DISPERSAL OF MOTILE BACTERIA FROM A PLANE LAYER

J. V. CRIDLAND AND P. C. THONEMANN
Department of Physics, University College of Swansea, Swansea, SA2 8PP, United Kingdom

ABSTRACT The dispersal of an initially well-defined concentration of the motile bacterium *Escherichia coli* was measured under nonchemotactic conditions. The distribution of bacteria along a glass observation cell was measured by recording the intensity of light scattered by the organisms. For comparison, the diffusion of fluorescein was also measured by determining the distribution of fluorescence throughout the observation cell. The dispersal of bacteria from a plane layer, under nonchemotactic conditions, can be adequately described by the Gaussian solution of the diffusion equation.

INTRODUCTION

When viewed microscopically, individual organisms in a culture of motile bacteria, exhibit a random motion that superficially resembles Brownian motion. Consequently, one expects an initially high concentration of motile microorganisms to disperse like particles that diffuse by Brownian motion. Consider an initial population of particles confined to a narrow layer at one end of a column of liquid: The solution of the diffusion equation under these conditions, as given by Ljunggren and Lamm (1), is

$$n(x, t) = [A \exp(-x^2/4Dt)]/(\pi Dt)^{1/2}, \quad (1)$$

where n is the number of particles per unit volume, A is the number of particles initially distributed uniformly over unit area at distance $x = 0$ and time $t = 0$, and D is the diffusion coefficient.

Adler and Dahl's (2) experimental results, which describe the dispersal of the bacterium *E scherichia coli (E. coli)* from an initial condition similar to that described above, do not agree with the solution of the diffusion equation given by Eq. 1. Considering the inconsistencies, Adler and Dahl suggested the following explanations: (*a*) that within the population, organisms have heterogeneous motilities, (*b*) that there is a nonuniform distribution of chemical substrates, and (*c*) that there is crowding at the origin. The first explanation was extensively discussed by Thonemann and Evans (3), who compared theoretical calculations with Adler and Dahl's experimental data. They proposed that more accurate experimental data are necessary to make further progress.

Segel et al. (4) described a simple quantitative technique to assess motility and strongly suggested that effective diffusivity is the best single measure of bacterial motility. Therefore, it would be interesting to determine if a single motility coefficient can be assigned to a population of microorganisms. Microscopic measurements do not provide sufficient data to average over the population of organisms.

In the present study, we investigated the diffusion equation's apparent failure to adequately describe the diffusion of bacteria. It was shown that the dispersal of bacteria could be described reasonably using the diffusion equation. The shortcomings of this description are small compared with the experimental errors due to the nonreproducibility in the samples of bacteria.

METHODS

Apparatus

The apparatus constructed was similar to that used by Dahlquist et al. (5) and is shown schematically in Fig. 1. The light, emitted from a 10-mW helium-cadmium laser (model 4110; Liconix, Sunnyvale, CA) at 442-nm wavelength, illuminated a small volume (0.8 mm in diameter and 5 mm long) of a glass observation cell with a 2 × 5-mm^2 cross section and 30 mm length. The observation cell contained the suspension of bacteria. The light scattered by the bacteria into a cone (10° half angle) at 90° to the incident beam and was collected by a 3× microscope objective and focused onto the exit slit located just in front of the photosurface of a photomultiplier tube (PMT). The exit slit crosssection was 3 × 17 mm^2, which gave a spatial resolution of 0.5 mm. The logarithm of the PMT output was displayed on a chart recorder.

Using a mechanical screw drive and electric motor, the observation cell could be moved vertically at rates of 0.25 mm/s or 0.9 mm/s, effecting total scan times of 1.8 min and 30 s, respectively, for a 27-mm scan. This way, the vertical distribution of bacteria could be recorded at various times.

In addition to the light-scattering apparatus a means of establishing the required initial conditions was necessary. To apply the Gaussian solution of the one-dimensional, time-dependent diffusion equation, the bacteria initially had to be distributed uniformly as a plane layer at one end of the observation cell. To do this, a centrifuge was constructed, consisting of a 600-W electric motor and an aluminium disk with a radius of 10 cm that

J.V. Cridland's present address is Control Laser, 9 Royal Oak Way South, Daventry Northamptonshire, United Kingdom.

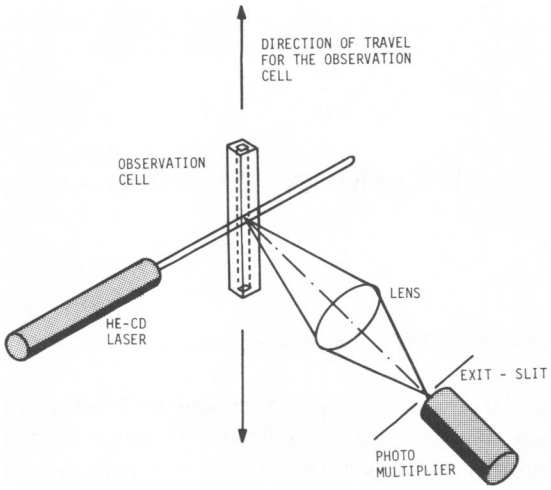

FIGURE 1 This is a schematic diagram to demonstrate the experimental apparatus used to measure the spatial distribution of bacteria.

held the observation cell in a horizontal position. The centrifuge was operated at 6,500 rpm.

Organism and Preparation

The bacteria were prepared using a variation of Adler and Dahl's (2) method. The bacteria, *E. coli K12-W1485*, obtained as freeze-dried cultures from N.C.I.B. Tory Research Centre (Aberdeen, United Kingdom), were motile and required methionine for growth. The cultures were inoculated into the liquid growth medium described below, grown up, and reinoculated onto nutrient agar (Difco Laboratories Inc., Detroit, MI) slopes, which were incubated at 30°C for 24 h and subsequently stored at +3°C. When required, the organisms were grown (at 30°C) with shaking to a density of $\sim 2 \times 10^8$ ml^{-1}. Organisms were harvested by low speed centrifugation, and the pellet was gently suspended in the filtered washing medium described below. The centrifugation and washing steps were repeated twice. This procedure produced samples in which 70–80% of the bacteria were motile.

Media

The specific growth medium contained 0.1 M K_2HPO_4, 0.1 M KH_2PO_4, 1.5×10^{-2} M $(NH_4)_2SO_4$, 10^{-3} M $MgSO_4 \cdot 7H_2O$, 2×10^{-5} M $FeCl_3 \cdot 6H_2O$, 0.5% (vol/vol) glycerol, and 2.7×10^{-3} M DL-methionine. The washing and experimental medium contained 0.01 M K_2HPO_4, 0.01 M KH_2PO_4, 7.5×10^{-4} M $(NH_4)_2SO_4$, 10^{-3} M $MgSO_4 \cdot 7H_2O$, 10^{-4} M ethylenediaminetetraacetic acid (EDTA), and 10^{-2} M L-serine. Glass double-distilled water was used. Serine was chosen as the energy source because it could supply energy for motility either aerobically or anaerobically.

RESULTS

The diffusion of an initial high concentration of fluorescein will be described. A comparison can then be made with the dispersal of bacteria. The initial conditions were established using a hypodermic syringe arrangement that could form a step of ~0.7 mm width. Providing that data recorded at sufficiently long times (i.e., >80 min) were

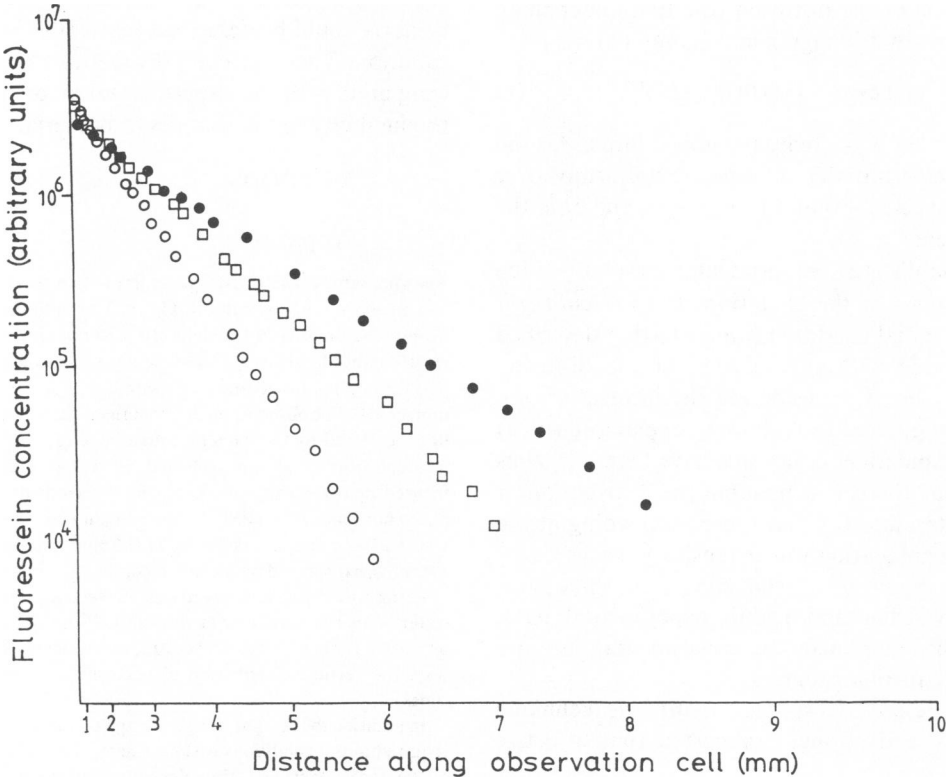

FIGURE 2 Fluorescein concentration (minus a background level) vs. the square of distance along the observation cell is plotted. The data for times of 54 (○), 85 (□), and 115 (●) min are shown.

considered, this step width was sufficiently narrow to ensure that little error (~5%) was introduced into the calculation of the diffusion coefficient.

Diffusion of Fluorescein

The distribution of fluorescein (in water) along the observation cell was measured by recording the intensity of fluorescence stimulated by the He-Cd laser. Fig. 2 shows the fluorescence intensity minus a background value plotted against the square of the distance along the observation cell. The diffusion coefficient was found from the slope to be 4.6×10^{-4} mm^2/s at 19°C using the method outlined in the Appendix. This value compared well with values calculated from the equation given by Arnold (6) and from data taken from the Chemical Engineers Handbook (7), which gave values of 5.0×10^{-4} and 4.4×10^{-4} mm^2/s, respectively.

Dispersal of *Escherichia coli*

This section presents the results of the dispersal experiments carried out with the strain W1485 of *E. coli* in the experimental medium described in the Methods. The observation cell was filled with a suspension of about 10^6–10^7 bacteria prepared as described previously. The cell was centrifuged for 6 min at 6,500 rpm, after which time the bacteria were evenly distributed over the base of the observation cell. The cell and holder were transferred to the light-scattering apparatus and a visual check was made for convection. The spatial distribution of bacteria was measured at various times. A single experiment lasted between 30 min and 1 h (typically 35–40 min), with the observation cell scanned at intervals ranging from 2 to 15 min.

Dispersal measurements were carried out with various initial numbers of bacteria. The outcome of these experiments were influenced not only by the number of bacteria introduced to the observation cell but also by their previous environmental history (e.g., the period of time they spent in the washing medium or growth medium). Chemotactic effects were generally observed with initial numbers of bacteria greater than 2×10^7. Moreover, traveling bands were always observed when the number of bacteria introduced to the observation cell was within the magnitude of 10^8. With fewer bacteria, the dispersal curves assumed forms that apparently depended on substrate concentration, but did not result in traveling bands. Further reduction in the bacterial number to levels in the region of 2×10^6 to 2×10^7, together with the use of fresh experimental medium saturated with air, provided conditions in which the dispersal of bacteria generally resembled that expected for diffusion. Reducing the bacteria concentration further did not alter the character of the dispersal curves; however, the signal-to-noise ratio was then unacceptable. Noise, in this case, was caused by scattering from dust particles suspended in the medium and from imperfections in the glass observation cell.

Fig. 3 shows the variation in the scattered light intensity (which is proportional to the bacterial number density) with distance along the observation cell. The curves closely resemble those that would be expected for the case when particles diffuse by Brownian motion from plane layer initial conditions. The data were replotted as the number density vs. the square of distance along the observation cell (Fig. 4). When plotted this way, the present data show a higher degree of linearity than those obtained by Adler and Dahl (2). Some possibilities were suggested by Adler and Dahl for their observations. We propose that the nonlinearity mainly resulted from a local depletion of critical substrate (e.g., oxygen) in the observation cell that created a spatial variation in motility. Curves that had a similar character to those obtained by Adler and Dahl were observed when experimental media that was depleted in oxygen was used.

The almost linear relationship between the logarithm of number density and the distance squared (Fig. 4) strongly suggests that a single motility coefficient can be assigned to describe the motility of a particular population of bacteria. Using the method described in the Appendix, the motility coefficients calculated at various time intervals for a single dispersal experiment are given in Table I. Motility coefficients, calculated from separate experiments, are shown Table II, along with various other parameters, such as temperature and bacterial number.

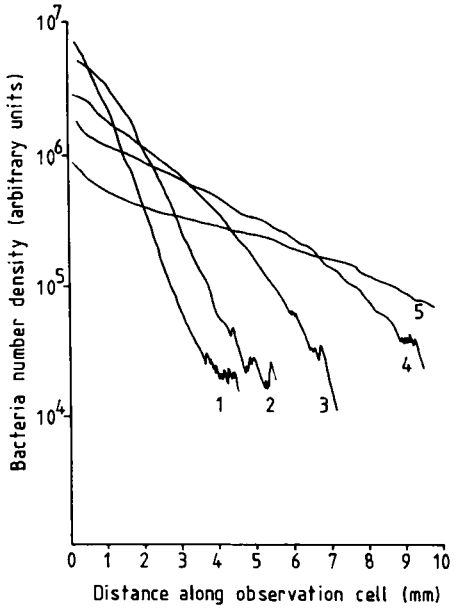

FIGURE 3 The dispersal of *E. coli* K12-W1485 under nonchemotactic conditions are shown. The observation cell contained ~2×10^7 bacteria in the experimental media (described under Methods) at a temperature of 20°C. Methionine was not present. The distribution at times of 2 min (*1*), 5 min (*2*), 17 min (*3*), 41 min (*4*), and 68 min (*5*) are shown.

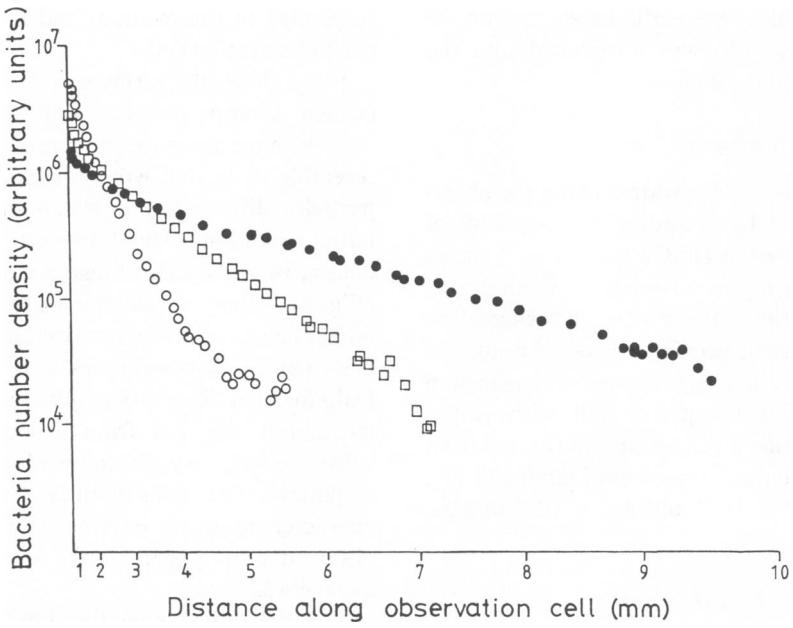

FIGURE 4 A portion of the data from Fig. 3 were replotted as the bacterial number density vs. the square of distance along the observation cell. The data for times of 5 (○), 17 (□), and 41 (●) min are shown.

DISCUSSION

Motility

A simple, well-known technique used to measure diffusion coefficients has been applied to the dispersal of *E. coli* *K*12-*W*1485. The technique provides a simple and accurate way to measure the degree of motility in a population of organisms. The initial conditions of a plane layer, were chosen because diffusion can be described by the Gaussian solution of the diffusion equation.

Under suitable conditions, we found that the dispersal of bacteria could be described adequately by Eq. 1. This supports the suggestion made by Segel et al. (4) that a single motility coefficient can provide an adequate description for the motility of a population of bacteria. To the best of our knowledge no previous measurements conclusively support this suggestion.

The motility coefficient (M) determined from the results of seven experiments yielded a mean value of 2.9×10^{-3} mm^2/s (0.1 cm^2/h). This is approximately half the value reported by Adler and Dahl (2) and by Segel et al. (4) for *E. coli* B275 and *Pseudomonas fluorescens*, respectively (see Table III). This discrepancy probably results from differences in the bacterial species and the methods of preparation. In addition, because Adler and Dahl calculated their motility coefficients from the bacteria that had moved the furthest (i.e., at a front), their values tend to be high and do not represent the population as a whole. Note,

TABLE I
MOTILITY COEFFICIENTS OF *ESCHERICHIA COLI* *K*12-*W*1485 DETERMINED FROM A SINGLE DISPERSAL EXPERIMENT

Time difference	Motility coefficient
min	$mm^2/s \times 10^{-3}$
7.0	1.97
11.0	2.34
13.3	2.50
15.0	2.43
19.0	2.37
38.3	2.56
66.0	3.19
Mean value	2.4 ± 0.4 (SD)

Motility coefficients that were calculated at various time intervals using the method described in the Appendix are shown. The time differences correspond to the quantity ($t_2 - t_1$) of Eq. A1. The temperature was 20°C.

TABLE II
AVERAGE MOTILITY COEFFICIENTS CALCULATED FROM THE DATA OF SEVERAL SEPARATE EXPERIMENTS

Experiment	Motility coefficient	Temperature	Number of bacteria
	$mm^2/s \times 10^{-3}$	°C	
R1	2.1	19.7	2×10^7
R2*	2.3	21.0	6×10^6
R3	2.4	19.9	2×10^7
R4	3.6	22.7	3×10^6
R5*	3.8	22.8	3×10^6
R6	4.2	22.2	2×10^6
R7	1.6	21.2	6×10^6

The asterisk (*) indicates that the sample used for the measurement was taken from the same experimental suspension as that used for the previous experiment.

TABLE III
COMPARISON OF PUBLISHED MOTILITY COEFFICIENTS

Reference	Organism	Mean free	Mean speed	Motility coefficient
		s	$mm/s \times 10^{-3}$	mm^2/s
11	*E. coli* wild type *AW*405	0.86 ± 1.18	14.2 ± 3.4	0.92×10^{-4} C*
	E. coli nonchemotactic	6.3 ± 5.2	20 ± 4.9	5.2×10^{-3} C
2	*E. coli K*12-*B*275	—	—	6.94×10^{-3} M‡
4	*Pseudomonas fluorescens*	—	—	5.6×10^{-3} M
9	*Salmonella typhimurium*	10.0	20.0	1.3×10^{-3} C
13	*Salmonella typhimurium*	—	—	4.4×10^{-3} C1§
16	*Salmonella typhimurium*	—	—	5.6×10^{-3} C1
15	*Salmonella typhimurium*	10.0	15.0	0.8×10^{-3} C
14	*E. coli* wild type	—	—	1.0×10^{-4} C
10	*E. coli K*12-*W*1485	10.0	30.0	5.0×10^{-3} C
This report	*E. coli K*12-*W*1485	—	—	2.9×10^{-3} M

*C represents values calculated from microscopic measurements using Eq. 2.
‡M represents values obtained from macroscopic measurements.
§C1 represents values calculated from chemotactic experiments.

however, that both the present study and that of Adler and Dahl discount nonmotile bacteria.

Inspection of Fig. 4 reveals that there is a slight nonlinearity in the data. This probably results because the population was described by a single motility coefficient, but actually should be described by a distribution of such coefficients (3, 8). Moreover, if the population has a Gaussian distribution of motility coefficients, as opposed to speeds, then Eq. 1 still should apply. This can be verified by application of Eq. 3 of Thonemann and Evans (3). Thus, the nonlinearity in the experimental data may suggest that there are slight deviations from a Gaussian distribution of motility coefficients.

To compare the motility coefficients measured in the present study with those obtained from microscopic measurements, we can use the expression (9):

$$M = U^2 T/3(1 - \overline{\cos\theta}), \qquad (2)$$

where T is the mean duration of a trajectory, U is the mean speed, and $\overline{\cos\theta}$ is the mean cosine of the angle of turn. Measurements of U and T have been made by King (10) using *E. coli* W1485 in the medium described in the Methods. The values obtained for U and T were 30×10^{-3} mm/s and 10 s, respectively. These are the mean values of 13 separate measurements. A value for $\cos\theta$ was not available so a value of 0.42 was taken from Berg and Brown (11). Substituting these numbers into Eq. 2 gave a value for M of 5×10^{-3} mm^2/s. This is in reasonable agreement with the measured value, considering that U and T were averaged over only 13 measurements.

Agreement with other reported data is generally good. Lovely and Dahlquist (12) and Nossal and Weiss (13) reported values of 1.3×10^{-3} mm^2/s and 4.4×10^{-3} mm^2/s, respectively. However, the values of M quoted for *E. coli* by Holz and Chen (14) are a factor of 30 less than the value reported here. This difference was caused by the individual motion of the bacteria (see the reference to Berg and Brown [11] in Table III).

Sources of Error

Possible sources of error in our measurements include sedimentation due to gravity, the dependence of bacterial swimming speed on size, and convection. The first might give an error of 10–20% in M, which is small compared with the range of motility coefficients measured (Table II). Regarding the second source of error, samples taken from various positions along the observation cell did not reveal any redistribution of size. Finally, convection was eliminated by using an observation cell with the appropriate cross-sectional area, which was determined from a series of tests.

APPENDIX

Calculation of the Motility and Diffusion Coefficients

The equation used to evaluate the motility and diffusion coefficients was:

$$M = (1/s_1 - 1/s_2)/4(t_2 - t_1), \qquad (A1)$$

where s_1 and s_2 are the slopes calculated from the data, presented as the logarithm of the number density vs. distance squared, at times t_1 and t_2, respectively, and M is the motility coefficient. This approach was adopted because it is not necessary to identify the initial start time of dispersal and the time of observation relative to this start time. Both these times were variable because the interval was variable between the time of sedimentation in the centrifuge and the time when observation began.

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