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THE DISPERSION OF BACTERIA

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of Francis G. Rust

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Francis G. Rust and Charles R. Wilke

September 1966

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September 1966

ABSTRACT

The dispersion of bacteria by their own motility was measured by three methods and was found to follow the behavior predicted by diffusion. The diffusivity was found to range from 2 to 3×10^{-5} cm^2/sec at 26° C in a uniform nutrient medium. Methods are presented for estimating the rate of dispersion of motile bacteria that are multiplying as they diffuse.

I. INTRODUCTION

On large scale, bacterial transport is accomplished by convection or sedimentation, both of which can be handled by conventional engineering methods. Some bacteria also move by their own motility so that in a uniform medium that is free from convection, bacteria will disperse by a random motion which could be characterized as a diffusional mechanism. The dispersion of bacteria by this diffusive process has not been investigated previously, and is the primary subject of this report. The dispersion of bacteria will be shown to follow the behavior predicted by diffusion provided that the following necessary conditions are met.

1. Suitable medium for motility
2. Absence of convection currents or force fields
3. Absence of areas depleted in a nutrient

Even in the absence of motility, bacteria will slowly diffuse by Brownian motion. Bacteria are so large that transport by this mechanism is not significant.

The diffusive transport of bacteria is of engineering interest in cracks, along the interior surfaces of pipes, in packed beds, and in any case where convective forces have been damped out by the presence of solid surfaces.

The maintenance of sterility can be of great importance in biological operations such as fermentation, for one bacterium can spoil the entire contents of a feed tank in less than a day. A knowledge of all mechanisms of bacterial transport is necessary to evaluate the probability of bacteria gaining access to a sterile process.

Three different experimental methods were used to measure the dispersion of bacteria. In the first experimental method, the location of individual bacteria was recorded at different times by multiple exposure photography. The displacement of the bacteria from their original position was measured, and the diffusivity was calculated

using the Einstein relationship.[1] The extent to which the motion of the bacteria had become random could also be evaluated in this experiment.

In the second experimental method, a steep bacterial concentration gradient was established on a microscope slide and was photographed at various times for counting. From these photographs bacterial concentration profiles were obtained. Different diffusivities were then used in a numerical solution to the diffusion equation, each diffusivity gave a different concentration profile. The calculated profiles were matched with the measured profile to see which diffusivity gave a profile that matched the measured profile.

In the third experimental method, a capillary tube was filled with a bacterial suspension and was immersed in stirred nutrient. After allowing time for diffusion of bacteria from the tube to occur, the tube was removed and the number of bacteria in the tube and the number of bacteria in the solution was determined by plating on nutrient agar and counting the colonies of bacteria that grew from each viable bacterium. The diffusivities were obtained by solving the partial differential equation for diffusion, sedimentation, and growth numerically to give concentration profiles at many different times. These concentration profiles were integrated to give the fraction of the bacteria remaining in the tube as a function of dimensionless time, Dt/l^2. The diffusivity could be determined from this function once the fraction of the bacteria remaining in the tube had been experimentally measured, provided that the time for diffusion, and the length of the tube were known.

The experimentally determined diffusivity of bacteria was used to calculate the rate at which contamination by motile bacteria would spread through a crack filled with quiescent medium. An example was also calculated for the diffusion along the inside surface of a feed line against the flow of medium.

II. CHARACTERISTICS OF THE BACTERIAL CULTURES

For the proposed experiments to be valid, it is essential that such properties as bacterial motility and multiplication rate be constant during an experiment. Scouting experiments were made on several strains of E. coli to determined how the multiplication rate, percent of bacteria that are motile and bacterial velocity were affected by temperature, stage of growth and composition of the medium.

The cultures used were three strains of E. coli obtained from the Department of Bacteriology at the University of California. They were:

E. coli U. C. 27, a highly motile strain isolated in 1958

E. coli 3000, motile but slightly less motile than U. C. 27

E. coli B (Hershey), a non motile strain.

E. coli U. C. 27 was selected for the diffusion measurements because the fraction of motile bacteria was highest in this strain.

Nutrient medium (5 g. peptone, 3 g. beef extract, 2 g. glucose, to 1 l. water) was used in all experiments because in the absence of peptone only about half of the bacteria were motile at 20° C. This decrease in motility was observed in all defined media regardless of sugar energy source and was not due to contamination, for the definitive test for E. coli was made on all samples. The disadvantage of nutrient medium was that the growth rate was so high that the diffusion experiments had to be limited to three hours. Anticonvectants such as gelatine, agar, and methyl cellulose were not used because they are reported to accumulate on flagella and make them large enough to be seen with a light microscope.[2] Since flagella are believed to be the motor organs of bacteria, the heavy deposites might affect bacterial motility and diffusivity.

The diffusion experiments in this report are based on the premise that bacteria move randomly. Bacteria would not be expected

to move in a random manner if there were zones in the medium that were depleted in some essential nutrient. Under conditions of unfavorable environment bacteria often undergo a "shock reaction", their motion is erratic and characterized by rapid, random changes in direction until they locate a favorable environment. The term chemotaxis is used to describe this ability of bacteria to locate and move preferentially toward a favorable environment. Adler[3] recently described the ability of E. coli to deplete the nutrient at one end of a tube and then migrate down the tube in a band following the oxygen and energy source front; a depleted region was left behind the migrating bacteria.

A typical growth curve based on optical counts is shown (Fig. 1) for E. coli U. C. 27 at 20°C in nutrient medium. The percent of bacteria that are motile can be seen to remain constant from early exponential phase to stationary phase. At the onset of stationary phase the velocity of the bacteria was observed to decrease rapidly. A growth rate discontinuity in which the bacteria ceased to divide for a two hour period can be seen in the growth curve at a concentration of 5×10^7 bacteria/ml. Catherine Fowler[4] has observed a similar discontinuity in the growth of E. coli at 37°C, and has demonstrated that this failure to multiply occurs coincident with the depletion of oxygen in the medium. At 37°C she observed that there was a 30 to 50 minute lag before growth continued in the absence of oxygen. After 70 minutes, the anerobic growth rate was the same as the previous aerobic rate. Based on the work of Adler[3] and Fowler,[4] it would be anticipated that nutrient medium (exposed to air for a week after autoclaving) would have sufficient dissolved oxygen for E. coli to grow aerobically to a concentration of about 5×10^7 bacteria/ml. The depletion of oxygen would result in the non-random behavior of chemotaxis, and would be signaled by failure of the E. coli to multiply.

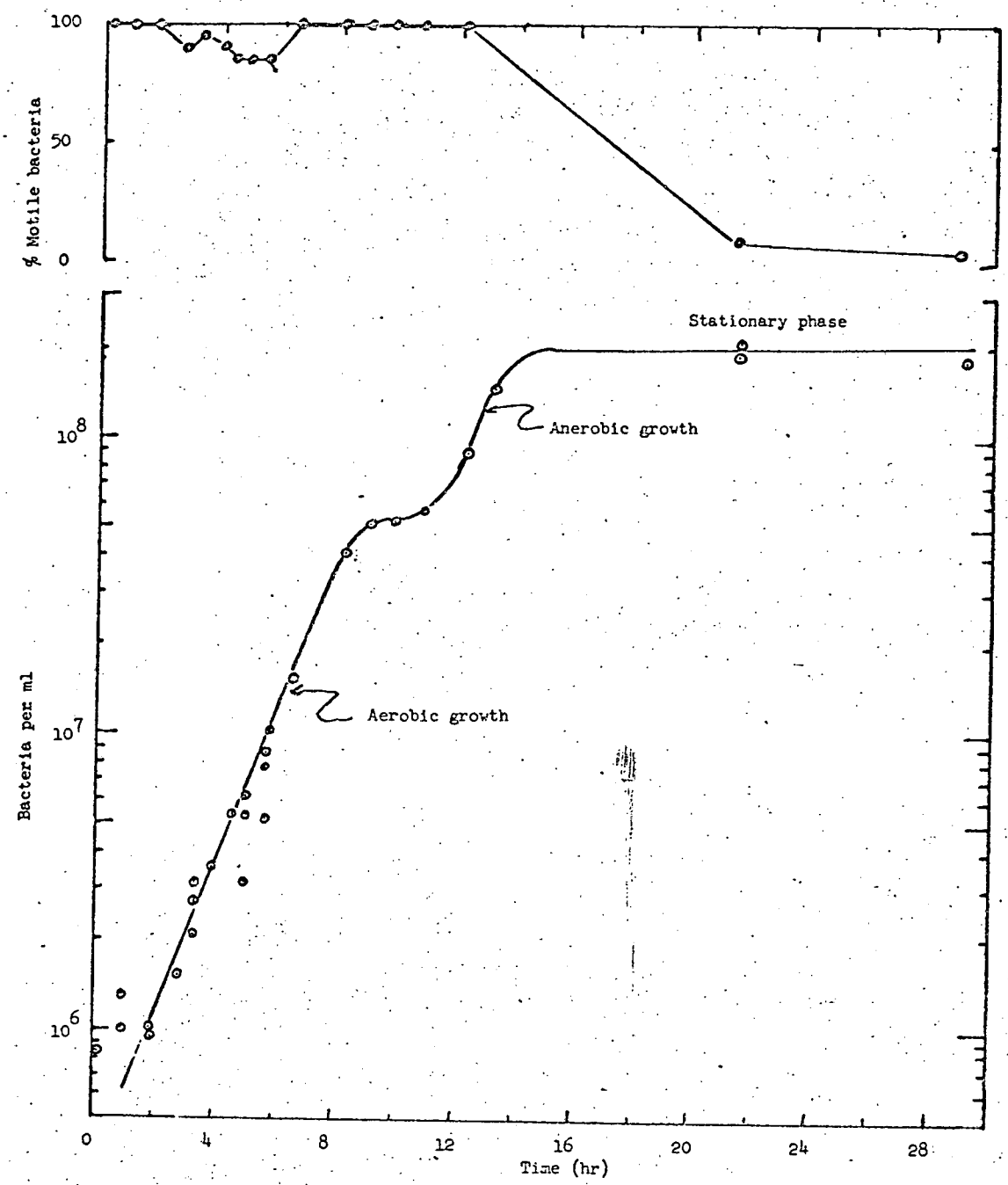

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Fig. 1. A typical growth curve from optical counts for E. coli 27 in nutrient medium at 20°C. Growth is aerobic until the oxygen is depleted. No further multiplication occurs until anerobic growth has started. The upper graph shows that a high percentage of the bacteria are motile until the onset of stationary phase.

The microscope lamp was found to stimulate motility during the onset of stationary phase. Multiple exposure photo-micrographs showed that about half of the bacteria were either stopped or moving very slowly. These bacteria accelerated to within 70 percent of their final velocity during the first second of exposure to the intense microscope lamp, which had been off for an hour. Continuous illumination was found to have no effect on the growth rate or motion during the majority of the exponential growth phase.

The heating caused by the microscope lamp was measured with a copper-constantan thermocouple in the medium between the slide and coverslip. The temperature rise for a 100 micron depth of medium was 0.2°C during multiple exposures, and 1.7°C during continuous exposure. The motion of nonmotile E. coli B in the slide chamber when it is vertical is evidence that the microscope lamp will cause convection currents in a vertical slide chamber.

A wide range of bacteria lengths was observed at all stages of growth. There was no significant effect of size on bacterial velocity. The majority of bacteria stay in the close proximity of a surface, vertical or horozontal, once they have encountered it. The surface might be expected to exert a drag on the moving bacteria; however, no significant change in velocity was observed at different distances from the surface.

III. DIFFUSIVITIES BY THE MEAN SQUARE DISPLACEMENT METHOD

Multiple exposure photographs were taken of bacteria in a thin film of nutrient medium between two microscope slides. The photographic images of a bacterium appeared as a series of dots (Fig. 2). From these photographs the distance that a bacterium traveled in a given time interval could be measured (Fig. 3). Thus sufficient information was available to treat the dispersion of bacteria in the same manner as Brownian motion had been originally treated by the Stokes-Einstein relationship.[1]

A. Theory

Diffusion can be thought of as a random walk of particles, and treated in the same manner as Brownian motion. At the turn of the century Einstein derived the relationship that relates the displacement of a particle that moves randomly to the diffusivity.[1] This relationship is:

$$\overline{X}^2 = 2Dt$$

or

$$\overline{r}^2 = 4Dt$$

where D = Diffusivity (cm^2/sec)

t = the time increment of observation (sec)

$\overline{X}^2$ = the mean of the squares of the displacement in a fixed arbitrary direction (cm^2)

$\overline{r}^2$ = the mean of the squares of the distances between the initial and final positions on a surface (cm^2)

For short times, before the particles have made their first turn, $\overline{X}$ is proportional to t, and the apparent D will increase linearly with t. As the particles make more turns their motion approaches random motion and the dependence of D on t decreases to the limiting case where motion is completely random and there is no dependence of D on t.

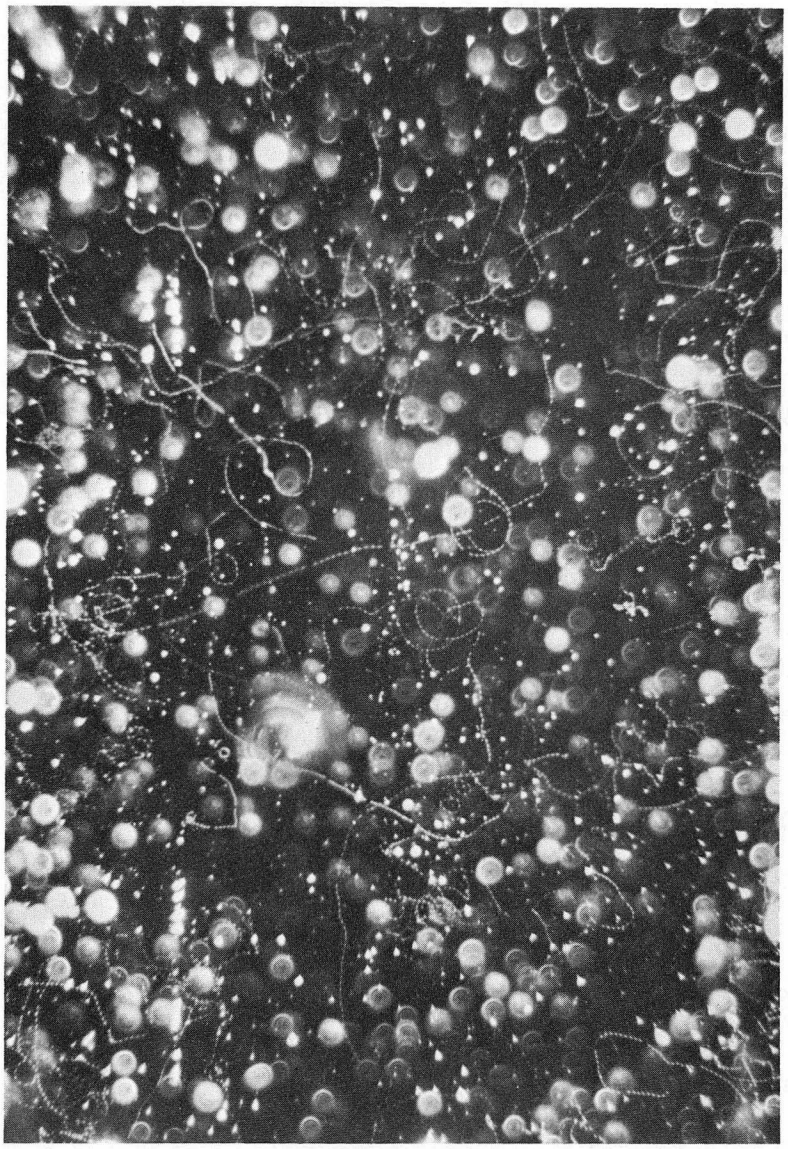

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Fig. 2. Multiple exposure photomicrograph of E. coli 27 in nutrient medium at 18 X lens magnification. The multiple images start with a 3 exposure sequence and end with a 5 exposure sequence. The 85 multiple exposures were taken one per second on pan film.

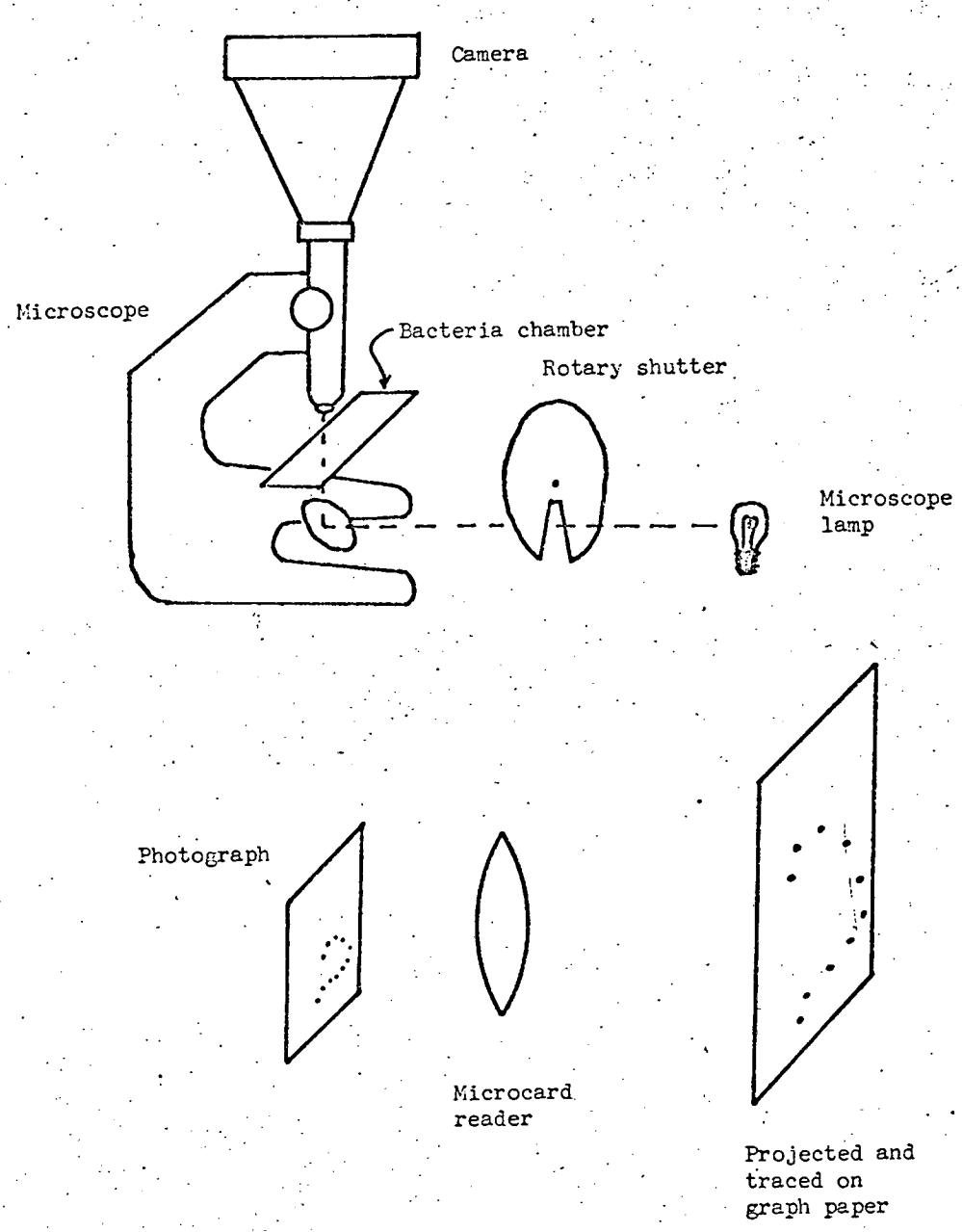

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Fig. 3. Schematic diagram showing the experimental procedure used in the mean square displacement method.

The calculations can easily be accomplished by hand. $\overline{x^2}$ is determined by squaring the x-components of all of the displacements at a given time, and averaging them. The diffusivity is obtained by dividing the $\overline{x^2}$ by twice the time interval for the displacements. The same calculations can be accomplished on a computer; a program to do this is given in Appendix A.

B. Equipment

The multiple exposure photomicrographs were made by using a rotary shutter to interrupt the beam of light from the microscope lamp. The rotary shutter was a disk with a slot cut in it; the disk was driven by a clock motor at 1 rps. With a dark field condenser, the bacteria appeared as bright spots moving against a black background. Every second the slot in the rotary disk permitted the light from the bacteria to reach the film. The image of a bacterium in motion is a string of bright spots. A good dark field condenser permits over 200 multiple exposures to be made on one negative, with no noticable washing out of the image by the background.

A Bausch and Lomb microscope with a low power dark field condenser and a large area light source was used for all runs in this experiment. Both high speed "Polaroid" film and medium speed panchromatic films were used with equal success. All optical systems were calebrated with a stage micrometer at the time of an experiment.

C. Experimental Procedure

Preliminary experiments quickly established that an individual bacterium would have to be followed for more than a minute for its motion to be random. During a minute an E. coli travels two milimeters. These long distances require that the magnification be decreased to the lower limit. At 18X the area of illumination limited further reduction in magnification. Construction of a large dark field condenser would permit the bacteria to be followed for longer periods of time.

The bacteria were grown for six hours in exponential phase by frequent dilution before they were placed in the microscope viewing chamber. These chambers (Fig. 4) were made by separating two carefully cleaned glass slides with a thin spacer. After partially coating the edges with hot paraffin, the spacer was removed, and the rest of the edge was coated except for filling and venting holes. Capillary action sucked the bacterial suspension into the chamber. After the fill and vent holes were coated; the suspension was completely enclosed in a chamber of uniform thickness.

Multiple exposure photomicrographs were taken (Fig. 2). Several reference points were made in the tracks by closing the mechanical shutter briefly during the multiple exposure photomicrographs. These reference points simplified measurements and permitted the direction of travel to be determined.

The displacements of a bacterium from the initial image to the image at time t was measured at times $t = 5, 10, 20, 40, 60$, and 80 sec by projecting the prints in a microcard reader and tracing the tracks on graph paper. A photomicrograph of the stage micrometer was also projected to give a scale factor. The introduction of bias was prevented by selecting only those tracks that could not have reached the edge of field if they had moved directly towards the nearest edge from their origin. The other tracks discarded were those that could not be followed at all because they moved in tight circles.

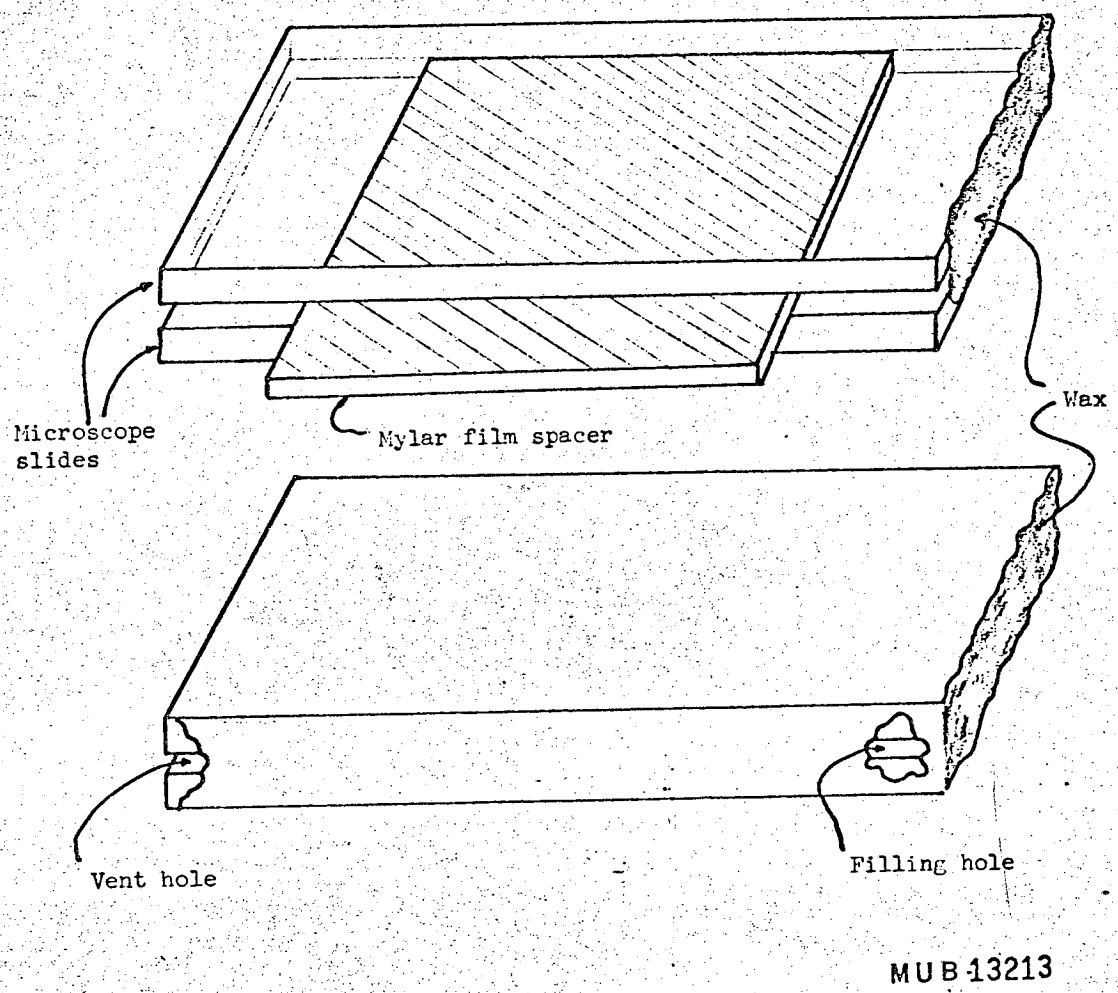

Fig. 4. Diagram showing the technique for the fabrication of the microscope chambers used for observing bacteria.

D. Discussion of Data

The diffusivities calculated from the mean square displacements are initially proportional to time (Fig. 5). As expected, the time dependence decreases with the increasing size of the time interval. The data are compared to the straight line that would have resulted if the bacteria had gone in a straight line at a velocity equal to the velocity measured over a 5 second interval.

Since the time increment could not be increased, the data was extrapolated to infinite time with the assumption of an exponential approach to the time independent diffusivity. The results are shown in (Fig. 6).

The data were tested for movement of the fluid by plotting the end points of the tracks on a scatter diagram where all tracks have the same origin (Appendix A). The centers of gravity of all of the scatter diagrams were very close to the origin. Bulk flow would have displaced the center of gravity. The data and calculations for one experiment are included in Appendix A.

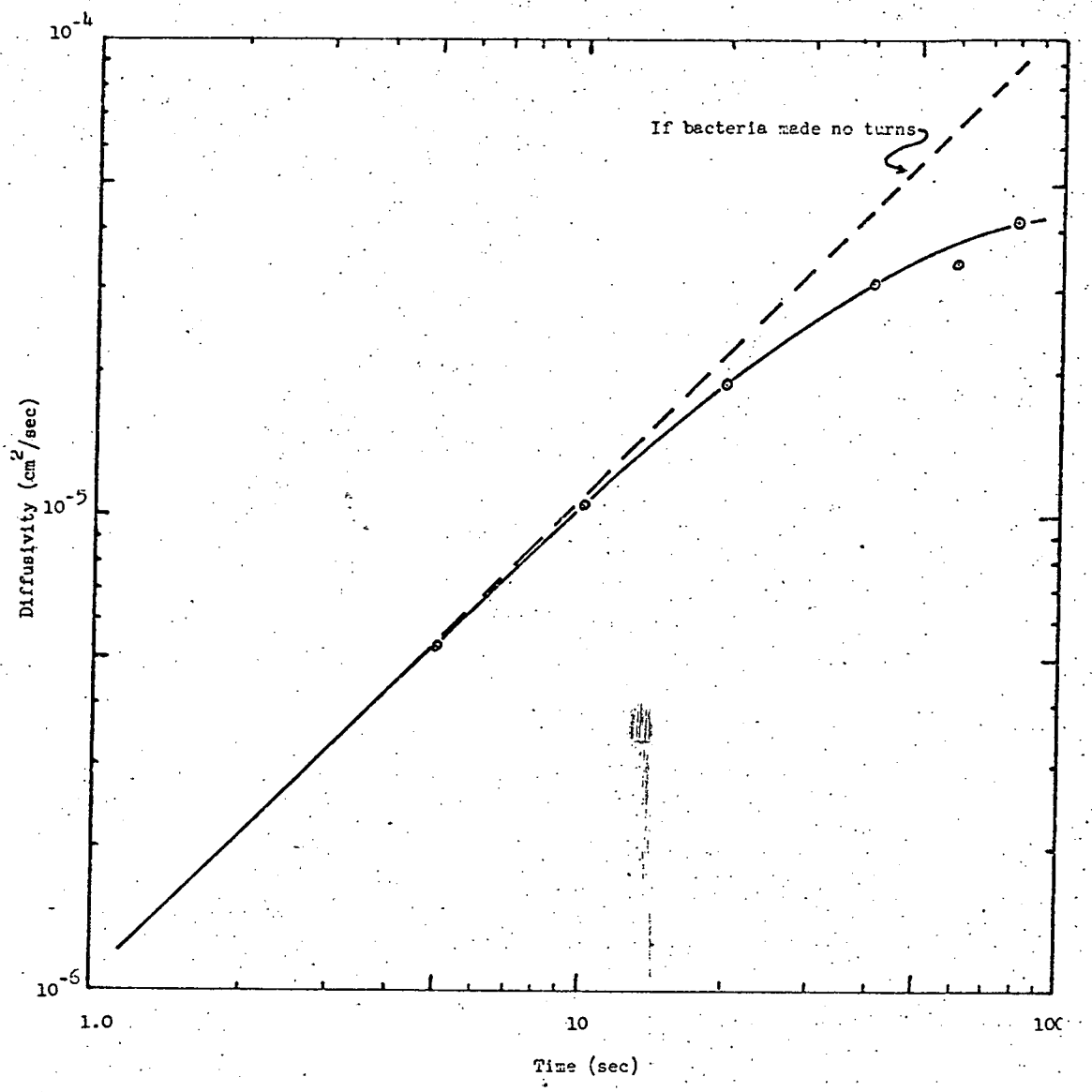

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Fig. 5. The diffusivities calculated from the mean square displacement of bacteria are plotted against the time interval over which the displacement was measured. As the number of direction changes increases, the diffusivity becomes less dependent on the size of the time increment. The average bacterial velocity is 20.7 μ/sec.

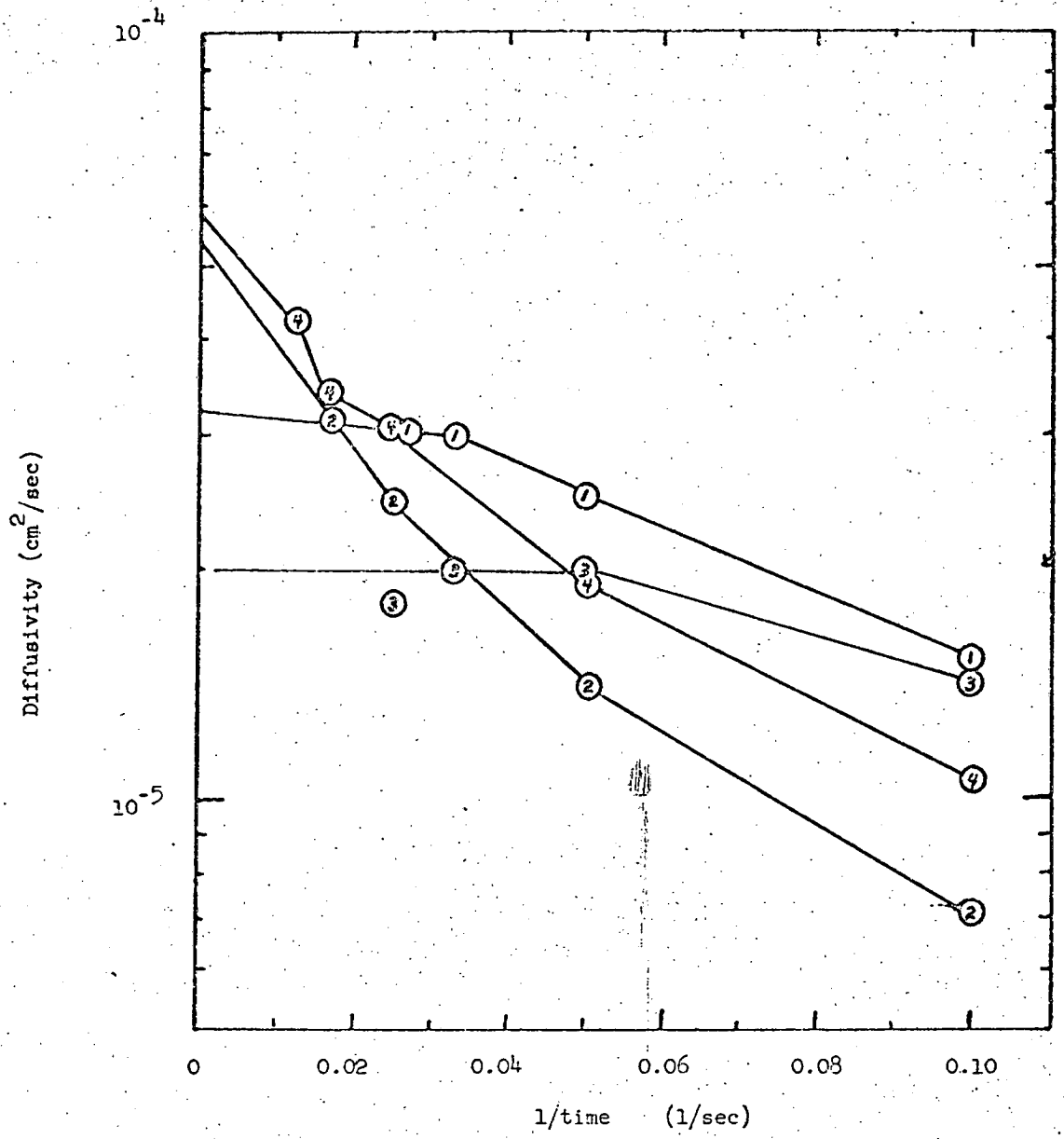

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Fig. 6. The log of the diffusivities from five different runs are plotted against reciprocal time and are extrapolated to an infinite time interval.

Run no.	D (cm^2/sec) at $t = \infty$
1	3.3×10^{-5}
2	5.4×10^{-5}
3	2.0×10^{-5}
4	5.8×10^{-5}
Ave.	4.1×10^{-5}

IV. DIFFUSION FROM MEASURED CONCENTRATION PROFILES

A. Experimental Procedure and Equipment

In this series of experiments microscope chambers were built that would permit direct observation of the diffusion of motile bacteria into a channel filled with nutrient medium. The concentration profiles of the bacteria were obtained by counting the number of bacteria on photographs taken at various positions along the channel. The diffusivities were obtained by matching the measured profiles to profiles calculated from different, known diffusivities.

The diffusion chambers for this experiment (Fig. 7) were similar in construction to the chambers in the previous experiment, except that "Mylar" spacers 2 or 3 mils thick were left in the chamber to form a channel at the right hand end of the slide. The open chamber on the left end formed an unstirred reservoir. The channel was then filled with fresh medium. Many chambers had to be discarded at stage because of overfilling or the inclusion of air bubbles. Once the channel was filled, a suspension of E. coli was added to the reservoir. The filling and vent holes were waxed over so that the entire chamber was sealed. The initial distribution was immediately photographed for subsequent counting.

A Reichert "Zetopan" research microscope was used in all of these experiments because the calibrated stage permitted the same locations to be photographed at different times. The objective lenses were stopped down by placing an aluminum foil, fixed aperature above the lens. This modification gave the lenses a depth of field of 100 microns without excessive loss of resolution. Two different magnifications were used for the counting, 256 X for high concentrations, and 48 X for low concentrations. This combination of lenses enabled a concentration range of ten thousand to be covered. The most accurate measurements were made at high concentrations where as many as two thousand bacteria were counted. For high counts the photograph was covered with a grid

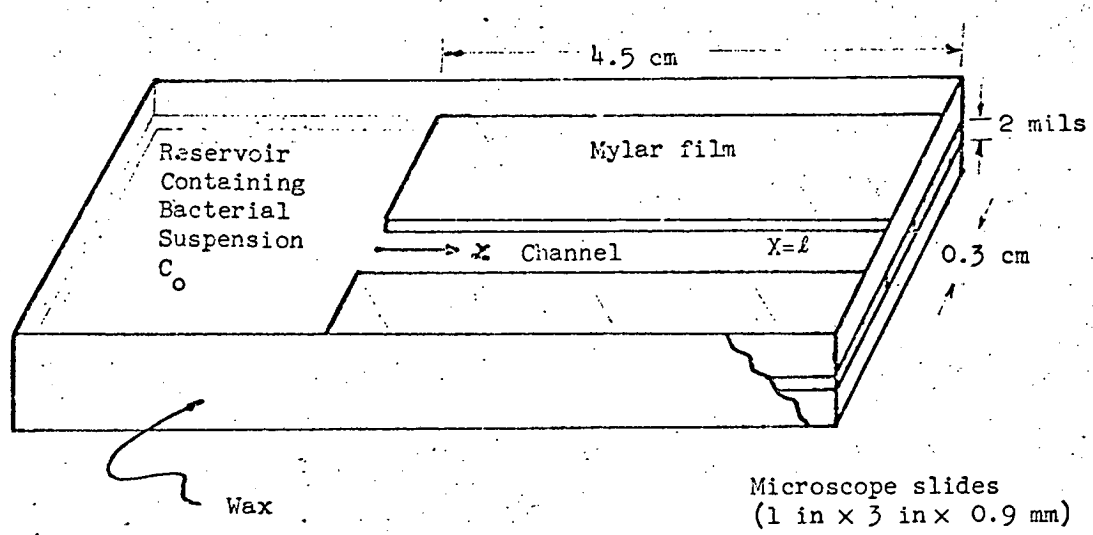

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Fig. 7. Chamber for the direct observation of bacterial concentration gradients with a microscope. The chamber was made by separating two microscope slides with thin sheets of "Mylar" then waxing the edges of the slides. The bacterial suspension was placed in the left hand chamber and fresh medium was placed in the channel on the right. Diffusion occured down the channel and could be recorded by photomicrographs.

and projected in a microcard reader. At low concentrations of bacteria the rotary shutter was used so that the characteristic short strings of images (Fig. 8) would positively identify the motile bacteria. In Fig. 8 the rotary shutter had two additional slots that were one fifth as large as the main slot. The smaller slots produced two additional dimmer images between the usual bright images. The additional images facilitated the identification of the tracks of a bacterium when many different tracks overlapped.

B. Calculation of Concentration Profiles

The diffusion chambers used for this experiment approximate the case of one dimensional diffusion into a finite slab that contains some of the diffusing substance. The partial differential equation that applies to this case which was used to calculate the concentration profiles at various times was:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} + R \cdot C$$

Boundary Conditions are:

Initial condition:	the measured initial distribution
Boundary condition no. 1	$C = C_o \cdot e^{Rt}$, $x < 0$, all t
Boundary condition no. 2	$\frac{dC}{dx} = 0$, $x = \ell$, all t

C = Concentration (number bacteria/ml)

t = Time (sec)

D = Diffusivity (cm^2/sec)

x = Distance from reservoir (cm)

R = Growth rate (1/sec)

ℓ = Total length of channel (cm)

m = Distance segment number

n = Time increment number

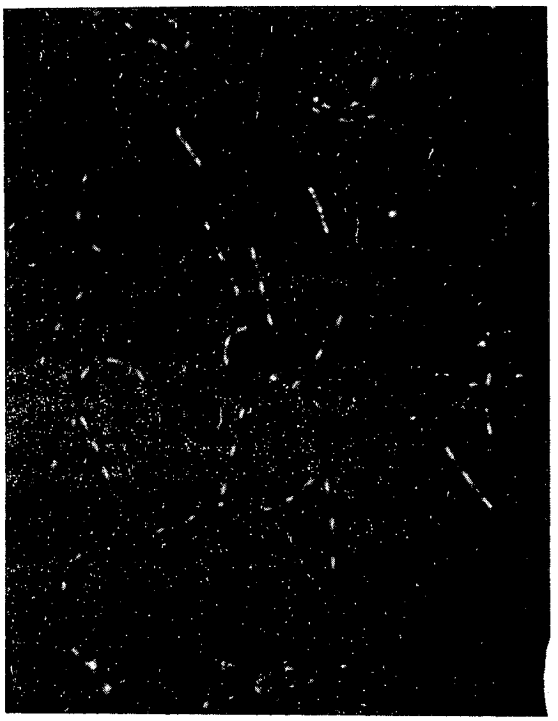

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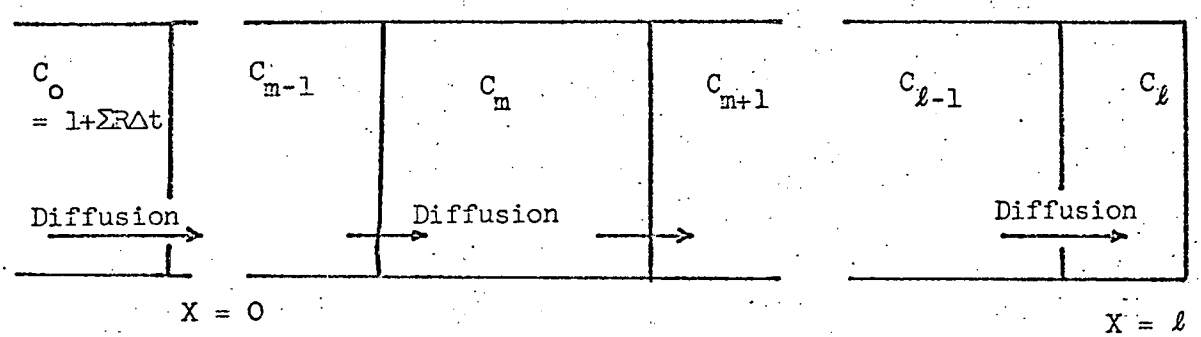
Fig. 8. A multiple exposure photomicrograph of E. coli in nutrient medium. Three exposures were taken per second at 256 X lens magnification on polaroid film. The shutter speed was 1/40 sec for the bright exposures.

Concentration profiles can be calculated numerically by finite difference equations which can be derived from a material balance around a typical section m.



	Diffusion	Growth
in:	$\frac{D\Delta t}{\Delta x}(C_{m-1,n} - C_{m,n})$	$R\Delta x\Delta t\ C_{m,n}$
out:	$\frac{D\Delta t}{\Delta x}(C_{m,n} - C_{m+1,n})$	
accumulation:	$\Delta x(C_{m,n+1} - C_{m,n})$	

Combining and collecting terms

$$C_{m,n+1} = \frac{D\Delta t}{\Delta x^2} \cdot C_{m-1,n} + (1.0 - \frac{2D\Delta t}{\Delta x^2} + tR)\cdot C_{m,n}$$

$$+ \frac{D\Delta t}{\Delta x^2}\ C_{m+1,m}$$

Accuracy of this method is greatest when $D\Delta t/\Delta x^2 = 1/6$. This substitution gives:

$$C_{m,n+1} = \frac{C_{m-1,n}}{6} + (2/3 + \Delta tR)\, C_{m,n} + \frac{C_{m+1,n}}{6} \qquad (1)$$

The channel of length CAPL was divided into 100 equal sections.

$$\Delta x = CAPL/100$$

since $D\Delta t/\Delta x^2 = 1/6$, then

$$\Delta t = TK = CAPL^2 10^{-4}/6D$$

The initial concentration profile was determined experimentally so that the concentration in all 100 sections, $C_{m,1}$, is initially known. The concentrations in all segments, $C_{m,2}$, at time Δt later can be calculated from $C_{m,1}$ by Eq.(1) except for the first segment concentrations which are equal to $(1.0 + \Sigma R\ \Delta t)$. In order to calculate the concentration in the last segment, $C_{1,2}$, the concentration in the segment $C_{1+1,1}$ must be known. For the boundary condition that no diffusion occurs through the end of the channel $C_{1+1} = C_{1-1}$. In like manner the concentration profiles after succeeding Δt's can be calculated by Eq. (1) from the previous concentrations. A computer program that permits calculation of concentration profiles at many different times from a given diffusivity and initial distribution is contained in Appendix B.

C. Discussion of Results

The concentration profiles measured at seven different times in one run are shown (Figs. 9 - 11). The solid lines are the calculated profiles at the indicated diffusivities. The dashed curves join the experimentally determined points. Short horozontal lines are drawn through the data points that were taken from low magnification photomicrographs; the length of the horozontal lines is the width of the photograph.

The similarity between the shape of the calculated profiles and the measured profiles is evidence that the dispersion of these motile bacteria is following the behavior predicted by diffusion. However, by 285 minutes (Fig. 10) the band of bacteria caused by chemotaxis is beginning to form in the region from 0 to 1.0 cm. By 330 minutes (Fig. 11) the band is very distinct and has moved to 1.5 cm; under these experimental conditions the bacteria should be following the receding oxygen supply, depleting it as they migrate.[3] By 285 minutes it can be concluded that the motion of the bacteria is no longer random, and diffusivities measured at the corresponding concentrations of 3×10^7 or greater are probably not valid.

The growth rates of the bacteria in the chamber reservoir were compared to the growth rates, obtained by optical counts, of the rest of the innoculum which was stored in a glass bottle at 26°C(Fig. 12). The slopes of the two curves, the growth rates, are the same within the same limit of experimental error. If there were effects of the high surface area, or if heating by the microscope lamp increased the cell temperature by more than 2°C, then the growth rates in the samples would have been significantly different. Fairly high concentrations were reached in the bottle before the multiplication rate slowed. Since the bottle contained some air and was shaken prior to sampling, oxygen starvation would be less significant in the bottle than in the chamber which was sealed from the air. At a concentration of 3×10^7

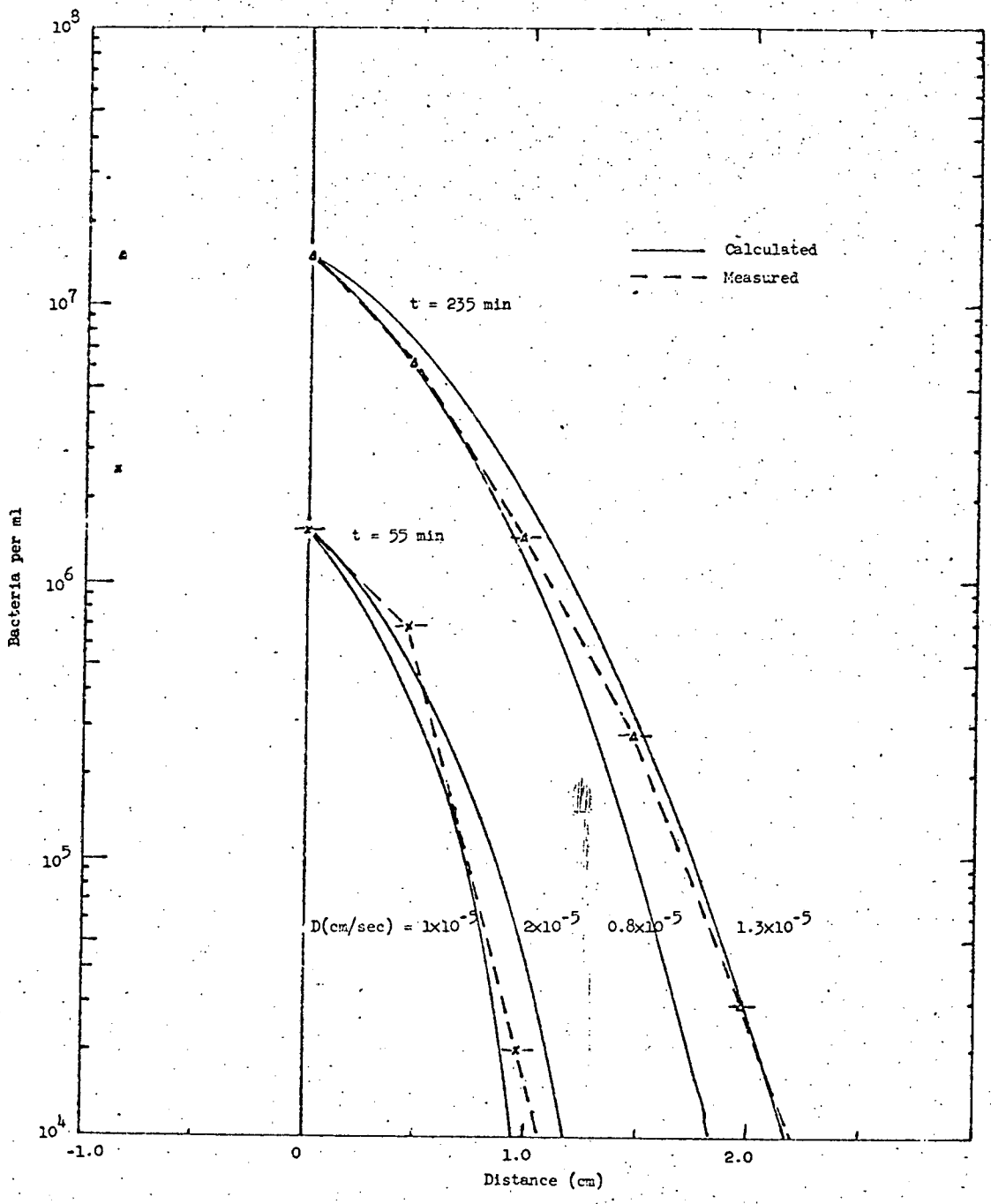


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Fig. 9. Comparison of observed sitributions of bacteria with calculated distributions at 55 and 235 min for a growth rate, R = 2.32×10^{-4}/sec.

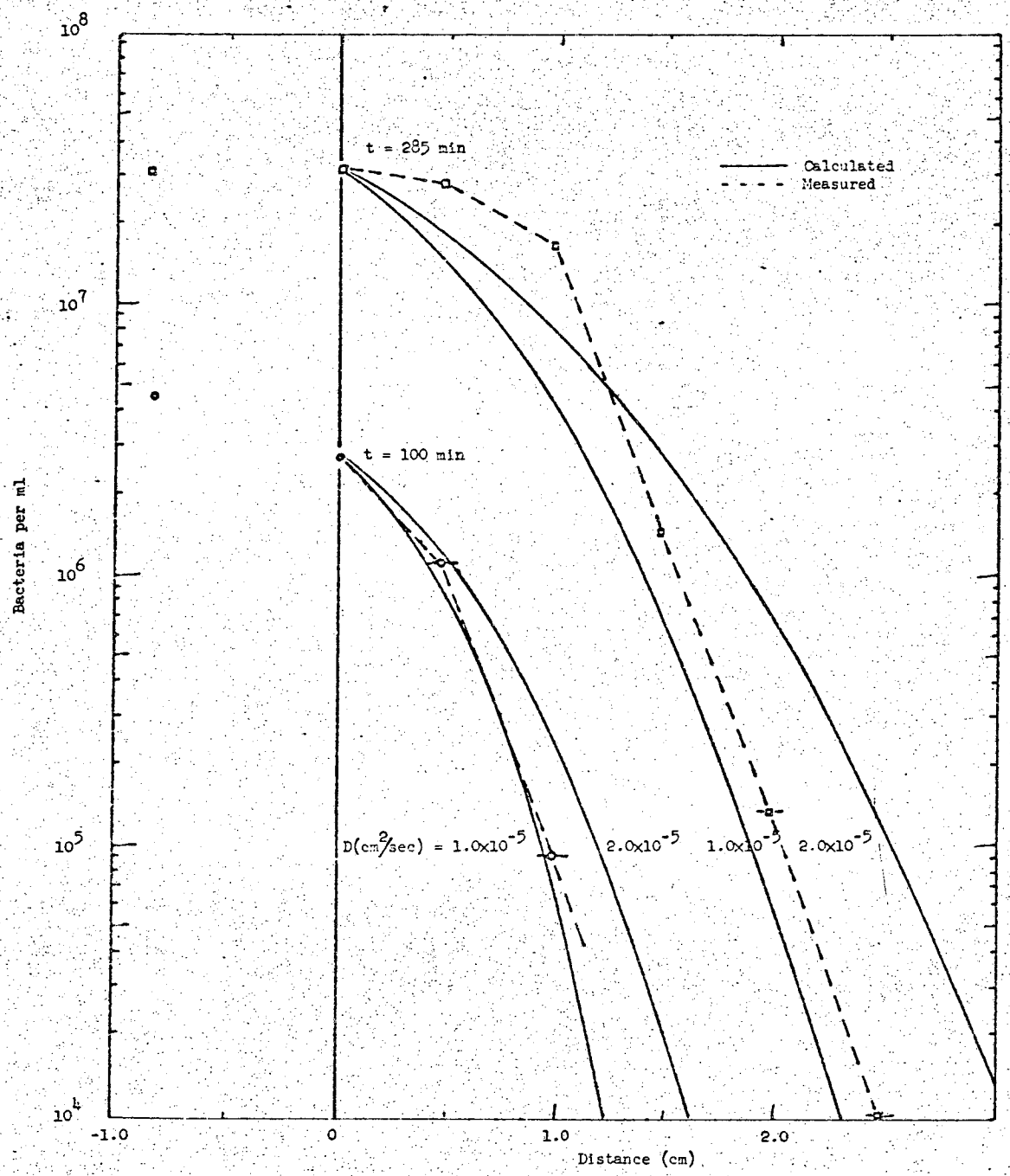

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Fig. 10. Comparison of observed distributions with calculated distributions of bacteria at 100 and 285 min, for a growth rate of $R = 2.32 \times 10^{-4}$/sec. At 285 min there is evidence of the start of a non diffusional migration down the channel following the oxygen.

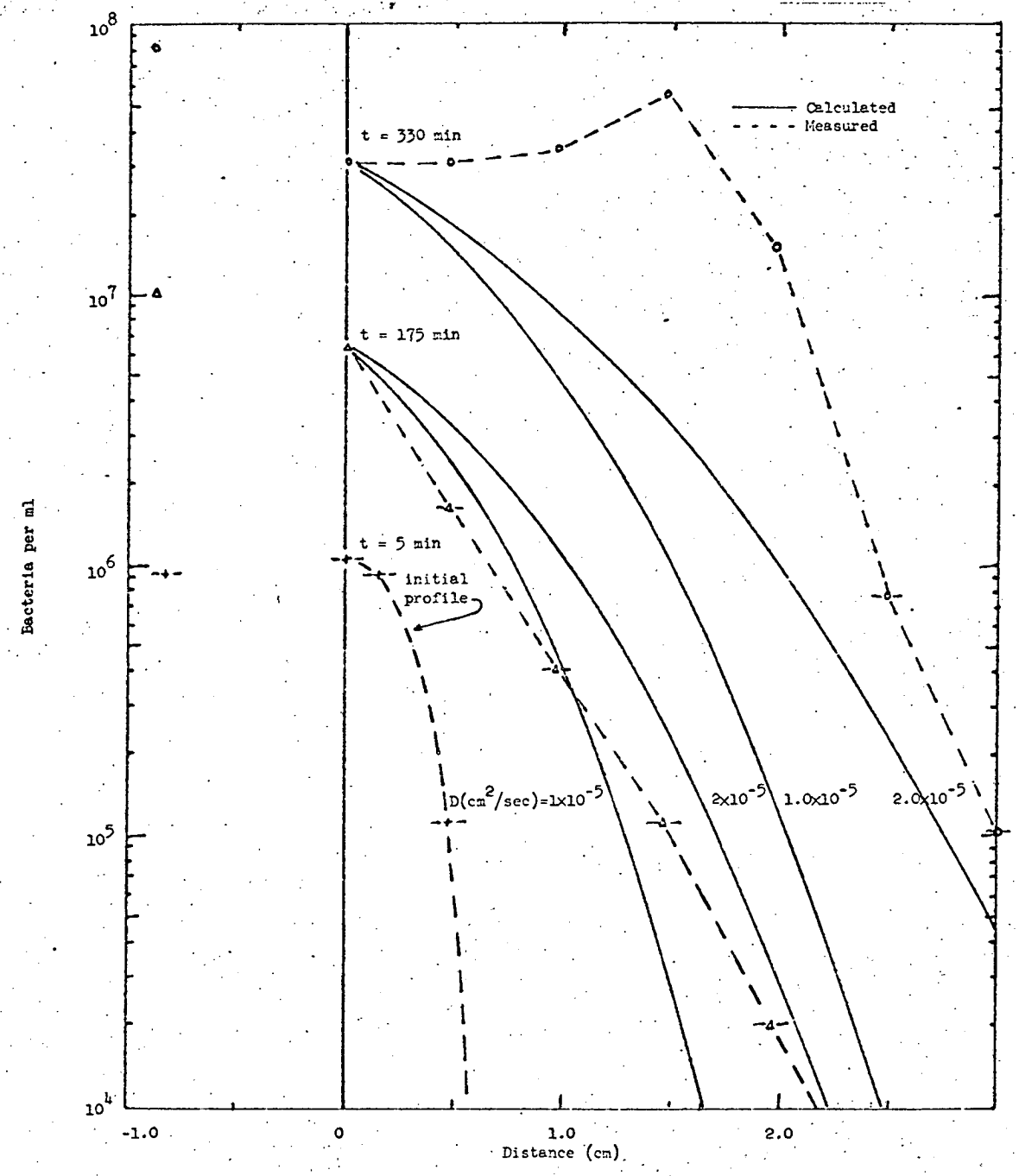

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Fig. 11. Comparison of the observed distribution of bacteria with the calculated distribution at 5, 175, 330 min, for a growth rate $R = 2.32 \times 10^{-4}$/sec. At 330 min there is a very pronounced migration of bacteria down the channel.

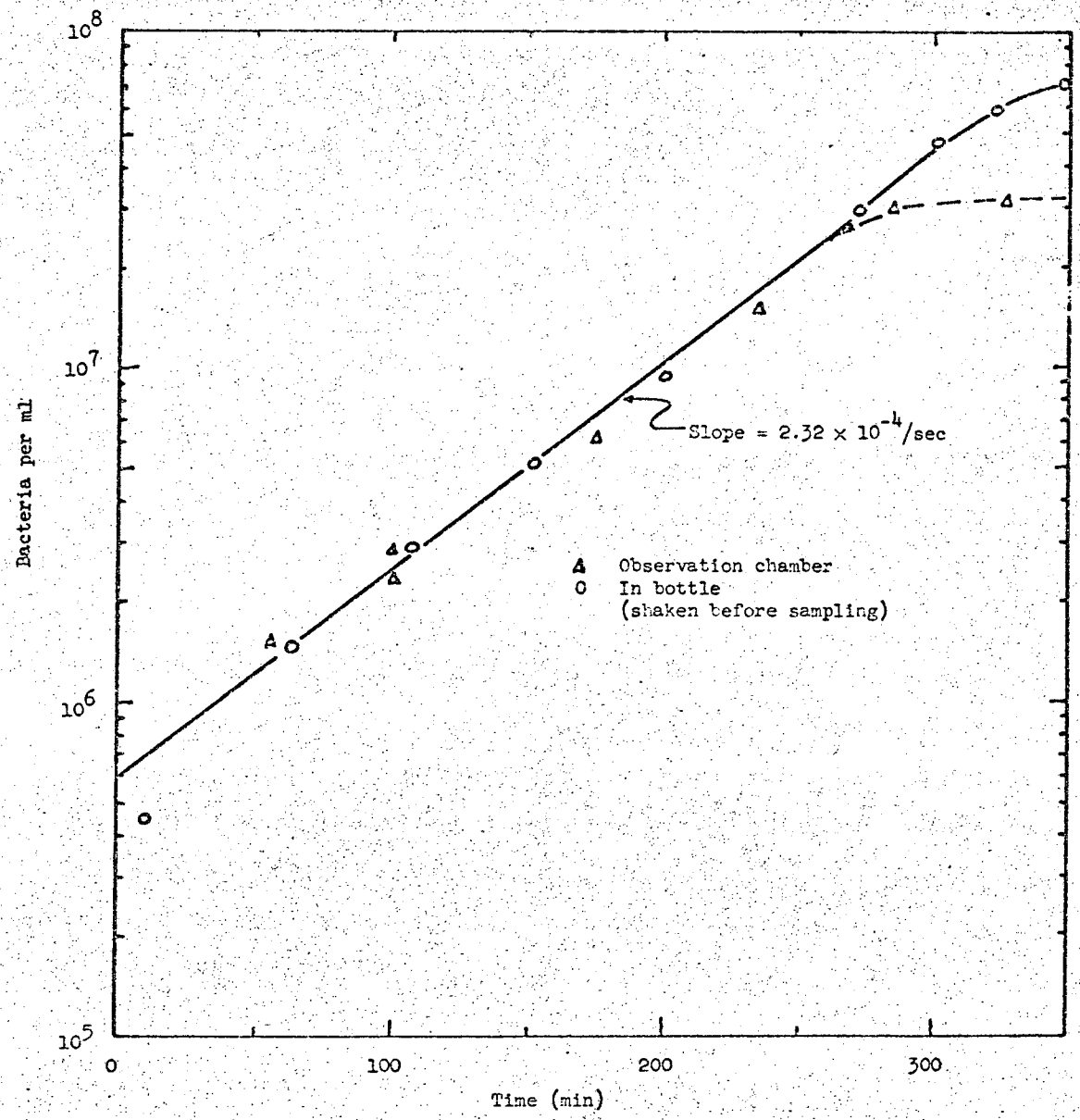

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Fig. 12. Comparison of the growth of E. coli in the microscope chamber to the growth rate of the rest of the sample in a bottle at 26°C. The agreement is good until apparent depletion of oxygen stopped multiplication in the chamber. Shaking the bottle prior to sampling replenished some oxygen consumed by the bacteria and permitted a higher concentration at 350 min in the bottle then in the chamber.

bacteria/ml, the bacteria in the chamber stopped multiplying. Fowler[4] has shown that the cessation of multiplication is caused by the depletion of oxygen in the medium. Adler's experiments[3] show that chemotaxis and the formation of bands of bacteria begin when oxygen in part of the medium is depleted. In this experiment the cessation of multiplication and the movement of the bacteria to form bands occur at the same time and are probably caused by oxygen starvation.

As was previously mentioned, the reservoir of the microscope chamber could not be stirred. This means that the bacteria that diffuse into the channel have to be replaced by the two dimensional diffusion of bacteria in the reservoir to the depleted zone around the opening of the channel. The lack of stirring in the reservoir would cause the measured diffusivities to be low.

The diffusivities calculated for the individual data points for three runs are shown on a summary graph (Fig. 13). There does not appear to be an effect of length of time for diffusion on the value of the diffusivity. The scatter in the data is much more pronounced at short distances than at long distances. This scatter at short distances is probably inherent in the method.

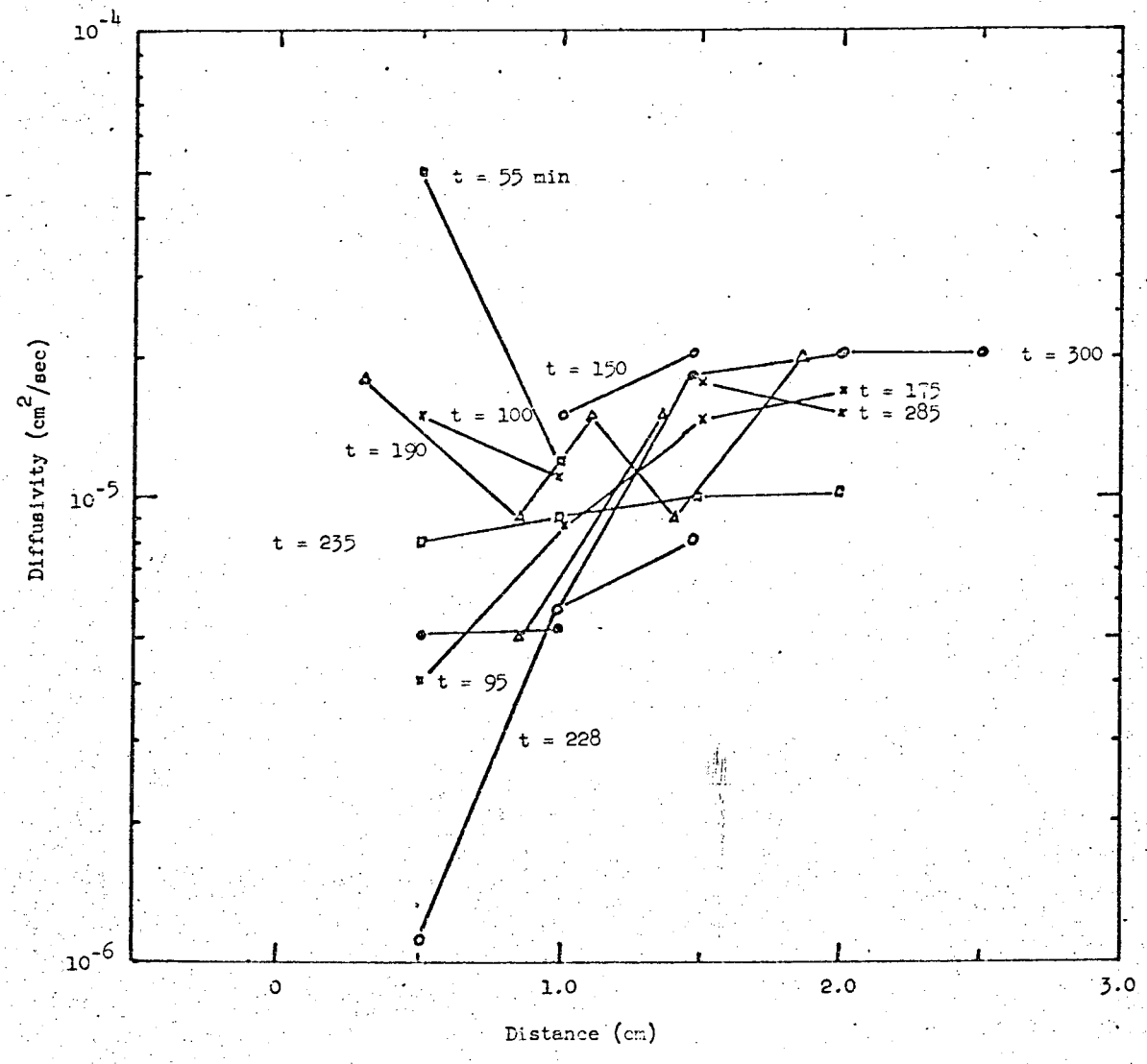

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Fig. 13. The diffusivities from three concentration gradient runs at times where migration was not significant are summarized on a single plot.

D. Experiment to Evaluate Flow in the Diffusion Chamber

The possible presence of convection currents in the diffusion chamber was evaluated by measuring the rate of dispersion of 1.3 micron diameter polyvinyl toluene latex spheres. The spheres were suspended in 8 wt/NaCl, a solution that had the same density as the spheres. The suspension was then centrifuged to remove anything that might settle or float during the experiment. A suspension of spheres was loaded into a microscope chamber in the same manner as the bacterial suspensions had been loaded.

The diffusion of these spheres of neutral density can be calculated from kinetic theory by the Stokes-Einstein equation.[5]

$$D = \frac{kT}{6\pi a\eta}$$

k = The gas constant for one molecule

T = The absolute temperature

a = The particle radius

η = The viscosity

For the experimental case of the 1.3 micron spheres, the diffusivity would be about 4×10^{-9} cm^2/sec.

The diffusivities of the spheres could not be measured accurately because the spheres stuck to the surface of the glass slide when they encountered it in the course of their random motion. After several days equal numbers of spheres were stuck to top and bottom surfaces. The experiment revealed a very slight flow of about 7×10^{-7} cm/sec into the channel. Flow of this magnitude would have no effect on bacterial diffusivities. The total displacement by flow in a three hour diffusion experiment would be less than the accuracy of position measurement. The distribution of spheres is shown in (Fig. 14). From the calculated concentration profiles, the solid lines, it can be seen that the diffusity is substantially less than $10^{-7}cm^2/sec$, but the calculated diffusivity of $4\times10^{-9}cm^2/sec$ cannot be verified.

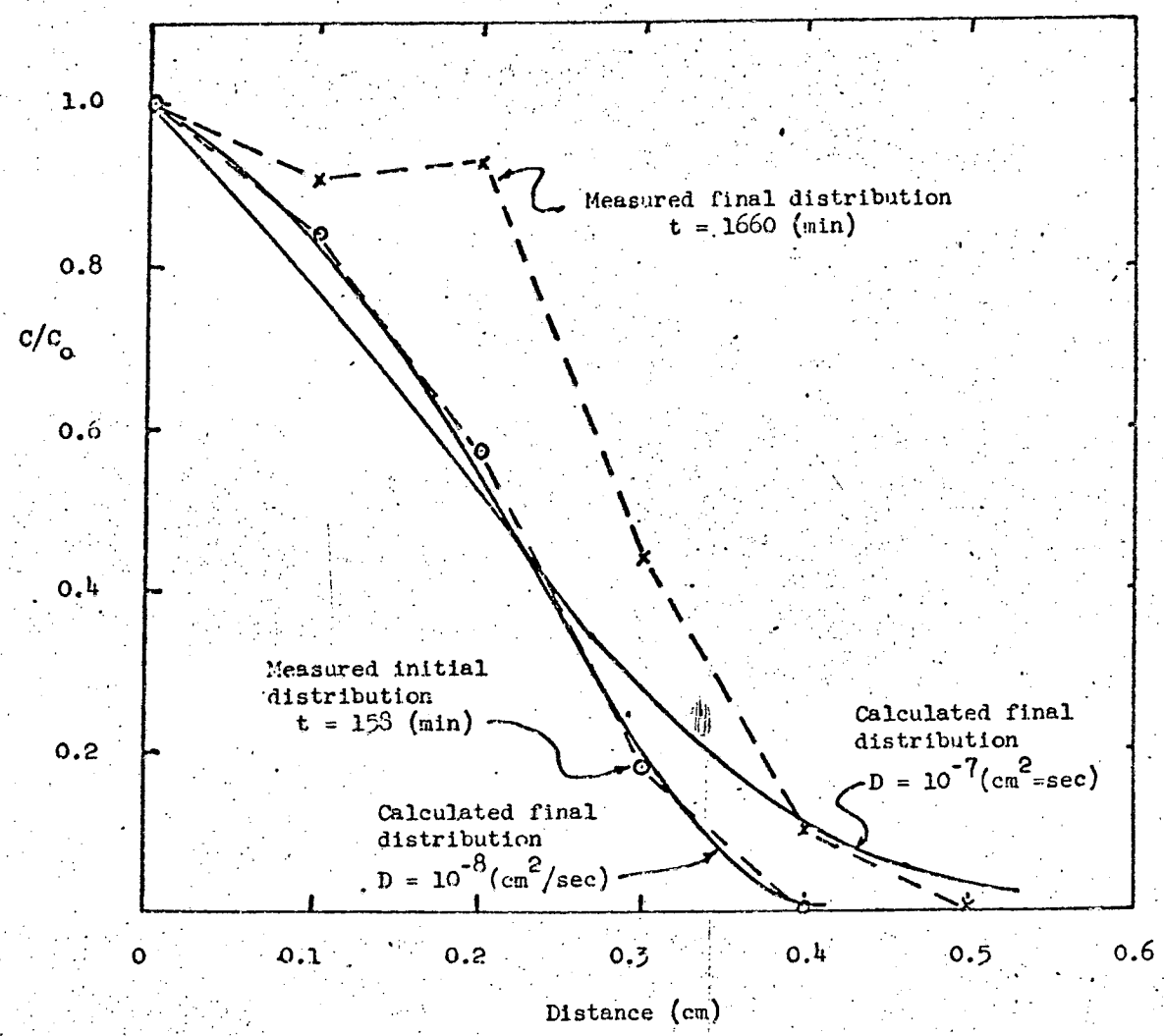

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Fig. 14. Dispersion of 1.3 micron latex spheres by Borwnian motion. The dotted lines show the measured initial and final distributions. The solid lines show the calculated final distributions if the dispersion were caused only by Brownian motion. The poor fit by the calculated curves and the shape of the curve for 1660 min shows that there must have been bulk flow of fluid down the channel as well as diffusion, and that the diffusivity must have been less than 10^{-7} cm^2/sec.

V. DIFFUSION FROM A CAPILLARY TUBE

In this set of experiments a capillary tube that was closed at the bottom was filled with a bacterial suspension and was immersed in bacteria free nutrient medium. Some of the motile bacteria diffused out of the capillary. After a known length of time the capillary tube was removed from the medium and the number of viable bacteria in the tube and in the medium was determined by plating on nutrient agar and counting the colonies that resulted. Thus for a known time for diffusion the fraction ϕ of the bacteria remaining in the tube could be experimentally measured. A diagram of the experimental procedure for determining ϕ is shown in (Fig. 15). The diffusivity was obtained by solving the partial differential equation for diffusion, sedimentation, and growth to give ϕ as a function of dimensionless time, Dt/l^2. Thus the diffusivity could be determined if the fraction remaining ϕ, the time for diffusion t, the capillary length l, sedimentation velocity, and growth rate were all known.

A. Experimental Procedure

The selection of experimental conditions was based on experiments using NaCl in place of bacteria. NaCl was used because of simplicity of analysis, well established diffusivity, and freedom from the requirements of sterility. The conditions finally selected were vertical capillary tubes 0.17 cm in diameter and 2 cm long with the bottom closed. Larger diameter tubes were not used because the salt solution was washed out of the end of the capillary tube by the motion of the bulk fluid. Smaller diameter tubes did not permit the diffusion of sufficient material for analysis. The stirring rate used was 2rps; higher stirring rates washed salt solution out of the end of the tube. Lower stirring rates caused a decrease in the measured diffusivity, probably because a concentration gradient was established in the bulk medium around the entrance of the capillary tube. When

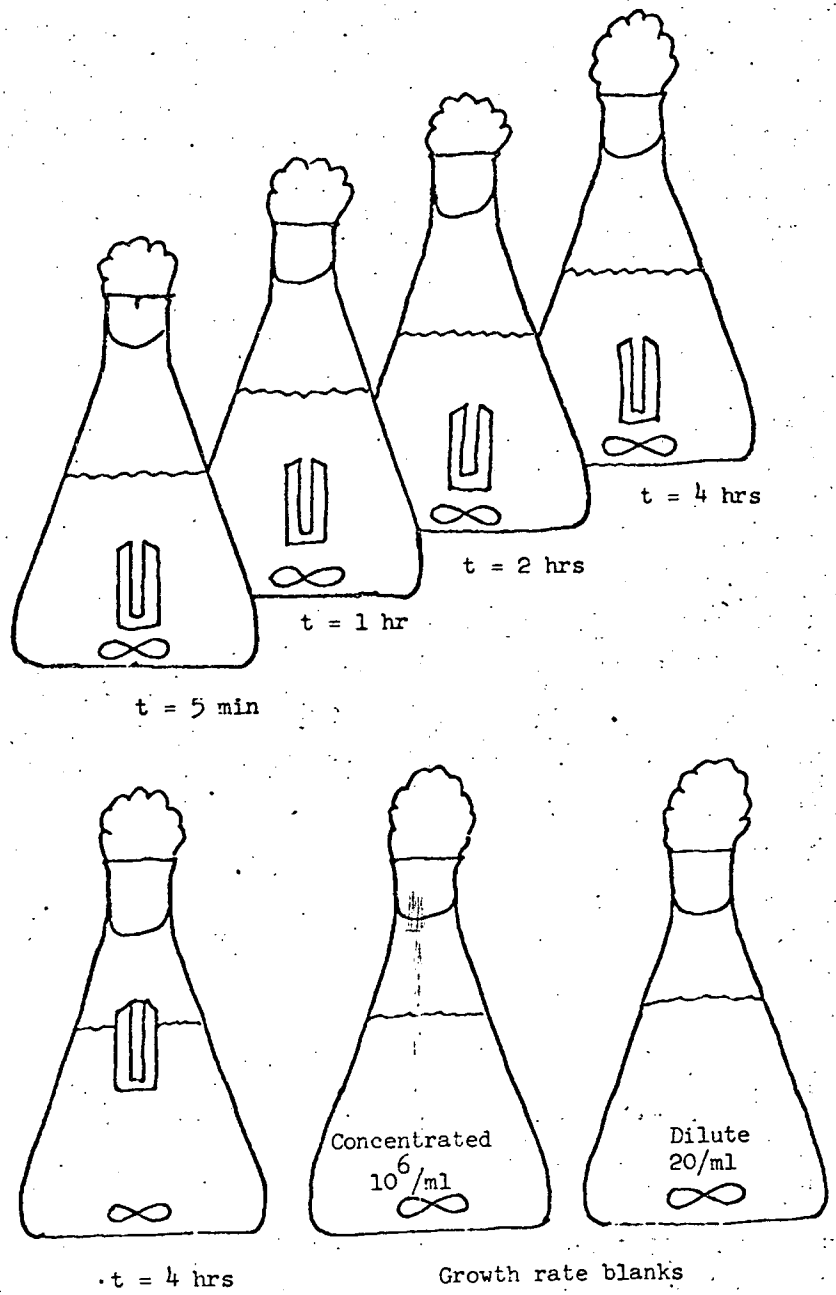

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Fig. 15. Diagram of the experiment used in the tube diffusion method. Usually the diffusivities were measured over four different time intervals, and the growth rate was measured in the two extremes of concentration.

bacteria were used, the tubes were kept vertical because inclined surfaces are reported to increase sedimentation rates by the Boycott effect.[6]

The procedure used in these experiments (Fig. 15) was to fill a sterilized capillary tube with a bacterial suspension of E. coli that was in exponential growth. The filled capillary was then partially immersed in 125 ml of sterile medium in a 150 ml Erlenmeyer flask. The system was allowed to come to thermal equilibrium with a 26°C constant temperature bath before the tube was completely immersed and the experiment started. The medium was sampled both before and after complete immersion of the capillary tube. Usually, five duplicate capillary setups were used with each experiment. One capillary was never immersed and the other four were immersed and removed at time intervals ranging from five minutes to four hours. The contents of the tubes and the concentrations in the flasks were measured by dilution and plating in nutrient agar. The fraction remaining in the tube was the measured contents of the tube divided by the total number of bacteria found in the tube and bulk medium. Some samples of the bulk medium were withdrawn at times prior to the removal of the tube from the medium. Since the contents of the tube could not be measured directly, the fraction remaining in the tube was based on the total number of bacteria originally placed in the tube and the known rate of multiplication of the bacteria.

If the growth rate in the tube were different from the growth rate in the bulk medium, the fraction remaining in the tube would be a function of the growth rate as well as the diffusivity. The growth rates were measured in both concentrated and dilute suspensions to ascertain that the multiplication rate was not a function of concentration in the range of the experiment. These growth rate blanks also indicated the depletion of oxygen and the end of the period of random motion of the bacteria.

B. Numerical Solution to the Diffusion Equation

In this experiment bacteria are multiplying as they diffuse up out of the capillary tube against the force of gravity (Fig. 16). The partial differential equation representing this system is:

$$\frac{\partial C}{\partial t} = \frac{D\partial^2 C}{\partial x^2} + \frac{U\partial C}{\partial x} + RC$$

The boundary conditions are:

Initial Condition	$C = C_o$	all x	$t = 0$
Boundary Condition 1	$C = 0$	$x \leq 0$	all t
Boundary Condition 2	$\frac{dC}{dx} = 0,\ U = 0$	$x = \ell$	all t

Program	Equation
C	C = Concentration (bacteria/ml)
	t = Time (sec)
D	D = Diffusivity (cm^2/sec)
U	U = Sedimentation velocity (cm/sec)
R	R = Growth rate constant (1/sec)
	x = Distance from the top of the capillary (cm)
CAPL	ℓ = Length of the capillary (cm)
K	m = Distance position
J	n = Time position
T	Δt = Time increment
H	Δx = Distance increment
THETA	Dt/ℓ^2 = Dimensionless time
PHI	ϕ = Fraction remaining in the tube
CTO	= Concentration in the tube if no diffusion
TMAX	= Total time for diffusion
AMOD	$\frac{D\Delta t}{\Delta x^2}$ = Dimensionless time increment

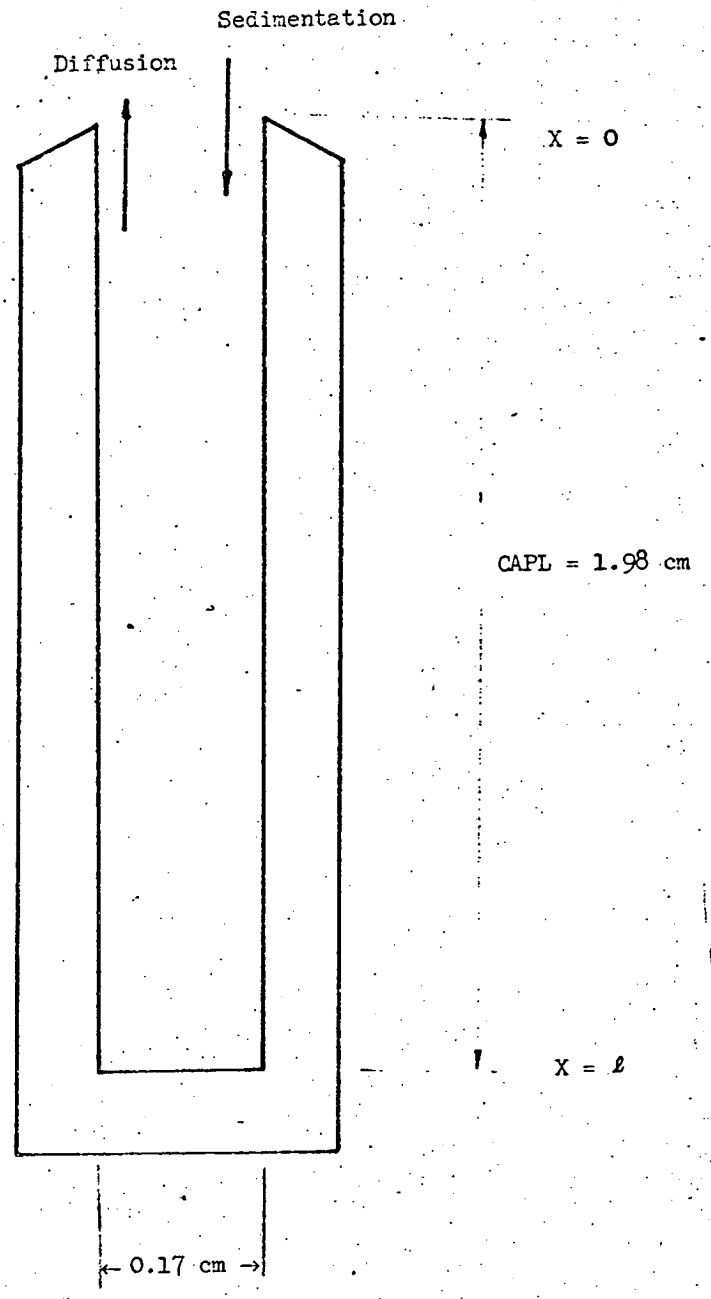

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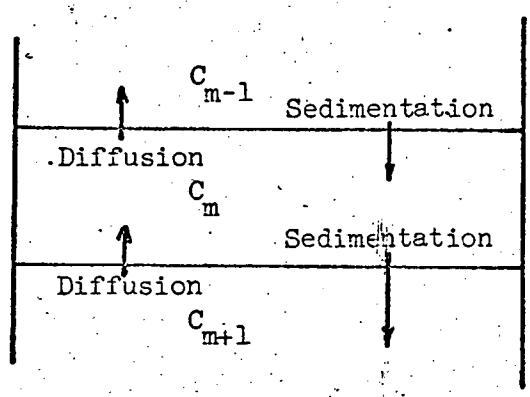
Fig. 16. Drawing of the capillary tube that was closed on one end and that was used in the tube diffusion method.

BMOD	$\frac{U\Delta t}{2\Delta x}$	= Dimensionless sedimentation velocity
DH		= Number of distance segments
DT		= Number of time increments

The procedure used in the numerical solution to the partial differential equation is to divide the capillary tube into DH equal segments. The finite difference equation is then derived from a material balance around a typical segment m. The finite difference equation can be used directly to calculate the concentration profiles at many different times. These concentration profiles can be integrated numerically to give the total number of bacteria in the tube. The fraction remaining in the tube, ϕ , was then calculated as a function of the dimensionless time Dt/ℓ^2 (Fig. 17). This plot permits evaluation of D from measured values of ϕ and t , from a tube of known ℓ.



The material balance around segment m is:

	Diffusion	Sedimentation	Growth
in:	$\frac{D\Delta t}{\Delta x}(C_{m+1,n} - C_{m,n})$	$+ \frac{U\Delta t}{2}(C_{m-1,n} + C_{m,n})$	$+ R\Delta x\Delta t C_{m,n}$
out:	$\frac{D\Delta t}{\Delta x}(C_{m,n} - C_{m-1,n})$	$+ \frac{U\Delta t}{2}(C_{m,n} + C_{m+1,n})$	
accumulation:	$\Delta x(C_{m,n+1} - C_{m,n})$		

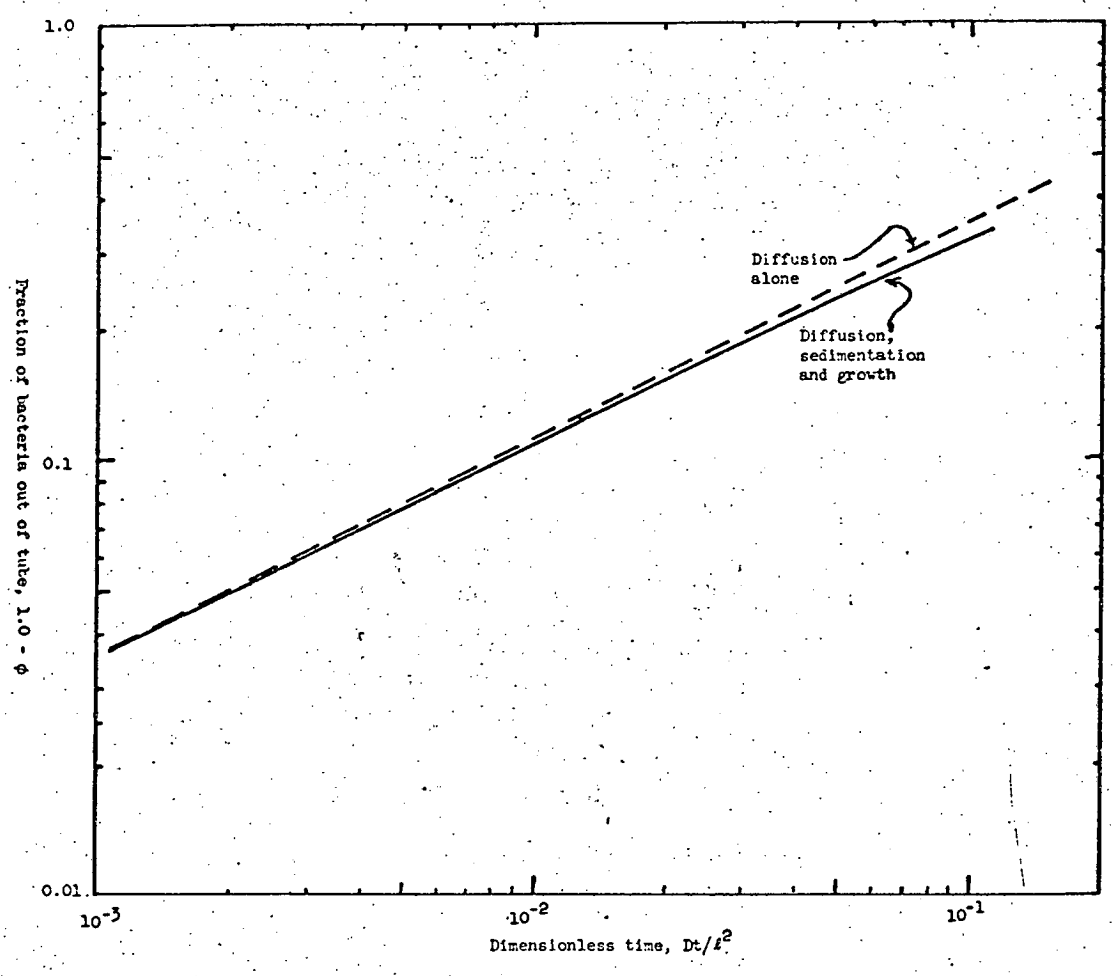


Fig. 17. The effect of sedimentation and growth on diffusion out of a tube is shown. The fraction of the bacteria calculated to be out of the tube is plotted against dimensionless time. For the conditions of these experiments there is little effect of sedimentation on diffusion. ($D = 2\times10^{-5}\,cm^2/sec$, $R = 2.8\times10^{-4}/sec$, $U = 1.0\times10^{-5}\,cm/sec$, $l = 1.98$ cm)

combining and collecting terms

$$C_{m,n+1} = \left(\frac{D\Delta t}{\Delta x^2} - \frac{U\Delta t}{2\Delta x}\right) C_{m+1,n} + \left(1.0 \quad \Delta tR - \frac{2D\Delta t}{\Delta x^2}\right) C_{m,n} + \left(\frac{D\Delta t}{\Delta x^2} + \frac{U\Delta t}{2\Delta x}\right) C_{m-1,n} \tag{2}$$

The boundary conditions for the tube diffusion method are that the initial concentration in the tube is one. The concentration at the entrance of the tube is zero at all times. The sedimentation of the bacteria causes accumulation in the bottom segment of the tube. The condition for accumulation was handled by assuming that accumulation occured in the bottom 5 percent of the capillary, and that this bottom 5 percent was well mixed. In actually the sedimentation zone would be smaller and the concentration gradient in the bottom of the tube would be very steep and would introduce errors into the subsequent numerical integration. The assumption that sediment collects in 5 percent of the volume will not affect the diffusion at the open end of the capillary tube. To satisfy the boundary condition of no transport through the bottom of the tube, the sedimentation velocity and the concentration gradient were set equal to zero.

The finite difference equation for the boundary condition at the bottom of the capillary can be derived from a material balance around the bottom segment of the capillary. The volume of the bottom segment is one twentieth of the tube volume, and is larger than the rest of the sections. Since the tube is divided into DH sections, the bottom segment contains DH/20 regular size sections. The finite difference equation is derived from the material balance around segment ℓ.

Accumulation = Diffusion out + sedimentation in

$$\frac{DH\Delta x}{20}(C_{\ell,n+1} - Cl,n) = \frac{\Delta tD}{\Delta x}(C_{\ell,n} - C_{\ell-1,n}) + \frac{\Delta tU}{2}(C_{\ell,n} + C_{\ell-1,n}) + \Delta x\Delta tR\frac{DH}{20} C_{\ell,n}$$

Combining and collecting terms gives the finite difference equation for the sedimentation section at the bottom of the tube.

$$C_{\ell,n+1} = \frac{20}{DH}\left\{\left(\frac{\Delta tD}{\Delta x^2} + \frac{\Delta tU}{2\Delta x}\right)C_{\ell-1,n} + \left(\frac{\Delta tU}{2\Delta x} - \frac{D\Delta t}{\Delta x^2}\right)C_{\ell,n}\right\} \qquad (3)$$

$$+ (1.0 + \Delta tR)C_{\ell,n}$$

The actual calculation of the concentration profiles is very simple. The initial concentration in all of the segments is known. The concentrations at time Δt later are zero for the first segment and calculated by Eq. (2) for the rest of the segments except for the last five percent of the segments which are all equal and are calculated by Eq. (3).

After the concentration profiles have been calculated the total number of bacteria contained in the tube was determined by summing and averaging the incremental concentrations. The total that would have been in the tube with growth but without diffusion, CTO, was calculated. The ratio of the contents of the tube with diffusion to the contents without diffusion is, PHI, the fraction remaining. The computer program for the above numerical calculations is included in Appendix C. The numerical calculations are summarized by the plot of $1.0 - \phi$ as a function of Dt/ℓ^2 in (Fig. 17). The calculated curve is compared to the curve that would have resulted had there been only diffusion. The analytical solution for diffusion into a semi-infinite slab $1.0 - \phi = 2(Dt/\pi\ell^2)^{1/2}$ is valid up to $Dt/\ell^2 = 0.3$ with an error of less than 1.3 percent.

The value of the sedimentation velocities used in the numerical solution were measured by allowing a culture of nonmotile <u>E. coli</u> B

to settle in a tube that was thermostated. The position of the interface between clear medium and suspension was measured at several time intervals. The sedimentation velocities ranged from 0.05 to 0.1 micron/sec.

C. Discussion of Results

The diffusivities obtained in the tube diffusion experiment are shown plotted against the length of time allowed for diffusion to occur (Fig. 18). The X's and the solid dots are based on actual measurement of the tube concentrations whereas the open dots are calculated from the number of bacteria loaded into the tubes. There does not appear to be any additional scatter caused by use of the calculated values. This observation is not surprising since the percent recovery of bacteria, based on the total number of bacteria accounted for with a correction for growth, ranged from 85 to 105 percent. The spread in the recovery rates is not unusual considering the inherent errors in both plating and in growth rate measurement.

The diffusivities measured over short periods of time are considerably higher than the diffusivities measured from runs lasting two hours or more. The short time diffusivity measurements would be much more sensitive to some mechanism such as the bulk flow of medium washing the suspension out of the top of the capillary tube. The diffusivity measurements made at longer periods of time should be more accurate.

The validity of this experiment is contingent on the condition that the growth rate in the tube at high concentration be the same as the growth rate of the diffused bacteria in the dilute medium. The measured growth rates agree within experimental error.

There are several significant differences between this set of experiments and the two previous methods. In this experiment diffusion occurs in three dimensions in the capillary tube instead of in two dimensions across a surface. In this experiment viable bacteria are counted rather than only motile bacteria. The selection of a strain that is highly motile minimizes the effect of this difference.

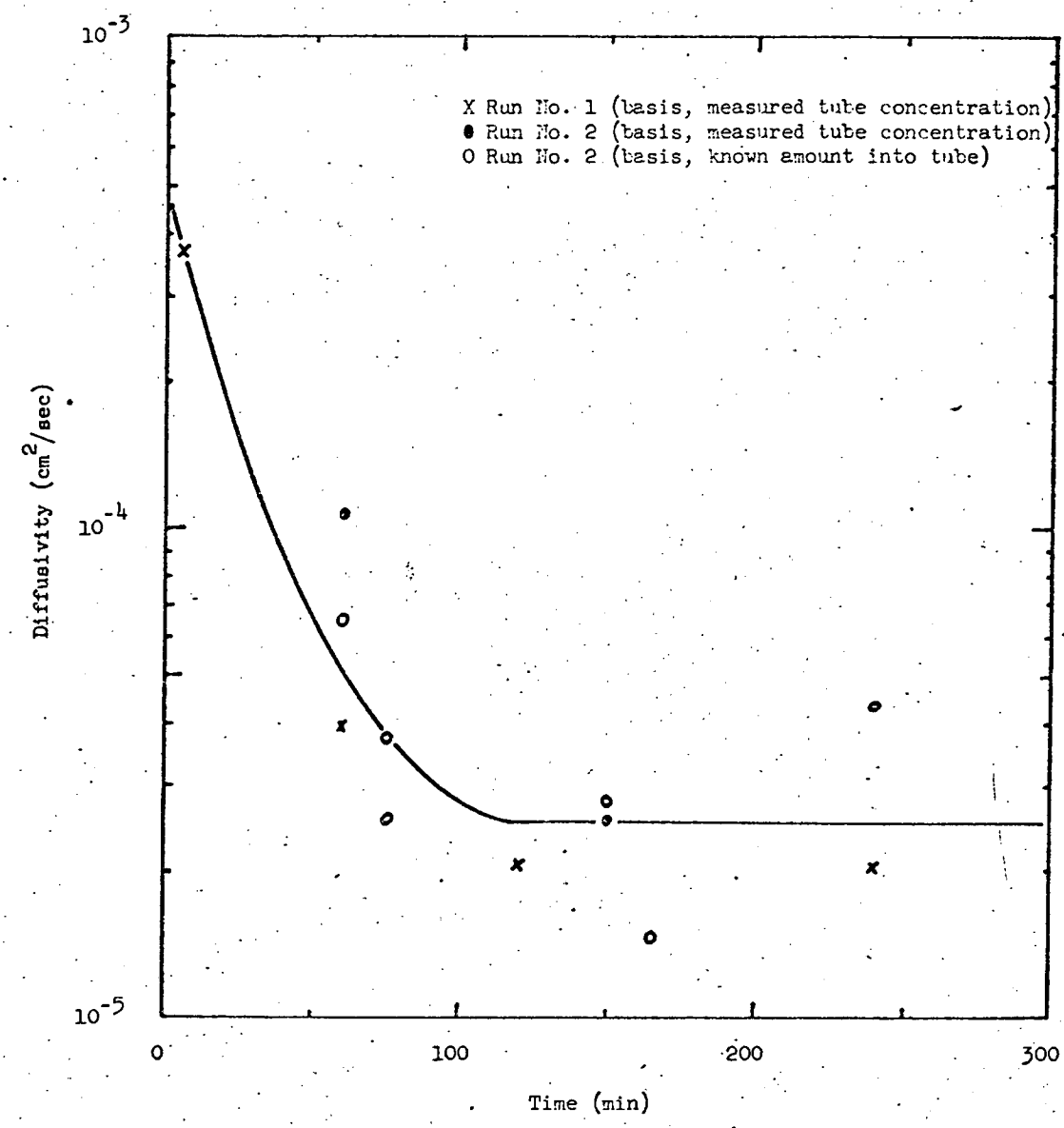


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Fig. 18. Diffusivities measured by the tube diffusion method for two runs are plotted against the length of time for diffusion. For very short times the measured diffusivities were high probably because bacteria were washed out of the tube.

VI. CONCLUSIONS

The lack of understanding of diffusive transport has led to some apparent contradictions in the literature. For example, Ôgiuti[7] measured the velocities of many strains of E. coli directly with a microscope and observed that the strains had velocities which fell into two groups differing by a factor of two. A different technique was used by Clowes, Furness, and Rowley[8,9] and independently by Mazloum and Abdel-Maguid[10] to measure the speed of E. coli. They measured the speeds by innoculating one end of a capillary filled with sloppy agar, incubating the capillary, then extruding and segmenting the agar to determine how far the bacteria had traveled. The speeds of the strains measured by this method differed widely rather than falling into two distinct groups. The agar experiment actually measured the diffusion of a growing culture. The differences in the two types of experiments are substantial. The first experiment measured bacterial velocity whereas the second experiment measured the diffusive transport which is a function of velocity and the frequency of direction change.

VII. EXAMPLES OF THE SPREAD OF BACTERIA BY DIFFUSION

From an engineering point of view the diffusion of bacteria is most significant in cracks, such as on a gasket seal, in a thin almost stagnant film along the inside surface of a feed line, or perhaps in a porous bed. The diffusivities that were measured in the previous sections of this report can now be used to determine the importance of diffusive transport in engineering problems. A rather general partial differential equation was solved which is very useful in estimating how fast motile bacteria can diffuse into a sterile system.

The equation solved in this section is the equation governing the dispersion of multiplying bacteria up a film of finite length, counter to bulk flow of the fluid. At the upper end of this film is a well stirred reservoir. The growth rate is G in the film and R in the reservoir. The boundary conditions, shown in (Fig. 19), are that one end of the film is at zero concentration and the other end is at a constant concentration, TOP. Growth occurs in the film, which is initially sterile, until it reaches concentration TOP. This behavior would be anticipated from a knowledge of batch cultures and is confirmed by experiments by Mazloum[10] in which he innoculated one end of a tube filled with sloppy agar, permitted diffusion to occur, then extruded the agar, sectioned it and measured the concentration of organisms. There is no back diffusion or loss from the reservoir; all bacteria entering the reservoir stay there and multiply at growth rate R.

The numerical solution to the partial differential equation is solved by first dividing the fluid film into a finite number of sections. The finite difference equations are then derived from the material balances around the individual sections. Section $C_{\ell-1}$ is typical for all sections from the second through the next to last section.

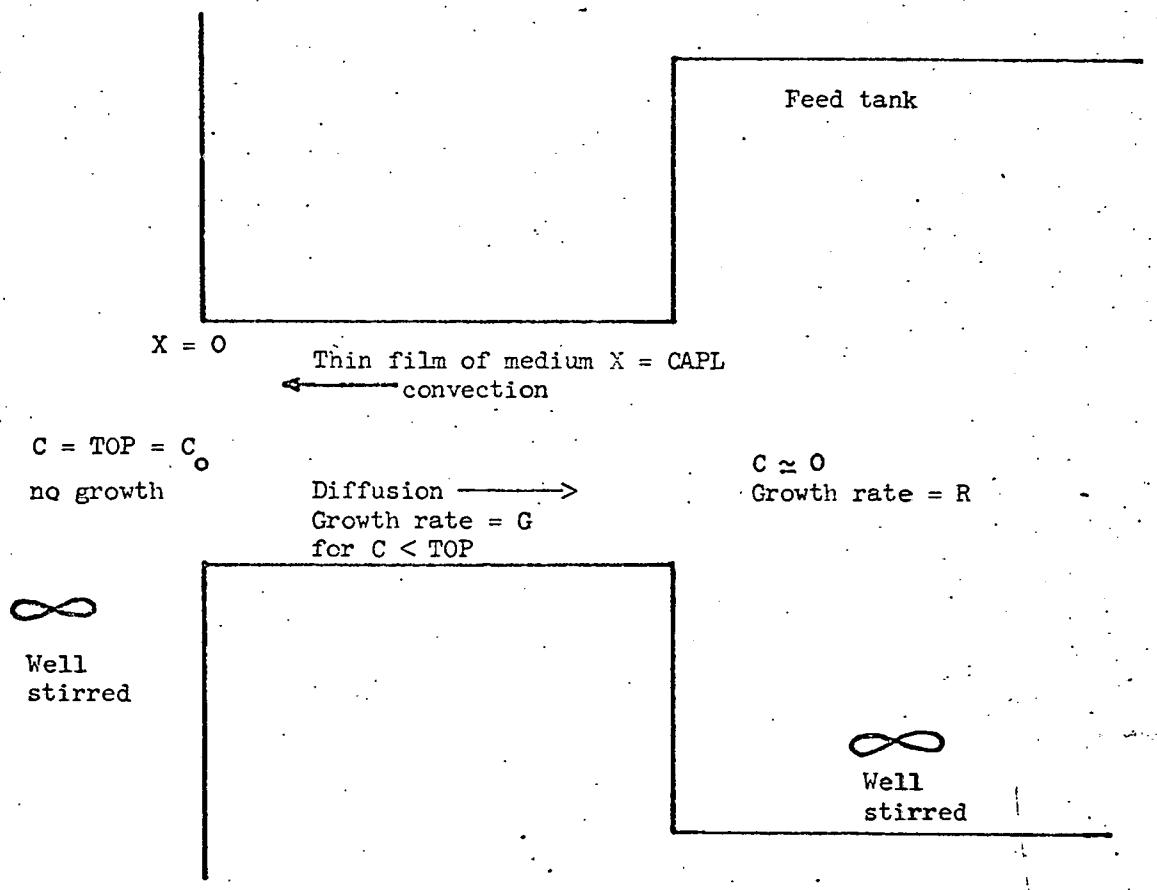

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Fig. 19. Schematic diagram of the conditions used in the numerical solution of the diffusion, convection, and growth in a small crack.

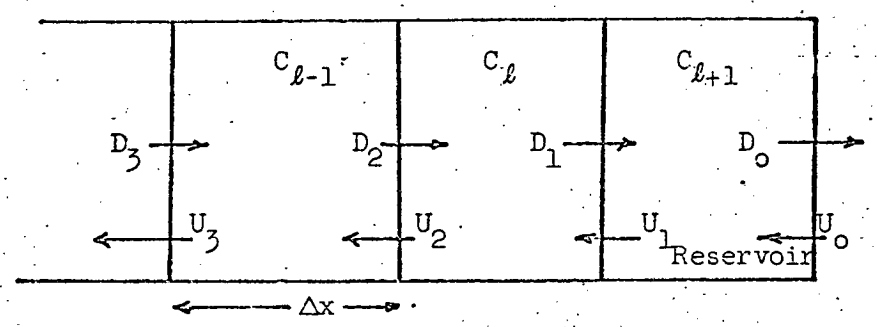

Diagram of Fluxs

Diffusive Flux	Convective Flux	Growth
$D_3 = \frac{\Delta t D}{\Delta x}(C_{\ell-2,n} - C_{\ell-1,n})$	$U_3 = \frac{\Delta t U}{2}(C_{\ell-2,n} + C_{\ell-1,n})$	$G_3 = \Delta x \Delta t G C_{\ell-1,n}$
$D_2 = \frac{\Delta t D}{\Delta x}(C_{\ell-1,n} - C_{\ell,n})$	$U_2 = \frac{\Delta t U}{2}(C_{\ell-1,n} + C_{\ell,n})$	$G_2 = \Delta x \Delta t G C_{\ell,n}$
$D_1 = \frac{\Delta t D}{\Delta x}(C_{\ell,n} - 0)$	$U_1 = \frac{\Delta t U}{2}(C_{\ell,n} + 0)$	$G_1 = \Delta x \Delta t G C_{\ell+1,n}$
$D_o = 0$	$U_o = 0$	

Material Balance for Typical Section $(C_{\ell-1})$

$$\Delta x(C_{\ell-1,n+1} - C_{\ell-1,n}) = (D_3 - D_2) + (U_2 - U_3) + G_3$$

substituting and simplifying

$$C_{\ell-1,n+1} = \left(\frac{\Delta t D}{\Delta x^2} - \frac{\Delta t U}{2\Delta x}\right) C_{\ell-2,n} + \left(1.0 - \frac{2\Delta t D}{\Delta x^2} + \Delta t G\right) C_{\ell-1,n} + \left(\frac{\Delta t D}{\Delta x^2} + \frac{\Delta t U}{2\Delta x}\right) C_{\ell,n} \qquad (4)$$

Material Balance for last Section (C_{ℓ})

$$\Delta x(C_{\ell,n+1} - C_{\ell,n}) = (D_2-D_1) + (U_1-U_2) + G_2$$

$$C_{\ell,n+1} = \left(\frac{\Delta tD}{\Delta x^2} - \frac{\Delta tU}{2\Delta x}\right)C_{\ell-1,n} + \left(1.0 - \frac{2\Delta tD}{\Delta x^2} + \Delta tG\right)C_{\ell,n} \qquad (5)$$

Material Balance for Reservoir ($C_{\ell+1}$)

$$\Delta x(C_{\ell-1,n+1}) = D_1 - U_1 + G_1$$

$$C_{\ell+1,n+1} = \left(\frac{\Delta tD}{\Delta x^2} - \frac{\Delta tU}{2\Delta x}\right)C_{\ell,n} + (1.0 + \Delta tR)C_{\ell+1,n} \qquad (6)$$

substiting

$$AMOD = D\Delta t/\Delta x^2$$
$$BMOD = \Delta tU/2\Delta x$$
$$AL = AMOD - BMOD$$
$$AN = AMOD + BMOD$$
$$AM = (1.0 - 2AMOD + \Delta tG)$$
$$CM = (1.0 + \Delta tR)$$

Typical Section

Eq. (4) $C_{m,n+1} = AL\ C_{m-1,n} + AM\ C_{m,n} + AN\ C_{m+1,n}$

Last Section

Eq. (5) $C_{\ell,n+1} = AL\ C_{\ell-1,n} + AM\ C_{\ell,n}$

Reservoir Section

Eq. (6) $C_{\ell+1,n+1} = AL\ C_{\ell,n} + CM\ C_{\ell+1,n}$

The above three equations are the equations used to calculate the concentrations along the path of diffusion at any given time, n+1 from the previous concentrations at time, n. For example at time Δt after the initial conditions, the concentration in the first section is zero, from the boundary condition. The concentration in the second through the next to last section can be directly calculated from Eq. (4). The concentration in the last section can be calculated from Eq. (5). The concentration in the reservoir, if it were the same volume as any typical section, can be calculated from Eq. (6). The explanation of the program is written directly in the program as comments. The program for the above calculations is included in Appendix D.

The first example is the case where motile E. coli diffuse through initially sterile medium in a crack at room temperature. Figs. 20 and 21 show the calculated distances at which a given low concentration of E. coli would be expected to travel in a given length of time. Mazloum's data[10] for several strains of E. coli diffusing in sloppy agar are shown in Fig. 20 and are in good agreement, considering that the sloppy agar slowed the bacteria.

Figures 20 and 21 can be used to make estimates of the progress of motile bacteria through a crack in the absence of flow of the bulk fluid. For example the concentration C of bacteria 5 cm from the entrance of a crack can be estimated at a time 5 hours after sterilization and innoculation of the crack entrance with a concentration C_o of 10^8 E. coli/ml. From Fig. 21, C/C_o is found to be 10^{-6} at 5 cm and 5 hours. The concentration C of bacteria at the desired location is then equal to 100 bacteria/ml.

In the event that the bulk fluid is in motion, the penetration rate of bacteria by diffusion and convection will be changed. The effect of a bulk flow rate of 1 micron/sec is shown in Fig. 22.

An interesting application of diffusion counter to convection is the dispersion of motile bacteria through the fluid along the wall

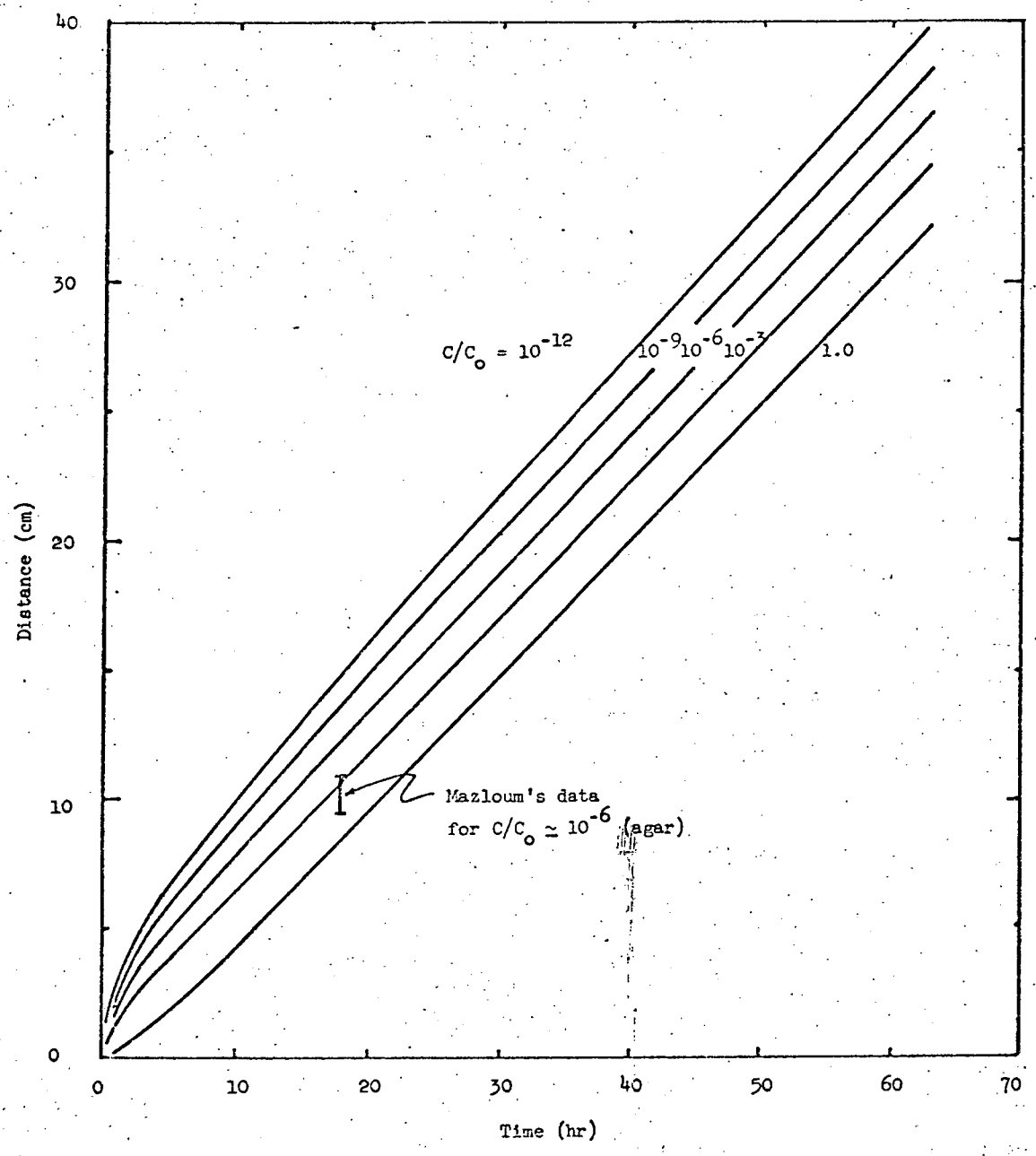

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Fig. 20. The diffusion of E. coli into quiescent medium was calculated for several values of C/C_o. ($D = 2\times10^{-5}\,cm^2/sec$, $R = 2.8\times10^{-4}/sec$).

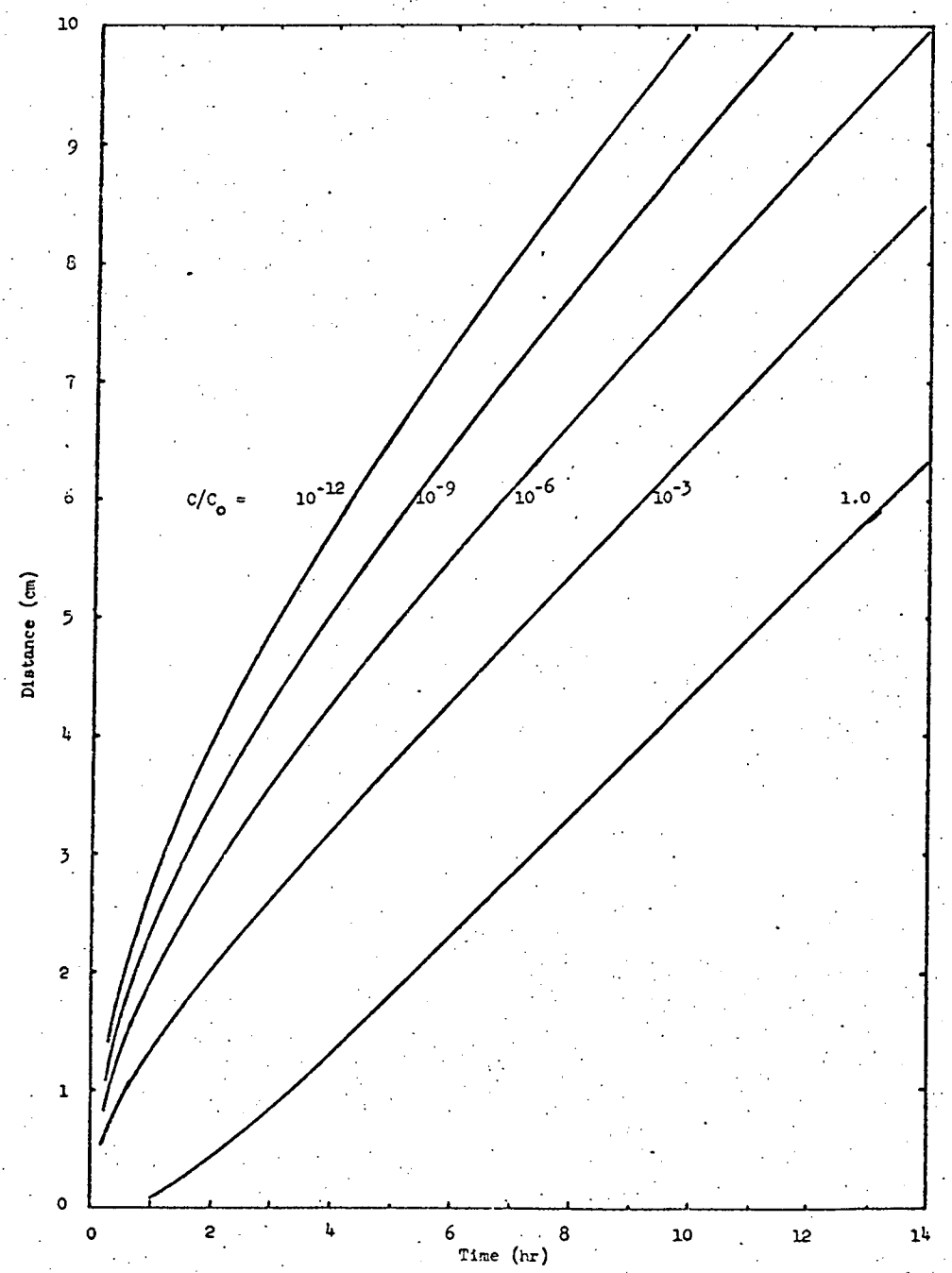

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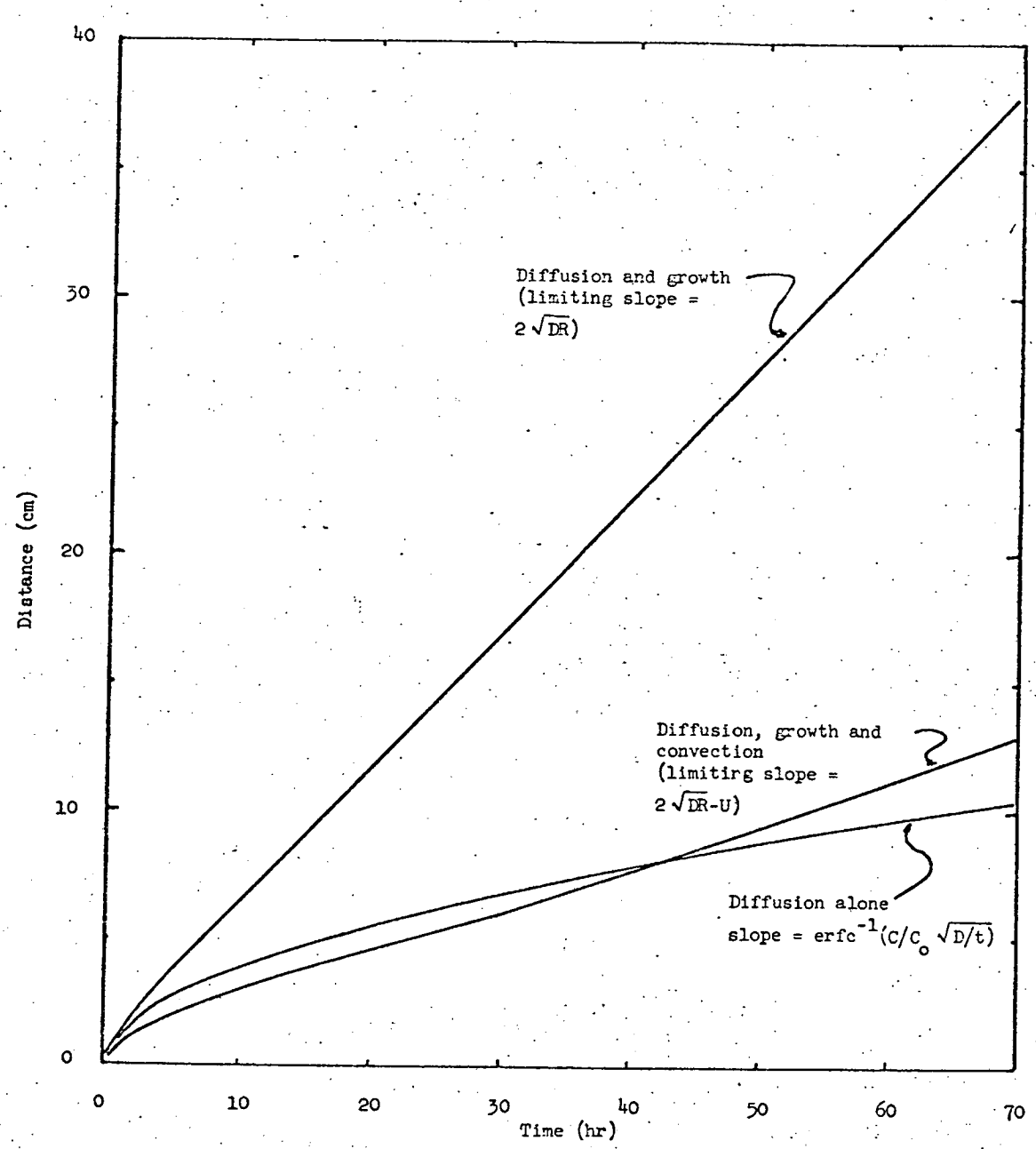
Fig. 21. The diffusion of E. coli into quiescent medium was calculated numerically for several values of C/C_o for short times.



MUB 13230

Fig. 22. Growth and convection effect the dispersion of bacteria. The effect of growth is to increase the dispersion of bacteria, where as bulk flow counter to diffusion decreases the dispersion. ($D = 2\times10^{-5}\,cm^2/sec$, $R = 2.8\times10^{-4}/sec$, $U = 1.0\times10^{-4}\,cm/sec$ $C/C_o = 10^{-3}$)

of a feed line counter to the bulk flow of the feed. The bacteria in the fluid nearest to the wall (5 to 10 microns) would disperse faster since the fluid velocity is slow there. For example laminar flow in a pipe has a parabolic velocity profile.

$$V_z \propto 1.0 - \left(\frac{r}{R}\right)^2$$

$V_{z\ max}$ occurs at $r = 0$

$$V_{z\ ave} = V_{zmax}/2$$

for a one inch pipe, $R = 1.333$ cm

$r = 1.3325$ cm, 5 microns from the wall

$$\frac{V_{5microns}}{V_{z\ max}} = 1 - \left(\frac{r}{R}\right)^2 = 0.00075$$

if $V_{5\ microns} = 1$ micron/sec then $V_{z\ max} = 0.133$ cm/sec and $V_{ave} = 2$ cm/min

Thus it can be seen that if the velocity in a 1 inch pipe is 2 cm/min, the velocity 5 microns from the wall will be 1 micron/sec. From Fig. 22 it can be seen that this low flow rate would decrease the distance traveled up a feed line in two days from 26 to 9 cm. It can therefore be concluded that the motility of bacteria normally would not be a major factor in the movement of bacteria through pipes counter to any appreciable flow in the pipes.

VIII. SUMMARY

The average diffusivities of E. coli obtained by the three different experimental methods were:

Mean square displacement method	4.1×10^{-5} cm^2/sec
Measured concentration gradient method	1.7×10^{-5} cm^2/sec
Diffusion out of a capillary tube	2.5×10^{-5} cm^2/sec

In the mean square displacement method the bacteria which moved in tight circles could not be evaluated. The exclusion of these bacteria would to make the diffusivity high. In the concentration gradient method the reservior of cells could not be stirred. The lack of stirring would make the measured diffusivities low. Probably the best range of values for the diffusivity of E. coli is 2 to $3 \times 10^{-5} cm^2/sec$. These values hold for E. coli subject to the following necessary conditions.

1. 90 to 100 percent motility
2. Cells in exponential growth at 26°C.
3. The medium have excess nutrients and no concentration gradients
4. Absence of convection

Several hundred individual bacteria were followed as they dispersed across a surface. Multiple exposure photomicrographs revealed that as a group their motion was random provided they met the above necessary conditions. It was found that when bacteria were subject to a gradient in bacterial concentration that they dispersed following typical diffusion behavior.

The diffusional transport of bacteria is usually secondary to convective transport. However, near surfaces, in cracks or in packed beds, the diffusion becomes potentially significant, particularly when rapid growth occurs in conjunction with the diffusion. The solution

of the partial differential equation for diffusion convection and growth yields a method of estimating the probability of contamination by diffusional transport. The examples calculated show that bacteria could diffuse through a crack to a sterile system in a few hours. It can also be concluded that bacteria would not make much headway back up a feed line against any significant flow of medium.

ACKNOWLEDGMENTS

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APPENDIX A

Data and Calculations for the Mean Square Displacement Method

The data that represents the X, Y, coordinates of an individual bacterium at six different times, were read in and corrected to centimeter units. The straight line distances and velocities between the time intervals were then calculated. Since the velocity of a given bacteria is fairly constant, a change in velocity was a good indication of an error in the data. For all the runs the diffusivities were calculated from the X and Y components and from the calculated directional displacement for each time interval by summing the squares and averaging them. The average total of the X and Y displacements were calculated. These averages should approach zero for a large random sample. The data were plotted on a scatter diagram for each time interval; one such figure is included in the following program.

The complete explanation of the program is included as comments written directly into the following program. The title contains the run number, date, organism, medium, microscope objective and eyepiece, and temperature in degrees centigrade.

The following program was used for calculating diffusivities in the mean square displacement method from the X, Y coordinates of data points from N bacteria tracks at six different times after the initial exposure. The circled numbers refer to the location of the listed step in the program.

1. Read in the data, the X, Y coordinates (relative to t = 0) for the location of each bacterium at t = 5, 10, 20, 40, 60, and 80 sec for all N tracks
2. Echo print data
3. Multiply input data by CF (converts input measurements to cm)
4. Print corrected data
5. Sum all X and Y values at each time

6. Square and sum X and Y values at each time
7. Calculate distance from origin to X, Y, and the sum $R = (X^2 + Y^2)^{1/2}$
8. Square and sum the R displacements for each time
9. Average all X, Y, R, X^2, Y^2, R^2
10. Calculate the diffusivities $D = \overline{X}^2/2t$, $D = \overline{R}^2/4t$
11. Calculate the incremental velocities, $R/\Delta t$
12. Print diffusivities
13. Print scatter diagram of data for each time

```
      PROGRAM CLICK    (INPUT,OUTPUT,TAPE 3= OUTPUT)

C     THIS PROGRAM SQUARES THE X-Y COORDINATES OF THE LOCATION OF
C     BACTERIA AT DIFFERENT TIMES AND CALCULATES THE DIFFUSIVITY
C     FROM THE MEAN SQUARE DISPLACEMENT

C     X(N,J) = X COORDINATE OF TRACK N AT TIME J
C     Y(N,J) = Y COORDINATE OF TRACK N AT TIME J
C     R(N,J) = DISTANCE FROM ORIGION TO THE POINT X-Y
C     T(J) = LENGTH ON TIME INCREMENT
C     AN(J) = NUMBER OF POINTS AT EACH TIME

      DIMENSION X(200,6),Y(200,6),SX(10),SY(10),SXQ(10 ),SYQ(10),
     $DR(10), AN(200),T(10),BX(10),BY(10),BXQ(10),BYQ(10),R2(200,6),
     $DX(10),R2S(100),R(200,6), V(200,6),DY(10),
     $RS(200),VS(200), RAV(10), VAV(10)
     $,XMIN(10), XMAX(10), YMIN(10), YMAX(10)
     $,CC(20),QT(20)
     $,SS(200,10) ,TS(10)

C     SET INITIAL VALUE OF ALL SUMMATIONS  = 0.0
      DATA (SX(J),SY(J),SXQ(J),SYQ(J),R2S(J),J=1,6)/30*0.0/
      DATA (AN(J), RS(J), VS(J), J=1,6)/18*0./
      DATA (TS(J), J=1,6)/6*0./
110   FORMAT (1H1,113H
C     THESE NEXT 3 CARDS WERE CHANGED FOR EACH RUN
     $RUN NO. 4  7/11/66  E COLI 27  NUTR.  2.*8.  TEMP= 24.          //)
      DATA (T(J), J=1,6)/5., 10.,20., 40., 60., 80./
      CF=5.33E-04

C     START PRINTING PAGE 1  ECHO PRINT OF DATA

      PRINT 110
      PRINT 104
      PRINT 106, (T(J), J=1,6)
      PRINT 109
      DO 15 N=1,200

C     READ IN DATA
      READ 1, ( X(N,J),  Y(N,J), J=1,6)
      IF (( X(N,1).EQ.0.).AND.( Y(N,1).EQ.0.))  GO TO 16

C     ECHO PRINT OF DATA
      PRINT 100,(X(N,J),  Y(N,J),  J=1,6)
15    CONTINUE
16    CONTINUE
      N=N-1

C     START PRINTING PAGE 2  CORRECTED DATA

      PRINT 105, CF
      PRINT 110
      PRINT 106, (T(J), J=1,6)
      PRINT 109
      DO 40 K=1,N
      DO 30 J=1,6
```

```
(3)       CONVERT RAW DATA BY CF TO CM UNITS
      X(K,J)=X(K,J)*CF
      Y(K,J)=Y(K,J)*CF

          FIND THE NUMBER OF POINTS AT EACH TIME
      AN(J)=AN(J)+1.
      IF ((X(K,J).EQ.0.).AND.(Y(K,J).EQ.0.))  AN(J)=AN(J)-1.

(5)       SUM OF ALL X VALUES, SUM OF ALL Y VALUES
      SX(J)=SX(J)+X(K,J)
      SY(J)=SY(J)+Y(K,J)

(6)       SUM OF X-SQUARES  AND Y-SQUARES
      SXQ(J)=SXQ(J)+X(K,J)*X(K,J)
      SYQ(J)=SYQ(J)+Y(K,J)*Y(K,J)

(7)       CALCULATE THE DISTANCE FROM ORIGION TO X-Y AND SUM
      R(K,J)=SQRT(X(K,J)*X(K,J)+Y(K,J)*Y(K,J))
      RS(J)=RS(J) + R(K,J)

          CALCULATE THE AVERAGE VELOCITY TO EACH POINT AND SUM
      V(K,J)=R(K,J)/T(J)
      VS(J)=VS(J) + V(K,J)

(8)       SQUARE AND SUM THE DISPLACEMENTS
      R2(K,J)=R(K,J)*R(K,J)
      R2S(J)=R2S(J)+R2(K,J)
   30 CONTINUE

(4)       PRINT CORRECTED VALUES FOR ALL X, AND Y
      PRINT 3, (X(K,J), Y(K,J), J=1,6)
   40 CONTINUE
      DO 50 J=1,6

(9)       FIND AVERAGES OF X, Y, R, VELOCITIES,  X-SQUARES, AND Y-SQUARES
      BX(J)=SX(J)/AN(J)
      BY(J)=SY(J)/AN(J)
      RAV(J)=RS(J)/AN(J)
      VAV(J)=VS(J)/AN(J)
      BXQ(J)=SXQ(J)/AN(J)
      BYQ(J)=SYQ(J)/AN(J)

(10)      CALCULATE DIFFUSIVITY
      DX(J)=BXQ(J)/(2.*T(J))
      DY(J)=BYQ(J)/(2.*T(J))
      DR(J)=R2S(J)/(4.*T(J)*AN(J))
   50 CONTINUE
      DO 19 K=1,N

(11)      CALCULATE THE INCREMENTAL VELOCITIES OF EACH BACTERIA
      SS(K,1)= SQRT(X(K,1)*X(K,1) + Y(K,1)*Y(K,1)) /T(1)
      DO 18 J=2,6
      SS(K,J)=SQRT((X(K,J)-X(K,J-1))**2+(Y(K,J)-Y(K,J-1))**2)
     $/(T(J)-T(J-1))
      IF ((X(K,J).EQ.0.).AND.(Y(K,J).EQ.0.))  SS(K,J)=0.
   18 CONTINUE
   19 CONTINUE
      DO 44 J=1,6
      DO 43 K=1,N

          SUM AND AVERAGE INCREMENTAL VELOCITIES
```

```
      TS(J)=TS(J)+SS(K,J)
   43 CONTINUE
      TS(J)=TS(J)/AN(J)
   44 CONTINUE
```

```
          PRINT PAGE 3, INCREMENTAL VELOCITIES AND AVERAGE

      PRINT 110
      PRINT 2
      PRINT 103, (T(J), J=1,6)
      PRINT 4,((SS(K,J), J=1,6),K=1,N)
      PRINT 111
      PRINT 4, (TS(J), J=1,6)
      PRINT 110
```

```
(12)      PRINT PAGE 4 SUMMARY OF DATA CALCULATED

      PRINT 9
      PRINT 103, ( T(J), J=1,6)
      PRINT 5
      PRINT 4,  ( SX(J), J=1,6)
      PRINT 4,  ( SY(J), J=1,6)
      PRINT 6
      PRINT 4,  (SXQ(J), J=1,6)
      PRINT 4,  (SYQ(J), J=1,6)
      PRINT 8
      PRINT 112, (AN(J), J=1,6)
      PRINT 10
      PRINT 4,  ( BX(J), J=1,6)
      PRINT 4,  ( BY(J), J=1,6)
      PRINT 11
      PRINT 4,  (BXQ(J), J=1,6)
      PRINT 4,  (BYQ(J), J=1,6)
      PRINT 12
      PRINT 4,  ( DX(J), J=1,6)
      PRINT 4,  ( DY(J), J=1,6)
      PRINT 4,  (DR(J), J=1,6)
      PRINT 107
      PRINT 4, (RAV(J), J=1,6)
      PRINT 108
      PRINT 4, (VAV(J), J=1,6)
      PRINT 111
      PRINT 4, (TS(J), J=1,6)

          PRINT PAGE 5,  DISPLACEMENTS FROM ORIGION AND AVERAGE
      PRINT 110
      PRINT 101
      PRINT 103, ( T(J), J=1,6)
      PRINT 4,((R(K,J),J=1,6),K=1,N)
      PRINT 107
      PRINT 4, (RAV(J), J=1,6)

          PRINT PAGE 6,  AVERAGE VELOCITY FROM THE ORIGION AND AVERAGE
      PRINT 110
      PRINT 102
      PRINT 103, ( T(J), J=1,6)
      PRINT 4,((V(K,J),J=1,6),K=1,N)
      PRINT 108
      PRINT 4, (VAV(J), J=1,6)
    1 FORMAT (9X,12F5.0)
```

```
  2 FORMAT (20X,20HINCREMENTAL VELOCITY)
  3 FORMAT (1X,12E10.2)
  4 FORMAT (1X,6E15.3)
  5 FORMAT(/20X,11HSUM OF DATA)
  6 FORMAT (/20X,21HSUM /F SQUARE OF DATA)
  8 FORMAT(/20X,21HNUMBER OF DATA POINTS)
  9 FORMAT (     /20X,21HTIME FOR DISPLACEMENT)
 10 FORMAT (20X,20HAVERAGE DISPLACEMENT)
 11 FORMAT(/20X,24HMEAN SQUARE DISPLACEMENT)
 12 FORMAT(/20X,11HDIFFUSIVITY)
100 FORMAT (1X,F6.1,11F10.1)
101 FORMAT (     //21HABSOLUTE DISPLACEMENT//)
102 FORMAT (     //20X,16HAVERAGE VELOCITY)
103 FORMAT (/1X,6F15.1,7H (SEC.)//)
104 FORMAT (       20X,18HECHO PRINT-OF DATA)
105 FORMAT (20X,21HX-Y DISPLACEMENT(CM.),5X,20H(CONVERSION FACTOR =,
   $E10.3,2X,7HCM/DIV))
106 FORMAT (/1X,10HTIME (SEC),F4.0,5F20.0)
107 FORMAT (//20X,16HMEAN RADIUS (CM)//)
108 FORMAT (//20X,22HMEAN VELOCITY (CM/SEC)//)
109 FORMAT (6X,1HX,9X,1HY,9X,1HX,9X,1HY,7H   ETC.)
111 FORMAT (/20X,28HAVERAGE INCREMENTAL VELOCITY//)
112 FORMAT (/1X,6F15.1//)

        IN THIS SECTION ROUGH PLOTS ARE MADE BY THE COMPUTER USING
        PLOTTING SUBROUTINE  PPLT

        PLOT PAGE 7  DIFFUSIVITY VS TIME

      DO 71 J=1,6
      QD(J)=DX(J)
   71 QT(J)=T(J)
      DO72 J=7,12
      QD(J)=DY(J-6)
   72 QT(J)=T(J-6)
      DO 74 J=13,18
      QT(J)=T(J-12)
   74 QD(J)=DR(J-12)
      DMAX=QD(1)
      DO 73 J=2,18
      IF (QD(J).GT.DMAX)  DMAX=QD(J)
   73 CONTINUE
      DMAX=1.2*DMAX
      TMAX=T(6)*1.2
      AB=0.
      NO=18

      CALL PPLT (QT, QD, AB, TMAX, AB, DMAX, NO)
        PLOT PAGES 8-13   SCATTER DIAGRAMS OF X-Y AT DIFFERENT TIMES

      DO 81 J=1,6
      XMIN(J)= X(1,J)
      XMAX(J)=X(1,J)
      YMIN(J)=Y(1,J)
      YMAX(J)=Y(1,J)
      DO 80 K=2,N
      IF (X(K,J).LT.XMIN(J))  XMIN(J)=X(K,J)
      IF (Y(K,J).LT.YMIN(J))  YMIN(J)=Y(K,J)
      IF (Y(K,J).GT.YMAX(J))  YMAX(J)=Y(K,J)
      IF (X(K,J).GT.XMAX(J))  XMAX(J)=X(K,J)
```

```
 80 CONTINUE
    EX=AMAX1 (ABS(XMAX(J)),ABS(XMIN(J)), ABS(YMAX(J)), ABS(YMIN(J)))
    YMAX(J)=EX
    YMIN(J)=-EX
C
C         CORRECT THE SCALES FOR THE FACT THAT THE VERTICAL SPACES ARE
C         NOT EQUAL TO HORIZONTAL SPACES
    XMAX(J)=1.2*EX
    XMIN(J)=-1.2*EX
    NUM=N
    CALL PPLT(X(1,J),Y(1,J),XMIN(J),XMAX(J),YMIN(J),YMAX(J),NUM)
 81 CONTINUE
    STOP
    END
```

```
      SUBROUTINE PPLT (X, Y, XMIN, XMAX, YMIN, YMAX, NUM)

      THIS SUBROUTINE, GIVEN A SET OF N X-Y COORDINATES, WILL PLOT THEM
      ON A 51-101 X-Y GRID, THUS MAKING APPARENT THE CHARACTERISTICS OF
      THE EQUATION FROM WHICH THEY WERE OBTAINED OR REPRESENT

           X = X COORDINATE OF EACH POINT
           Y = Y COORDINATE OF EACH POINT
           XMIN = MINIMUM VALUE FOR X SCALE
           YMIN = MINIMUM VALUE FOR Y SCALE
           XMAX = MAXIMUM VALUE OF X SCALE
           YMAX = MAXIMUM VALUE OF Y SCALE
           NUM = NUMBER OF DATA POINTS

      DIMENSION X(1), Y(1), XGRID(11), YGRID(11), GRID(101)
      DATA BLNK,XXXXX/1H ,1HX/
      T1= (XMAX-XMIN)/10.
      T2 = (YMAX - YMIN) / 10.
      XGRID(1) = XMIN
      YGRID(1) = YMAX
      DO 25 I = 2, 11
      XGRID(I) = XGRID(I - 1) + T1
   25 YGRID(I) = YGRID(I - 1) - T2
      WRITE (3, 35)
   35 FORMAT (1H1)
      DO 40 I = 1, 3
   40 WRITE (3, 45)
   45 FORMAT (20X, 1H*, 10(9X, 1H*))
      L = 1
      M = 1
      DO 65 K = 1, 10
      DO 50 I = 1, 101
   50 GRID(I) = BLNK
      A = FLOAT(M)
      Q = (YMAX * (51. - A) + YMIN * (A - 1.))/ 50.
      DO 53 IL = 1, NUM
      IF (ABS(Q - Y(IL)) - (YMAX - YMIN) / 100.) 41, 53, 53
   41 IXP = 100. * (X(IL) - XMIN) / (XMAX - XMIN) + 1.5
      GRID(IXP) = XXXXX
   53 CONTINUE
      WRITE (3,75) YGRID(L),(GRID(I), I = 1, 101)
      N = M + 1
      M = N + 3
      DO 60 J = N, M
      DO 55 I = 1, 101
   55 GRID(I) = BLNK
      A = FLOAT(J)
      Q = (YMAX * (51. - A) + YMIN * (A - 1.))/ 50.
      DO 57 IL = 1, NUM
      IF (ABS(Q - Y(IL)) - (YMAX - YMIN) / 100.) 46, 57, 57
   46 IXP = 100. * (X(IL) - XMIN) / (XMAX - XMIN) + 1.5
      IF (IXP .GT. 101)  PRINT 4682, IXP
 4682 FORMAT(6H IXP =,O22)
      GRID(IXP) = XXXXX
   57 CONTINUE
   60 WRITE (3,76)(GRID(I), I = 1, 101)
      M = M + 1
   65 L = L + 1
      DO 66 I = 1, 101
```

```
66 GRIC(I) = BLNK
   DO 72 IL = 1, NUM
   IF (ABS(YMIN - Y(IL)) - (YMAX - YMIN) / 100.) 69, 72, 72
65 IXP = 100. * (X(IL) - XMIN) / (XMAX - XMIN) + 1.5
   GRIC(IXP) = XXXXX
72 CONTINUE
   WRITE (3,75) YGRID(11),(GRIC(I), I = 1, 101)
75 FORMAT (10X, 1PE9.2, 1X, 101A1)
76 FORMAT (20X, 101A1)
   DO 80 I = 1, 3
80 WRITE (3, 45)
   WRITE (3,85) (XGRID(I), I = 1, 11)
85 FORMAT (16X, 11(1PE9.2, 1X))
   RETURN
   END
```

RUN NO. 4 7/11/66 E COLI 27 NUTR. 2.*8. TEMP= 24.

TIME FOR DISPLACEMENT

5.0	10.0	20.0	40.0	60.0	80.0 (SEC.)

SUM OF DATA

-4.460E-02	-3.312E-02	-1.232E-01	8.350E-02	-2.146E-02	-7.044E-02
6.624E-02	1.343E-01	3.028E-01	4.236E-01	2.799E-01	1.493E-02

SUM OF SQUARE OF DATA

3.655E-03	1.359E-02	5.075E-02	1.559E-01	2.387E-01	1.899E-01
4.718E-03	1.879E-02	6.961E-02	2.171E-01	1.868E-01	1.438E-01

NUMBER OF DATA POINTS

80.0	80.0	80.0	79.0	56.0	29.0

AVERAGE DISPLACEMENT

-5.575E-04	-4.140E-04	-1.539E-03	1.057E-03	-3.832E-04	-2.429E-03
8.280E-04	1.685E-03	3.784E-03	5.362E-03	4.998E-03	5.148E-04

MEAN SQUARE DISPLACEMENT

4.574E-05	1.698E-04	6.343E-04	1.973E-03	4.262E-03	6.548E-03
5.897E-05	2.349E-04	8.702E-04	2.748E-03	3.336E-03	4.960E-03

DIFFUSIVITY

4.574E-06	8.491E-06	1.586E-05	2.467E-05	3.552E-05	4.093E-05
5.897E-06	1.174E-05	2.175E-05	3.435E-05	2.780E-05	3.100E-05
5.235E-06	1.012E-05	1.881E-05	2.951E-05	3.166E-05	3.596E-05

MEAN RADIUS (CM)

5.883E-03	1.956E-02	3.726E-02	6.402E-02	7.909E-02	9.702E-02

MEAN VELOCITY (CM/SEC)

1.977E-03	1.956E-03	1.863E-03	1.601E-03	1.318E-03	1.213E-03

AVERAGE INCREMENTAL VELOCITY

1.977E-03	2.110E-03	2.006E-03	1.771E-03	1.723E-03	1.832E-03

RUN NO. 4 7/11/66 E COLI 27 NUTR. 2.*S. TEMP= 24.

X-Y DISPLACEMENT(CM.) (CONVERSION FACTOR = 9.330E-04 CM/DIV)

TIME (SEC)	5		10		20		40		60		80	
X	Y	X	Y	ETC.								
-8.86E-02	3.64E-03	-1.87E-02	3.73E-03	-3.73E-02	-3.73E-03	-6.90E-02	-2.33E-02	-9.80E-02	-1.21E-02	-1.09E-01	2.61E-02
7.46E-03	-4.66E-03	1.49E-02	-6.06E-03	9.80E-03	1.40E-03	5.60E-03	-3.45E-02	5.60E-03	-6.90E-02	3.73E-03	-1.02E-01
-1.03E-02	3.73E-03	-1.35E-02	1.26E-02	-8.40E-03	1.87E-02	3.27E-03	4.20E-03	-1.87E-03	-2.43E-02	1.40E-02	-4.20E-02
2.33E-03	7.46E-03	4.66E-03	1.68E-02	9.33E-03	2.38E-02	3.55E-02	1.77E-02	5.88E-02	5.13E-03	9.52E-02	1.12E-02
-5.33E-03	-1.40E-03	-1.59E-02	-4.20E-03	-3.55E-02	-8.40E-03	-1.59E-02	-5.60E-03	-4.20E-02	7.46E-03	-4.01E-02	4.11E-02
8.86E-03	1.40E-03	2.10E-02	2.33E-03	3.64E-02	-1.40E-03	2.52E-02	-4.48E-02	0.	0.	0.	0.
-1.31E-02	-8.40E-03	-2.52E-02	-1.68E-02	-5.13E-02	-2.61E-02	0.	0.	0.	0.	0.	0.
1.87E-03	-8.86E-03	4.66E-03	-1.77E-02	1.59E-02	-2.89E-02	6.53E-03	-3.17E-02	0.	0.	0.	0.
7.46E-03	8.40E-03	1.31E-02	1.73E-02	3.17E-02	2.99E-02	6.72E-02	4.43E-02	0.	0.	0.	0.
-1.03E-02	-5.59E-03	-1.12E-02	-9.33E-03	-2.89E-02	-2.80E-02	-5.50E-02	-4.20E-02	-4.48E-02	-3.97E-02	0.	0.
5.05E-03	7.74E-03	1.31E-02	1.77E-02	1.21E-02	4.20E-02	4.11E-02	5.88E-02	1.59E-02	4.29E-02	0.	0.
-4.66E-03	-6.53E-03	-7.46E-03	-1.31E-02	-1.87E-03	-1.31E-02	-1.31E-02	1.49E-02	-2.80E-02	4.29E-02	-7.46E-03	7.37E-02
-3.27E-03	9.80E-03	-1.03E-02	1.59E-02	-2.43E-02	3.64E-02	-6.25E-02	3.69E-02	-2.33E-02	3.83E-02	2.80E-03	4.39E-02
0.	-5.33E-03	9.33E-04	-1.96E-02	-1.40E-03	-3.73E-02	-1.96E-02	-5.32E-02	0.	0.	0.	0.
1.40E-03	1.21E-02	4.66E-03	2.33E-02	1.40E-02	4.39E-02	3.55E-02	8.21E-02	0.	0.	0.	0.
5.33E-03	7.00E-03	2.15E-02	1.31E-02	4.40E-02	3.45E-02	9.33E-02	3.36E-02	1.35E-01	1.49E-02	0.	0.
6.53E-03	-1.21E-02	1.21E-02	-2.80E-02	1.96E-02	-5.13E-02	1.96E-02	-1.09E-01	0.	0.	0.	0.
-1.12E-02	-4.66E-03	-2.05E-02	-1.40E-02	-3.92E-02	-3.55E-02	-5.60E-02	-5.41E-02	-5.88E-02	-5.32E-02	0.	0.
6.53E-03	1.03E-02	1.87E-02	1.21E-02	4.29E-02	1.82E-02	8.58E-02	3.83E-02	8.30E-02	8.58E-02	0.	0.
-1.03E-02	4.66E-04	-2.15E-02	-2.80E-03	-4.11E-02	-2.15E-02	-8.02E-02	-5.13E-02	0.	0.	0.	0.
2.80E-03	1.07E-02	7.46E-03	1.96E-02	-3.27E-03	2.24E-02	-3.92E-02	4.66E-03	-6.53E-02	-2.15E-02	-9.24E-02	-4.48E-02
3.73E-03	1.03E-02	8.86E-03	1.96E-02	2.52E-02	3.36E-02	6.72E-02	2.99E-02	0.	0.	0.	0.
-1.03E-02	1.87E-03	-2.05E-02	3.27E-03	-4.20E-02	4.66E-03	-8.12E-02	-2.33E-03	-1.19E-01	-1.87E-02	0.	0.
5.33E-04	5.33E-03	4.20E-03	1.82E-02	8.40E-03	3.36E-02	8.40E-03	5.50E-02	1.26E-02	2.33E-02	0.	0.
-4.66E-04	8.12E-03	9.33E-04	1.54E-02	1.40E-02	1.73E-02	3.36E-02	3.73E-02	4.01E-02	2.52E-02	3.17E-02	1.87E-02
-2.27E-03	-8.40E-03	-6.53E-03	-1.68E-02	-2.33E-02	-2.15E-02	-3.08E-02	-5.13E-02	-1.49E-02	-4.29E-02	0.	0.
-4.66E-03	-3.73E-03	-4.66E-03	-1.31E-02	-1.87E-03	-2.80E-03	-1.87E-03	-1.68E-02	-3.73E-03	-1.87E-02	0.	0.
-1.12E-02	2.33E-03	-2.43E-02	-2.80E-03	-4.66E-02	3.73E-03	-8.77E-02	-9.33E-04	0.	0.	0.	0.
-7.46E-03	-5.33E-04	-1.40E-02	-9.33E-04	-2.89E-02	8.40E-03	-5.50E-02	2.71E-02	-4.11E-02	6.05E-02	-4.76E-02	1.04E-01
5.33E-04	7.46E-03	7.46E-03	1.96E-02	5.60E-03	3.83E-02	1.87E-02	7.46E-02	0.	0.	0.	0.
7.46E-03	0.	2.52E-02	2.80E-03	4.11E-02	1.31E-02	8.58E-02	2.80E-02	1.28E-01	3.92E-02	1.32E-01	7.84E-02
1.87E-03	-5.33E-03	3.73E-03	-1.87E-02	7.00E-03	-3.45E-02	2.43E-02	-6.90E-02	0.	0.	0.	0.
2.33E-03	5.33E-03	1.40E-02	1.49E-02	3.64E-02	1.59E-02	7.74E-02	3.17E-02	7.28E-02	7.65E-02	0.	0.
1.87E-03	-1.12E-02	-1.87E-03	-1.68E-02	-1.96E-02	-2.05E-02	-2.43E-02	-2.71E-02	0.	0.	0.	0.
-1.87E-03	-1.31E-02	-4.66E-03	-2.43E-02	9.33E-04	-4.48E-02	3.55E-02	-5.50E-02	7.16E-02	-3.08E-02	8.96E-02	1.40E-02
5.13E-03	5.33E-03	1.31E-02	1.87E-02	3.03E-02	3.83E-02	4.66E-02	8.02E-02	9.14E-02	9.89E-02	0.	0.
0.	-1.12E-02	-3.73E-03	-2.15E-02	-1.59E-02	-4.48E-02	-4.48E-02	-6.90E-02	-9.14E-02	-5.97E-02	-1.05E-01	-1.49E-02
5.33E-03	3.73E-03	1.96E-02	9.80E-03	3.41E-02	2.57E-02	5.69E-02	5.60E-02	0.	0.	0.	0.
-1.03E-02	-3.73E-03	-1.87E-02	-8.40E-03	-2.99E-02	-2.33E-02	-6.06E-02	-6.34E-02	-8.86E-02	-9.42E-02	-1.21E-01	-1.16E-01
-2.73E-03	5.33E-03	-5.13E-03	1.96E-02	-3.27E-03	4.20E-02	-7.46E-03	8.77E-02	-4.76E-02	1.09E-01	0.	0.
-8.40E-03	-3.73E-03	-1.77E-02	-9.33E-04	-2.15E-02	1.77E-02	1.12E-02	3.08E-02	2.24E-02	-6.53E-03	0.	0.
-4.66E-04	1.45E-02	1.87E-03	2.80E-02	7.00E-03	5.32E-02	1.12E-02	1.07E-01	0.	0.	0.	0.
-1.21E-02	5.60E-03	-1.87E-02	1.54E-02	-2.61E-02	4.11E-02	9.33E-04	8.02E-02	0.	0.	0.	0.
6.53E-03	5.60E-03	1.21E-02	1.31E-02	1.49E-02	2.80E-02	3.17E-02	5.60E-02	0.	0.	0.	0.
-5.33E-04	-1.03E-02	-7.93E-03	-1.87E-02	-2.61E-02	-2.52E-02	-2.89E-02	5.13E-03	-1.49E-02	3.27E-02	0.	0.
-5.60E-03	5.33E-04	-4.20E-03	-9.33E-03	9.33E-04	-2.71E-02	2.80E-03	-6.44E-02	-5.60E-03	-6.81E-02	0.	0.
-1.87E-03	7.53E-03	-5.13E-03	1.31E-02	-3.73E-03	3.08E-02	-1.03E-02	6.44E-02	-2.99E-02	9.05E-02	0.	0.
-7.93E-03	-4.66E-04	-6.53E-03	9.33E-03	-1.49E-02	2.80E-02	1.68E-02	3.08E-02	-2.15E-02	0.	0.	0.
-4.66E-03	0.	1.12E-02	4.66E-03	2.47E-02	1.40E-02	3.78E-02	4.94E-02	7.56E-02	5.88E-02	0.	0.
7.46E-03	-5.33E-03	1.12E-02	-1.87E-02	1.21E-02	-3.73E-02	-9.33E-04	-6.62E-02	2.80E-03	-1.03E-01	1.87E-02	-1.45E-01
6.53E-03	5.13E-03	1.26E-02	1.03E-02	2.43E-02	2.57E-02	5.41E-02	4.66E-02	0.	0.	0.	0.
-2.73E-03	1.21E-02	-8.40E-03	2.52E-02	-2.99E-02	2.61E-02	-2.15E-02	-2.71E-02	0.	0.	0.	0.
-1.40E-03	-1.40E-03	-1.12E-02	-1.40E-03	-5.13E-03	1.96E-02	1.31E-02	8.40E-03	-1.31E-02	2.80E-02	-2.80E-02	6.72E-03
6.53E-03	5.33E-04	1.54E-02	1.87E-03	3.17E-02	1.31E-02	6.16E-02	3.92E-02	8.77E-02	6.90E-02	1.13E-01	1.02E-01
-1.21E-02	-5.13E-03	-2.43E-02	-9.33E-03	-4.85E-02	-1.21E-02	-9.80E-02	-2.99E-02	-1.42E-01	-3.83E-02	0.	0.

-5.33E-04	-1.12E-02	-1.12E-02	-1.59E-02	-3.45E-02	-2.61E-02	-3.36E-02	-1.96E-02	-6.81E-02	-5.13E-02	-4.76E-02	-4.20E-02
4.66E-04	-1.40E-03	-5.60E-03	-1.31E-02	-1.21E-02	-3.36E-02	-2.52E-02	0.	0.	0.	0.	0.
5.33E-04	-6.40E-03	4.66E-04	-1.66E-02	-6.53E-03	-3.08E-02	-1.59E-02	-6.16E-02	0.	0.	0.	0.
0.	-7.46E-03	1.87E-03	-1.45E-02	1.49E-02	-1.77E-02	3.55E-02	-2.80E-02	3.27E-02	-5.41E-02	0.	0.
-2.33E-03	-6.06E-03	9.33E-04	-1.40E-02	-4.66E-03	-3.73E-02	-1.69E-02	-8.21E-02	-1.87E-02	-1.15E-01	-1.12E-02	-1.42E-01
2.33E-03	1.87E-03	3.27E-03	1.12E-02	-9.33E-04	2.99E-02	-2.71E-02	2.33E-02	-5.97E-02	2.05E-02	0.	0.
6.53E-03	4.20E-03	-4.20E-03	7.93E-03	-1.77E-02	1.87E-03	-2.15E-02	1.40E-03	-3.83E-02	-2.33E-02	-4.29E-02	-4.39E-02
1.35E-02	-3.73E-03	2.24E-02	-1.21E-02	2.24E-02	-4.20E-02	9.33E-04	-9.24E-02	0.	0.	0.	0.
1.07E-02	4.66E-03	2.15E-02	9.33E-03	4.20E-02	1.49E-02	2.24E-02	2.24E-02	7.93E-03	1.45E-02	4.94E-02	1.77E-02
-4.66E-03	5.33E-03	-6.53E-03	2.05E-02	-1.59E-02	4.01E-02	-2.61E-02	8.86E-02	0.	0.	0.	0.
1.40E-03	-6.66E-03	-1.87E-03	-1.59E-02	-1.12E-02	-2.89E-02	-1.49E-02	-3.73E-02	3.73E-03	-4.76E-02	2.15E-02	-2.61E-02
7.00E-03	8.46E-03	1.21E-02	1.77E-02	2.33E-02	3.27E-02	9.33E-04	5.60E-02	-3.27E-02	7.46E-02	0.	0.
2.33E-03	5.60E-03	6.53E-03	1.87E-02	1.31E-02	3.64E-02	2.52E-02	7.00E-02	6.25E-02	7.00E-02	0.	0.
5.33E-03	5.33E-03	1.12E-02	2.33E-02	2.52E-02	3.45E-02	6.34E-02	2.61E-02	8.40E-02	-1.77E-02	0.	0.
-4.66E-03	5.33E-03	-3.73E-03	1.87E-02	1.87E-03	4.01E-02	1.96E-02	7.84E-02	4.85E-02	1.09E-01	0.	0.
-2.80E-03	-1.21E-02	-4.20E-03	-2.89E-02	-1.40E-02	-5.41E-02	9.33E-03	-8.02E-02	4.94E-02	-5.13E-02	7.65E-02	-9.33E-03
-7.46E-03	2.80E-03	-2.80E-03	7.46E-03	-2.80E-03	-1.03E-02	1.35E-02	-2.61E-02	2.89E-02	-3.83E-02	0.	0.
1.87E-02	1.12E-02	8.40E-03	2.05E-02	1.12E-02	4.01E-02	2.80E-03	8.30E-02	2.52E-02	1.14E-01	0.	0.
-8.40E-03	0.	-1.68E-02	-1.87E-03	-3.08E-02	-8.40E-03	-5.97E-02	-1.03E-02	-8.21E-02	6.53E-03	-9.61E-02	3.64E-02
2.73E-03	5.33E-03	5.60E-03	1.96E-02	1.12E-02	4.29E-02	5.41E-02	7.65E-02	9.70E-02	1.06E-01	1.18E-01	1.56E-01
-1.21E-02	-4.66E-03	-2.15E-02	-5.13E-03	-4.48E-02	-2.80E-03	-9.52E-02	3.73E-03	-1.40E-01	5.13E-03	-1.68E-01	-3.64E-02
-7.00E-03	-8.40E-03	-1.59E-02	-1.31E-02	-2.99E-02	-2.52E-02	-3.36E-02	-6.34E-02	4.66E-03	-6.53E-02	-3.27E-03	-2.89E-02
7.46E-03	-4.66E-03	1.07E-02	-1.40E-02	1.59E-02	-3.55E-02	-1.03E-02	-5.69E-02	-4.66E-02	-3.45E-02	-6.34E-02	9.33E-04
5.80E-03	0.	2.05E-02	1.40E-03	4.20E-02	9.33E-03	7.65E-02	2.80E-03	1.12E-01	8.40E-03	1.46E-01	1.59E-02
-3.73E-03	1.03E-02	-1.03E-02	1.96E-02	-1.59E-02	3.92E-02	-1.12E-02	8.02E-02	0.	0.	0.	0.

RUN NO. 4 7/11/66 E COLI 27 NUTR. 2.*8. TEMP= 24.

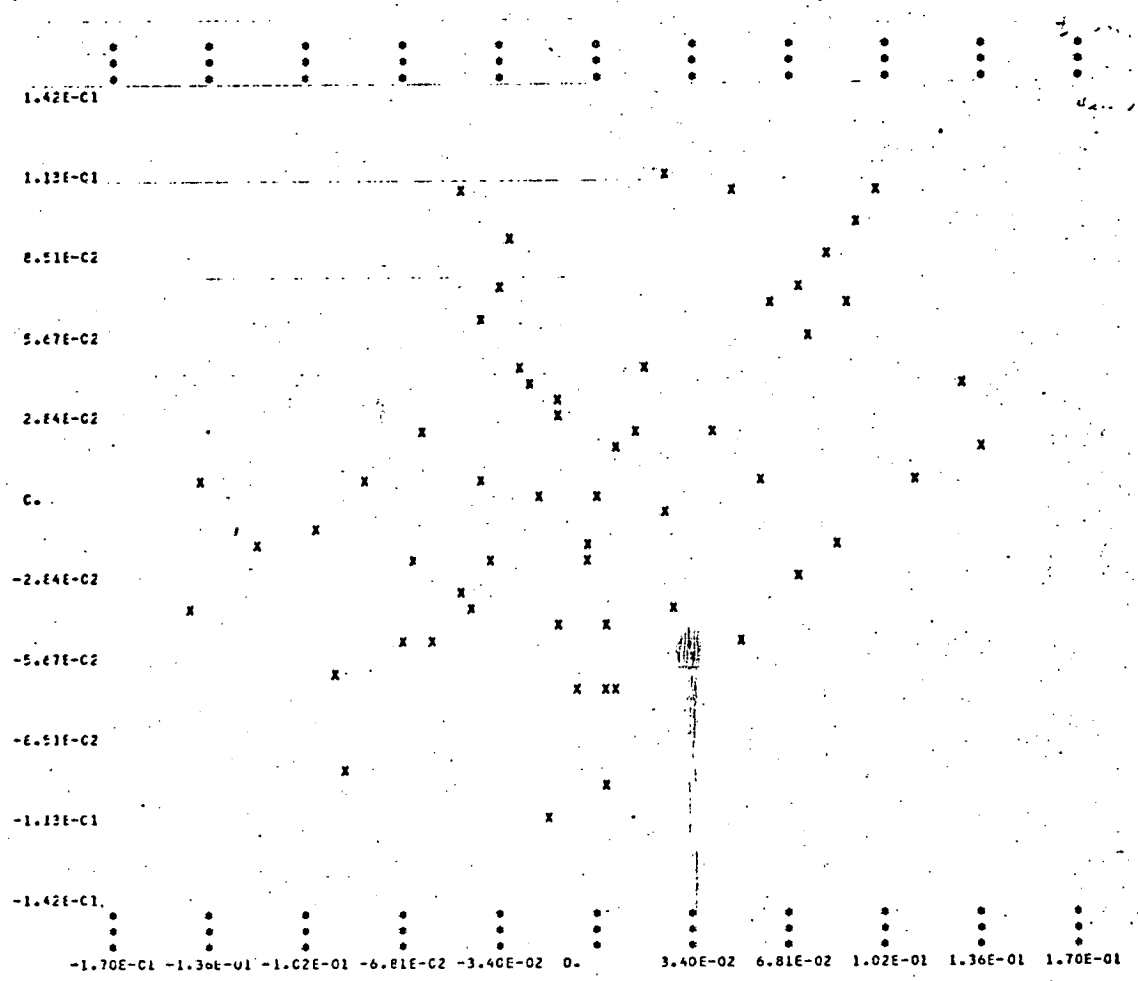
INCREMENTAL VELOCITY

5.0	10.0	20.0	40.0	60.0	80.0 (SEC.)
1.916E-03	1.959E-03	2.010E-03	1.864E-03	1.551E-03	1.993E-03
1.760E-03	1.519E-03	9.058E-04	1.805E-03	1.726E-03	1.635E-03
2.184E-03	1.889E-03	7.944E-04	9.289E-04	1.446E-03	1.189E-03
1.564E-03	1.923E-03	8.410E-04	1.341E-03	1.325E-03	1.844E-03
1.887E-03	1.421E-03	2.004E-03	9.896E-04	1.460E-03	1.682E-03
1.755E-03	2.433E-03	1.584E-03	2.240E-03	0.	0.
3.106E-03	2.950E-03	2.774E-03	0.	0.	0.
1.812E-03	1.859E-03	1.583E-03	4.870E-04	0.	0.
2.247E-03	2.097E-03	2.251E-03	1.914E-03	0.	0.
2.375E-03	6.972E-04	2.574E-03	1.482E-03	5.262E-04	0.
2.382E-03	2.152E-03	2.428E-03	1.672E-03	1.488E-03	0.
1.605E-03	1.421E-03	5.598E-04	1.507E-03	1.586E-03	1.850E-03
2.065E-03	1.852E-03	2.484E-03	1.913E-03	1.961E-03	1.336E-03
1.866E-03	2.061E-03	1.788E-03	1.207E-03	0.	0.
2.442E-03	2.332E-03	2.255E-03	2.193E-03	0.	0.
2.332E-03	2.712E-03	3.169E-03	2.426E-03	2.297E-03	0.
2.921E-03	3.189E-03	2.449E-03	2.892E-03	0.	0.
2.426E-03	2.639E-03	2.844E-03	1.255E-03	1.475E-04	0.
2.433E-03	2.454E-03	2.500E-03	2.369E-03	2.383E-03	0.
2.055E-03	2.332E-03	2.706E-03	2.463E-03	0.	0.
2.218E-03	2.003E-03	1.109E-03	2.003E-03	1.847E-03	1.786E-03
2.184E-03	2.130E-03	2.150E-03	2.108E-03	0.	0.
2.066E-03	2.072E-03	2.150E-03	1.990E-03	2.080E-03	0.
1.875E-03	1.889E-03	1.596E-03	1.073E-03	1.600E-03	0.
1.626E-03	1.482E-03	1.319E-03	1.402E-03	6.888E-04	5.319E-04
1.802E-03	1.802E-03	1.743E-03	1.535E-03	8.973E-04	0.
1.155E-03	1.866E-03	1.064E-03	6.997E-04	1.319E-04	0.
2.287E-03	2.807E-03	2.332E-03	2.066E-03	0.	0.
1.504E-03	1.306E-03	1.760E-03	1.605E-03	1.819E-03	2.217E-03
1.504E-03	2.755E-03	1.875E-03	1.933E-03	0.	0.
1.453E-03	3.589E-03	1.889E-03	2.360E-03	2.173E-03	1.973E-03
1.503E-03	1.903E-03	1.619E-03	1.930E-03	0.	0.
1.923E-03	2.587E-03	2.241E-03	2.200E-03	2.251E-03	0.
2.270E-03	1.346E-03	1.812E-03	4.013E-04	0.	0.
2.639E-03	2.308E-03	2.128E-03	1.801E-03	2.187E-03	2.408E-03
2.130E-03	2.449E-03	2.611E-03	2.252E-03	2.426E-03	0.
2.239E-03	2.184E-03	2.629E-03	1.887E-03	2.379E-03	2.346E-03
2.010E-03	2.384E-03	2.146E-03	1.899E-03	0.	0.
2.184E-03	1.921E-03	1.866E-03	2.529E-03	2.081E-03	1.954E-03
2.010E-03	2.072E-03	2.247E-03	2.295E-03	2.275E-03	0.
1.838E-03	1.948E-03	1.903E-03	1.759E-03	1.948E-03	0.
2.587E-03	2.654E-03	2.571E-03	2.714E-03	0.	0.
2.672E-03	2.355E-03	2.672E-03	2.381E-03	0.	0.
1.720E-03	1.866E-03	1.519E-03	1.632E-03	0.	0.
2.061E-03	2.186E-03	1.933E-03	1.523E-03	1.544E-03	0.
1.135E-03	2.072E-03	1.845E-03	1.868E-03	4.594E-04	0.
1.629E-03	1.216E-03	1.778E-03	1.711E-03	1.633E-03	0.
1.589E-03	1.979E-03	2.046E-03	1.592E-03	2.455E-03	0.
9.330E-04	3.307E-03	1.643E-03	1.889E-03	1.946E-03	0.
2.350E-03	2.010E-03	1.868E-03	1.587E-03	1.829E-03	2.244E-03
1.661E-03	1.589E-03	1.931E-03	1.825E-03	0.	0.
2.717E-03	2.599E-03	2.148E-03	2.692E-03	0.	0.
3.958E-04	1.959E-03	2.185E-03	1.068E-03	1.633E-03	2.097E-03
1.319E-03	1.782E-03	1.980E-03	1.984E-03	1.984E-03	2.062E-03

2.634E-03	2.567E-03	2.442E-03	2.627E-03	2.232E-03	0.
2.247E-03	2.255E-03	2.548E-03	3.299E-04	2.344E-03	1.127E-03
2.950E-04	2.629E-03	2.154E-03	1.802E-03	0.	0.
1.650E-03	1.682E-03	1.565E-03	1.609E-03	0.	0.
1.493E-03	1.448E-03	1.346E-03	1.147E-03	1.314E-03	0.
1.300E-03	1.715E-03	2.399E-03	2.320E-03	1.635E-03	1.403E-03
5.974E-04	1.875E-03	1.913E-03	1.346E-03	1.639E-03	0.
1.553E-03	2.272E-03	1.483E-03	1.881E-04	1.494E-03	1.052E-03
2.607E-03	2.442E-03	2.986E-03	2.738E-03	0.	0.
2.340E-03	2.340E-03	2.128E-03	1.048E-03	8.247E-04	2.082E-03
2.086E-03	2.270E-03	2.170E-03	2.479E-03	0.	0.
1.795E-03	1.544E-03	1.605E-03	4.594E-04	1.065E-03	1.392E-03
2.255E-03	2.048E-03	1.866E-03	1.617E-03	1.921E-03	0.
2.097E-03	1.859E-03	1.889E-03	1.786E-03	1.866E-03	0.
2.635E-03	2.824E-03	1.792E-03	1.958E-03	2.421E-03	0.
2.086E-03	1.875E-03	2.216E-03	2.108E-03	2.112E-03	0.
2.672E-03	3.185E-03	2.703E-03	1.751E-03	2.473E-03	2.497E-03
1.554E-03	1.319E-03	1.773E-03	1.138E-03	9.799E-04	0.
2.270E-03	2.278E-03	1.979E-03	2.187E-03	1.904E-03	0.
1.675E-03	1.720E-03	1.544E-03	1.449E-03	1.399E-03	1.649E-03
2.010E-03	2.086E-03	2.399E-03	2.725E-03	2.614E-03	2.695E-03
2.555E-03	1.868E-03	2.344E-03	2.540E-03	2.240E-03	2.504E-03
2.186E-03	2.003E-03	1.852E-03	1.922E-03	1.915E-03	1.862E-03
1.760E-03	1.977E-03	2.206E-03	1.690E-03	2.136E-03	1.962E-03
1.959E-03	2.164E-03	2.288E-03	1.757E-03	1.795E-03	1.766E-03
2.184E-03	2.278E-03	2.038E-03	2.066E-03	0.	0.

AVERAGE INCREMENTAL VELOCITY

1.977E-03	2.110E-03	2.006E-03	1.771E-03	1.723E-03	1.832E-03


1.42E-01
1.13E-01
8.51E-02
5.67E-02
2.84E-02
0.
-2.84E-02
-5.67E-02
-8.51E-02
-1.13E-01
-1.42E-01
-1.70E-01 -1.36E-01 -1.02E-01 -6.81E-02 -3.40E-02 0. 3.40E-02 6.81E-02 1.02E-01 1.36E-01 1.70E-01

APPENDIX B

The following program was used in the concentration gradient method for calculating the concentration profiles from a given initial profile and from an assumed value for the diffusivity.

1. Read in values for the constants used
   a. Trial value of bacterial diffusivity (cm^2/sec)
   b. Growth rate of the bacteria (1/sec)
   c. Time limit, TMAX, for the duration of the diffusion (min)
2. Set the values of the initial concentration in all sections, if not zero use measured values.

Repeat

3. Calculate the concentration in all sections Δt later than the last profile using the boundary conditions and Eq. (1)

   Section number m =

   1 $\quad C_{\ell,n+1} = C_{\ell,n}(1.0 + \Delta tR)$

   2 through l $\quad C_{m,n+1} = C_{m+1,n}/6 \quad (2/3 + \Delta tR)C_{m,n} + C_{m-1,n}/6$

   l+1 $\quad C_{\ell+1,n+1} = C_{\ell-1,n+1}$
4. Calulate elapsed time for diffusion

   Time (n+1) = Time (N)+ $\frac{\Delta t}{60}$ (min)
5. Print every fiftieth profile
6. Set $C_{m,n} = C_{m,n+1}$
7. When time = TMAX, STOP

```
      PROGRAM TAM      (INPUT,OUTPUT)

      DIFFUSION INTO A FINITE SLAB WITH GIVEN INITIAL DISTRIBUTION

         D=  DIFFUSIVITY  (CM*CM/SEC)
         R = GROWTH RATE  (1/SEC)
         TMAX = TOTAL TIME FOR DIFFUSION  (MIN)
         CAPL = LENGHT OF DIFFUSION CHANNEL  (CM)
         FL(J) = DISTANCE LOCATION OF PRINTS  (PERCENT OF CAPL)
         C(M,N) = CONCENTRATION AT DISTANCE M AND TIME N
         TN = NUMBER OF PRINTS  (1/50 TIME INCREMENTS)
         TK = LENGTH OF TIME INCREMENT  (SEC),  BASED ON D*TK/(H*H) = 1/6
         NUMBER OF DISTANCE INCREMENTS = 100


      DIMENSION  C(105,2), FL(16), CM(16), P(17)

(1)      READ IN DATA
    6 READ 9,  D,R,TMAX
      B=0.
      C(1,1)=1.0
      DATA (FL(J), J=1,15)/0., 3., 6.,10., 15., 20., 25., 30.,
     $35., 40., 45., 50., 60., 70., 100./
      IF (TMAX.EQ.0.0)  STOP
   10 FORMAT (1H1,113H


         THE FOLLOWING RUN SECTION IS CHANGED WITH EACH RUN

     1 RUN NO.  E   8/7/66   E. COLI 27  NUTR  .32*4.5*.0050
      CAPL=4.5
      TIME=5.0

(2)      THE OBSERVED INITIAL DISTRIBUTION IS PROGRAMED ASSUMING LINEAR
         VARIATION BETWEEN DATA POINTS
      DO 42 M=2,4
      C(M,1)=C(M-1,1)-0.019
      IF (C(M,1).LT.0.0)  C(M,1)=0.0
   42 CONTINUE
      DO 43 M=5,12
      C(M,1)=C(M-1,1)-0.120
      IF (C(M,1).LT.0.0)  C(M,1)=0.0
   43 CONTINUE
      DO 44 M=13,102
      C(M,1)=C(M-1,1)-0.00906
      IF (C(M,1).LT.0.0)  C(M,1)=0.0
   44 CONTINUE
         END OF RUN SECTION

         ECHO PRINT OF DATA
      PRINT 10
      PRINT 8,  D,R,CAPL,TMAX

         CALCULATE CONSTANTS USED IN PROGRAM
      TK = CAPL*CAPL/( 6.0E+04*D)
      TN=1.2*TMAX/TK
      DO 41 J=1,15
   41 CM(J)= CAPL*FL(J)/100.

         PRINT PERCENT OF LENGTH AND ACTUAL LENGTH
      PRINT 90, (FL(J), J=1,15)
```

```
      PRINT 91, (CM(J), J=1,15)
      PRINT 92
      P(1)=TIME
      DO 60 J=1,15

         SELECT AND PRINT CONSTANTS USED IN PROGRAM
      M=FL(J)+1.
   60 P(J+1)=C(M,1)
      PRINT 100, (P(J), J=1,16)

(3)      START MAIN TIME LOOP, TIME INCREASES BY T EACH TIME THROUGH

      DO 83 N=1,4000

         CALCULATE BOUNDARY CONCENTRATION AT OPEN END OF DIFFUSION CHANNEL
      C(1,2)=C(1,1)+TK*R*C(1,1)
      DO 55 M=2,101

         BOUNDARY CONDITION THAT DIFFUSION FLUX EQUALS ZERO CLOSED END
      C(102,1)=C(100,1)

         CALCULATE CONCENTRATION , C(M,2) IN ALL DISTANCE INCREMENTS
         FROM PREVIOUS CONCENTRATIONS,  C(M,1)
   55 C(M,2)=C(M+1,1)/6. +(2./3.+TK*R)*C(M,1) +C(M-1,1)/6.
      B=B+1.
(4)   TIME=TK/60. +TIME
(7)   IF (TIME.GT.TMAX)  GO TO 87

         SELECT 50 PROFILES FOR PRINTING
(5)   IF (B.LT.FN)  GO TO 81
      B=0.
      P(1)=TIME
      DO 61 J=1,15
      M=FL(J)+1.
   61 P(J+1)=C(M,2)

         PRINT CONCENTRATION PROFILES
      PRINT 100, (P(J), J=1,16)
   81 CONTINUE
      DO 62 M=1,102
(6)62 C(M,1)=C(M,2)
   83 CONTINUE
         END OF TIME LOOP


   87 CONTINUE

         PRINT THE TOTAL NUMBER OF TIMES THROUGH THE TIME LOOP
      PRINT 7, N
      GO TO 6
    7 FORMAT (/10X,I6,2X,10HITERATIONS)
    8 FORMAT (/1X,  2HU=,E10.3,3X,2HR=,E10.3,3X,5HCAPL=,F7.3,3X,
     $5HTMAX=,F7.1 //)
    9 FORMAT (1X,2E10.3,F10.3)
   90 FORMAT (  1X,7HPERCENT,2X,15F8.1)
   91 FORMAT (/1X,7HLENGTH CM,2X,15F8.3 /)
   92 FORMAT (1X,10HTIME (MIN))
  100 FORMAT(1X, F9.2,15F8.4)
      END
```

RUN NO. E 8/7/66 E. COLI 27 NUTR .3244.5*.0050

E= 2.1111E-05 H= 2.3200E-04 CAPL= 4.500 TMAX= 400.0

PERCENT	C.	3.0	6.0	10.0	15.0	20.0	25.0	30.0	35.0	40.0	45.0	50.0	60.0	70.0	100.0
LENGTH CM	C.	.135	.270	.450	.675	.900	1.125	1.350	1.575	1.800	2.025	2.250	2.700	3.150	4.500
TIME (MIN)															
5.00	1.0000	.9430	.5030	.1030	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.
13.16	1.1200	.9213	.6322	.2260	.0174	.0002	.0000	.0000	.0000	0.	0.	0.	0.	0.	0.
21.31	1.2544	.9930	.8909	.4203	.0605	.0044	.0001	.0000	.0000	.0000	.0000	.0000	.0000	0.	0.
29.47	1.4049	1.0944	1.1008	.6059	.1112	.0163	.0012	.0000	.0000	.0000	.0000	.0000	.0000	.0000	0.
37.62	1.5734	1.2301	1.2930	.8954	.1673	.0355	.0045	.0003	.0000	.0000	.0000	.0000	.0000	.0000	.0000
45.78	1.7622	1.3533	1.4190	.9936	.2295	.0615	.0110	.0013	.0001	.0000	.0000	.0000	.0000	.0000	.0000
53.94	1.9136	1.5561	1.6031	.7034	.2991	.0942	.0212	.0033	.0004	.0000	.0000	.0000	.0000	.0000	.0000
62.09	2.2164	1.7575	1.3272	.8273	.3778	.1319	.0357	.0070	.0010	.0001	.0000	.0000	.0000	.0000	.0000
70.25	2.4757	1.9614	1.5132	.9673	.4673	.1813	.0551	.0128	.0023	.0003	.0000	.0000	.0000	.0000	.0000
78.41	2.7727	2.2349	1.7234	1.1259	.5643	.2375	.0798	.0213	.0044	.0007	.0001	.0000	.0000	.0000	.0000
86.56	3.1054	2.5102	1.9605	1.3054	.6859	.3035	.1108	.0329	.0079	.0015	.0002	.0000	.0000	.0000	.0000
94.72	3.4720	2.8377	2.2279	1.5086	.8192	.3807	.1489	.0483	.0129	.0028	.0005	.0001	.0000	.0000	.0000
102.87	3.8553	3.1901	2.5291	1.7360	.9714	.4709	.1951	.0682	.0200	.0048	.0010	.0002	.0000	.0000	.0000
111.03	4.2620	3.5965	2.8003	1.9588	1.1453	.5758	.2506	.0934	.0296	.0079	.0016	.0003	.0000	.0000	.0000
119.19	4.6851	4.0502	3.2591	2.2931	1.3436	.6975	.3169	.1248	.0423	.0122	.0030	.0006	.0000	.0000	.0000
127.34	5.4723	4.5271	3.6749	2.6259	1.5702	.8364	.3956	.1635	.0588	.0182	.0045	.0011	.0000	.0000	.0000
135.50	6.1289	5.1201	4.1033	3.0021	1.8252	1.0012	.4885	.2106	.0797	.0263	.0076	.0019	.0001	.0000	.0000
143.66	6.8844	5.7090	4.7012	3.4271	2.1222	1.1890	.5977	.2676	.1061	.0370	.0113	.0030	.0001	.0000	.0000
151.81	7.6876	6.4011	5.3190	3.9072	2.4567	1.4052	.7257	.3361	.1384	.0509	.0165	.0047	.0003	.0000	.0000
159.97	8.5102	7.2050	6.0070	4.4493	2.8373	1.6539	.8753	.4180	.1791	.0686	.0234	.0071	.0004	.0000	.0000
168.12	9.4453	8.1009	6.7808	5.0614	3.2700	1.9395	1.0495	.5153	.2284	.0910	.0325	.0104	.0007	.0000	.0000
176.28	10.4504	9.1908	7.6507	5.7521	3.7615	2.2670	1.2521	.6305	.2881	.1190	.0443	.0148	.0012	.0001	.0000
184.44	12.5502	10.3343	8.6260	6.5315	4.3195	2.6423	1.4671	.7664	.3601	.1537	.0594	.0207	.0018	.0001	.0000
192.59	13.5470	11.6074	9.7260	7.4106	4.9529	3.0717	1.7591	.9262	.4465	.1964	.0786	.0286	.0028	.0002	.0000
200.75	15.1730	13.0354	10.9659	8.4021	5.6712	3.5627	2.0735	1.1135	.5497	.2486	.1027	.0387	.0041	.0003	.0000
208.91	16.5535	14.6302	12.3521	9.5200	6.4856	4.1255	2.4363	1.3325	.6724	.3120	.1328	.0517	.0060	.0005	.0000
217.06	19.0325	16.4304	13.9165	10.7801	7.4084	4.7635	2.8543	1.5881	.8178	.3866	.1700	.0683	.0085	.0007	.0000
225.22	21.3106	18.4511	15.6057	12.2002	8.4537	5.4935	3.3353	1.8656	.9895	.4807	.2157	.0892	.0119	.0011	.0000
233.37	23.8736	20.7114	17.6335	13.8034	9.6372	6.3253	3.8601	2.2312	1.1918	.5911	.2716	.1153	.0163	.0017	.0000
241.53	26.7356	23.2473	19.9942	15.6031	10.9767	7.2726	4.5227	2.6321	1.4295	.7228	.3395	.1478	.0222	.0024	.0000
249.69	29.9961	26.0907	22.5324	17.6336	12.4924	8.3505	5.2505	3.0962	1.7080	.8794	.4217	.1880	.0298	.0035	.0000
257.84	33.5251	29.2744	25.1257	19.9263	14.2063	9.5768	6.0942	3.6329	2.0337	1.0650	.5207	.2373	.0397	.0049	.0000
266.00	37.5635	32.8591	28.0612	22.4330	16.1442	10.9707	7.0365	4.2524	2.4137	1.2844	.6396	.2975	.0522	.0069	.0000
274.16	42.0302	36.8047	31.7624	25.3936	18.3347	12.5543	8.1299	4.9659	2.8564	1.5431	.7818	.3708	.0681	.0095	.0000
282.31	47.1179	41.3607	35.7303	28.6562	20.8097	14.3526	9.3771	5.7697	3.3711	1.8474	.9513	.4596	.0881	.0129	.0000
290.47	52.7713	46.4020	40.1703	32.3282	23.6356	16.3937	10.8013	6.7363	3.9066	2.2045	1.1526	.5667	.1131	.0174	.0000
298.62	59.1026	52.0544	45.1012	36.4802	26.7631	18.7094	12.4263	7.8242	4.6612	2.6228	1.3917	.6954	.1443	.0232	.0000
306.78	66.1942	58.3420	50.7377	41.1092	30.3280	21.3355	14.2794	9.0731	5.4630	3.1117	1.6742	.8497	.1828	.0308	.0001
314.94	74.1204	65.4975	57.0405	46.3394	34.3519	24.3124	16.3912	10.5058	6.3900	3.6823	2.0075	1.0341	.2303	.0404	.0001
323.09	83.0215	73.4058	64.0942	52.2225	38.8430	27.6857	18.7964	12.1477	7.4603	4.3470	2.3996	1.2537	.2886	.0526	.0001
331.25	92.6938	82.3950	72.0098	58.8394	44.0164	31.5067	21.5344	14.0280	8.6945	5.1202	2.8607	1.5146	.3598	.0681	.0002
339.41	104.1515	92.4076	80.8955	66.2607	49.7957	35.8334	24.6494	16.1795	10.1173	6.0123	3.4012	1.8240	.4464	.0876	.0003
347.56	116.6476	103.0323	90.6005	74.0482	56.3135	40.7312	28.1916	18.6397	11.7545	7.0601	4.0338	2.1899	.5514	.1120	.0005
355.72	130.6436	116.2157	102.0012	84.0502	63.6628	46.2733	32.2178	21.4509	13.6372	8.2671	4.7730	2.6217	.6784	.1423	.0007
363.87	146.3180	130.3217	114.6221	94.6330	71.9481	52.5433	36.7919	24.6611	15.8003	9.6637	5.6356	3.1303	.8314	.1799	.0010
372.03	163.8744	146.1344	128.7170	106.5227	81.2870	59.6346	41.9104	28.3248	18.2835	11.2790	6.6434	3.7282	1.0152	.2263	.0014
380.19	183.5263	163.8954	144.5550	119.8070	91.8117	67.6522	47.8829	32.5045	21.1317	13.1420	7.8096	4.4300	1.2355	.2833	.0020
388.34	205.5771	183.7209	162.2633	134.9074	103.6708	76.7149	54.5738	37.2671	24.3963	15.2920	9.1680	5.2522	1.4988	.3532	.0028
396.50	230.2112	206.0006	182.1974	151.7878	117.0313	86.9564	62.1633	42.6945	28.1355	17.7676	10.7446	6.2141	1.8128	.4386	.0039

14CS ITERATIONS

APPENDIX C

The following program was used in the tube diffusion method for calculating the fraction remaining, ϕ , as a function of the dimensionless time, Dt/ℓ^2

1. Read in the values of the constants used.
   a. Bacterial diffusivity, D (cm^2/sec)
   b. Multiplication rate of bacteria, R (1/sec)
   c. Sedimentation velocity of bacteria, U (cm/sec)
   d. Length of capillary tube, CAPL (cm)
   e. Length of time for diffusion, TMAX (sec)
   f. Number of length segments in the tube, DH
   g. Number of Δt iterations, DT

   DT was usually selected by setting

$$1/6 = AMOD = \frac{\Delta t D}{\Delta x^2} \quad \text{or} \quad \frac{DH^2 \cdot TMAX \cdot D}{CAPL^2 \cdot DT}$$

   AMOD must be greater than EMOD,

$$\frac{\Delta t U}{2\Delta x}, \quad \text{or} \quad \frac{TMAX \cdot DH}{2 \cdot DT \cdot CAPL}$$

2. Print AMOD and echo print input
3. Calculate constants used in program for Eq. (2)

$$AL = \frac{D\Delta t}{\Delta x^2} - \frac{\Delta t U}{2\Delta x}, \quad AM = 1 \quad R\Delta t - \frac{2D\Delta t}{\Delta x^2}$$

$$AN = \frac{D\Delta t}{\Delta x^2} + \frac{\Delta t U}{2\Delta x}$$

4. Set initial concentrations in the sections of the tube equal to 1.0

Calculate the concentrations, $C_{m,n+1}$, from the known concentrations $C_{m,n}$ for all sections, m, of the tube at time, n+1, Δt later than time n.

5. First section, $C_{m,n+1} = 0.0$
6. Second through the first 95% of the sections

   Eq. (2) $C_{m,n+1} = AN\ C_{m-1,n} + AM\ C_{m,n} + AL\ C_{m+1,n}$
7. Last 5% of sections are equal to:

   Eq. (3) $C_{m,n+1} = (AN\ C_{m-1,n} - AL\ C_{m,n})\ 20/DH + (1 + R\Delta t)\ C_{m,n}$

≤ 50 8. After calculating every fiftietn profile make the following calculations

9. Calculate elapsed time

   time (min) = $\Sigma\Delta t/60$
10. Calculate THETA

    Theta = $D\ t/l^2 = D$ time(sec)/$CAPL^2$
11. Integrate the profile and find average concentration

    $C_{ave} = \Sigma C_{m,n}/m$
12. Calculate PHI $PHI = C_{ave}/\exp(R \cdot time)$
13. Print final concentration profile
14. Print: time, PHI, THETA

```
PROGRAM BONES   (INPUT,OUTPUT)

     RESTRICTED DIFFUSION OF BACTERIA OUT OF A CAPILLARY TUBE
     WITH SEDIMENTATION AND GROWTH


       D = DIFFUSIVITY
       R = GROWTH RATE ( 1/SEC )
       U = SEDIMENTATION RATE ( CM/SEC )
       C(M,N) = CONC. AT POSITION M AT TIME N
       CAPL = LENGTH OF CAPILLARY TUBE (CM )
       CAVE = AVERAGE CONCENTRATION IN TUBE
       CTO = AMOUNT IN TUBE IF NONE ESCAPE
       PHI = FRACTION LEFT IN TUBE
       THETA = D * TIME / CAPL * CAPL
       TMAX = TOTAL TIME FOR DIFFUSION ( SEC )
       DH = NUMBER OF DISTANCE INCREMENTS
       DT = NUMBER OF TIME INCREMENTS


DIMENSION C(999,2),TIME(100),CAVE(100),CTO(100),PHI(100),
1THETA(100),CSOLV(100),PHIC(100)

(1)    READ IN DATA
6 READ 9,D,R,U,CAPL,TMAX,DH,DT
IF((TMAX.EQ.0.).OR.(CAPL.EQ.0.)) STOP
H=CAPL/DH
T=TMAX/DT
AMOD=T*D/(H*H)
BMOD = U*T/(2.*H)
IF (AMOD.GT.0.5)  GO TO 6

(2)    ECHO PRINT OF DATA
PRINT 8, D,R,U,CAPL,TMAX,AMOD,DH,DT

(3)    CALCULATE CONSTANTS USED IN PROGRAM
AL = AMOD - BMOD
AM=1.-2.*AMOD+T*R
AN = AMOD + BMOD

       SET COUNTERS EQUAL TO ZERO
A=0.
B=0.
L=0

       STARTING VALUE SELECTED AT INTERSECTION OF BOUNDARY CONDITION
       C(M,1) = 1.0  AND C(1,N) = 0.0
C(1,1)=.5
I=DH+1.
IF (I.GT.1000)  GO TO 84

(4)    SET INITIAL CONDITIONS THAT  TUBE CONC EQUALS  1.0
DO 45 M=2,I
45 C(M,1)=1.
CT=1.0
J=DT


       START MAIN TIME LOOP,  TIME INCREASES BY T EACH TIME THROUGH

DO 83 N=1,J
```

```
(5)   C(1,2)=0.
      I=DH + 1.0
      MO=0.95*DH

         CALCULATE CONCENTRATION, C(M,2) IN ALL DISTANCES INCREMENTS
         FROM PREVIOUS CONCENTRATIONS, C(M,1)
      DO 54 M=2,MO
(6)54 C(M,2)=AN*C(M-1,1)+AM*C(M,1)+AL*C(M+1,1)
      MY=MO+1
(7)   C(MY,2)= (AN*C(MY-1,1) - AL*C(MY,1))*20./DH +(1.+R*T)*C(MY,1)
      DO 57 M=MY,I
   57 C(M,2)=C(MY,2)
      CT=(1.0+T*R)*CT
   50 A=A+1.
      B=B+1.

         SELECT FIFTY EVENLY SPACED CONCENTRATION  PROFILES FOR PRINTING
(8)   IF  (B.LT.DT/50.)  GO TO 81
      L=L+1
(9)   TIME(L)=(A*T)
      TH= TIME(L)/60.
(10)74 THETA(L)=D*TIME(L)/CAPL**2
      B=0.
      CTO(L)=CT
      I=DH

         INTEGRATE THE SELECTED CONCENTRATION PROFILES
(11)  SUM=C(I+1,2)/2.
      DO 78 M=1,I
   78 SUM=SUM+C(M,2)

         CALCULATE AND STORE QUANTITIES BASED ON INTEGRATION
      CAVE(L)=SUM/DH
      CSOLV(L)=CTO(L)-CAVE(L)
(12)  PHI(L)=CAVE(L)/CTO(L)
      PHIC(L)=1.0-PHI(L)
   81 CONTINUE
      I=DH+1.
      DO 82 M=1,I
   82 C(M,1)=C(M,2)
   83 CONTINUE
         END OF TIME LOOP

   84 CONTINUE
      PRINT 99
      M=DH/10.+1.
      J=DH/10.
      K=DH+1.

(13)     PRINT CONCENTRATION PROFILE
      PRINT 100, (C(I,2),I=2,6), (C(I,2),I=M,K,J)
      J=L
      PRINT 101

(14)     PRINT INTEGRATED QUANTATIES
      PRINT 102,       (TIME(L),CAVE(L),CTO(L),PHI(L),THETA(L),PHIC(L),
     $CSOLV(L),L=1,J)
      CE=EXP(R*TMAX)
      CR=100.*(CT-CE)/CE

         PERCENT ERROR CAUSED BY USING FINITE DIFFERENCE INSTEAD OF AN
         EXPONENTIAL TO CALCULATE GROWTH
```

```
      PRINT 103, CR
      I=DI + 1.0
    8 FORMAT(1H1,2HD=,E10.3,3X,2HR=,E10.3,3X,
     $2HU=,E10.3,3X,5HCAPL=,F7.3,3X,5HTMAX=,
     $E10.3,3X,5HAMOD=,E10.3,2X,3HDH=,F7.0,2X,3HDT=,F7.0//)
    9 FORMAT (1X,3E10.3,F10.3, E10.3,2F10.1)
   99 FORMAT (3X,2H1.,6X,2H2.,6X,2H3.,6X,2H4.,5X,3H 5.,5X,3H10.,
     $5X,3H20.,5X,3H30.,5X,3H40.,5X,3H50.,
     $5X,3H60.,5X,3H70.,5X,3H80.,5X,3H90.,4X,4H100. /)
  100 FORMAT  (1X, F7.4, 13F8.3, F8.2//)
  101 FORMAT(        15X,4HTIME,11X,4HCAVE,12X,3HCT3,12X,3HPHI,12X,
     $5HTHETA,8X,7H1.0-PHI,10X,5HCSOLV /)
  102 FORMAT  (8X,7E15.5)
  103 FORMAT(/80X,23HPERCENT ERROR IN TIME =,E10.3)
      GO TO 6
      END
```

D= 2.500E-05 R= 2.800E-04 J= 1.000E-05 SAPL= 1.993 TMAX= 1.800E+06 ANOD= 1.693E-31 DT= 27123

1.	2.	3.	4.	5.	10.	20.	30.	40.	50.	60.	70.	80.	90.	100.
1.0399	2.082	3.129	4.179	5.236	21.419	43.675	65.706	87.007	107.064	125.553	142.963	159.233	174.793	191.55

TIME	DAVE	CTD	PHI	THETA	1.0-PHI	CSDLV
9.63998E+02	1.06749E+00	1.10617E+00	9.45951E-01	2.23972E-03	5.37692E-02	5.86914E-02
7.23796E+02	1.18252E+00	1.22361E+00	9.25561E-01	4.59665E-03	7.44332E-02	9.13165E-02
1.08119E+03	1.23055E+00	1.35352E+00	9.33351E-01	6.89657E-03	9.06627E-02	1.22696E-01
1.44159E+03	1.34126E+00	1.49722E+00	8.95894E-01	9.19289E-03	1.04152E-01	1.55393E-01
1.80199E+03	1.45411E+00	1.55617E+00	9.84232E-01	1.14911E-02	1.15968E-01	1.92063E-01
2.16239E+03	1.60016E+00	1.83201E+00	8.73443E-01	1.37393E-02	1.25557E-01	2.31953E-01
2.52279E+03	1.75345E+00	2.02651E+00	8.63775E-01	1.60876E-02	1.36224E-01	2.76039E-01
2.88319E+03	1.91626E+00	2.24155E+00	8.56831E-01	1.43858E-02	1.45152E-01	3.25404E-01
3.24358E+03	2.03732E+00	2.47965E+00	8.46495E-01	2.08609E-02	1.55334E-01	3.33539E-01
3.60398E+03	2.30335E+00	2.74292E+00	8.33552E-01	2.29872E-02	1.61344E-01	4.42554E-01
3.96438E+03	2.52207E+00	3.03413E+00	8.31233E-01	2.52935E-02	1.53757E-01	5.12363E-01
4.32478E+03	2.75617E+00	3.35625E+00	8.24182E-01	2.75787E-02	1.75818E-01	5.93349E-01
4.68518E+03	3.03647E+00	3.71258E+00	8.17454E-01	2.98759E-02	1.82344E-01	6.77716E-01
5.04558E+03	3.33251E+00	4.10674E+00	8.11012E-01	3.21751E-02	1.88988E-01	7.75125E-01
5.40597E+03	3.65611E+00	4.54275E+00	8.04926E-01	3.44734E-02	1.95175E-01	8.86534E-01
5.76637E+03	4.01436E+00	5.02504E+00	7.98866E-01	3.67716E-02	2.01134E-01	1.01379E+00
6.12677E+03	4.40857E+00	5.55854E+00	7.93117E-01	3.92598E-02	2.06883E-01	1.16937E+00
6.48717E+03	4.84244E+00	6.14953E+00	7.87555E-01	4.13680E-02	2.12444E-01	1.33529E+00
6.84757E+03	5.31991E+00	6.80148E+00	7.82172E-01	4.35552E-02	2.17828E-01	1.48157E+00
7.20796E+03	5.84939E+00	7.52358E+00	7.76942E-01	4.59665E-02	2.23058E-01	1.67819E+00
7.56836E+03	6.42371E+00	8.32295E+00	7.71962E-01	4.82627E-02	2.28138E-01	1.89556E+00
7.92876E+03	7.06019E+00	9.20592E+00	7.66918E-01	5.05609E-02	2.33332E-01	2.14573E+00
8.28916E+03	7.75070E+00	1.01833E+01	7.62101E-01	5.28591E-02	2.37899E-01	2.42260E+00
8.64956E+03	8.51171E+00	1.12644E+01	7.57409E-01	5.51576E-02	2.42593E-01	2.73278E+00
9.00996E+03	9.39033E+00	1.24604E+01	7.52813E-01	5.74556E-02	2.47187E-01	3.05326E+00
9.37035E+03	1.03144E+01	1.37833E+01	7.48327E-01	5.97539E-02	2.51673E-01	3.46997E+00
9.73075E+03	1.13426E+01	1.52466E+01	7.43939E-01	6.20520E-02	2.56061E-01	3.90635E+00
1.00912E+04	1.24743E+01	1.68653E+01	7.39562E-01	6.43503E-02	2.60359E-01	4.39173E+00
1.04515E+04	1.37201E+01	1.86559E+01	7.35439E-01	6.66485E-02	2.64573E-01	4.93578E+00
1.08119E+04	1.53915E+01	2.06366E+01	7.31503E-01	6.89467E-02	2.68700E-01	5.54534E+00
1.11723E+04	1.66012E+01	2.28275E+01	7.27767E-01	7.12449E-02	2.72753E-01	6.22529E+00
1.15327E+04	1.82632E+01	2.52411E+01	7.23266E-01	7.35432E-02	2.76734E-01	6.93783E+00
1.18931E+04	2.00929E+01	2.79119E+01	7.19354E-01	7.59414E-02	2.80646E-01	7.83697E+00
1.22535E+04	2.21373E+01	3.08974E+01	7.15508E-01	7.81396E-02	2.84492E-01	8.79337E+00
1.26139E+04	2.43251E+01	3.41777E+01	7.11724E-01	8.04379E-02	2.88276E-01	9.85252E+00
1.29743E+04	2.67669E+01	3.78263E+01	7.07999E-01	8.27360E-02	2.92331E-01	1.10375E+01
1.33347E+04	2.94522E+01	4.18232E+01	7.04331E-01	8.50343E-02	2.95669E-01	1.23669E+01
1.36951E+04	3.24159E+01	4.62601E+01	7.00717E-01	8.73325E-02	2.99283E-01	1.33669E+01
1.40555E+04	3.56744E+01	5.11715E+01	6.97154E-01	8.96307E-02	3.02846E-01	1.54971E+01
1.44159E+04	3.92631E+01	5.66043E+01	6.93661E-01	9.19289E-02	3.06339E-01	1.73419E+01
1.47763E+04	4.32165E+01	6.26139E+01	6.90174E-01	9.42272E-02	3.09826E-01	1.93331E+01
1.51367E+04	4.75656E+01	6.92615E+01	6.86753E-01	9.65254E-02	3.13247E-01	2.16950E+01
1.54971E+04	5.23567E+01	7.66149E+01	6.83375E-01	9.88236E-02	3.16625E-01	2.42587E+01
1.58575E+04	5.75376E+01	8.47490E+01	6.80039E-01	1.01122E-01	3.19961E-01	2.71154E+01
1.62179E+04	6.34473E+01	9.37466E+01	6.76742E-01	1.03420E-01	3.23258E-01	3.03063E+01
1.65783E+04	6.91422E+01	1.03700E+02	6.73484E-01	1.05718E-01	3.26516E-01	3.33595E+01
1.69387E+04	7.60853E+01	1.14709E+02	6.73263E-01	1.08017E-01	3.29737E-01	3.78239E+01
1.72991E+04	8.45439E+01	1.26888E+02	6.67077E-01	1.10315E-01	3.32923E-01	4.22639E+01
1.76595E+04	9.31891E+01	1.40359E+02	6.63925E-01	1.12613E-01	3.36074E-01	4.71711E+01

PERCENT ERROR IN TIME =-6.692E-02

APPENDIX D

The following program was used for calculating the diffusion of E. coli into a crack filled with nutrient medium.

1. Read in the values of the constants used
   a. Bacterial diffusivity, D (cm^2/sec)
   b. Multiplication rate of the bacteria in the reservoir, R (1/sec)
   c. Multiplication rate of the bacteria in the crack G (1/sec)
   d. Velocity of the flow of the bulk medium, U(cm/sec)
   e. Length of time for diffusion, TMAX (sec)
   f. Length of capillary crack, CAPL (cm)
   g. Number of length segments in crack, DH
   h. Number of Δt iterations, DT
      DT was usually selected by setting AMOD = 1/6 and AM OD > BMOD, as in Appendix C
2. Print AMOD and echo print input constants
3. Calculate constants used in the program.
4. Set the values of the initial concentrations in all sections equal to zero, except for the first section which equals 1.0 at all times.
5. Calculate the concentration in all sections Δt later than the last profile using Eqs. (4,5,6)
6. Calculate the concentration in section 2 through section ℓ-1, use Eq. (4)
7. Calculate the concentration in the last section, Eq.(5)
8. Calculate the concentration in the reservoir, Eq. (6)
9. Set any concentrations that are greater than the entrance concentration equal to the entrance concentration.
10. Print every fiftieth concentration profile
11. Locate the increment number and position of the first section whose concentration is less than 1, 10^{-3}, 10^{-6}, 10^{-9}, and 10^{-12}

(12) Print the increment number and the distance to the above concentrations.

```
      PROGRAM LEAK    (INPUT,OUTPUT)

      DIFFUSION OF BACTERIA COUNTER TO CONVECTIVE FLOW

           D= DIFFUSIVITY (CM/SEC)
           R= GROWTH RATE IN RESERVOIR  (1/SEC)
           G= GROWTH RATE IN THE TUBE  (1/SEC)
           TMAX = TOTAL TIME FOR DIFFUSION  (SEC)
           CAPL= LENGTH OF CAPILLARY TUBE  (CM)
           DH = NUMBER OF LENGTH INCREMENTS
           DT = NUMBER OF TIME INCREMENTS
           FL(J) =LOCATION FOR PRINTOUTS, PERCENT OF LENGTH

      DIMENSION C(1005,2), FL(15), P(17), Q(100,11), Z(5)
(1)6  READ 9, D,R,G,U,TMAX,CAPL,DH,DT
      IF (TMAX.EQ.0.)  STOP
      DATA (FL(J),J=1,15)/0., 5., 10., 15., 20., 25., 30., 40.,
     $50., 60., 70., 80., 90., 100., 101./
      H=CAPL/DH
      T=TMAX/DT
      AMOD=T*D/(H*H)
      IF (AMOD.GT.0.49)  GO TO 6
(2)   PRINT 8, D,R,G,U,TMAX,CAPL,AMOD,DH,DT
      PRINT 90, (FL(J), J=1,15)
      PRINT 92

(3)        CALCULATE CONSTANTS USED IN THIS PROGRAM

      BMOD=U*T*0.5/H
      AL=AMOD-BMOD
      AM=1.0-2.*AMOD+T*G
      AN=AMOD+BMOD
      CM=(1.0+T*R)
      I=DH+2.0

(4)        SET INITIAL VALUES FOR ALL CONCENTRATION INCREMENTS
      DO 45 M=2,I
   45 C(M,1)=0.
      C(1,1)=1.0

           SET CONSTANTS AND COUNTERS
      TOP=1.0
      TIME=0.
      IT=0
      B=0.
      JQ=DT

           MAIN TIME LOOP, EACH TIME BY HERE INCREASES TIME BY DT

      DO 83 N=1,JQ
      C(1,2)=C(1,1)+R*T
      I=DH

           START CALCULATING THE CONCENTRATIONS, C(M,2), FOR THE NEXT
(5)        TIME INTERVAL FROM THE CONCENTRATIONS,C(M,1), ATTHE
           PREVIOUS TIME INTERVAL
```

```
      DO 54 M=2,I
(6)   C(M,2)=AN*C(M+1,1)+AM*C(M,1)+AL*C(M-1,1)
   54 CONTINUE
(7)   C(I+1,2)=AM*C(I+1,1)+AL*C(I,1)
(8)   C(I+2,2)=CM*C(I+2,1)+AL   *C(I+1,1)
      I=DH+2.0

           TEST MAGNITUDE OF NEW CONCENTRATIONS
      DO 57 M=1,I
(9)   IF (C(M,2).GT.TOP)  C(M,2)=TOP
      IF (C(M,2).LT.0.)  C(M,2)=0.
   57 CONTINUE
      TIME=TIME+T
      B=B+1.

           PRINT ONLY 50  OF THE CALCULATED PROFILES
(10)  IF (B.LT.DT/50.)   GO TO 81
      B=0.
      P(1)=TIME/3600.
      DO 60 J=2,15

           LOCATE THE INCERMENTS TO BE PRINTED
      M=FL(J-1)*DH/100.+1.0
   60 P(J)=C(M,2)
      P(16)=C(M+1,2)

           PRINT THE SELECTED CONCENTRATIONS
      PRINT 100, (P(J), J=1,16)
      IT=IT+1
      Q(IT,1)=TIME/3600.
      I=DH+2.0
      DO 813 M=1,I

(11)       LOCATE INCREMENT NUMBER OF THE FIRST CONC. LESS THAN  1.0
      IF  (C(M,2).LT.TOP)   GO TO 814
  813 CONTINUE
  814 Z(1)=M
      JL=M
      DO 815 M=JL,I

(11)       LOCATE INCREMENT NUMBER OF THE FIRST CONC. LESS THAN 1.E-03
      IF (C(M,2).LT.1.E-03)  GO TO 816
  815 CONTINUE
  816 Z(2)=M
      JL=M
      DO 817 M=JL,I

(11)       LOCATE INCREMENT NUMBER OF THE FIRST CONC. LESS THAN 1.E-06
      IF (C(M,2).LT.1.E-06)  GO TO 818
  817 CONTINUE
  818 Z(3)=M
      JL=M
      DO 819 M=JL,I

(11)       LOCATE INCREMENT NUMBER OF THE FIRST CONC. LESS THAN 1.E-09
      IF (C(M,2).LT.1.E-09)  GO TO 820
  819 CONTINUE
  820 Z(4)=M
      JL=M
      DO 821 M=JL,I
```

```
(11)        LOCATE INCREMENT NUMBER OF THE FIRST CONC. LESS THAN 1.E-12
      IF (C(M,2).LT.1.E-12)  GO TO 822
  821 CONTINUE
  822 Z(5)=M
      DO 824  MT=2,6

          CALCULATE THE DISTANCE TO THE CONC. OF INTEREST
      Q(IT,MT)= (Z(MT-1) -1.5)*4
      Q(IT,MT+5)= Z(MT-1) -1.5
  824 CONTINUE
   81 CONTINUE
      I=DH+2.0
      DO 82 M=1,I

          CALL NEW CONC. OLD CONC. AND REPEAT TIME LOOP
   82 C(M,1)=C(M,2)
   83 CONTINUE


          PRINT HEADINGS

      PRINT8 ,D,R,G,J,TMAX,CAPL,AMOD,DH,DT
      PRINT 101
      PRINT 102

(12)      PRINT TIME, DISTANCES, AND INCREMENT NUMBERS
      PRINT 103,  ((Q(L,K),K=1,11), L=1,IT)
      PRINT 104, IT
      GO TO 6
    9 FORMAT (1X,E9.2, 4E10.2, F10.3 ,2F10.1)
    8 FORMAT (/1H1,2HD=,E9.2,3X,2HR=,E9.2,3X,2HG=,E9.2,3X,2HJ=,E9.2,
     $3X,5HTMAX=,E9.2,3X,5HCAPL=,F5.2,3X, 5HAMOD=,E9.2,
     $3X,3HDH=,F5.0, 3X,3HDT=,F6.0//)
   90 FORMAT (  1X,7HPERCENT,2X,15F8.1)
   92 FORMAT (1X,10HTIME (HRS))
  100 FORMAT(1X, F9.2,15E8.1)
  101 FORMAT  (          2X,10HTIME (HRS), 5X,13HDISTANCE (CM),
     $                          50X,16HINCREMENT NUMBER)
  102 FORMAT  (/5X,9HLOG C/CO=,2X,2H0.,8X,2H3.,8X,2H6.,8X,2H9.,7X,3H12.,
     $  23X,2H0.,8X,2H3.,8X, 2H6., 8X,2H9., 7X,3H12. //)
  103 FORMAT (1X,F9.3, 5E10.2, 13X, 5F10.1)
  104 FORMAT (//1X,3HIT=, I6 )
      END
```

D= 2.00E-05 R= 2.PCE-04 G= 2.80E-04 U= 1.00E-04 TMAX= 2.88E+04 CAPL= 10.00 AMOD= 1.65E-01 DH= 400 DT= 5600

PERCENT TIME (HRS)	0.	5.0	10.0	15.0	20.0	25.0	30.0	40.0	50.0	60.0	70.0	80.0	90.0	100.0	101.0
.16	1.0E+00	3.1E-04	4.1E-12	5.5E-25	8.9E-43	2.9E-68	0.	0.	0.	0.	0.	0.	0.	0.	0.
.32	1.0E+00	6.5E-03	3.1E-07	7.7E-14	9.0E-23	4.2E-34	6.3E-49	5.8E-84	4.4-135	0.	0.	0.	0.	0.	0.
.48	1.0E+00	2.0E-02	1.5E-05	3.4E-10	2.4E-16	4.5E-24	7.3E-33	8.4E-57	4.5E-97	4.4-125	8.1-173	3.0-236	0.	0.	0.
.64	1.0E+00	3.4E-02	1.1E-04	7.5E-08	4.1E-13	4.6E-19	3.6E-26	6.7E-44	1.6E-66	3.9E-94	3.9-127	3.5-166	1.0-212	1.8-268	5.1-270
.80	1.0E+00	4.4E-02	3.5E-04	3.4E-07	3.8E-11	5.0E-16	7.7E-22	2.9E-36	1.7E-54	1.4E-76	1.2-102	7.3-133	1.4-167	2.9-207	3.0-208
.96	1.0E+00	7.3E-02	5.1E-04	2.0E-06	8.1E-10	5.4E-14	6.2E-19	3.7E-31	1.6E-46	4.9E-65	8.8E-87	6.0-112	2.8-140	2.4-172	4.8-173
1.12	1.0E+00	8.9E-02	1.7E-03	7.6E-06	7.4E-09	1.6E-12	7.6E-17	1.7E-27	8.1E-41	7.7E-57	1.4E-75	4.4E-97	2.1-121	1.2-149	3.7-149
1.28	1.0E+00	1.1E-01	2.4E-03	2.1E-05	4.0E-08	2.1E-11	2.5E-15	9.9E-25	1.6E-36	1.1E-50	3.3E-67	4.1E-86	1.9-107	3.0-131	1.3-131
1.44	1.0E+00	1.3E-01	4.3E-03	4.6E-05	1.5E-07	1.6E-10	5.0E-14	1.4E-22	3.4E-33	6.7E-46	1.0E-60	1.3E-77	1.7E-96	7.1-119	4.0-118
1.60	1.0E+00	1.4E-01	6.0E-03	8.9E-05	4.6E-07	9.2E-10	5.0E-13	7.8E-21	1.6E-30	4.6E-42	1.7E-55	8.0E-71	4.9E-88	3.1-107	2.2-107
1.76	1.0E+00	1.6E-01	8.1E-03	1.5E-04	1.1E-06	3.2E-09	3.4E-12	2.1E-19	2.6E-28	6.4E-39	3.1E-51	2.9E-65	5.7E-81	1.5E-98	1.3E-98
1.92	1.0E+00	1.7E-01	1.0E-02	2.5E-04	2.5E-06	1.0E-08	1.7E-11	3.3E-18	1.9E-26	2.8E-36	1.1E-47	1.3E-60	3.9E-75	2.5E-91	2.6E-91
2.08	1.0E+00	1.9E-01	1.3E-02	3.9E-04	4.8E-06	2.7E-08	6.7E-11	3.5E-17	6.7E-25	4.7E-34	1.2E-44	1.1E-56	3.5E-70	3.2E-85	4.0E-85
2.24	1.0E+00	2.1E-01	1.6E-02	5.4E-04	8.6E-06	6.3E-08	2.2E-10	2.7E-16	1.5E-23	3.9E-32	4.7E-42	2.6E-53	6.3E-66	5.5E-80	8.1E-80
2.40	1.0E+00	2.2E-01	1.9E-02	7.5E-04	1.4E-05	1.3E-07	6.2E-10	1.6E-15	2.2E-22	1.8E-30	8.5E-40	2.2E-50	3.1E-62	1.9E-75	3.3E-75
2.56	1.0E+00	2.4E-01	2.2E-02	1.0E-03	2.3E-05	2.6E-07	1.6E-09	7.4E-15	2.4E-21	5.4E-29	8.1E-38	8.1E-48	5.5E-59	1.8E-71	3.5E-71
2.72	1.0E+00	2.6E-01	2.6E-02	1.3E-03	3.5E-05	4.8E-07	3.6E-09	3.0E-14	2.0E-20	1.1E-27	4.6E-36	1.5E-45	4.0E-56	5.9E-69	1.3E-67
2.88	1.0E+00	2.8E-01	3.0E-02	1.7E-03	5.0E-05	8.3E-07	7.5E-09	1.0E-13	1.3E-19	1.6E-26	1.7E-34	1.6E-43	1.4E-53	7.9E-65	2.0E-64
3.04	1.0E+00	3.0E-01	3.5E-02	2.1E-03	7.1E-05	1.4E-06	1.5E-08	3.2E-13	7.3E-19	1.7E-25	4.2E-33	1.1E-41	2.7E-51	5.0E-62	1.4E-61
3.20	1.0E+00	3.1E-01	3.9E-02	2.4E-03	9.8E-05	2.1E-06	2.7E-08	8.9E-13	3.4E-18	1.5E-24	7.7E-32	4.6E-40	3.2E-49	1.6E-59	5.1E-59
3.36	1.0E+00	3.3E-01	4.4E-02	3.7E-03	1.3E-04	3.3E-06	4.8E-08	2.3E-12	1.4E-17	1.1E-23	1.1E-30	1.4E-38	2.3E-47	3.2E-57	1.1E-56
3.52	1.0E+00	3.5E-01	5.0E-02	3.8E-03	1.7E-04	4.8E-06	8.1E-08	5.3E-12	5.0E-17	6.6E-23	1.2E-29	3.2E-37	1.2E-45	3.8E-55	1.4E-54
3.68	1.0E+00	3.7E-01	5.5E-02	4.6E-03	2.3E-04	6.9E-06	1.3E-07	1.2E-11	1.6E-16	3.4E-22	1.1E-28	5.6E-36	4.3E-44	3.1E-53	1.3E-52
3.84	1.0E+00	4.0E-01	6.1E-02	5.4E-03	2.9E-04	9.7E-06	2.1E-07	2.4E-11	4.8E-16	1.6E-21	8.6E-28	7.7E-35	1.2E-42	1.7E-51	7.7E-51
4.00	1.0E+00	4.2E-01	6.8E-02	6.3E-03	3.6E-04	1.3E-05	3.1E-07	4.8E-11	1.3E-15	6.4E-21	5.6E-27	8.8E-34	2.4E-41	6.9E-50	3.4E-49
4.16	1.0E+00	4.4E-01	7.5E-02	7.3E-03	4.5E-04	1.8E-05	4.7E-07	9.0E-11	3.4E-15	2.4E-20	3.2E-26	8.3E-33	4.1E-40	2.1E-48	1.1E-47
4.32	1.0E+00	4.7E-01	8.2E-02	8.5E-03	5.5E-04	2.4E-05	6.7E-07	1.6E-10	8.1E-15	8.0E-20	1.6E-25	6.7E-32	5.5E-39	5.1E-47	2.9E-46
4.48	1.0E+00	4.9E-01	9.0E-02	9.7E-03	6.7E-04	3.1E-05	9.6E-07	2.8E-10	1.8E-14	2.5E-19	7.4E-25	4.7E-31	6.3E-38	9.9E-46	6.1E-45
4.64	1.0E+00	5.2E-01	9.8E-02	1.1E-02	8.1E-04	4.0E-05	1.3E-06	4.8E-10	3.9E-14	7.3E-19	3.0E-24	2.9E-30	6.1E-37	1.6E-44	1.0E-43
4.80	1.0E+00	5.4E-01	1.1E-01	1.3E-02	9.7E-04	5.1E-05	1.8E-06	7.9E-10	8.1E-14	2.0E-18	1.1E-23	1.6E-29	5.2E-36	2.1E-43	1.5E-42
4.96	1.0E+00	5.7E-01	1.2E-01	1.4E-02	1.2E-03	6.4E-05	2.5E-06	1.3E-09	1.6E-13	5.1E-18	4.0E-23	7.8E-29	3.8E-35	2.3E-42	1.8E-41
5.12	1.0E+00	6.0E-01	1.3E-01	1.6E-02	1.4E-03	8.0E-05	3.3E-06	2.0E-09	3.1E-13	1.2E-17	1.3E-22	3.5E-28	2.5E-34	2.3E-41	1.8E-40
5.28	1.0E+00	6.3E-01	1.4E-01	1.8E-02	1.6E-03	9.8E-05	4.3E-06	3.0E-09	5.6E-13	2.9E-17	3.9E-22	1.5E-27	1.5E-33	1.9E-40	1.7E-39
5.44	1.0E+00	6.6E-01	1.5E-01	2.0E-02	1.9E-03	1.2E-04	5.6E-06	4.5E-09	1.0E-12	6.3E-17	1.1E-21	5.6E-27	7.7E-33	1.4E-39	1.3E-38
5.60	1.0E+00	6.9E-01	1.6E-01	2.3E-02	2.2E-03	1.5E-04	7.2E-06	6.6E-09	1.7E-12	1.3E-16	3.0E-21	2.0E-26	3.8E-32	9.7E-39	9.5E-38
5.76	1.0E+00	7.2E-01	1.7E-01	2.5E-02	2.5E-03	1.8E-04	9.1E-06	9.5E-09	3.0E-12	2.8E-16	7.8E-21	6.6E-26	1.7E-31	5.9E-38	6.1E-37
5.92	1.0E+00	7.5E-01	1.8E-01	2.9E-02	2.9E-03	2.1E-04	1.1E-05	1.4E-08	4.9E-12	5.5E-16	1.9E-20	2.1E-25	7.0E-31	3.3E-37	3.6E-36
6.08	1.0E+00	7.8E-01	2.0E-01	3.1E-02	3.3E-03	2.5E-04	1.4E-05	1.9E-08	7.9E-12	1.0E-15	4.5E-20	6.1E-25	2.7E-30	1.7E-36	1.9E-35
6.24	1.0E+00	8.1E-01	2.2E-01	3.5E-02	3.8E-03	3.0E-04	1.8E-05	2.4E-08	1.2E-11	2.0E-15	1.0E-19	1.7E-24	9.8E-30	7.8E-36	9.6E-35
6.40	1.0E+00	8.4E-01	2.3E-01	3.8E-02	4.3E-03	3.6E-04	2.2E-05	3.6E-08	1.9E-11	3.6E-15	2.2E-19	4.7E-24	3.7E-29	3.4E-35	4.4E-34
6.56	1.0E+00	8.7E-01	2.5E-01	4.2E-02	4.9E-03	4.2E-04	2.7E-05	4.8E-08	3.0E-11	6.3E-15	4.7E-19	1.2E-23	1.1E-28	1.4E-34	1.9E-33
6.72	1.0E+00	8.9E-01	2.7E-01	4.6E-02	5.6E-03	4.9E-04	3.3E-05	6.5E-08	4.5E-11	1.1E-14	9.5E-19	3.0E-23	3.2E-28	5.4E-34	7.6E-33
6.88	1.0E+00	9.2E-01	2.9E-01	5.1E-02	6.3E-03	5.7E-04	4.0E-05	8.6E-08	6.6E-11	1.8E-14	1.9E-18	7.1E-23	9.6E-28	1.9E-33	2.9E-32
7.04	1.0E+00	9.4E-01	3.1E-01	5.6E-02	7.1E-03	6.7E-04	4.8E-05	1.1E-07	9.6E-11	3.1E-14	3.6E-18	1.6E-22	2.7E-27	6.6E-33	1.0E-31
7.20	1.0E+00	9.6E-01	3.3E-01	6.1E-02	8.0E-03	7.7E-04	5.7E-05	1.5E-07	1.4E-10	5.0E-14	6.9E-18	3.6E-22	7.2E-27	2.1E-32	3.6E-31
7.36	1.0E+00	9.8E-01	3.5E-01	6.7E-02	9.0E-03	8.9E-04	6.8E-05	1.9E-07	2.0E-10	8.0E-14	1.3E-17	7.8E-22	1.9E-26	6.7E-32	1.2E-30
7.52	1.0E+00	9.9E-01	3.8E-01	7.4E-02	1.0E-02	1.0E-03	8.1E-05	2.4E-07	2.8E-10	1.3E-13	2.3E-17	1.6E-21	4.6E-26	2.0E-31	3.6E-30
7.68	1.0E+00	1.0E+00	4.0E-01	8.0E-02	1.1E-02	1.2E-03	9.6E-05	3.1E-07	3.9E-10	2.0E-13	4.0E-17	3.3E-21	1.1E-25	5.6E-31	1.1E-29
7.84	1.0E+00	1.0E+00	4.3E-01	8.9E-02	1.3E-02	1.4E-03	1.1E-04	3.9E-07	5.3E-10	3.0E-13	6.9E-17	6.6E-21	2.6E-25	1.5E-30	3.0E-29
8.00	1.0E+00	1.0E+00	4.5E-01	9.5E-02	1.4E-02	1.5E-03	1.3E-04	4.9E-07	7.3E-10	4.5E-13	1.2E-16	1.3E-20	5.8E-25	4.0E-30	8.3E-29

D= 2.00E-05 R= 2.80E-04 G= 2.80E-04 U= 1.00E-04 TMAX= 2.88E+04 CAPL= 10.00 ANOD= 1.65E-01 DH= 400 DT= 560

TIME (HRS) DISTANCE (CM)

LOG C/CO=	0.	3.	6.	9.	12.
.160	1.25E-02	4.62E-01	4.87E-01	9.87E-01	1.04E+00
.320	1.25E-02	4.12E-01	5.62E-01	1.31E+00	1.44E+00
.480	1.25E-02	7.37E-01	1.14E+00	1.46E+00	1.71E+00
.640	1.25E-02	8.37E-01	1.22E+00	1.66E+00	1.94E+00
.800	1.25E-02	9.12E-01	1.44E+00	1.94E+00	2.14E+00
.960	1.25E-02	9.87E-01	1.56E+00	1.99E+00	2.34E+00
1.120	1.25E-02	1.05E+00	1.65E+00	2.14E+00	2.56E+00
1.280	1.25E-02	1.11E+00	1.76E+00	2.24E+00	2.63E+00
1.440	1.25E-02	1.15E+00	1.84E+00	2.36E+00	2.81E+00
1.600	1.25E-02	1.24E+00	1.94E+00	2.49E+00	2.96E+00
1.760	1.25E-02	1.29E+00	2.01E+00	2.59E+00	3.03E+00
1.920	1.25E-02	1.34E+00	2.09E+00	2.69E+00	3.21E+00
2.080	1.25E-02	1.39E+00	2.14E+00	2.79E+00	3.31E+00
2.240	1.25E-02	1.43E+00	2.24E+00	2.84E+00	3.41E+00
2.400	1.25E-02	1.46E+00	2.29E+00	2.94E+00	3.56E+00
2.560	1.25E-02	1.51E+00	2.34E+00	3.04E+00	3.64E+00
2.720	1.25E-02	1.54E+00	2.41E+00	3.11E+00	3.71E+00
2.880	1.25E-02	1.59E+00	2.45E+00	3.19E+00	3.81E+00
3.040	1.25E-02	1.61E+00	2.54E+00	3.26E+00	3.91E+00
3.200	1.25E-02	1.65E+00	2.59E+00	3.34E+00	3.99E+00
3.360	1.25E-02	1.69E+00	2.66E+00	3.41E+00	4.06E+00
3.520	1.25E-02	1.74E+00	2.69E+00	3.49E+00	4.16E+00
3.680	1.25E-02	1.74E+00	2.74E+00	3.54E+00	4.24E+00
3.840	1.25E-02	1.79E+00	2.81E+00	3.61E+00	4.31E+00
4.000	1.25E-02	1.84E+00	2.84E+00	3.69E+00	4.39E+00
4.160	1.25E-02	1.84E+00	2.89E+00	3.74E+00	4.46E+00
4.320	1.25E-02	1.89E+00	2.94E+00	3.79E+00	4.54E+00
4.480	1.25E-02	1.94E+00	2.99E+00	3.86E+00	4.61E+00
4.640	1.12E-01	1.96E+00	3.04E+00	3.91E+00	4.66E+00
4.800	1.12E-01	1.99E+00	3.09E+00	3.96E+00	4.74E+00
4.960	1.37E-01	2.04E+00	3.14E+00	4.04E+00	4.81E+00
5.120	1.62E-01	2.04E+00	3.16E+00	4.09E+00	4.86E+00
5.280	1.87E-01	2.09E+00	3.21E+00	4.14E+00	4.96E+00
5.440	1.87E-01	2.11E+00	3.26E+00	4.19E+00	5.01E+00
5.600	2.12E-01	2.16E+00	3.31E+00	4.24E+00	5.06E+00
5.760	2.37E-01	2.19E+00	3.36E+00	4.29E+00	5.11E+00
5.920	2.62E-01	2.21E+00	3.39E+00	4.34E+00	5.19E+00
6.080	2.87E-01	2.24E+00	3.41E+00	4.39E+00	5.24E+00
6.240	3.12E-01	2.26E+00	3.44E+00	4.44E+00	5.31E+00
6.400	3.12E-01	2.31E+00	3.51E+00	4.49E+00	5.36E+00
6.560	3.37E-01	2.34E+00	3.54E+00	4.54E+00	5.41E+00
6.720	3.62E-01	2.36E+00	3.59E+00	4.59E+00	5.46E+00
6.880	3.87E-01	2.39E+00	3.61E+00	4.64E+00	5.54E+00
7.040	4.12E-01	2.41E+00	3.66E+00	4.69E+00	5.59E+00
7.200	4.37E-01	2.44E+00	3.69E+00	4.74E+00	5.66E+00
7.360	4.62E-01	2.49E+00	3.74E+00	4.79E+00	5.69E+00
7.520	4.62E-01	2.51E+00	3.76E+00	4.81E+00	5.74E+00
7.680	4.87E-01	2.54E+00	3.81E+00	4.86E+00	5.79E+00
7.840	5.12E-01	2.56E+00	3.84E+00	4.91E+00	5.84E+00
8.000	5.37E-01	2.59E+00	3.89E+00	4.96E+00	5.89E+00

INCREMENT NUMBER

	0.	3.	6.	9.	12.
.5	18.5	27.5	35.5	41.5	
.5	24.5	38.5	48.5	57.5	
.5	29.5	45.5	58.5	68.5	
.5	33.5	51.5	66.5	78.5	
.5	36.5	57.5	73.5	86.5	
.5	39.5	62.5	79.5	94.5	
.5	42.5	66.5	85.5	101.5	
.5	44.5	70.5	90.5	107.5	
.5	47.5	73.5	94.5	112.5	
.5	49.5	77.5	99.5	118.5	
.5	51.5	80.5	103.5	123.5	
.5	53.5	83.5	107.5	128.5	
.5	54.5	86.5	111.5	132.5	
.5	56.5	89.5	114.5	136.5	
.5	58.5	91.5	118.5	141.5	
.5	60.5	94.5	121.5	145.5	
.5	61.5	96.5	124.5	148.5	
.5	63.5	99.5	127.5	152.5	
.5	64.5	101.5	130.5	156.5	
.5	66.5	103.5	133.5	159.5	
.5	67.5	105.5	136.5	162.5	
.5	69.5	107.5	139.5	166.5	
.5	70.5	110.5	141.5	169.5	
.5	71.5	112.5	144.5	172.5	
1.5	73.5	114.5	147.5	175.5	
1.5	74.5	115.5	149.5	178.5	
2.5	75.5	117.5	151.5	181.5	
3.5	77.5	119.5	154.5	184.5	
4.5	78.8	121.5	156.5	186.5	
4.5	79.5	123.5	158.5	189.5	
5.5	81.5	125.5	161.5	192.5	
6.5	82.5	126.5	163.5	194.5	
7.5	83.5	128.5	165.5	197.5	
7.5	84.5	130.5	167.5	200.5	
8.5	86.5	132.5	169.5	202.5	
9.5	87.5	133.5	171.5	204.5	
10.5	88.5	135.5	173.5	207.5	
11.5	89.5	136.5	175.5	209.5	
12.5	90.5	138.5	177.5	212.5	
12.5	92.5	140.5	179.5	214.5	
13.5	93.5	141.5	181.5	216.5	
14.5	94.5	143.5	183.5	218.5	
15.5	95.5	144.5	185.5	221.5	
16.5	96.5	146.5	187.5	223.5	
17.5	97.5	147.5	189.5	225.5	
18.5	99.5	149.5	191.5	227.5	
18.5	100.5	150.5	192.5	229.5	
19.5	101.5	152.5	194.5	231.5	
20.5	102.5	153.5	196.5	233.5	
21.5	103.5	155.5	198.5	235.5	

IT= 50

REFERENCES

1. E. H. Kennard, Kinetic Theory of Gases (Mc Graw-Hill Book Co., Inc., New York, New York, 1938), p. 280.
2. A Pijper, "Methyl Cellulose and Bacterial Motility", J. Bact. 53, 257 (1957).
3. Julius Adler, "Chemotaxis in Bacteria", Science 153, 708 (1966).
4. Catherine B. Fowler, "Relationship between Fermentation and Enzymatic Adaption", Biochim. Biophys. Acta. 7, 563 (1951).
5. W. Jost, Diffusion in Solids, Liquids, Gases, (Academic Press Inc., New York, 1960), 283.
6. K. W. Pearce, "Settling in the Presence of Downward-Facing Surfaces", Interaction Between Fluids and Particles (London Instn. Chem. Engrs, 1962), p. 30.
7. Dr. K. Ôgiuti, "Untersuchungen über de Geschwindigkeit der Eigenbewegung von Bakterien", Japan J. of Expt. Med. 14, 19 (1936).
8. R. C. Clowes and D. Rowley, "Small Colony Varients of E. coli", J. Gen. Microbiol. 13 461 (1955).
9. R. C. Clowes, G. Furness and D. Rowley, "Measurements of the Speed of Motility in E. coli", J. Gen. Microbiol. 13 (1955)
10. H. A. Mazloum, R. Abdel-Maguid, "Factors and Drugs Affecting Bacterial Motility as Measured by the Capillary Tube Method", J. Egypt. Med. Assoc. 42, 309 (1959)

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