time scale of the convection zone, τ_c ($\sim 10^5$ years), then $\tau = \tau_\infty$ and the change in luminosity will depend on the rate of change of α ($\delta L \propto d\alpha/dt$). Since α has little effect on the structure of the central regions of the sun, the luminosity returns to its original value on the time scale τ_c if the efficiency of convection stops changing.

Faster changes in α ($\tau_\alpha < \tau_c$) lead to different behavior. The internal energy lost (or gained) because of a change of α cannot be replaced (or leak out) on time scales shorter than τ_c. In this case $\tau = \tau_c$ and $\delta L \propto \delta\alpha$ or

$$\delta \log L \cong 0.43 \frac{GM\Delta m}{LR^2} \frac{H}{\tau_c} \delta\alpha \cong 0.2\delta\alpha$$

for a standard solar model, where $H = 10^{10}$ cm is the pressure scale height; M is the mass of the sun, $M_\odot$; Δm is $0.008\ M_\odot$; $R = 6 \times 10^{10}$ cm is the radius at the base of the convection zone; L is the solar luminosity, $L_\odot$; and τ_c is 10^5 years. This behavior has been observed in detailed numerical models with reasonable quantitative agreement (see Fig. 1). If there is a single rapid change in α, the luminosity will quickly change by an amount $\sim 0.2\ \delta\alpha$, and then decay to the original luminosity on a time scale as shown in Fig. 2. However, if α continuously changes on a short time scale, the luminosity will accurately track the change in α. Our model yields a relation $dL = 4 \times 10^4\ d\alpha/dt$ in the case where the convective efficiency changes on a long time scale ($\gtrsim 10^5$ years). This is a slightly larger change than that suggested by Ulrich (*8*), but the difference can be accounted for by uncertainties in the actual structure of the convection zone (differences in solar models).

Thus it is seen that changes in the solar constant of 1 percent can result from varying α by as little as 0.02. One wonders then whether it is reasonable for solar convection to be so precisely characterized by an average quantity like α, or whether small changes in the effective mixing length (efficiency of convection) might naturally occur (*9*).

The present understanding of convection is inadequate to prove the existence of time-varying convective efficiency, but such variation is not out of the question. Abbott's belief that he had detected a time variation of the solar constant has been widely doubted, but modern observations cannot exclude variations in the solar constant of 0.1 to 1 percent on a time scale of years to decades. In view of the extreme sensitivity of global climate models to changes in the solar constant (*10–15*) and the results of our analysis, which indicate that variations in the solar constant can occur on short time scales, it would seem that an observational program to monitor the solar constant over a period of years to 0.1 percent or better is in order.

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Investigation of Electron Tunneling Between Cytochrome c Peroxidase and Cytochrome c

Abstract. *The nature of electron transfer between the bound complex cytochrome c and cytochrome c peroxidase has been investigated. Experimental verification of the predicted charge-transfer band provides evidence of electron tunneling as the mechanism of transfer between these molecules in solution at room temperature. The measured transfer distance is ≃7 angstroms between heme edges, which results in a distance of ≃15 to 20 angstroms between iron atoms.*

We report photoinduced electron transfer between cytochrome c (C) and cytochrome c peroxidase (CP) at room temperature, which has been predicted by the theory of nonadiabatic electron tunneling in biological molecules (*1*). This model predicts a new charge-transfer band (*2*) whose intensity and width provide a definitive verification of electron tunneling, yield information about the transfer distance, and characterize the properties of the electron tunneling process. Previous experiments by Potasek and Hopfield (*3–5*) have verified this process in a model system consisting of cytochrome c–iron hexocyanide [C-Fe(CN)$_6$]. In this report, nonadiabatic electron tunneling is verified between CP and C, a biological donor-acceptor pair found in mitochondria.

Cytochrome c peroxidase catalyzes the oxidation of ferrocytochrome c (C^{II}) to ferricytochrome c (C^{III}) in the presence of hydroperoxide. The binding between CP and C is electrostatic, with two binding sites for C on CP (*6*), and depends on ionic strength and pH.

Electronic wave function overlap enables electron tunneling to occur between CP and C and also allows for the direct optical excitation of an electron from one molecule to the other without first exciting the electron on one molecule. The molar extinction coefficient for this charge-transfer band is given by (*2*)

$$\epsilon(E) = 1.22 \times 10^{19} \frac{T^2a^2}{E\sigma} \exp\left[-\frac{(E - E_{peak})^2}{2\sigma^2}\right] \quad (1)$$

in which E is energy, E_{peak} is the peak position of the Gaussian charge-transfer band, σ is the standard deviation, T is the tunneling matrix element and is related to the distance transferred, and a is the distance between the centers of gravity of the donor and the acceptor.

However, the magnitude of the charge-transfer band is on the order of 10^6 times smaller than other sample absorbances, which renders static spectroscopy unfeasible. A method of excitation spectroscopy was used to detect the band. The experimental apparatus has been described previously (*4*). It includes an excitation source, a filtered tungsten lamp, the light from which is chopped by a variable-speed chopper and then focused on the sample. The change in sample absorbance is monitored by light from a xenon arc lamp that is passed through a monochromator.

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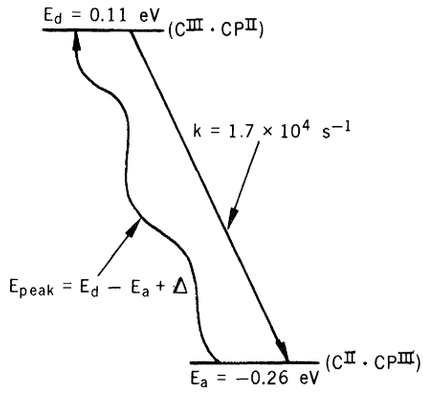

Fig. 1. Energy level diagram for cytochrome c peroxidase–cytochrome c.

After passing through the sample, the monitoring beam is detected by a photomultiplier tube (PMT). This beam is measured by a two-phase lock-in amplifier. For the system used in these studies, the intensity of the signal is given by

$$I = \frac{4.8 \times 10^{-26} \Delta\epsilon}{(k^2 + \omega^2)^{1/2}} \int_0^\infty N(E)\, \epsilon(E)\, dE \qquad (2)$$

where $N(E)$ is the number of photons per second per square centimeter per erg from the excitation source; k is the rate of electron transfer; ω is the chopping angular frequency of the excitation source and is related to the phase angle, θ, and the rate, k, by the expression $\omega = k \tan \theta$; $\Delta\epsilon$ is the change in molar extinction coefficient of the sample when an electron is transferred from one molecule to another; and $\epsilon(E)$ is given by Eq. 1. The phase angle is a measure of the lag between the signal measured by the PMT and the chopped excitation light from the tungsten lamp. The tangent of θ is obtained by dividing the out-of-phase signal from the lock-in amplifier by the in-phase signal.

In order to have a cyclic process for modulation and a stable ground state, the redox levels between the +2 and +3 states on CP and C were selected. The reaction is given by the expression

$$CP^{III} \cdot C^{II} \rightleftarrows CP^{II} \cdot C^{III} \qquad (3)$$

The forward reaction is the photoinduced electron transfer. Figure 1 shows the redox energy of the states involved. The charge-transfer band will occur at photon energy (*3*) $E_{peak} = E_d - E_a + \Delta$, where $E_d - E_a$ is the difference in redox energy of the system and Δ is the amount of energy lost to nuclear vibrations.

The signal from an anaerobic sample consisting of 55 μM yeast CP^{III} and ~ 115 μM C^{II} in 0.01M tris HCl in D_2O at pH 5.6 was measured as a function of chopping angular frequency, monitoring beam wavelength, and excitation energy. The "downhill" electron transfer rate can be determined from the expression $k = \omega/\tan \theta$. At a chopping angular frequency of ω = 9420 sec^{-1} the measured phase angle is $\theta = 29°$, yielding the rate $k = 1.7 \times 10^4$ sec^{-1}.

Measurements of the signal as a function of monitoring wavelength demonstrate that the electron is actually transferring between the states in question and rule out incorrect electron transfer processes. The change in extinction coefficient, $\Delta\epsilon$, is given by

$$\Delta\epsilon = (\epsilon_{CP^{II}} - \epsilon_{CP^{III}}) + (\epsilon_{C^{III}} - \epsilon_{C^{II}}) \qquad (4)$$

in which the first term is the difference (reduced − oxidized) spectrum of CP and the second term is the difference (oxidized − reduced) spectrum of C. These difference spectra were measured on a spectrophotometer; the millimolar extinction coefficients are given in Fig. 2a. The solid line is the difference spectrum of CP and the dashed line is the difference spectrum of C. The signal was measured as a function of monitoring wavelength, and the observed signal is given in Fig. 2b. The solid line represents the sum of the two difference spectra in Fig. 2a. The data agree well with the predicted spectrum for electron transfer between C and CP.

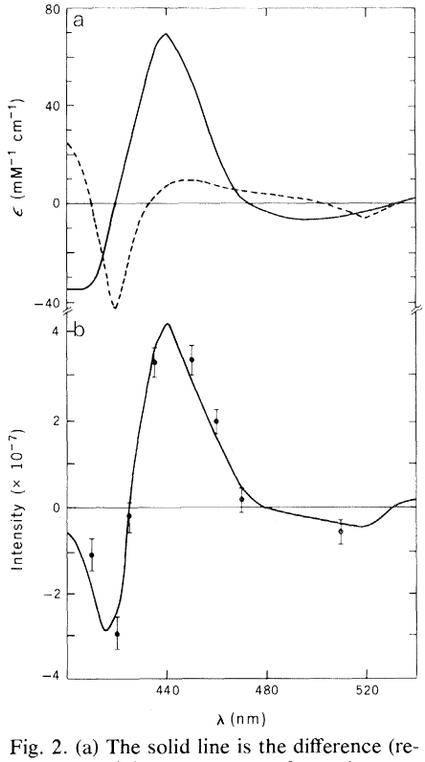

Fig. 2. (a) The solid line is the difference (reduced − oxidized) spectrum of cytochrome c peroxidase. The dashed line is the difference (oxidized − reduced) spectrum of cytochrome c. (b) The solid line is the sum of the difference spectra in the upper portion. The data points are photoinduced electron transfer.

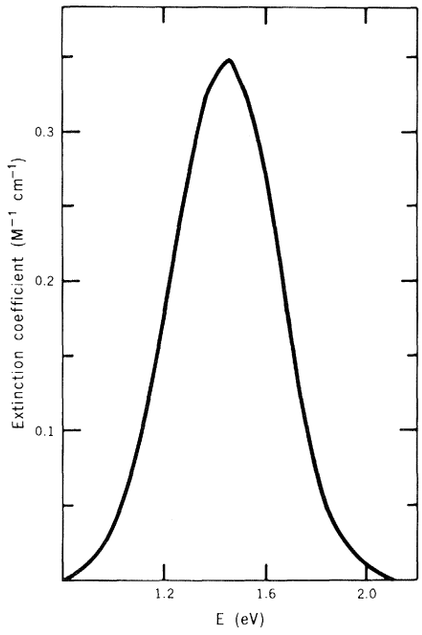

Fig. 3. New charge-transfer band present in the bound complex between cytochrome c peroxidase and cytochrome c.

The properties of the charge-transfer band were investigated by filtering the excitation light. Interference filters that transmit long wavelengths and cut out short wavelengths were used. By spacing the cut-on points of the filters, the shape and location of the band can be determined. These measurements were made at a monitoring wavelength of 450 nm and a chopping angular frequency of ω = 3140 sec^{-1}.

Figure 3 gives the evaluated charge-transfer band (*7*). The peak height is 0.35 M^{-1} cm^{-1}, quite small compared to Soret peaks ~ 10^5 M^{-1} cm^{-1}. The position and width of the band are E_{peak} = 1.44 ± 0.08 eV and σ = 0.21 ± 0.04 eV. The CP^{III} has absorption bands with extinction coefficients on the order of 600 M^{-1} cm^{-1} at 1.33 and 1.18 eV as measured on a Cary 14 spectrophotometer. The new charge-transfer band is well separated from these bands. The C^{II} has no electronic excitations in this region. For $E_d - E_a \simeq 0.37 \pm 0.15$ eV, a value of Δ = 1.07 ± 0.17 eV is obtained, very similar to the Δ = 0.97 ± 0.12 eV obtained for C-Fe(CN)$_6$ (*3*). A width of σ = 0.21 ± 0.04 eV has been measured and is similar to the values predicted for biological electron transfer (*1*) and to the value σ = 0.19 ± 0.02 eV directly measured for C-Fe(CN)$_6$ (*3*).

The peak intensity of the new band is related to the tunneling matrix element. From Eq. 2 and considerations about

the size and orientation of the proteins involved, the matrix element is $T = 9 \pm 3 \times 10^{-4}$ eV. Using an approximate estimate (*1*)

$$T = \frac{2.7}{(N_d N_a)^{1/2}} \exp\left(-\frac{R}{1.4}\right) \quad (5)$$

T can be related to $N_d(N_a)$, the number of atoms over which the electron is delocalized on the donor (acceptor) molecule, and R, the distance between the heme groups on CP and C. For electronic wave functions delocalized over the heme groups, $N_d = N_a = 20$ and Eq. 5 gives the value $R \simeq 7$ Å. This is consistent with values predicted from the theory for other biological systems and similar to the value $R \simeq 7$ to 10 Å for C-$Fe(CN)_6$ (*3*). The distance between iron atoms is ~15 to 20 Å, similar to distances measured by fluorescence techniques (*8*).

The values of T and Δ obtained in these experiments are similar to those inferred in electron transfer processes in bacterial photosynthesis. Depending on which molecules are involved, the theory predicts values of T between 2×10^{-4} and 3×10^{-3} eV. Values of Δ between 0.4 and 1.6 eV are predicted for a series of electron transfers in bacterial photosynthesis. The CP-C value of $\Delta = 1.07$ eV is within this range.

The electron transfer rate, k, can be directly calculated from the parameters Δ, σ, and T (*1*). A value of $k \simeq 1 \times 10^{7 \pm 3}$ sec^{-1} is calculated for the measured parameters $E_d - E_a - \Delta = 0.70 \pm 0.22$ eV, $\sigma = 0.21 \pm 0.04$ eV, and $T = 9 \pm 3 \times 10^{-4}$ eV. The large error is due to the exponential behavior of the rate expression.

These results show the existence of a new charge-transfer band in the bound complex CP-C which is not present in the individual components. This new band and the parameters obtained are significant demonstrations of the validity of nonadiabatic electron tunneling.

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Monensin and the Prevention of Tryptophan-Induced Acute Bovine Pulmonary Edema and Emphysema

Abstract. *3-Methylindole, a ruminal fermentation product of tryptophan, induces acute pulmonary edema and emphysema in cattle, and 3-methylindole is present in the ruminal fluid and blood of cows with a natually occurring form of this disease. Monensin, a polyether antibiotic and widely used feed additive for beef cattle, prevented tryptophan-induced acute bovine pulmonary edema and emphysema. Monensin acted by reducing the ruminal conversion of* L-*tryptophan to 3-methylindole both in vitro and in vivo. Lasalocid, also a polyether antibiotic, showed similar effects in vitro. These results provide a promising approach to prevention of this major respiratory disease of cattle.*

Acute bovine pulmonary edema and emphysema (ABPE) or fog fever is a naturally occurring disease of adult cattle characterized by sudden onset of acute respiratory distress soon after a change to lush forage, usually in the fall (*1*). The disease occurs in many parts of the world including the United States, Canada, and Europe (*1, 2*), and it is the most prevalent respiratory disease associated with groups of pastured beef cattle (*3*). The incidence of ABPE appears to be increasing in parallel with changes to more intensive range and pasture management. This and the absence of proven methods of treatment or prophylaxis results in a significant economic loss to beef cattle producers. Effective prevention would allow more efficient utilization of improved pastures and provide economic benefits contributing toward more profitable red meat production.

Previously (*4*) we demonstrated that 3-methylindole, a ruminal fermentation product of tryptophan, would cause acute pulmonary lesions in cattle, similar to those of ABPE. Additional work has shown that the pathogenesis of the disease probably involves ruminal conversion of L-tryptophan, a naturally occurring amino acid and constituent of forage, to indoleacetic acid which is then converted to 3-methylindole (*5, 6*). We have also found that ruminal microorganisms convert L-tryptophan to indoleacetic acid (*7*) and that a *Lactobacillus* sp. isolated from the rumen metabolizes indoleacetic acid to 3-methylindole (*8*). This process occurs in vivo, as indicated by the presence of 3-methylindole in ruminal fluid and plasma of cows given tryptophan to induce ABPE (*6*). In contrast to tryptophan or indoleacetic acid, both intravenous and oral administration of 3-methylindole results in pulmonary lesions typical of ABPE (*4, 9*). In addition to the pathological similarity between the experimentally induced and naturally occurring diseases, the presence of 3-methylindole in ruminal fluid (*10, 11*) and peripheral blood (*10*) has been demonstrated in cows that developed ABPE after a move from relatively dry to lush, green pasture. These observations lend support to the view that naturally occurring ABPE results from ruminal metabolism of tryptophan. In this report we describe experiments that demonstrate that monensin, a widely used feed additive, can inhibit the production of 3-methylindole and prevent experimentally induced ABPE. These results provide a promising approach to possible prevention of naturally occurring ABPE.

Previous work in our laboratory demonstrated that 3-methylindole production in ruminal fluid could be decreased in vitro and in vivo by the use of antibiotics (*7*), but the effectiveness of these antibiotics at concentrations low enough for practical application in live animals was not investigated. Therefore, we undertook experiments to screen several compounds for their ability to reduce 3-methylindole production. By means of in vitro techniques (*7*) and analytical methods devised in our laboratory (*12*), several antimetabolites were screened for their ability to reduce the conversion of L-tryptophan to 3-methylindole in vitro. Mixtures of 23 ml of ruminal fluid, 10 mg of L-tryptophan (10 mg of L-tryptophan per milliliter of H_2O), and 0.625 mg or 0.125 mg of test compound (25 or 5 μg per milliliter, final concentration) were incubated in triplicate in 50-ml Erlenmeyer flasks fitted with rubber caps. The test compounds were dissolved in 1 ml of H_2O, 0.1*N* aqueous NaOH, or absolute methanol. Each flask was flushed with CO_2 for 1 hour, then incubated at 37°C for another 23 hours. The results obtained were compared to those for con-

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