gests that water and solutes moved into and out of the grains (as do the results from the air abrasion experiment with ²²Na⁺). We infer that solute diffusion occurs from the pore space surrounding the grain to the interior where ion-exchange reactions occur. We further infer that this time-dependent process can create a chemical disequilibrium that results in increased dispersion of the solute plume. If our evaluation is correct, these results have important ramifications for simulating solute transport in aquifers with intragranular porosity. (i) There are more reaction sites than indicated by shortterm laboratory tests for distribution coefficients. (ii) It will be necessary to include a diffusion expression in numerical codes used to simulate reactive solute transport. (iii) The total porosity of the aquifer is larger than estimated by normal grain size and packing techniques. (iv) The apparent dispersion of a nonreactive ion may not be applicable in simulating the transport of a reaction ion.

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- 15. We evaporated to near dryness 2 ml of a ²²Na⁺ tracer solution with an activity of 1 μ Ci in a HCl base and added it to 4 liters of Cape Cod ground water. The pH of the solution was adjusted to approximately 7 with a few drops of Na2CO3 solution; 250-

ml subsamples of this stock solution were placed in covered Teflon bottles with Teflon-covered magnetic stirring bars. To these solutions, a known weight of the various mineral grains was added. The grains had been previously soaked in a 1N solution of LiBr for 21 days, rinsed in distilled water, and oven-dried at 50°C. The solutions were stirred continuously. At specific time intervals 1.000-ml samples were extracted and placed on Al foil-covered planchettes and evaporated to dryness. The $^{22}Na^+$ activity on the foil was counted at 511 keV for 1 hour with a Ge-Li detector. Control samples consisted of unweathered, freshly ground orthoclase and biotite of the same size range and treated the same way as the samples. Because of the short time period of the experiments relative to the half-life of $^{22}Na^+$ (2.6 years), no correction was made for its radioactive decav.

16. The column consisted of a clear plastic tube (1 m by 5 cm) to which had been fitted inflow and overflow tubes, a sediment support screen of stainless steel, and a brass exit flow control valve. Dried, bulk Cape Cod aquifer (1500.0 g) were placed in the column, and a slow upflow of native Cape Cod ground water was pumped through the sediments for 24 hours to

remove the air trapped between the grains. The native water in the column above the sediment was then drained until it was at the sediment interface (43.5 cm from the bottom). Cape Cod ground water, which had been tagged with Li^+ (6.75 mg/liter) as LiBr and LiF, was introduced and allowed to flow down through the sediments at a rate of 4.4 ml/min. Effluent was collected in 25.0-ml samples and analyzed for Li⁺ and Br⁻ by atomic absorption and specific ion electrodes, respectively. The experiment was conducted at 24°C.

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Orientational Dielectric Relaxation of **Collisionless Molecules**

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The generation of the orientation component of the polarization of matter in an electric field has previously been thought to require interaction of molecules with their neighbors. It is demonstrated that, even in the absence of collisions between neighboring molecules, hot isolated polyatomic molecules can reorient in response to an external field, thereby giving rise to the orientation component of polarization. This reorientation occurs through the interaction of rotation with molecular vibrations, which provides a heat bath to establish thermal rotational equilibrium. This effect is demonstrated for o-diffuorobenzene, o-dichlorobenzene, and p-chlorotoluene, with an inhomogeneous electric field used to deflect molecular beams of these molecules.

TUDY OF THE DIELECTRIC RESPONSE has contributed to many topics in of matter to an applied electric field chemical and materials science, including, for example, the measurement of molecular dipole moments (1) and the investigation of the dynamics of liquid crystals (2), polymers (3), and disordered inorganic materials (4). When an electric field is applied to a substance, it induces a dipole moment per unit volume or polarization P consisting of two components. The polarizability component arises from the distortion of the electronic distribution of the substance. The orientation component arises from a very slight redistribution of the orientation of the constituent molecules to favor lower energy orientations according to a Boltzmann distribution. The orientation component generally develops over a relaxation time deter-

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mined by interactions with neighboring molecules, which exert a torque to reorient the molecules and provide a heat bath that gives rise to the Boltzmann distribution.

In this report, we demonstrate that, even in the absence of collisions, hot isolated polyatomic molecules can reorient in response to an external field to attain their equilibrium polarization. This reorientation occurs through the interaction, brought about by Coriolis and centrifugal forces, of each molecule's rotation with its own vibrations. The vibrations provide a heat bath to establish thermal rotational equilibrium. Within the constraints of energy and angular momentum conservation, each molecule rotates ergodically.

When a static electric field E is applied to a gas of molecules with permanent dipole moment μ and polarizability α , the ensemble-average electric dipole per molecule induced along the field direction is (1)

$$\langle d \rangle = \frac{P}{N} = E \left(\frac{\mu^2}{3kT} + \alpha \right)$$
 (1)

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where N is the number density of the gas, k is the Boltzmann constant, and T is the absolute temperature. The orientation and polarizability components of the polarization arise from the first and second terms in parentheses, respectively.

Although our conclusions will apply to any nonlinear polyatomic molecule except those with a significant component of dipole moment along the B axis (the axis of intermediate moment of inertia), for simplicity we now select for discussion symmetric top molecules, in which the dipole moment μ points along the symmetry axis. If such a molecule rotates as a rigid body, μ precesses about the rotational angular momentum J, maintaining a fixed angle with respect to J (Fig. 1a). The rotational energy is an even function of $\cos(\mu, J)$ for a particular angular momentum. In an electric field E, J in turn precesses about E, maintaining a fixed angle with respect to E. The time-averaged effective electric dipole of this molecule points along E and has a value

$$d = \mu \cos(\mathbf{E}, \mathbf{J}) \cos(\mathbf{\mu}, \mathbf{J})$$
(2)

The interaction energy of this dipole with the field is

$$V = -\mu E \cos(\mathbf{E}, \mathbf{J}) \cos(\mathbf{\mu}, \mathbf{J})$$
(3)

Although the field alters the energy of a rotational trajectory of the molecule by the addition of V, it induces no net orientation in the gas until collisions equilibrate the rigid gas molecules to trajectories that favor positive values of $\cos(\mathbf{E}, \mathbf{J}) \cos(\mathbf{\mu}, \mathbf{J})$. This Boltzmann weighting of the dipole of Eq. 2 leads directly to the first term of Eq. 1.



Fig. 1. Rotational motion of an isolated molecule with a dipole moment along the symmetry axis. The time between frames is many rotational periods. (a) Cold molecule undergoing rigid-body rotational motion. (b) Hot molecule undergoing IVRET. In the absence of a field, the molecule would equally sample motions described by the first and last panels of (b). In the presence of a field component along J, one of these orientations is slightly favored.



Fig. 2. Electric deflection apparatus. The displacement D of the scanning slit is a measure of the force exerted on a molecule as it passes through the field. The beam flight path is about 1 m long.

In collisionless internally hot molecules, the simple rotational motion on which Eq. 2 is based may be complicated by Coriolis and centrifugal interactions with vibration. To determine the response of such molecules to an electric field, we observe the deflection of a molecular beam caused by an inhomogeneous field (5, 6). Our apparatus is shown in Fig. 2. A related focusing technique has been used to determine molecular geometries (7). After effusing in thermal equilibrium at temperature T from an oven, the molecules are collimated by a narrow scanning slit. As each molecule passes through the field, its trajectory is curved proportionally to its effective dipole d. Trajectories of a particular curvature enter the narrow slit of a mass-selective detector. The detected signal I(D) is measured as a function of the displacement D of the central slit, which compensates for the curvature; D is proportional to the effective dipole d, averaged over the flight time in the field:

$$D = A \frac{\partial E}{\partial x} \frac{1}{T} d \qquad (4)$$

The constant A depends only on instrumental geometry, $\partial E/\partial x$ is the field gradient perpendicular to the beam, and T accounts for the velocity dependence of the deflection.

Using this apparatus, we have investigated the deflection of a large number of compounds as a function of temperature (5, 6). Near room temperature, all those compounds with a permanent dipole undergo a significant deflection, characterized by a broad deflection distribution and a decrease in the normalized signal at the center of the beam (Fig. 3a). This deflection is typical of the rotational behavior of a rigid rotor. Each molecule leaves the oven with its own value of the angles $\angle(\mathbf{E}, \mathbf{J})$ and $\angle(\mathbf{\mu}, \mathbf{J})$, and these values are subsequently conserved because there are no collisions. The distribution of deflections arises from the resultant distribution of d values, according to Eqs. 2 and 4.

For asymmetric tops, the residual peak at zero deflection is due to a significant fraction of molecules that are undergoing rigid body trajectories qualitatively different from those of symmetric tops. The trajectoryaveraged dipole of such molecules is exactly zero.

As we have reported elsewhere (5), deflection essentially ceases at higher temperatures (Fig. 3a), at which the average density of molecular vibrational states is on the order of 10⁷ states/cm⁻¹ or more. We attribute this change to statistical intramolecular vibration-rotation energy transfer (IVRET). In highly excited molecules, strong Coriolis and centrifugal couplings cause energy to flow between vibration and rotation. Since $\angle(\mu, J)$ depends on the rotational energy, which varies over time, this angle can vary over time as well, as indicated in Fig. 1b. In the limit of statistical IVRET, the molecule will spend as much time with a particular positive value of $\cos(\mu, J)$ as with its negative. As implied by Eqs. 2 and 4, the timeaveraged force exerted on such molecules is zero, so there is no deflection. Magnetic spin relaxation has been discussed in magnetic deflection experiments analogous to ours (8,

Upon closer examination, we find that the



Fig. 3. Detected beam intensity versus slit displacement D for o-diffuorobenzene. These plots essentially present the distribution of flight time-averaged molecular dipole moments along the deflection field. The field-off plots show the instrumental resolution in the absence of deflection. (a) Deflection distribution at 8.0 kV at 300 and 1095 K. (b) Deflection distribution at 12.0 kV at 1090 K, with expanded horizontal axis. Included is a typical error bar spanning 4 SD.

deflection of hot molecules is not exactly zero but is in fact very slightly shifted toward the regions of higher field, indicating that, averaged over the flight time in the field, the dipole is oriented to slightly favor a lowered interaction energy V. Since the molecular distribution is isotropic before entering the field, it is the field itself that orients the dipole. The time-averaged dipole is much larger than what is expected from the polarizability term of Eq. 1 alone. It is, in fact, exactly what is expected from Eq. 1 including the orientation term, even though no collisions can occur to orient the molecules. In the electric field a particular hot molecule does not, as suggested above, spend exactly as much time with one value of $\cos(\mu, J)$ as with its opposite value. Rather, the ratio of times it spends in these orientations is slightly different from unity and is determined by thermal equilibration with the molecule's own vibrations.

Figure 3b shows the small shift for odifluorobenzene at 1090 K. For three compounds at various combinations of high temperature and voltage, we have carefully measured the shifts $\langle D \rangle$ by integrating over the deflection profiles such as those shown in Fig. 3b. These shifts are presented in normalized form in Table 1. The normalization is performed because the extent of the deflection depends on the field strength, which in turn depends on the alignment of the beam in the field region. For a variety of reasons, this alignment is not as well known as would be preferred, but, because the field dependence of $\langle D \rangle$ is the same for all compounds, the ratio of the deflection for two substances is independent of the alignment. We have therefore used the deflection distributions of benzene and carbon tetrachloride (CCl₄) at room temperature as a calibration

and normalized our data to the data for these compounds. Since neither has a permanent dipole, their induced dipoles arise solely from the electronic polarizability, regardless of their rotational motion. (Because the absolute deflections of CCl₄ and benzene differ by -11 and -31%, respectively, from the values we calculate from the field strength corresponding to the nominal experimental geometry and their known polarizabilities, this normalization amounts to only a small correction.) In Table 1, each deflection entry R is the average of the ratios of the deflections of compound *i* under consideration to the deflection of the compounds used for calibration:

$$R = \frac{1}{2} (\langle D_i \rangle / \langle D_{\text{benzene}} \rangle + \langle D_i \rangle / \langle D_{CCl_4} \rangle)$$

For comparison to experiment, each line of Table 1 contains two theoretical R values calculated according to two extreme limits of the rotational dynamics, based on known values of μ and α . In one limit, that without orientational relaxation, it is assumed that IVRET occurs but that the field has no effect on the rotational motion, only electronically polarizing the molecule. In the other limit, that with thermal orientational relaxation, it is assumed that, as IVRET occurs, the rotational motion relaxes through coupling with the molecular vibrations to generate the full thermal equilibrium polarization with respect to the field. These limiting values are computed from Eqs. 1 and 4, with the μ^2 term in Eq. 1 omitted or included for the two cases. If we compare the measured R values in Table 1 with the two calculations, it is clear that the deflections observed are much greater than those expected from electronic polarizability alone and agree much better with the values

Table 1. Measured normalized deflection compared to model calculations with and without orientational relaxation with respect to the field. The uncertainties correspond to 2 SD in the experimental data. The measured deflections are much larger than those calculated without orientational relaxation and are in considerably better agreement with those calculated with orientational relaxation.

Tom	Voltage (kV)	Number of deter- minations	Normalized deflection R			
perature (K)			Experiment	Calculated with reorientation	Calculated without reorientation	
		0-	Difluorobenzene			
1090	12.0	6	1.22 ± 0.07	1.31	0.54	
1095	8.0	8	0.58 ± 0.05	0.58	0.24	
855	8.0	6	0.83 ± 0.14	0.86	0.31	
		0-	Dichlorobenzene			
1165	12.0	10	0.86 ± 0.12	1.31	0.68	
870	12.0	6	1.56 ± 0.04	2.03	0.91	
1150	8.0	4	0.79 ± 0.06	0.59	0.31	
		p	-Chlorotoluene			
1200	12.0	8	0.84 ± 0.20	1.12	0.66	
1220	8.0	6	0.62 ± 0.17	0.49	0.29	
775	5.1	6	0.46 ± 0.10	0.39	0.19	

predicted from complete orientational relaxation.

Although we believe that vibration-rotation interaction can bring $\cos(\mu, J)$ of an isolated molecule into thermal equilibrium with the applied field, by contrast the distribution of $\cos(\mathbf{E}, \mathbf{J})$ is not affected by the applied field, which simply causes J to precess about E. It may therefore seem surprising that the equilibrium average dipole $\langle d \rangle$ after dielectric relaxation can be the same in the molecular beam as in a gas, in which there are collisions to change $\cos(\mathbf{E}, \mathbf{J})$. The explanation is that, because $\cos(\mu, J)$ and cos(E, J) appear as a product in Eq. 3, the effect of the field will be to induce a positive correlation between these two factors, a correlation that is the same regardless of whether just one or both angles are allowed to vary.

In summary, our data provide dramatic evidence that isolated highly excited molecules can orientationally relax in response to an external field to give a polarization characteristic of a thermal ensemble. This arises because the angle $\angle(\mu, J)$ can relax by the intramolecular conversion of energy between vibration and rotation in response to the field, as shown in Fig. 1. In essence, $\angle(\mu, J)$ behaves ergodically: for a single molecule, the distribution over time of this angle is that of a microcanonical ensemble with the same energy and J. Orientational relaxation both in gases and in condensed phases has been previously thought to require interactions with neighboring molecules. The effect we have reported should be directly observable by the more conventional methods of monitoring the dielectric response of a low-pressure gas to an oscillating field, as long as the frequency of the field is slower than IVRET but faster than the collision rate. It may be relevant to the process of field ionization (10), in which an enhancement of the ionization rate occurs, due to the attraction of gas molecules into a strong inhomogeneous field.

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The Actin-Binding Protein Profilin Binds to PIP₂ and Inhibits Its Hydrolysis by Phospholipase C

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Profilin is generally thought to regulate actin polymerization, but the observation that acidic phospholipids dissociate the complex of profilin and actin raised the possibility that profilin might also regulate lipid metabolism. Profilin isolated from platelets binds with high affinity to small clusters of phosphatidylinositol 4,5-bisphosphate (PIP₂) molecules in micelles and also in bilayers with other phospholipids. The molar ratio of the complex of profilin with PIP₂ is 1:7 in micelles of pure PIP₂ and 1:5 in bilayers composed largely of other phospholipids. Profilin competes efficiently with platelet cytosolic phosphoinositide-specific phospholipase C for interaction with the PIP₂ substrate and thereby inhibits PIP₂ hydrolysis by this enzyme. The cellular concentrations and binding characteristics of these molecules are consistent with profilin being a negative regulator of the phosphoinositide signaling pathway in addition to its established function as an inhibitor of actin polymerization.

HE INHIBITION OF THE INTERACtion of actin with profilin, the most abundant actin-binding protein, by the membrane phospholipid PIP_2 (1) is one of a growing number of examples where phospholipids can influence cytoplasmic proteins (2). In the case of profilin and actin, it has been assumed that PIP₂ binds to the profilin. PIP2 is also the precursor of two second messengers, inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol, which regulate a variety of cellular processes (3). We have characterized the interaction of human platelet profilin with PIP₂ in vitro and present evidence that profilin can regulate phospholipid metabolism.

In a gel filtration assay (Fig. 1, A to E, and Table 1), 12 molecules of platelet profilin bound to micelles composed of 83 molecules of PIP₂ (4). This corresponds to 1 profilin molecule bound to 7 lipid molecules. The profilin that was bound to micelles eluted in the void volume and was well-separated from the free profilin. As the molar ratio of profilin bound to PIP2 was

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the same (1:7) at all micelle concentrations tested, the affinity of the interaction must be relatively high. The amount of profilin trailing behind the micelle peak was small, which is further evidence that the complex has a dissociation constant (K_d) in the submicromolar range. The amount of profilin bound was the same in buffer with and without 75 mM KCl. Micelles of phosphatidylinositol 4-phosphate (PIP) gave similar results as micelles of PIP₂ (5), whereas much higher concentrations of small unilamellar vesicles (SUVs) of pure phosphatidylinositol (PI) were required to bind profilin.

At a 1:7 binding ratio, the profilin molecules should be tightly packed on the surface of a PIP₂ micelle. This conclusion is based on the dimensions of the profilin molecule $[\sim 3.0 \text{ nm by } 3.0 \text{ nm by } 3.5 \text{ nm } (6)]$ and the 5.5- to 6.0-nm diameter of PIP₂ micelles (4). Thus, steric hindrance is likely to limit the binding of more profilin molecules to the micelles and to result in an overestimate of the number of PIP2 molecules actually associated with each profilin molecule.

To obtain a better estimate of the molecular stoichiometry of the complex, we carried out parallel experiments with small amounts of PIP₂ incorporated into large unilamellar vesicles composed of other lipids. We used large unilamellar vesicles produced by the extrusion technique (LUVETs) (7) that were composed of a 5:1 molar ratio of phosphatidylcholine (PC) to PIP₂. Electron microscopy of negatively stained vesicles showed that they were unilamellar and had a mean diameter of 0.12 μ m (SD = 0.03 μ m, n = 31). Freeze-fracturing (7) showed that these vesicles were unilamellar, and nuclear magnetic resonance (8) showed that PIP₂ partitions between the two leaflets of such vesicles. Thus, we have assumed that 50% of the PIP₂ in the LUVETs is exposed to the medium and available for binding to profilin.

In the gel filtration assay, all concentrations of PC-PIP₂ LUVETs that we tested bound the same number of profilin mole-

Table 1. Characterization of the PIP2-profilin complex. Stoichiometry and Kd were estimated by fitting theoretical curves (obtained by varying these two parameters independently) to the data from the binding filtration assay (22) and the PLC inhibition assay (25) (Fig. 1, E and F; Fig. 2, B and C). Because the bound and free profilin are separated during the gel filtration assay, the equilibrium is perturbed, causing the complex to dissociate and profilin to trail behind the micelles and vesicles. In the PLC inhibition assay, the equilibrium is also perturbed, as PIP2 is hydrolyzed when profilin comes off the PIP₂ clusters. Consequently, the K_d 's estimated by these assays are maximum values. The 95% confidence intervals (in parentheses) were calculated from 8 to 12 separate filtrations for the binding filtration assay, and from two separate experiments (each representing eight individual time courses) for the PLC inhibition assay.

Lipid composition	Stoichiometry (number of PIP ₂ molecules per profilin molecule)		$K_{\mathbf{d}}$ (μM)	
	Filtration	PLC assay	Filtration	PLC assay
Micelles of PIP ₂	7.4 (6.7 to 8.2)	10.0	<0.1	<0.1
LUVETs of PIP ₂ :PC (1:5)	5.4 (3.4 to 7.3)		<1.0	
LUVETs of PIP ₂ :PC:PE (1:1:1)		4.8 (3.9 to 5.8)		<1.0
LUVETs of PIP ₂ :PC (1:12)		5.0		<5.0
LUVETs of PIP ₂ :PC:PE (1:5:5)		5.0		<1.0
LUVETs of PI:PC (1:5)			<1000	
SUVs of PI			<1000	

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