of the $\mu$-$N_2$ of **2** is observed, an indication that the terminal dinitrogen ligands are playing roles beyond that of mere spectators in the reaction of **1** with HCl.

The data require a reaction sequence mediated by a symmetric species in which one terminal $N_2$ and the $\mu$-$N_2$ have become equivalent.[9] While a number of mechanisms satisfying this requirement could be formulated, we favor one involving protonation of a terminal dinitrogen of **1**, loss of the other terminal $N_2$, and generation of the symmetric reaction intermediate $(C_5Me_5)_2Zr(N_2H)_2$ (**3**).[10] Consistent with the labeling experiments, **3** would then lead to 1 mol each of $N_2$ and $N_2H_4$ (eq 3).

$$(C_5Me_5)_2Zr(N_2H)_2 + 2HCl \rightarrow (C_5Me_5)_2ZrCl_2 + N_2 + N_2H_4 \quad (3)$$

Generation of the neutral, monomeric species **3** from **1** would require a formal two-electron transfer to the $N_2$-bearing Zr accompanied by release of the other zirconium in the fully oxidized state, i.e., as $(C_5Me_5)_2ZrCl_2$. Strong electronic coupling of the two Zr(II) centers through the $\mu$-$N_2$ of **1** as suggested by its structural features, ir, and visible spectra should facilitate such a Zr-to-Zr charge transfer.

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## References and Notes

(1) J. M. Manriquez and J. E. Bercaw, *J. Am. Chem. Soc.*, **96**, 6229 (1974).
(2) Notable exceptions are complexes of the type $ML_4(N_2)_2$ (M = Mo, W; L = $PMe_2Ph$, $PMePh_2$), which have recently been shown to liberate up to 1.8 mol of ammonia in acid methanol: J. Chatt, A. J. Pearman, and R. L. Richards, *Nature (London)*, **253**, 39 (1975); J. Chatt, *J. Organomet. Chem.*, **100**, 17 (1975).
(3) J. M. Manriquez, D. R. McAlister, E. Rosenberg, A. M. Shiller, K. L. Williamson, S. I. Chan, and J. E. Bercaw, manuscript in preparation.
(4) Colorimetric determinations of ammonia (indophenol; J. A. Russell, *J. Biol. Chem.*, **156**, 457 (1944)), and hydrazine (*p*-dimethylaminobenzaldehyde; I. D. Snell and C. T. Snell, "Colorimetric Methods of Analysis", Vol. IIA, D. Van Nostrand, New York, N.Y., 1959, p 707), indicate that the reduced $N_2$ consists of 0.86 mol of $N_2H_4$ and 0.24 mol of $NH_3$ per mol of 1. Treatment of **1** with anhydrous $N_2H_4$ in toluene at −80° (instantaneously) yields $N_2$ (3 mol) and $NH_3$ (2 mol), thus indicating that ammonia could arise from interference of this reaction during the latter stages of the reaction with HCl.
(5) A small correction for residual $N_2$ trapped in the frozen toluene (0.0185 mmol as determined by a blank) was made in calculating the expected fraction of $^{15}N_2$ evolved in the addition of HCl.
(6) Since (1) the reaction proceeds only 86% according to eq 2 (footnote 4), and (2) iodate oxidizes $N_2H_4$ but not $NH_3$ to $N_2$, hydrazine labeling should be somewhat more reliable.
(7) **2** is prepared by treatment of **1** with CO at −23° and isolated as metallic green crystals. On the basis of analytical data, ir ($\nu$(CO) 1902 (ms), 1860 (s); $\nu$(NN) 1682 (ms)), and NMR ((toluene-$d_8$) s, $\delta$ 1.80 (30 H); s, 1.82 (30 H)), the structure of **2** is believed to be identical with **1** with carbonyl substituted for the terminal dinitrogen ligands.
(8) Apparently some carbon monoxide is reduced when **2** is treated with HCl. The identity of the reduction product(s) is presently under investigation.
(9) This intermediate need only be symmetric on the reaction time scale, so that a monoprotiated species such $[(C_5Me_5)_2Zr(N_2)(N_2H)]^-$ is a possibility, providing proton transfer between dinitrogen ligands is sufficiently rapid. No significant variation in $X(^{15}N_2)$ was observed with DCl (Table II), so that if this is the case such a proton transfer cannot be so slow as to be comparable to the rate of subsequent reaction steps.
(10) Our data do not exclude the possibility that this symmetric intermediate could, in fact, be dimeric (e.g., $[(C_5Me_5)_2Zr(\mu\text{-}N_2H)_2Zr(C_5Me_5)_2]^{2+}$, $[(C_5Me_5)_2Zr(\mu\text{-}N_2H_2)_2Zr(C_5Me_5)_2]^{4+}$); however, in view of the high charges necessarily associated with such dimers, we favor the neutral monomer **3**.

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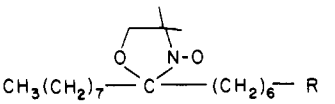
## A New Measurement of Surface Charge in Model and Biological Lipid Membranes

*Sir:*

We report that the distribution of charged, amphiphilic spin labels,[1] **1** and **2**, between lipid membranes and buffered salt solutions is related quantitatively to the surface charge density of the membrane. Previous measurements of conductance across model bilayer membranes[2] and of surface potentials of lipid monolayers[3] demonstrated that the relation between surface charge density and binding of electrolytes at the lipid–water interface is described to a first approximation by the Gouy–Chapman double layer analysis.[4,5] The principal aims of the study reported here are to develop a technique whereby changes in surface potential may be measured under similar conditions in both model and biological membranes and at the same time to detect changes in lipid fluidity. This approach should also be useful in assessing the bilayer free energy in a lipid phase separation.[6]




$R = -CH_2\overset{+}{N}(CH_3)_3$ (1)

$= -COO^-$ (2)

Two overlapping paramagnetic resonance signals are evident in Figure 1. A sharp, three-line spectrum (indicated by arrows) arises from label **1** tumbling rapidly in aqueous solution. The portion of label bound to membranes contributes the broad signal. The relative intensities of the two signals are a function of lipid surface charge density, total lipid concentration, and lipid fluidity.

In typical measurements, samples initially containing $10^{-4}$ M label and $10^{-2}$ M multilayered lipid liposomes[7] in buffer (0.1 M NaCl, 0.05 M Tris, pH 8.0) are diluted with buffer and allowed to equilibrate overnight. Quantitative determination of sharp signal intensity is made by comparison of lipid samples with a standard containing only label and buffer. Figure 2 shows that the distribution of label **1** between lipid and aqueous solution is linear over a 20-fold lipid concentration range, and increases as the fraction of lipid bearing a net negative charge increases.

At low ratios of label to lipid, the relation between binding of label and membrane surface potential, $\Psi$, is

$$\text{label bound/label free} = k \exp(\pm F\Psi/RT) \quad (1)$$

Assuming that the lipid suspensions contain large particles (~1000 Å diameter[7]) so they may be treated as flat diffusely charged layers, the Gouy–Chapman analysis may be applied:

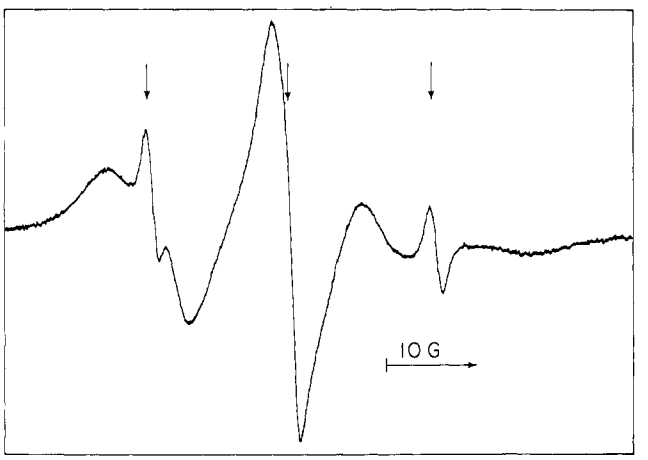



**Figure 1.** Typical EPR signal of label **1** in 1% (w/w) aqueous, lipid dispersion.

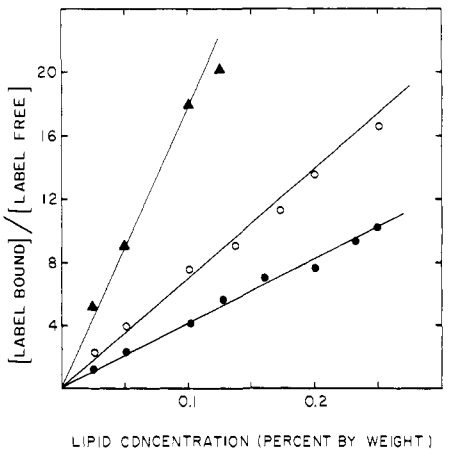



**Figure 2.** Binding at 27 °C of **1** vs. lipid concentration for egg lecithin plus 0% (●), 5 mol % (○), and 10 mol % cardiolipin (▲). Cardiolipin bears two negative charges/molecule.

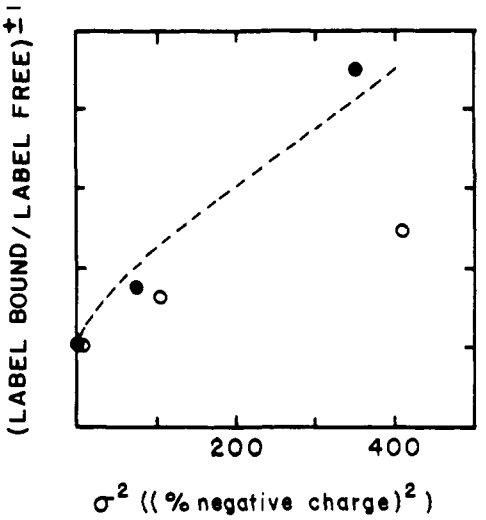



**Figure 3.** Normalized slopes of the type of plot shown in Figure 2 vs. $\sigma^2$, for label **1** (closed circles) and label **2** (open circles). Data are corrected for surface charge imparted by bound label. Dashed line is binding calculated for 60 Å$^2$/molecule.

$F\Psi/2RT = \sinh^{-1} x$ ($x = \sqrt{\sigma^2\pi/2\epsilon RT \sum_i c_i}$ for monovalent electrolytes of concentration $c_i$; $\sigma$ = surface charge density; $\epsilon$ = dielectric constant of medium). With $\sinh^{-1} x = \ln(x + \sqrt{x^2+1})$ eq 1 becomes:

$$\text{label bound/label free} = k(x + \sqrt{x^2+1})^{\pm 2} \qquad (2)$$

For negatively charged lipid, the positive exponent applies to label **1** and the negative exponent to label **2**.

Figure 3 shows that the distribution of label **1** vs. $\sigma^2$ (filled circles) agrees well (within the limits of uncertainty of area/molecule) with binding calculated from eq 2 for a bilayer with 60 Å$^2$/molecule (dashed line). Label **2** binding falls below the theoretical curve and may indicate that the labels are not completely ionized in the membranes. (Label **2**, unlike **1**, does not give linear plots of the type shown in Figure 2 until buffer concentration ≥0.05 M.)

As predicted in eq 2, the binding of **1** to red blood cell ghosts is linear with $1/\sum_i c_i$ over a 20-fold concentration range. Lipid fluidity affects $k$ in eq 2 in the same direction for both labels:[8] rigidity imparted by cholesterol decreases the bound/free ratio and sonication produces a slightly enhanced ratio.

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## References and Notes

(1) **1** = 2-(7-trimethylammoniumheptyl)-4,4-dimethyl-2-octyl-3-oxazolidinyl oxyl (synthesis to be reported later). **2** = 2-(6-carboxyhexyl)-4,4-dimethyl-2-octyl-3-oxazolidinyl oxyl (W. L. Hubbell and H. M. McConnell, *J. Am. Chem. Soc.*, **93**, 314 (1971)).
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## Stereospecific Synthesis of Penicillins. Conversion from a Peptide Precursor

*Sir:*

The antibiotic penicillin **1**[1] is a remarkable substance, both therapeutically and chemically. One of the more notable aspects of its chemistry, which is perhaps less well recognized, is the paucity of successful total syntheses of this molecule. At present the literature contains four claims,[2-4] none of which represents a stereocontrolled synthesis.[5] On the other hand, the less strained cephalosporin molecule and its derivatives have been the target of a number of successful stereospecific syntheses.[6-11] The existence of this situation undoubtedly results from the coincidence within the penicillin molecule of both ring strain and a very high concentration of functionality. We now present the first stereocontrolled total synthesis of a penicillin system.

Recently we described a stereospecific conversion of a dipeptide into β-lactam systems, for example **2**.[12] An extension of the original scheme[12] has now enabled conversion of a dipeptide into a penicillin, as follows. In order to close the thiazolidine ring in derivatives of **2** we required a suitably functionalized valine unit. We chose D-isodehydrovaline (**3**), which was readily obtained from methyl 2-nitrodimethylacrylate by deconjugation of the potassium salt (potassium hydride, THF, 0°) with aqueous hydrochloric acid to the β,γ-unsaturated ester **4** (at 0°, bp 115–116° (24 mm), 96%)[13] which was reduced with tin/hydrochloric acid at 95° to racemic **3** (mp 206–208° dec, 74%). Resolution of the chloroacetyl derivative of **3** with hog acylase 1, (Sigma Chemical Co.), gave, after hydrolysis (hot aqueous HCl) D-isodehydrovaline (**3**) (mp 202–205° dec, $[\alpha]^{27}$D −104.7° (*c* 3, $H_2O$) 60%).[14] This was coupled, as its methyl ester, with the thiazolidine acid **5**[12] (EEDQ, quinoline, $CH_2Cl_2$, 0°) to the dipeptide **6** (X = H, mp 185–186°, $[\alpha]^{27}$D −177.3 (*c* 1.1, $CHCl_3$), 28%). Stereospecific functionalization α to the sulfur atom was achieved with benzoyl peroxide (carbon tetrachloride, reflux) to the benzoate **6** (X = OCOPh, mp. 179–181°, 40%), which on treatment with hydrogen chloride ($CH_2Cl_2$, 0°) gave the chloride **6** (X = Cl, mp 137–138° dec, $[\alpha]^{28}$D −39.2 (*c* 1.2, $CHCl_3$), 94%). The stereochemistry of this series was proved by the NMR spectra. For example, in **6** (X = Cl) the coupling constant between the two vicinal thiazolidine protons was 0 Hz; a rationale for this has been previously presented.[12]

Closure of **6** (X = Cl) to the β-lactam was achieved smoothly with NaH in $CH_2Cl_2$/DMF at 0° yielding **7** (oil, purified by chromatography on silica gel; 82%, $[\alpha]^{27}$D −309° (*c* 2.6, $CHCl_3$); NMR δ 5.53 and 5.73 (2 H, AB quartet, $J$ = 5 Hz); $\nu_{max}$ ($CHCl_3$) 1769, 1740, 1655 cm$^{-1}$), which was ox-