THE NEUROTOXIN OF *SHIGELLA SHIGAE*.

2. Examination of the Toxin in the Oil-turbine Ultracentrifuge.

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A sample of Shiga neurotoxin, prepared by van Heyningen and Gladstone (1953), was furnished for physico-chemical examination. The difficulty of obtaining more than a few mg. of this toxin made it desirable to obtain as much information as possible from few measurements. It has proved possible to obtain the concentration, the weight-average sedimentation coefficient, the distribution of sedimentation coefficient, and a mean diffusion coefficient, all from two ultracentrifuge experiments made on a single (0·7 ml.) sample of solution. The contributions of diffusion and of heterogeneity to the spread of a boundary in the ultracentrifuge can be sorted out, and both the diffusion coefficient and the distribution of sedimentation coefficient obtained, by making use of the different way in which these two vary with time.

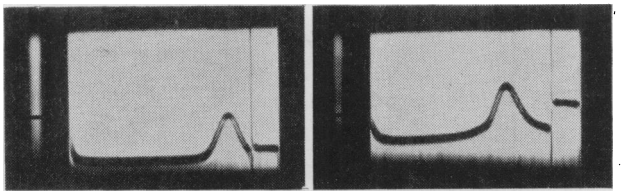

Fig. 1.—Schlieren diagrams of the sedimentation of Shiga neurotoxin (0·5 g./100 ml.). The fine vertical line to the right of the sedimenting peak represents the meniscus between toxin solution and liquid paraffin (*a*) after sedimentation for 55 min. at 650 rev./sec. (*b*) after sedimentation for 58 min. at 900 rev./sec. Much of the skewness is due to buffer sedimentation.

The diffusion coefficient is found by plotting an " apparent diffusion coefficient," D^* (Williams, Baldwin, Saunders and Squire, 1952), against time and extrapolating to zero time. For a homogeneous substance this plot will be a horizontal line. When the boundary includes substances of differing sedimentation coefficient, D^* will increase with time in a nearly linear fashion ; the slope of this plot is a measure of the degree of heterogeneity. The distribution of sedimentation coefficient is found by a method comparable to that of finding the diffusion coefficient (Williams *et al.*, 1952 ; Gosting, 1952) : an " apparent distribution of sedimentation coefficient " is obtained from each boundary record and these are again extrapolated, but now to infinite time.

EXPERIMENTAL.

Toxin, 4·5 mg., was dissolved in 0·65 ml. of buffer (0·2 M NaCl, 0·0077 M Na_2HPO_4, 0·0023 M KH_2PO_4 ; pH measured by glass electrode = 7·25 at 20°).

This volume was sufficient nearly to fill a 12 mm. ultracentrifuge cell: the solution was covered with a layer of liquid paraffin. Two runs were performed in the Svedberg oil-turbine centrifuge, the first at 650 rev./sec.; after this run the cell contents were mixed by stirring, and a second run was performed at 900 rev./sec. The procedure was that of Cecil and Ogston (1948), except that the edge-schlieren optical system was replaced by a bar-schlieren system (Fig. 1). This was adjusted so as to give a narrow boundary trace, whose centre could be measured with high precision. Values of X and dn/dx were measured with a two-way travelling microscope. Base lines were separately determined, and base line correction was made to the values of dn/dx.

RESULTS AND DISCUSSION.

1. The area under the boundary curves was obtained by summation of dn/dx; this gave a refractive increment for the original solution of $0{\cdot}97 \times 10^{-3}$ ($\pm$ 1 per cent). Assuming a value for the specific refractive increment of $1{\cdot}85 \times 10^{-3}$ (this is unlikely to be wrong by more than 3 per cent), the concentration of the

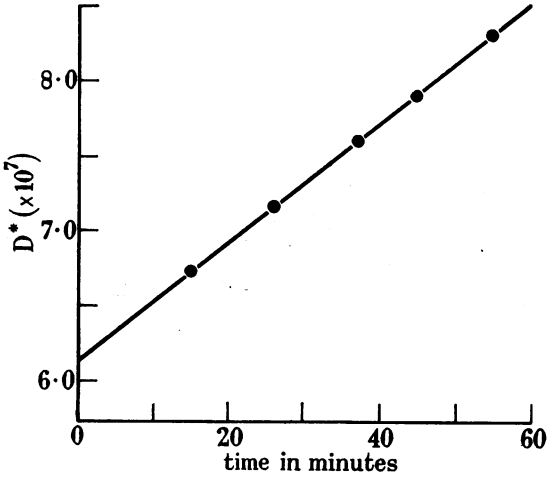

FIG. 2.—Extrapolation of the "apparent diffusion coefficients" from the centrifuge run at 650 rev./sec. to zero time. The values shown refer to the viscosity of the buffer at the temperature of the centrifuge run.

solution is 0·52 g./100 ml., compared with an expected value (from the wt. of toxin used) of 0·69 g./100 ml. It is possible, however, that some part of the toxin might sediment too fast or too slowly to appear in the sedimentation diagram.

2. For obtaining the diffusion coefficient the centrifuge run at 650 rev./sec. was used, since diffusion is relatively more pronounced at lower speed. The extrapolation of D^* to zero time is shown in Fig. 2. D^* was calculated from the boundary curves by a method to be described in detail elsewhere; in addition to the factors discussed by Williams *et al.* (1952), explicit allowance was made for the dependence of sedimentation coefficient upon concentration and for the possibility of convection while the centrifuge is coming up to speed. D^* values were obtained by the "height-area" method, since the boundary did not depart

significantly from Gaussian shape during the time these measurements were taken. The value obtained for the diffusion coefficient, $D_{20,\,w}$, is $5{\cdot}7 \times 10^{-7}$ cm.^{2} sec.$^{-1}$ with an estimated uncertainty of $\pm$ 5 per cent.

3. For obtaining the distribution of sedimentation coefficient, by extrapolation to infinite time, the centrifuge run at 900 rev./sec. was used. The resulting distribution is shown in Fig. 3. The weight-average value of the sedimentation coefficient, $S_{m20,\,w}$, is $4{\cdot}80 \pm 0{\cdot}05$ Svedberg units, and the standard deviation of its distribution 0·83 S.

4. Using the mean values of S and D, and assuming the partial specific volume to be 0·75 ml. per gm., the value for molecular weight M is 82,000. It is not

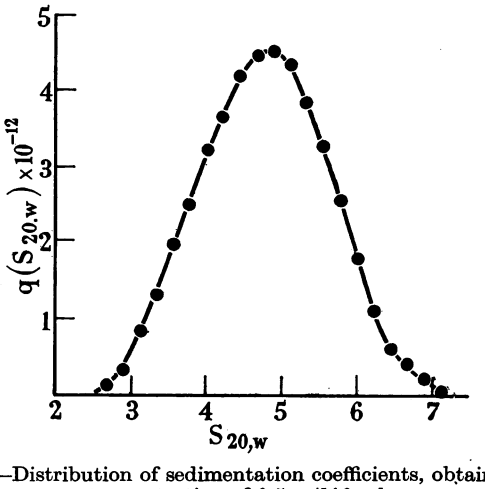

FIG. 3.—Distribution of sedimentation coefficients, obtained at a concentration of 0·5 g./100 ml.

possible to say from these data whether the toxin possesses a distribution of molecular weight comparable to the distribution of sedimentation coefficient, since the latter could be caused by variation in shape (*i.e.*, frictional coefficient) as well as molecular weight; the mean value of molecular weight given here is not a simply defined average. Additional uncertainty in the molecular weight arises from the fact that the measurements were made at finite concentration. Since the concentration was only 0·5 g./100 ml. and the value of f/f_0 only 1·26, it is unlikely that the last source of error is important.

One general comment is required. The breadth of the distribution of sedimentation coefficient found here should be a warning against judging, from the appearance of a single, fairly symmetrical boundary in the ultracentrifuge, that the substance is homogeneous in sedimentation.

SUMMARY.

The concentration, the mean sedimentation coefficient and its distribution and a mean diffusion coefficient of a sample of Shiga neurotoxin have been determined from two experiments in a Svedberg ultracentrifuge. Only 4·5 mg. of material was required for these measurements.

I would like to acknowledge Dr. A. G. Ogston's helpful advice and suggestions throughout the course of this work, which is part of a thesis to be presented for the degree of Doctor of Philosophy at Oxford University.

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