then washed with a salt solution (containing, per liter, 9 g of NaCl, 0.25 g of $MgSO_4$, 1.8 mg of $MnCl_2$, 0.22 mg of $ZnSO_4$, 0.08 mg of $CuSO_4$, and 0.005 mg of $FeCl_3$). Portions of the lyophilized cellular preparations were then converted to protoplast. Protoplast preparations of *M. luteus* were made in a hypertonic solution containing lysozymes, as described by Mitchell and Moyle (*8*) and Brown (*9*). *Azotobacter* sp. protoplast was prepared by the method of Jose and Wilson (*10*) in an aqueous solution of the following composition: 33 m*M* tris(hydroxymethyl)aminomethane, 0.06*M* sucrose, 13.3 mg of lysozyme per milliliter, and 5.32 mg of ethylenediaminetetraacetic acid tetrasodium salt per milliliter. Protoplast preparations were separated from cell wall digests by centrifugation and then washed and recentrifuged twice. The washings were added to the cell wall digests. Protoplast preparations were then lysed with cold distilled water (*8, 9*). The cytoplasmic fraction was separated from the membrane fractions by centrifugation, washed, and centrifuged again. These washings were added to the cytoplasmic fraction. Isolated cellular subfractions were chemically characterized by determining the amounts of protein (*11*), lipids (*12*), RNA (*9, 13*), and hexosamines (*14*). The quantities determined are typical for membrane preparations (*9*). Each cellular subfraction was then analyzed for lead by dissolution in concentrated nitric acid followed by atomic absorption spectrometric analysis with a Varian Techtron AA-5 spectrophotometer. The amounts of lead found in various cellular cultures and cellular fractions of *Micrococcus* and *Azotobacter* are listed in Table 1. *Azotobacter* cultures (*p*H 7.0 to 7.5) were incubated for 7 days, while *Micrococcus* cultures (*p*H 6.8 to 7.0) were incubated for 2 days to reach the same early stationary growth phase. Amounts of lead taken up by whole cells may thus be dependent upon both incubation period and culture *p*H. Considerably more lead is found in inoculated broth components than in uninoculated controls. Lead transport through the dialyzing membrane may be affected by both cellular structure and the composition of the culture medium. The distinctive feature of both sets of data is the appreciable uptake of lead by the microbial cells. The relatively greater effectiveness of *Azotobacter* for immobilizing lead may be related to the large quantity of capsular material surrounding the cells, which has a tendency to accumulate biological and nonbiological substances.

Table 2. Distribution of lead in cellular subfractions. Values are averages for four separate culture preparations for each species. Included in cell wall and cytoplasmic fractions are washes of the protoplast and membrane preparations, respectively.

Substrate	Total lead recovered			
	Micrococcus luteus		*Azotobacter* sp.	
	Micrograms	Percentage	Micrograms	Percentage
Cell wall fraction	111	9.5	11,505	37.6
Membrane fraction	1,048	89.8	18,833	61.5
Cytoplasmic fraction	8	0.7	273	0.9

The distribution of lead among cellular subfractions is reported in Table 2. The large amount of capsular material associated with *Azotobacter* may account for the difference between the amounts of lead found in the cell wall fractions of the two systems. Recovery of lead from the noncapsulated *Micrococcus* is essentially complete (Tables 1 and 2). Incomplete lead recovery for *Azotobacter* may be due to scavenging of the lead by the surface of the glass container during protoplast preparation. The striking feature of these data is the small amount of lead found in the cytoplasmic fractions for both systems. However, the amount found in the cytoplasmic fractions may be associated with finely fragmented membranes. Total lipid extraction of membrane preparations with methanol : chloroform : water, 10 : 5 : 4 by volume (*12*), results in extraction of the major portion of lead found in those fractions. Adjustment of the solvent mixture to 10 : 10 : 9 by volume (*12*) results in essentially all of the extracted lead being transferred to the methanol : water phase.

A major conclusion of this study is that the microbial systems investigated are highly capable of abstracting substantial quantities of inorganic lead. A second major finding is that lead thus immobilized is largely associated with cell membranes, and virtually none is found associated with the cytoplasmic fractions. Immobilization of lead by microbial systems may have importance in terms of transfer through the food chain.

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Dielectrophoresis of Macromolecules: Determination of the Diffusion Constant of Poly-γ-Benzyl-L-Glutamate

Abstract. *The translational diffusion constant of poly-γ-benzyl-L-glutamate in ethylene dichloride was measured by dielectrophoresis. The method is rapid, precise to about ± 5 percent, and yields values in good agreement with those obtained by standard methods.*

Dipolar molecules experience a net force in a strongly inhomogeneous electric field and migrate toward the region of maximum absolute field strength. This migration, called dielectrophoresis (*1*), will continue until a concentration gradient reflecting equilibrium with diffusion is attained. It is the dipolar

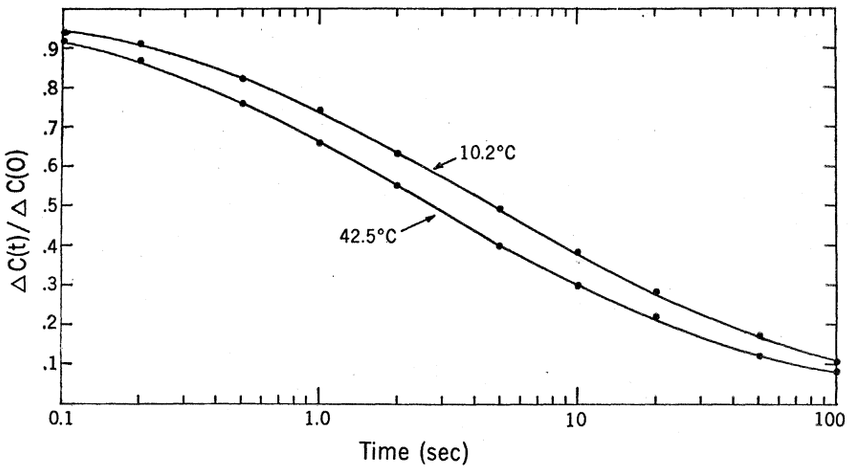

Fig. 1. Relative capacitance change plotted against time after the removal of excitation voltage for a 0.05 percent solution of PBLG, weight average molecular weight 118,000, in pure ethylene dichloride.

analog of electrophoresis, and offers a new basis for structural characterization or separation of macromolecules. We now show how a dielectrophoretically established concentration gradient can be used for the rapid determination of the translational diffusion constant of dipolar polymers.

Unlike electrophoresis, dielectrophoresis will occur in a-c as well as d-c fields provided that the frequency is low enough for the dipolar orientation to follow the field reversals. An experimentally observable effect can only be expected, however, if the change in electric field over the length of a macromolecule is of the order of the electric fields used in electrophoresis, or about 1 volt/cm. Even for large polymers, such fields require rather high voltages and electrodes of small radius of curvature. Despite difficulties in achieving these conditions while avoiding electrode reactions and ohmic heating, several groups of investigators have reported dielectrophoresis of induced and permanent dipolar molecules (*2*). The effect is much greater for particles greater than 1 μm, and Pohl and Crane (*1*) have observed dielectrophoretic migration with such materials.

Our experiments are carried out in a cell consisting of nine platinum cylindrical electrodes, 0.3 cm in diameter and 2 cm long, each with an axial inner electrode which is a platinum wire 10^{-3} cm in diameter. The inner and outer sets of electrodes are connected in parallel. A moderate a-c voltage is applied for a sufficient time to perturb the initial uniform distribution of concentration and achieve an equilibrium concentration gradient. This results in an increased capacitance of the cell. This a-c voltage is turned off, the cell is switched to a low-voltage, a-c bridge circuit, and the decay of the gradient is observed by continual recording of the capacitative unbalance, beginning within 10 msec of switching. Although the frequency of either circuit may be varied independently from 0.5 to 500 khz, we have generally operated both at 1 to 5 khz. The bridge unbalance voltage is measured with a phase-sensitive lock-in detector which separates capacitative from resistive changes.

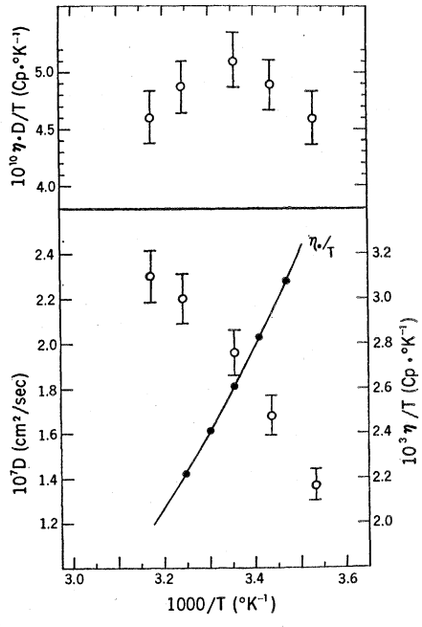

Fig. 2. (Bottom) Measured diffusion (D) constant of PBLG, as in Fig. 1, and viscosity data of ethylene dichloride, η_0/T, (*8*) plotted against the reciprocal temperature; the error bars are $\pm$ 5 percent. (Top) $\eta_0 D/T$ plotted against reciprocal temperature.

Fine wire electrodes offer several advantages. First, effective field gradients are established with only about 50 volts peak between electrodes. Second, the volume required for each of the nine cell components can be much less than 1 ml, as the following considerations show. At equilibrium the concentration at r, for $\bar{\mu}E < kT$, is given by

$$n(r) = n_0 \exp(\mu E/2kT) = n_0 \exp(\mu^2 E^2/6k^2T^2)$$

where n_0 is the initial uniform concentration, r is the distance from the wire axis, $\bar{\mu}$ is the thermally averaged polymer dipole moment, k is Boltzmann's constant, and E is the electric field strength. At r, the latter is

$$E(r) = V_0[2.30r \log(r_2/r_1)]^{-1}$$

where V_0 is the voltage between electrodes and r_1 and r_2 are the inner and outer electrode radii. Because of the inverse square dependence on r in the exponential, the concentration differs appreciably from n_0 only within a few wire radii of the inner electrode, which is also the minimal order of size of the outer electrode. Further, since the capacitance between the electrodes is largely determined by this very region, capacitance serves as a sensitive monitor of the migration.

Third, the time scale of the experiment is determined by the time needed to diffuse a distance of a few wire diameters. For a diffusion constant of 10^{-7} cm^2/sec, this is of the order of 10 seconds, so that the time needed to establish the equilibrium gradient and measure its decay is several hundred seconds, far shorter than that required for standard methods of diffusion measurement, and about as rapid as that based on the spectral width of scattered laser light, recently applied to poly-γ-benzyl-L-glutamate (PBLG) (*3*).

Our results were obtained for a PBLG sample (*4*) with an average molecular weight of 118,000 (viscosity method), and the sample was used as a 0.05 percent solution in pure ethylene dichloride (EDC). Excitation was at 40 volts peak and 3.5 khz for 500 seconds, and measurement of ΔC was at 4 khz and 2.5 volts peak. Figure 1 shows decay data for two temperatures. It is expressed as the ratio of the capacitance change at time t, after the field gradient is turned off at $t = 0$, to the capacitance change at $t = 0$. Both changes are

with respect to the capacitance corresponding to the initial uniform concentration in the cell.

We have solved numerically the differential equation of diffusion for an initial exponential concentration gradient and have obtained solutions for the decay of the capacitance in terms of only one adjustable parameter, the average diffusion constant. The solutions agree with those obtained by MacCallum (*5*) using other methods for the same problem. The fit of the theoretical curve to the results at two temperatures is shown in Fig. 1. The corresponding diffusion constants (D), for five temperatures, are shown in Fig. 2; from replicate experiments we estimate their precision to be about ± 5 percent.

At 25°C we find D to be 2.0×10^{-7} cm²/sec. By classical methods, Tsvetkov *et al.* (*6*) found 2.2×10^{-7} cm²/sec for PBLG of molecular weight 114,000 in dimethylformamide at 21°C, and Spach *et al.* (*7*) found 2.7×10^{-7} cm²/sec for PBLG of molecular weight 135,000 in dimethylformamide (*8*) at 20°C. Since the viscosity, η_0, of dimethylformamide at 20°C, 0.84 centipoise, is almost identical to that of EDC (*9*), 0.83 centipoise, the agreement of our results with the data of the other two groups appears satisfactory.

Evidence that PBLG behaves as a hydrodynamic particle in solution is shown in Fig. 2. Although both D and η_0/T vary by almost a factor of 2 over the temperature range of measurements, their product is constant to within 10 percent, characteristic of a Stokes' law dependence.

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Immunological Studies on Urinary Bladder Tumors of Rats and Mice

Abstract. *Human neoplasms derived from the same tissue have been previously shown to have tumor associated antigens characterizing that tissue type. Evidence is now presented for the existence of analogous antigens common to both rat bladder papillomas and carcinomas, and for antigens common to mouse bladder carcinomas. Rats immunized with syngeneic urinary bladder papillomas, then challenged with a methylcholanthrene pellet inserted into the bladder, develop (4 to 6 months later) fewer primary bladder tumors than rats immunized with normal bladder tissue.*

The study, both by transplantation techniques in vivo and by tests in vitro, of antigens associated with chemically induced tumors suggests that such tumors have individually unique antigens that do not usually cross-react with other tumors induced by the same chemical (*1*). Tumor antigens associated with virus induced tumors, in contrast, are highly cross-reactive if two tumors are associated with the same virus. Tumor antigens associated with different viruses do not cross-react (*1*). Human tumors behave still otherwise; cross-reacting tumor associated antigens have been demonstrated for neoplasms from the same histological type of tissue; such antigens do not cross-react with antigen from neoplasms of different histological types (*2*). We refer to such antigens as tissue type specific (TTS) antigens.

We now report (i) that there are cross-reacting tumor associated antigens in methylcholanthrene induced bladder carcinomas of mice and in methyl-

Table 1. Effect of lymph node cells from mice sensitized against (or bearing) urinary bladder carcinomas, the same or different from the target tumors; C, carcinoma; S, sarcoma.

Target cells	Lymphocytes	No. of tumor cells per well (mean ± S.E.)	No. of wells	Reduction (%)	*P*
		Experiment 1			
5102 (C)	Normal	79.6 ± 4.6	32		
	5102 (C)	42.6 ± 3.7	16	46.5	<.001
	5591 (C)	46.7 ± 4.7	16	41.3	<.001
	5664 (S)	89.2 ± 7.2	24	−12.1	*
		Experiment 2			
5591 (C)	Normal	46.8 ± 3.0	32		
	5591 (C)	32.7 ± 3.3	16	42.4	<.001
	5102 (C)	44.8 ± 4.5	16	21.2	<.01
	5664 (S)	59.4 ± 5.2	24	−26.9	<.01
		Experiment 3			
5102 (C)	Normal	34.9 ± 1.6	24		
	5102 (C)	25.5 ± 1.8	24	26.9	<.001
	5331 (C)†	35.3 ± 3.6	16	− 1.1	*
	5664 (S)	47.1 ± 2.1	24	−35.0	<.01
		Experiment 4			
5331 (C)	Normal	19.7 ± 1.3	24		
	5331 (C)†	16.1 ± 1.1	24	18.4†	<.01
	5102 (C)	9.9 ± 0.9	16	49.9	<.001
	5664 (S)	17.2 ± 1.0	24	12.7	*
		Experiment 5			
7922 (S)	Normal	36.8 ± 1.8	24		
	7922 (S)	23.1 ± 1.7	24	37.2	<.001
	5331 (C)	35.4 ± 2.2	24	3.8	*
		Experiment 6			
7922 (S)	Normal	35.4 ± 1.8	16		
	7922 (S)	23.0 ± 1.3	24	35.2	<.001
	9206 (C)	33.2 ± 2.5	16	6.4	*
Fibroblasts	Normal	46.8 ± 2.3	8		
	7922 (S)	45.6 ± 2.0	8	2.6	*
	9206(C)	53.8 ± 2.7	8	−15.0	*
		Experiment 7			
9206 (C)	Normal	34.6 ± 1.3	16		
	9206 (C)	24.3 ± 1.3	24	29.8	<.001
	7922 (S)	39.5 ± 1.9	16	−14.2	*
Fibroblasts	Normal	36.9 ± 3.1	8		
	9206 (C)	43.1 ± 1.7	8	−16.8	*
	7922 (S)	40.3 ± 2.6	8	− 9.2	*

* Not significant ($P > .05$). † Lymph node cells obtained from animals irradiated with 300 r before the tumors were transplanted.