DIFFUSION RATES IN DISRUPTED BACTERIAL CELLS

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ABSTRACT The viscosity of the material resulting from squeezing *Escherichia coli* cells through an orifice in a French pressure cell has been shown to be very high and variable with temperature. Diffusion constants in this medium have been determined for sucrose, dextran, and beta galactosidase. The values found are: 1.07×10^{-6} cm²/second for sucrose, 0.36×10^{-6} cm²/second for dextran, and 0.025×10^{-6} cm²/second for beta galactosidase. The results agree with the idea that there is much interstitial space available for diffusion of small molecules in the cell medium in spite of the high viscosity, but that large molecules will be transported less readily.

INTRODUCTION

The viscosity of the medium inside bacterial cells is very high. Some preliminary measurements made in the Yale University Biophysics Department by Dr. W. Gardner indicated that the viscosity of material resulting from the extrusion of *E. coli* under pressure is not only high but also varies in a characteristic way with temperature. In view of this high viscosity, the question arises as to the method of transport of material within the cell. Some calculations based on the idea that random collision and specific selection are responsible for the rate of synthesis of nucleic acids and proteins, indicated that the presence of slow diffusion for small metabolites would make it very difficult to understand the way in which the cell executes very rapid synthetic processes. (Pollard, 1961, 1963). If the macroscopic viscosity controlled the diffusion rate, then for this very viscous material diffusion would be very slow indeed. In view of the requirement that diffusion be relatively rapid, at least for small substances, it seemed worthwhile to study diffusion in the medium produced by extruding cells under pressure in a French pressure cell. In order to do this, three classes of molecules were studied: first, C^{14}-labeled sucrose, which corresponds to the ordinary size of a metabolite, such as a nucleoside triphosphate; second, C^{14}-labeled dextran, which corresponds in size to a transfer RNA molecule; and third, beta galactosidase, which corresponds in size roughly to that of a messenger RNA. The results indicate that for all metabolites the diffusion

rates were much higher than expected theoretically and were, in fact, not much less than one would expect for these molecules to diffuse in water. It is found that for small metabolites there is a slightly diminished rate of diffusion from that in pure water, but that this diminished rate is not very serious and would correspond almost precisely to the filling up of space by the network-like material which must be part of the cell extrudate. For the larger substances there is a progressively lower value of the diffusion constant as compared to that in water, until the rate for beta galactosidase is some 10 times smaller than would be expected in water but several magnitudes greater than that predicted by Fick's law for a medium of this viscosity. The results, therefore, indicate that the relatively fast synthetic rates do not, at least as far as as diffusion is concerned, require any special mechanism to achieve them, providing the concentrations are adequate.

METHODS

Viscosity Studies *E. coli* bacteria, strain B, obtained from the Department of Bacteriology of the Pennsylvania State University, were grown in 20 liter cultures of Roberts' C minimal medium. (NH_4Cl 2 gm, Na_2HPO_4 6 gm, KH_2PO_4 3 gm, NaCl 3 gm, $MgCl_26H_2O$ 62 mg, Na_2SO_4 80 mg, glucose 5 gm per liter.) Five-gallon pyrex bottles were used as culture flasks and aeration was provided by an aquarium-type bubbler. Upon reaching a concentration of approximately 10^9 cells per ml, as measured by the optical density of the culture, the cells were harvested in a continuous flow centrifuge. The yield was about 30 gm (wet weight) of cells per 20 liters of culture. The centrifuged pellets, while still in the tubes, were drained, frozen, and then removed from the centrifuge tubes and stored at −18°C.

The bacteria were broken open by use of a French pressure cell. They were run through the pressure cell four times at a pressure held between 5000 and 15,000 pounds/inch^2. This procedure decreased the viable cells, measured by plate count, from 10^{12} per cc to less than 10^9 per cc. This material is referred to as a cell "extrudate." It contains "cytoplasm,"–contents of the nuclei, cell wall, and membranes.

Viscosity measurements were made with Ostwald viscosimeters. These were calibrated by the manufacturer (The Cannon Instrument Co., Boalsburg, Pennsylvania) and the kinetic viscosity, ν, is obtained by measuring the efflux time, in seconds, between two etched marks and multiplying by the viscosimeter constant. The absolute viscosity, η, is related to the kinetic viscosity by the relation $\eta = \rho\nu$ where ρ is the density of the liquid. Thus, the absolute viscosity is, for the temperature range covered, essentially a constant times the efflux time. As only relative viscosities are of interest, the data are presented in terms of efflux time which is, hereafter, considered equivalent to the viscosity. The viscosity measurements were made with the viscosimeters in a constant temperature bath, which maintained temperatures within 0.1°C. The viscosity of the pure cell extrudate was too high to be read with the available viscosimeters and had to be diluted with distilled water to less than a 30 per cent solution by weight in order to get efflux times which could be measured over a reasonable period.

Diffusion Measurements Preliminary studies were made with the use of Polson-Shepard diffusion cells (Polson and Shepard, 1949), which consist of triple compartmented cylindrical cells in which the upper two can be rotated with respect to the lower

two. The results obtained by these means were not very satisfactory, largely because of severe difficulties in filling the cells to avoid any kind of bubbling and also, particularly, in extracting the material out of them. The diffusion constant of sucrose, quoted later in Table III was measured in this way. It was found to be much more informative to have some idea of the whole distribution of the diffusion material; and a very simple and inexpensive method was found in terms of plastic cylinders obtainable on the market as "drinking straws." We have found them to be reasonably uniform in diameter and adequately suitable for use as diffusion chambers. They could be frozen and cut into small sections with a razor blade, so that the concentration of the diffusing molecule could be determined for an arbitrary number of adjacent slices along the column instead of only three sections as with a Polson device. With many sections taken, the concentration gradient along the length of the chamber could be determined and, therefore, the diffusion constant could be measured. The straws were filled with a special loading device which operated on the principle of a caulking gun, except that in this case the plunger was held stationary and the container tube moved up on it. The only critical dimensions are the diameters of the plunger and the copper tubing which forms the "gun barrel." In the one constructed for this experiment the diameter of the plungers was slightly less than 12 mm so that when a rubber "O" ring was placed in a groove on the edge, it accurately fitted a half inch Pyrex glass thick-walled centrifuge tube. The copper tubing had an outside diameter slightly less than the inside diameter of the straws. Four tubes of extrudate were prepared. One was mixed with C^{14}-sucrose, one with dextran–carboxy-C^{14}, one with beta galactosidase, and one with pure extrudate. The straws were loaded by slipping them on the end of the copper tubing. As the test tube was forced against the plunger the extrudate was pushed through the copper tubing into the straw. The viscosity of preparations was so high that the juice came out, filled the straw evenly, and pushed it back simultaneously. When the straw was approximately half full, it was removed from the gun containing the unlabeled preparation and slipped onto the barrel of the gun loaded with the material containing the desired diffusing molecule. The time at which this phase of loading began was recorded as the beginning of the diffusion time. The straw was filled within 0.5 inches of the end and sealed with wax to prevent drying. The straws were then wrapped in waxed paper, placed in screw-top test tubes, and held in the cold room at 4°C for the duration of the diffusion time.

Assay Procedure The straws were cut into equal length sections by placing the straws in a groove in a metal plate having three slots to guide the knife edge. It was placed on a rectangular slab of ice which was placed on chips of dry ice to obtain a temperature of −15°C. At this temperature the extrudate was frozen so firmly that it would slice cleanly without squashing the straw with the pressure of the razor blade. At temperatures below −15°C the frozen extrudate had a tendency to shatter.

The radioactive sections were placed in planchets, and the material was diluted with distilled water in order to get it out of the plastic shell, as well as to give a thin layer upon drying and thus minimize self-absorption. The planchets were weighed before filling and after drying to determine the net weight of the extrudate. An end window Geiger-Muller tube was used for counting.

The beta galactosidase was obtained by opening under pressure *E. coli* cells which had been grown on lactose. The material obtained after opening the cells was diluted slightly and centrifuged to remove the cell debris. The beta galactosidase remained in the supernatant, which was drawn off and evaporated in a vacuum desiccator to about 5 per cent of the initial volume to increase the enzyme concentration. The assay is a

colorimetric method and involves the use of orthonitrophenyl β-D-galactopyranoside (ONGP) as a substrate. As the ONGP molecule is hydrolyzed by beta galactosidase, the product, orthonitrophenol, produces color. The intensity of color is proportional to the amount of enzyme, provided there is an excess of substrate.

EXPERIMENTAL RESULTS

Viscosity Measurements Fig. 1 shows the viscosity of a dilute solution of *E. coli* grown at 37°C plotted as a function of temperature. The chronological order of the observation is of some importance as the viscosity does not remain constant with time. The first measurements were made at 39°C and are indicated by crosses.

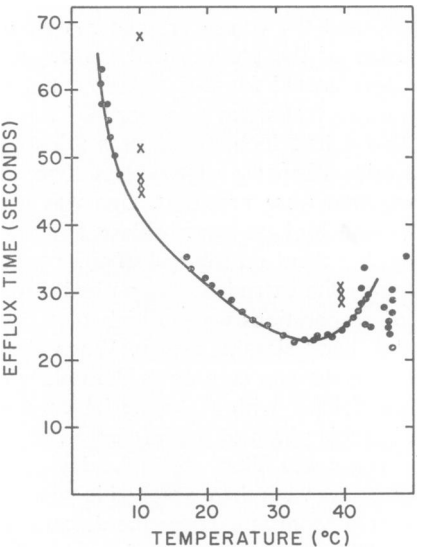

FIGURE 1 Variation of the efflux time of a solution at *E. coli* extrudate as a function of temperature. The circles represent one consecutive set of measurements starting at low temperatures. The crosses are measurements made at one temperature, indicating that the viscosity of a fresh extrudate is changing.

Next the series indicated by crosses at 10°C were taken, after which the temperature was lowered to 5°C and the sequence of observations indicated by circles was taken. From the graph one can see that the viscosity goes through a minimum at 35°C. At approximately 45°C repeated measurements began showing a high degree of variation as shown on the graph. The material also lost its uniform appearance and began to leave a solid residue on the inside of the viscosimeter capillary instead of flowing freely as it had been doing up to that temperature.

In order to investigate any possible relationship between growth rate and viscosity

a psychrophilic organism, *Pseudomonas flourescens* (obtained from the Department of Bacteriology at Pennsylvania State University) was studied. This organism has its optimum growth rate at about 20°C and will not grow at 37°C. It was found that there is certainly no minimum of viscosity at or near 20°C, the temperature of the maximum growth rate of the organism. It was also found that the viscosity, which fell as the temperature increased, did not recover its high value on cooling, thus indicating that factors other than temperature are affecting the viscosity. We found that in a period of 8 hours at 24°C the viscosity fell by a factor of 4.

Absolute Viscosity of E. coli Extrudate Some indication of the magnitude of the absolute viscosity of *E. coli* extrudate can be obtained by extrapolation from the fact that a 32 per cent solution has a viscosity of 20 centipoise at 25°C, and comparison with glycerol-water solutions. A 32 per cent solution of glycerol has a viscosity of 2.5 centipoise, while 100 per cent glycerol has a viscosity of 1000 centipoise. These relative figures suggest that the viscosity of *E. coli* extrudate is at least 100 poise at 25°C.

Relation of Growth Rate to Viscosity at Various Temperatures If the growth rate is dependent on diffusion rates and if these are controlled by the macroscopic viscosity, then the growth rate should vary inversely with viscosity, and the product of growth rate and viscosity should be a constant. Measurements of relative growth rates for *E. coli* bacteria were made, in C minimal medium by Miss Sue Kohlhepp. The product of relative growth rate and viscosity is given, as a function of temperature, in Table I.

TABLE I

Temperature	Product of growth rate and viscosity
°C	
16	36
20	44
24	74
28	102
32	131
36	149
40	157
44	144

Perhaps, in the range from 32-44°C the relation might be true. However, in this range neither viscosity nor growth rate is changing very rapidly. The evidence is therefore against the idea that the macroscopic viscosity of the extrudate controls the rate of growth of the cells.

Diffusion Experiments To obtain the diffusion constant the relation between activity and length along the plastic tube is needed, as a function of the diffusion time. As has been described, the plastic tubes and contents were sectioned to produce units 0.234 cm long. Measurement of 62 sections indicated a length of 0.234 ± 0.012 cm. The average dried mass of 306 of the sections was 0.00653 gm, equivalent to 0.028 gm per cm of tube length. Since weighing the samples was routine procedure, the data were first expressed in activity *versus* total mass and then converted to activity *versus* length.

The integrated diffusion equation for a one dimensional diffusion process is

$$C = \frac{Co}{2}\{1 + \text{erf}\,(x/\sqrt{4Dt}\}$$

where C is the concentration at a point x cm from the midpoint, Co the initial concentration before diffusion, D is the diffusion constant, t is the time for diffusion. $C = Co$ for $x > 0$ and $C = 0$ for $x < 0$ at $t = 0$. The error function, "erf" is given by

$$\text{erf}\, y = \frac{2}{\sqrt{\pi}}\int_0^y e^{-z^2}\, dz$$

and is tabulated in mathematical tables.

A function which behaves as the above can be plotted on "probability paper," in which the ordinate scale divisions are so extended as to make the above function plot as a straight line. Such plots are shown in Figs. 2, 3, and 4. Advantage can be

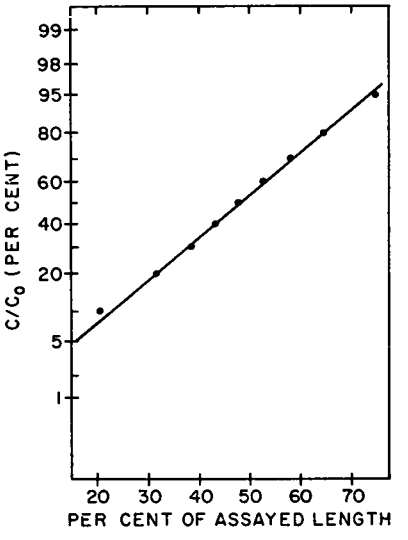

FIGURE 2 Probability plot of the concentration of C^{14} sucrose along the length of a plastic tube. Diffusion time 362 hours. Diffusion constant 0.63×10^{-6} cm²/sec.

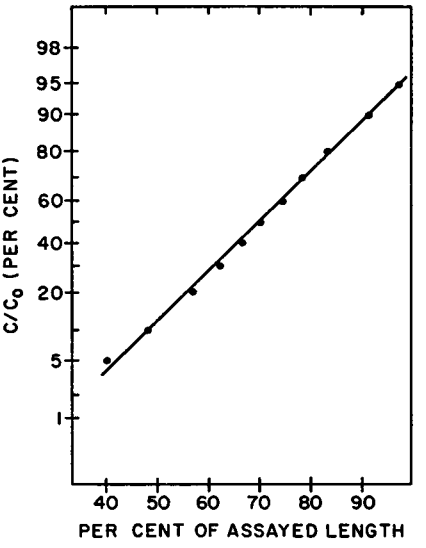

FIGURE 3 Probability plot of the concentration of C^{14} dextran along the length of a plastic tube. Diffusion time 362 hours. Diffusion constant 0.33×16^{-6} cm²/sec.

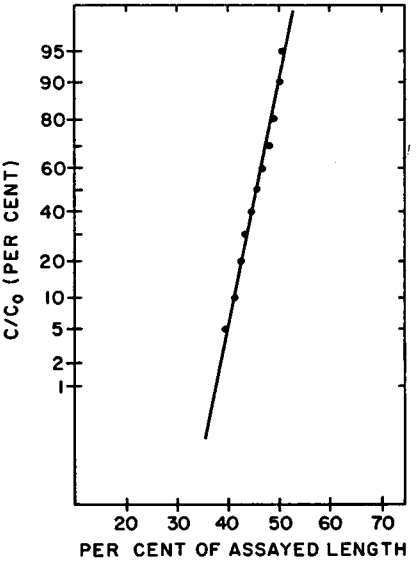

FIGURE 4 Probability plot of the concentration of C^{14} beta galactosidase along the length of a plastic tube. Diffusion time 362 hours. Diffusion constant 0.022×10^{-6} cm²/sec.

taken of the straight line plot by reading off the x-displacement at the $0.1C_0$ and $0.9\ C_0$ levels. If these values are x_1 and x_2, then

$$0.9C_0 = \frac{C_0}{2}\left\{1 + \text{erf}\frac{x_1}{\sqrt{4Dt}}\right\}$$

$$0.1C_0 = \frac{C_0}{2}\left\{1 + \text{erf}\frac{x_2}{\sqrt{4Dt}}\right\}$$

so that

$$0.8C_0 = \frac{C_0}{2}\left\{\text{erf}\frac{x_1}{\sqrt{4Dt}} - \text{erf}\frac{x_2}{\sqrt{4Dt}}\right\}$$

Substituting values from tables of the error function and putting in the actual numbers we find

$$D = \frac{x^2}{4(1.82)^2 t}$$

which is the relationship used to determine the diffusion constant. The results of the diffusion experiments on sucrose, dextran, and beta galactosidase are summarized in Table II.

The values of D for sucrose and dextran were found as described above. For beta galactosidase the sections were assumed to be uniform in length and each 0.234 cm. There is thus a slightly greater possibility of error in the measurement for beta galactosidase.

The values shown in Table II clearly show that this kind of medium is not the most uniform or consistent in behavior. Nevertheless, it is possible to conclude that the diffusion constant, in all cases, has been measured within a factor of two, and probably within 25 per cent.

Discussion of Experimental Results Perhaps the most striking feature of these results is shown by comparison of the diffusion constants of the molecules in the bacterial extrudate with those of similar molecules in water. Table III gives this comparison.

It is interesting to note from the table that the diffusion constants through this thick bacterial extrudate are at rates surprisingly close to those of similar molecules in water. Water has a viscosity of 1 centipoise and the extrudate has a viscosity estimated as at least 10^4 centipoises, yet the beta galactosidase molecule has a diffusion constant which is only 1/14 that of urease in water. Clearly the diffusion rates in the cytoplasm are not closely controlled by the high apparent viscosity of the extrudate. Apparently the amorphous mixture of macromolecules, cell wall debris, and water composing the extrudate impede the progress of smaller diffusing molecules only slightly, which means that the diffusing molecules "see" a viscosity which is considerably lower than the apparent viscosity.

TABLE II

DIFFUSION CONSTANTS OF SUCROSE, DEXTRAN, AND BETA GALACTOSIDASE IN *E. COLI* EXTRUDATE

Molecule	Diffusion time	Assayed length	Diffusion constant × 10^6
	hrs.	*cm*	*cm²/sec*
Sucrose	115	3.8	1.06
"	115	5.0	1.40
"	212	6.5	1.30
"	212	5.9	0.93
"	362	6.5	0.93
"	362	6.6	0.76
"	362	6.7	0.63
"	648	9.6	1.54
			Average 1.07
Dextran	115	4.2	0.42
"	115	4.4	0.44
"	212	4.0	0.29
"	212	6.1	0.43
"	362	5.2	0.27
"	362	5.4	0.30
"	362	5.9	0.33
"	648	9.3	0.43
			Average 0.36
Beta galactosidase	115	2.7	0.026
" "	115	5.4	0.033
" "	212	7.1	0.014
" "	212	6.4	0.020
" "	362	6.4	0.030
" "	362	6.9	0.034
" "	362	6.9	0.022
" "	648	6.9	0.027
" "	648	7.4	0.018
" "	648	7.4	0.027
			Average 0.025

The ratios of diffusing constants of the comparable sized molecules in water and cell extrudate from Table II are also interesting. Sucrose and dextran diffuse about one-third as fast in the cytoplasm as in water, while the beta galactosidase is down by a factor of 14 from the rate of urease in water even though urease is supposedly 50 per cent larger in value. Ferry (1959) has discussed this sort of phenomenon which occurs in synthetic high polymer systems and treats two kinds of viscosity: the effective local viscosity, and the apparent macroscopic viscosity. For low concentra-

TABLE III

A COMPARISON OF DIFFUSION RATES OF SUCROSE, DEXTRAN, AND BETA GALACTOSIDASE IN *E. COLI* EXTRUDATE WITH THE DIFFUSION RATES OF COMPARABLE MOLECULES IN WATER

Diffusing molecule	Molecular weight	Solvent	Temperature	Diffusion constant	References
			°C	$10^{-6} cm^2/sec.$	
Sucrose	342	Water	1.0	2.42	*
Sucrose	342	Extrudate	5.0	1.1	
Cytochrome C	13,000	Water	20.0	1.0	‡
Dextran	15,000-17,000	Extrudate	5.0	0.36	
Myoglobin	17,200	Water	20.0	1.1	‡
Urease	480,000	Water	20.0	0.35	‡
Beta galactosidase	250,000-400,000	Extrudate	5.0	0.025	

*Measured with the Shepard-Polson cell. Several different values under various conditions are given by Jost (1952).
‡Setlow and Pollard (1962).

tions of a high polymer solution, the two values are nearly the same, but with increasing polymer concentration both increase, with the macroscopic viscosity increasing most rapidly. This would seem to be in agreement with our findings.

We may say in conclusion that neither the growth rates nor the diffusion rates of molecules in the extrudate are closely dependent upon the apparent viscosity of the material formed by extruding cells. It is felt that the results are in agreement with the calculations mentioned earlier as to synthetic rates. Since the diffusion rates are not controlled by the apparent viscosity one would not necessarily expect the growth rates to be closely related to the apparent viscosity even if the molecules were synthesized according to the scheme proposed. Measured rates of diffusion in the cell extrudate appear to be adequate to account for the rates of synthesis assumed previously in setting up a hypothetical model, with the proviso that adequate concentrations of reacting molecules are present.

While the cell extrudate is admittedly not the same as the living cell, it would seem that the values of diffusion constants would be higher in the living system if changed at all. If the cell has any internal structure then one would expect greater ease of motion in spaces between the structures. Thus the values of diffusion rates found in these experiments are in a sense lower limiting values, and if these values are adequate to account for the rate of synthesis, then there appears to be an excellent probability that the proposed method of synthesis could occur in the living cell.

We wish to acknowledge the start given to the viscosity studies by Dr. W. Gardner. We were very effectively helped in part of the measurements by Mrs. Suzanne Woodyatt. Miss Sue Kohlhepp contributed the data on growth rates.

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REFERENCES

FERRY, J. D., 1959, A study program *in* Biophysical Science, (J. L. Oncley, editor), New York, John Wiley and Sons, Inc., 130.

JOST, W., 1952, Diffusion in Solids, Liquids, and Gases, New York, Academic Press.

POLSON, A., and SHEPARD, C. C., 1949, *Biochim. et Biophysica Acta*, **3**, 137.

POLLARD, E. C., 1961, *J. Theoret. Biol.*, **1**, 328.

POLLARD, E. C., 1963, *J. Theoret. Biol.*, **4**, 98.

SETLOW, R. B., *and* POLLARD, E. C., 1962, Molecular Biophysics, Reading, Massachusetts, Addison-Wesley Publishing Co.