sulfonamides in otherwise adequate highly purified diets.[1, 2, 3] The depression of growth rate and the development of pantothenic acid deficiency, which are seen under these circumstances, can be effectively counteracted by dosage with crystalline biotin and con-

[9] D. G. Sharp, A. R. Taylor, I. W. McLean, Jr., D. Beard and J. W. Beard. To be published.

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[1] S. Black, J. M. McKibbin and C. A. Elvehjem, *Proc. Soc. Exp. Biol. and Med.*, 47: 308, 1941.

[2] A. D. Welch, *Fed. Proceedings*, 1: 171, 1942.

[3] F. S. Daft, L. L. Ashburn and W. H. Sebrell, SCIENCE, 96: 321, 1942.

centrates containing "folic acid."[4, 5, 6, 7] Severe leucopenia and granulocytopenia, similarly produced, have been cured promptly by the administration of very small doses of a crystalline folic acid or of vitamin B_c.[8]

The exceedingly low content of "folic acid" in milk[9] suggested that suitable reinforcement of its content of vitamins and minerals and its supplementation with a poorly absorbed sulfonamide should provide a simple diet for the production of "folic acid" deficiency. Accordingly, powdered whole milk (Klim) was fortified as follows (per 100 grams): $FeSO_4 \cdot 7H_2O$, 25 mg; $CuSO_4 \cdot 5H_2O$, 7.8 mg; thiamine chloride, 0.8 mg; riboflavin, 1.6 mg; pyridoxine hydrochloride, 0.8 mg; nicotinic acid, 4.0 mg; calcium pantothenate, 4.4 mg; choline chloride, 100 mg; and a source of vitamins A, D and E, 100 mg [corn oil, 82 mg; A and D concentrate, 14 mg (6300 units A and 1250 units D); alpha-tocopherol, 4 mg]. Assays with *Lactobacillus casei* ε have shown that such a diet contains from 0.7 to 1.8 μg of "folic acid" per 100 gm; typical highly purified diets have contained from 0.5 to 1.4 μg of "folic acid" per 100 gm; assays with *Streptococcus lactis R* have given similar results.

The inclusion of succinylsulfathiazole in the dried milk diet, in amounts as large as 10 per cent., in contrast to the effects produced by levels of only 1 or 2 per cent. in highly purified diets, caused no evidence of nutritional deficiency in rats during a period of 14 weeks following weaning. The growth rate of the animals given the milk-sulfonamide ration was not inferior to that of animals on the milk ration alone, and leucopenia did not develop. At the end of the period of feeding, total leucocyte counts of 5,000 to 22,000 were observed, while on the milk diet alone the counts ranged from 7,000 to 13,000.

Assays for "folic acid" in the tissues of these and other rats showed that considerably larger amounts of microbiologically active material were present in the hepatic tissue of animals fed a whole milk ration than were found in the liver of rats given a highly purified diet "contaminated" with a comparable amount of "folic acid." In each case the addition of succinylsulfathiazole to the diet caused a marked reduction in the "folic acid" content of the liver; however, the reduction was notably greater in the case of animals given the sulfonamide in highly purified diets.

The above observations suggest that one (or more) of the components of milk may be utilized by the rat for growth and other purposes in lieu of material possessing the microbiological activity of "folic acid." Whether the material in milk is structurally related to microbiologically active factors (the various folic acids,[10] vitamin B_c,[11] and the factor of Keresztesy, *et al.*[12]) or whether it is in some manner concerned with the metabolism of "folic acid" is now under investigation.

It is readily apparent that the use of various microorganisms for the assay of foods and other natural materials may fail to measure their total content of various factors having vitamin activity in animals.

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THE HERBICIDAL ACTION OF 2,4 DICHLOROPHENOXYACETIC AND 2,4,5 TRICHLOROPHENOXYACETIC ACID ON BINDWEED[1]

THE use of growth-regulating substances on plants has been directed mostly towards improving their performance in terms of usefulness, such as increasing the set of fruit, preventing the premature dropping of fruit, speeding the rootings of cuttings and developing fruits which are seedless. It is well known, however, that growth substances may be toxic to plants in concentrations greater than those used to secure these desirable responses.

Kraus,[2] and more recently Mitchell and Hamner,[3] have suggested the possibility of growth-regulating substances as selective herbicides, since species and varieties of plants show wide differences in the degree to which they react or respond to the various compounds. Some of the more potent of these compounds are the substituted phenoxy compounds.[4]

[4] Folic acid, as defined (H. K. Mitchell, E. E. Snell and R. J. Williams, *Jour. Am. Chem. Soc.*, 63: 2284, 1941) refers to a factor essential for the growth of *Streptococcus lactis R*. Since there appear to be several entities with activity for that organism, we have used the term folic acid to include factors with microbiological activity for *L. casei* ε, as well as *Strep. lactis R*.

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