part of the Carbondale Formation. (iv) During later Desmoinesian time lycopods still dominated younger floras, but ferns and pteridosperms were second and third in abundance. (v) At the Desmoinesian-Missourian boundary, *Lycospora*-producing lepidodendrids terminated abruptly. (vi) In Late Pennsylvanian time, tree ferns were dominant, except in several coal floras that contained abundant *Sigillaria* and *Polysporia*; seed ferns and calamites were subdominant.

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Spectroscopy of Biological Compounds with Inelastic Electron Tunneling

Abstract. *Metal-insulator-metal electron tunnel junctions can be doped with a solution of an organic compound by placing a drop of the solution on the insulator and spinning off the excess. Electrical measurement of the second derivative of voltage with respect to current, as a function of applied voltage, then gives a spectrum of vibrational modes equivalent to an infrared or Raman spectrum, but with the use of only micrograms of sample.*

Inelastic electron tunneling can be used to detect vibrational modes of organic compounds that are active in both the infrared and Raman regions (*1*, *2*). The method can be applied systematically to a wide range of organic compounds and can be used successfully as an analytical tool (*3*, *4*). The compound to be investigated is placed on the insulator of a metal-insulator-metal electron tunnel junction. Inelastic electron tunneling events, in which an electron tunnels from one metal to the other with the excitation of a vibrational mode of the organic compound, then produce an abrupt change in the dynamic resistance, dV/dI, and a peak in the second derivative, d^2V/dI^2, at an applied voltage $V = \hbar\omega/e$, where $\hbar\omega$ is the characteristic energy of the mode excited, e is electronic charge, and I is current (*5*). The primary disadvantage of tunneling spectroscopy over infrared and Raman spectroscopy is that cryogenic temperatures are required. The primary advantage is that roughly 1/100 of the amount of sample is required: < 10 μg rather than $\gtrsim 1$ mg.

We report here preliminary results on a new method of doping junctions with organic compounds that is applicable to all organic compounds, including those of biological interest. The technique consists of placing a drop (2 to 10 μl) of a solution of the compound directly onto the insulating layer and then spinning the junction at approximately 3000 rev/min in order to remove the excess. Solvents such as water, alcohol, benzene, and chloroform have been used successfully. The concentration of the solution is only critical to within a factor of 2, and appropriate concentrations have been in the range 0.1 to 1.0 mg/ml.

Previous methods have used vacuum evaporation of the compound and were limited to compounds which evaporate before decomposing. The method discussed here also produces junctions with generally better resolution, which is probably connected with a more uniform doping of the junction since vacuum evaporation is difficult to control precisely.

The tunnel junctions were Al-Al_2O_3-Pb sandwiches fabricated in the conventional (*6*) crossed film geometry on microscope slides (1 by 3 inches). The Al bottom electrodes were evaporated in a high-vacuum evaporator and then oxidized for roughly 5 minutes in a laminar flow bench. The oxidized Al strips were then doped as described in essence above and in detail elsewhere (*7*). Finally, the slides were returned to the vacuum evaporator for evaporation of the Pb top electrode.

The completed junctions were tested with a low-voltage ohmmeter (*8*). The undoped junctions had resistances in the range 15 to 40 ohms, while acceptable doped junctions had resistances in the range 1 to 100 kilohms. Connections were made to the electrodes with silver paint, and the junctions were then immersed in liquid helium. Curves of d^2V/dI^2 versus V were plotted by applying an a-c modulation current at a frequency of approximately 1000 hertz and measuring the voltage at the second harmonic frequency with a lock-in amplifier.

Figure 1 shows spectra obtained from the amino acid L-phenylalanine in water and the pyrimidine base uracil in water. Tunneling spectra for these two compounds have been obtained by the vacuum evaporation technique (*4*), but the spectra in Fig. 1 show considerably improved resolution and generally resolve all modes that are resolved in the corresponding infrared spectra. The full width at half maximum of the sharper peaks in Fig. 1 is approximately 20 cm^{-1}, which is determined by the magnitude of the modulation, $(2\ mv)_{rms}$ (rms = root mean square); the peak broadening due to a modulation voltage V_m is $1.22 \times 8.065\ (cm^{-1}/mev) \times eV_m$ (*2*). [Recent measurements with modulation voltages of $(0.6\ mv)_{rms}$ have given full widths at half maximum as small as 7 cm^{-1} (*7*).] At higher temperatures the line broadening is dominated by the thermal broadening of 5.4 kT

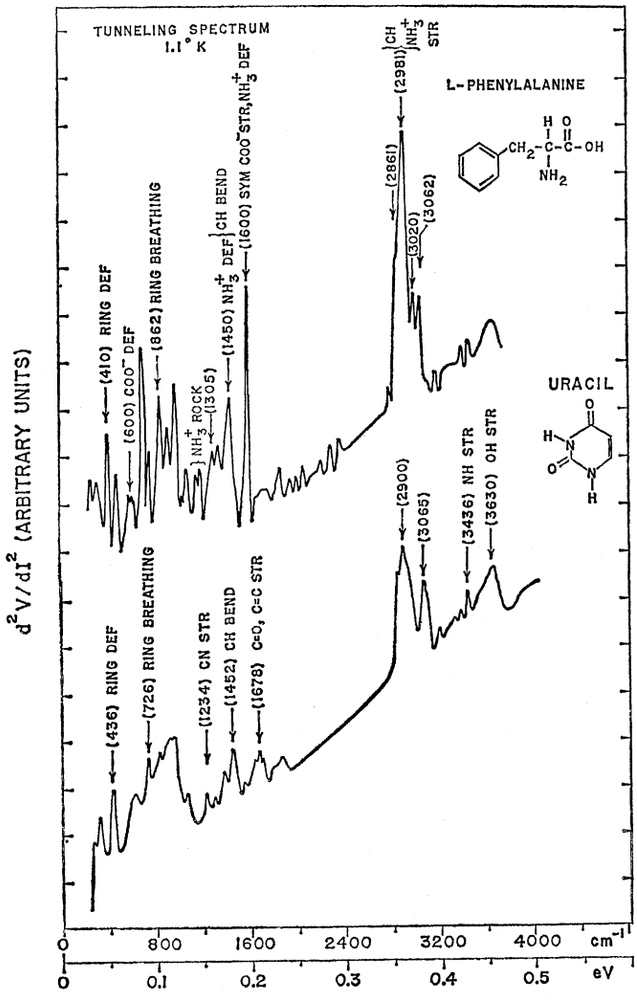
(*1*, *2*), where k is the Boltzmann constant and T is temperature. For example, at 77°K this broadening is approximately 300 cm^{-1}.

The tunneling spectrum of L-phenylalanine shows at least four bands in the region of 3000 cm^{-1} with three of them well resolved, in complete agreement with the infrared spectrum. These are to be identified with C-H and NH_3^+ stretching modes, the NH_3^+ mode arising since the α-amino acids either in the solid state or at their isoelectric point in aqueous solution exist almost entirely as dipolar ions. The asymmetric stretching mode of the COO^- group (carboxyl ion) at 1600 cm^{-1} and a deformation mode of the NH_3^+ in the range 1590 to 1660 cm^{-1} (amino acid I band) may both contribute to the very intense mode observed at 1600 cm^{-1} in the tunneling spectrum. Other specific modes resolved in the infrared spectra of the α-amino acids are an NH_3^+ deformation mode at 1550 to 1480 cm^{-1} (amino acid II band), an unidentified band at 1300 cm^{-1}, and a band near 880 cm^{-1} due to a rocking motion of the NH_3^+ groups. Sharp lines near these wave numbers are observed in the tunneling spectrum and are indicated in Fig. 1. Many additional modes are well resolved in the case of L-phenylalanine and correspond to those observed in the infrared spectrum.

The tunneling spectrum of uracil shown in Fig. 1 is in almost perfect agreement with the modes resolved in both the infrared and Raman spectra (*4*). The major modes are identified and labeled in Fig. 1.

To demonstrate that the technique is applicable to even the largest organic molecules we show results for calf thymus DNA and transfer RNA (tRNA) from *Escherichia coli* in Fig. 2. The upper curve shows the tunneling spectrum of pure calf thymus DNA dissolved in distilled deionized water, and characteristic modes are identified. The tunneling spectrum of tRNA is almost identical to the DNA spectrum, as it should be.

A detailed comparison with the infrared spectra of DNA and tRNA should await further development of the technique, but some general comments can be made. Both the DNA and tRNA tunneling spectra are remarkably similar and exhibit identical characteristic modes. A comparison of the tunneling data with infrared data on oriented films of calf thymus DNA (*9*) and tRNA (*10*) taken in various states of relative humidity indicates that most of the loosely adsorbed water is probably gone in the tunneling experiments. The intense band usually present at 3400 cm^{-1} and assigned to the OH stretching vibration of adsorbed water molecules is not observed in the tunneling spectra. The tunneling spectra of DNA and tRNA show much more intense bands at $\sim$ 2900 cm^{-1},



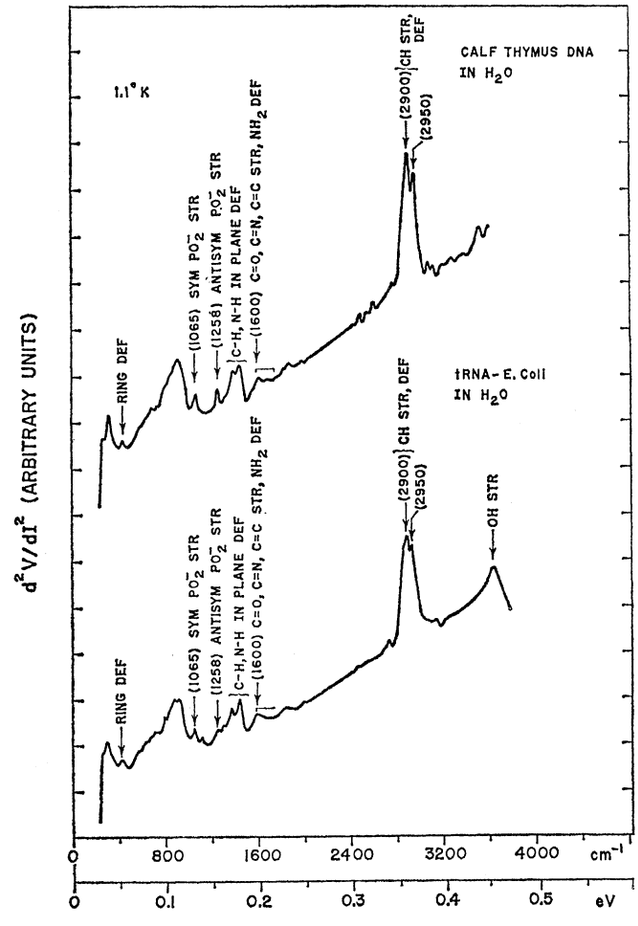


Fig. 1 (left). Tunneling spectra of L-phenylalanine dissolved in water (upper curve) and uracil dissolved in water (lower curve). The experimental apparatus measures d^2V/dI^2. Conversion to d^2I/dV^2 can be accomplished by multiplying by minus the dynamic resistance cubed $-(dI/dV)^3$, which is essentially constant over the range measured here. Abbreviations: *DEF*, deformation; *STR*, stretch; *SYM*, symmetric. Fig. 2 (right). Tunneling spectra of calf thymus DNA and tRNA from *E. coli*.

which are undoubtedly due to C-H stretching modes associated with deoxyribose or ribose groups as well as to C-H stretching modes associated with the base residues. In this respect, they are in almost exact agreement with the Raman spectrum of solid calf thymus DNA (*11*), which also shows two intense broad C-H stretching bands at 2900 and 2950 cm^{-1}. Plane deformation modes of C-H and N-H in the base residues also give rather strong peaks in the tunneling spectrum between 1300 and 1500 cm^{-1}.

Characteristic modes due to the symmetric and antisymmetric stretching vibrations of the PO_2^- ion are identified at $\sim$ 1050 cm^{-1} and 1180 to 1240 cm^{-1}, respectively, in the infrared spectrum. The tunneling spectra show fairly strong peaks at $\sim$ 1065 cm^{-1} and $\sim$ 1258 cm^{-1}, which is quite consistent with the infrared identification, although this should be considered tentative since other modes may contribute in this region.

The tunneling spectra of both DNA and tRNA show modes of medium intensity between 1600 and 1700 cm^{-1}. These can reasonably be assigned to C=O, C=N, and C=C double bond stretching and NH_2 deformation vibrations of the base residues.

These results demonstrate that the solution doping technique should be applicable to any soluble organic compound. The inelastic tunneling spectra are comparable in information content to infrared or Raman spectra and have great potential as a new analytic tool—especially where only microgram quantities of sample are available.

Important questions remain for further detailed investigation. What is the effect of the vacuum dehydration and cooling on the vibrational spectra of macromolecules? Are the macromolecules damaged by the evaporation of the Pb top electrode? A comparison of the tunneling spectra with the infrared and Raman spectra suggests that frequency shifts due to surface adsorption on the alumina and due to the evaporated Pb electrode are small in large classes of compounds (*3, 4*). However, there are other compounds that undergo surface reactions (*2, 7, 12*). Can general rules be formulated to predict this behavior?

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Peptide Regulation of Bursting Pacemaker Activity in a Molluscan Neurosecretory Cell

Abstract. *Vasopressin and related peptides (10^{-9} to 10^{-6} molar) induced bursting pacemaker potential activity and altered the current-voltage relations of the membrane in a specific molluscan neurosecretory cell. These effects long outlasted the period of application of the peptides. Sensitivity of the cell to these peptides was primarily localized on the axon hillock region. The observed effects do not resemble conductance changes evoked by conventional neurotransmitters, but rather suggest a membrane regulatory role for these peptides, and thus may be indicative of a new form of information transfer in the nervous system.*

The presence of a seasonal rhythm in the electrophysiology and biochemistry of a specific neurosecretory cell in the land snail, *Otala lactea* (*1, 2*), has prompted us to investigate the effects of vertebrate peptide hormones as putative neurohormones mediating this rhythm. We report here that lysine-vasopressin (LVP) and related peptides have potent and specific regulatory effects on the electrophysiology of this neurosecretory cell. Results demonstrate that specific peptides can regulate electrical activity of neurons in a different manner than conventional synaptic transmitters; this may represent a new form of neuronal modulation.

Specimens of *Otala lactea* were obtained from Scozzaro, Inc. (Brooklyn, New York) and were maintained in well-aerated plastic containers in a dormant, semidormant, or activated state, according to methods previously described (*2, 3*). In a typical experiment the fused ganglia were removed from the snail and pinned to Sylgard in snail saline (*4*), and the protective sheaths covering the large nerve cells were carefully excised. One or two micropipettes filled with 3*M* KCl were placed in identified cells (*1*), and the electrical activity of these cells was recorded by using conventional techniques. The LVP and related peptides (*5*) were made up as 1 m*M* stock solutions in distilled water. Each stock solution was either diluted for bath application or placed in a micropipette to be iontophoresed (as the cation). Acetylcholine (ACh) was treated in a similar fashion.

Cell 11 from dormant (or semidormant) snails either was electrically inactive (Fig. 1, A1), or exhibited spontaneous activity characterized by a beating pattern of spikes at a fairly constant frequency (Fig. 1, B1, B2, and D). The current-voltage relations of the membranes of these cells were linear (Fig. 1, A2), and changes in membrane potential by injections of transmembrane current could not produce bursting pacemaker potential (BPP) activity (for example, see Fig. 1, A1, control). Bath application of $10^{-9}M$ LVP rapidly induced BPP activity (Fig 1, A1), and altered the current-voltage relation of the membrane from linear to nonlinear (Fig. 1, A2) (*6*). Identical results were obtained with various similar peptides (10^{-9} to $10^{-6}M$), including arginine-vasopressin, homolysine-vasopressin, 8-L-homonorleucine-vasopressin, and oxytocin. The effects of these peptides were dose-dependent and long-lasting, and prolonged washing (about 1 to 4 hours) in peptide-free saline was required to restore the cell's membrane properties to control values (Fig. 1, A1 and A2). Higher concentrations of LVP (and related peptides) caused