STUDIES OF PHOTOSYNTHESIS USING A PULSED LASER

I. TEMPERATURE DEPENDENCE OF CYTOCHROME OXIDATION RATE IN CHROMATIUM. EVIDENCE FOR TUNNELING

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ABSTRACT The rate of oxidation of cytochrome following absorption of a short pulse of light from a ruby laser in the photosynthetic bacterium *Chromatium* has been measured spectrophotometrically. The half-time is about 2 μsec at room temperature increasing to 2.3 msec at about 100°K and constant at the latter value to 35°K or below. The temperature dependence above 120°K corresponds to an activation energy of 3.3 kcal/mole; that below 100°K to less than 80 cal/mol: essentially a temperature-independent electron transport reaction. Since the slowness below 100°K indicates the presence of a barrier, the lack of activation energy is taken to mean penetration by quantum-mechanical "tunneling."

The light-induced oxidation of cytochrome in *Chromatium* is remarkable for its relative insensitivity to temperature (1). Studies of the phenomenon have included: difference spectra at room temperatures (2, 6) and at 77°K (1, 77); measurements of light-limited rates of oxidation at various temperatures, aerobic and anaerobic (1, 3, 4); other kinetic studies including the "light-off" reaction (1, 2, 4, 5) and the effects of various additions or modifications such as the effect of glycerol (4, 7), various substrates, and inhibitors (2, 6, 8); and a detailed study at room temperature of the effects of starvation and subsequent additions (6). A temperature-insensitive cytochrome photooxidation has also been observed in green plants (9).

Chance, Schleyer, and Legallais (10) first applied the optical maser or "laser" to studies of photosynthesis, and Chance and Schoener (11) applied it to *Chromatium* without, however, measuring rates. The present work is a continuation of these studies in which a faster spectrophotometer and other auxiliary apparatus necessary to measure rates have been developed (13). The results reported in this paper concern the temperature dependence of the rates of cytochrome oxidation induced

in *Chromatium* with laser flashes of wavelength 694.3 nm. Preliminary reports have appeared in several places (12–16).

APPARATUS AND METHODS

The laser apparatus used in these measurements, which was constructed with the help of Mr. Armin Weiss, was partly described previously (13) and will be described more completely elsewhere. The lay-out of the apparatus is shown in Fig. 1. The chief features are:

1. A pulsed ruby laser,[1] employing a ruby rod 7.3 × 1 cm in diameter "pumped" with a 700 joule flash from a straight flash lamp placed beside the ruby. A partially

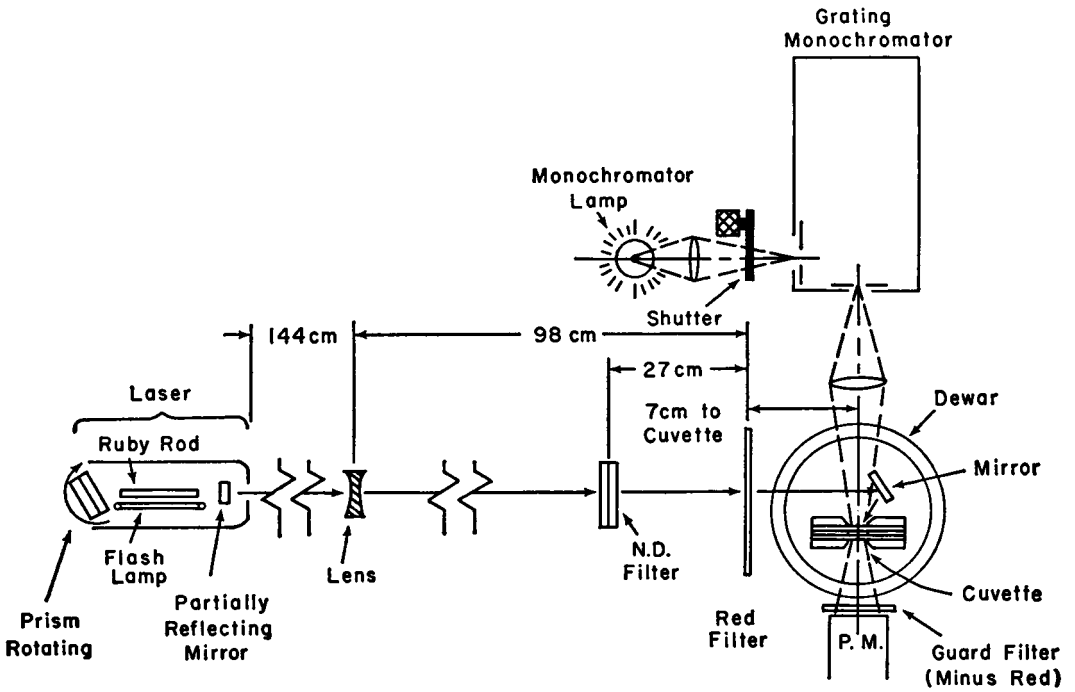

FIGURE 1 General arrangement of the apparatus for photosynthesis with laser pulses. The reactions are followed spectrophotometrically with monochromator, photomultiplier, and oscilloscope.

transmitting, flat mirror in front and a totally reflecting prism in the rear provide optical resonance for the laser action. In "normal" mode the prism is fixed in position and the laser action lasts roughly 0.5 msec. In the "Q-switched" mode the prism rotates 500 cycles/sec and is synchronized with the flash so that the laser action cannot start until the ruby is fully pumped. When the prism rotates into position, the laser discharge takes place completely in less than 0.1 μsec (one, or occasionally perhaps two or three, pulses

[1] Model 104, purchased and somewhat modified from TRG Inc., Melville, Long Island, New York.

of 10 nsec each). The output was about ⅔ joule in normal mode and ⅓ to ½ joule, Q-switched.

2. Attenuation of laser beam by negative lenses and neutral density filters (the latter at low, already partially attenuated levels only). In most experiments just enough laser intensity was used to nearly saturate the cytochrome oxidation (0.4 to 1.5 millijoules or 2 to 9 nanoeinsteins/cm^2). The distance between the laser and the lens attenuates the pump flash relative to the laser light as does the red filter (Corning No. 2-64) also.

3. A Bausch and Lomb 250 mm grating monochromator for the (single beam) spectrophotometric measurements. A slit width of 2 mm (6.6 nm spectral bandwidth) was used.

4. A 34 watt tungsten incandescent lamp as light source for the monochromator, "boosted" to nearly twice its rated voltage for a few milliseconds during the fastest measurements (near room temperature) to provide extra signal-to-noise ratio. This required dc coupling from photomultiplier to oscilloscope and careful adjustment of an offset voltage so that the boosted trace would appear on the oscilloscope screen with high vertical sensitivity. The careful preadjustment required for this procedure made it impractical for the experiments at lower temperature because too much preexposure to monochromator light could cause cytochrome oxidation from which the sample would not recover. Fortunately, at the lower temperatures the effects to be measured are slower and one can make the measurements without boosting the lamp. In this case ac coupling was used between photomultiplier and oscilloscope. The monochromator light shutter was opened ahead of the laser shot only long enough to allow the transients caused in the ac coupling by the opening to die out. The dc measurement of total signal was made afterwards.

5. A photomultiplier (EMI Type 9524B) registered the monochromator light transmitted through the cuvette. It was protected from laser light by two thicknesses of Corning No. 4-96 blue glass.

6. Preamplifier, oscilloscope, and camera to record the changes in spectrophotometer signal. The bandwidth of preamplifier and oscilloscope was dc to 200 k hertz, corresponding to a "rise-time" constant of 0.8 μsec. Since this is not much smaller than the smallest reaction times measured, there is a possibility that some of the latter may have been influenced. For the slower measurements a filter was used to decrease bandwidth and reduce noise.

7. Circuitry for synchronizing and controlling the various actions.

The laser energies were measured with a bolometer manufactured by TRG Inc. It absorbs the laser pulse in a silver cone and measures the temperature rise with a series of ten thermocouple junctions using a similar silver cone as reference. The manufacturer's calibration was accepted. The measurements are not critical for the conclusions made in this paper, but determine the scale factor used for the abscissas of Fig. 5.

The cuvette used in all experiments except those below liquid nitrogen temperature had 1.6 mm thick Plexiglas windows spaced 1.6 mm apart. The area observed by the monochromator beam was about 1.6 cm^2. It could be surrounded by an unsilvered Dewar flask and cooled by means of a metallic "fin" dipped into a cooling bath such as liquid nitrogen or a solid CO_2-alcohol mixture, or by means of cold, dry nitrogen gas circulated through copper tubing soldered to the fin. In the latter case, the stream of nitrogen was also directed against the cuvette window as it emerged from the copper tube. The laser beam struck the cuvette window at an angle of about 34° from the normal, after reflection from a metallic mirror.

In some of the earlier experiments with the cuvette fin dipped in liquid nitrogen, the

temperature is estimated to be 85 ± 3°K by comparison with temperatures measured in later experiments under similar conditions. Otherwise, except in the experiments below liquid nitrogen temperatures, the temperature was measured with a copper-constantan thermocouple inserted directly into the material in the cuvette.

The experiments below liquid nitrogen temperatures were done in a cryostat[2] in which a cuvette, in an evacuated chamber, was cooled by contact with a cold finger within which hydrogen was being liquified by Joule-Thompson effect. Again, the cuvette light path was 1.6 mm and the same area was illuminated by the monochromator beam. The windows, however, in the case of the points below 40°K, were sapphire, 2 mm thick, to take advantage of the superior heat conductivity of this material at these temperatures. The temperature was then measured by means of a thermistor inserted in the copper frame of the cuvette at nearly the maximum possible distance from the point of attachment to the cold finger. Uncertainties in this measurement are estimated at ±5°K. The fabrication of the cuvettes and the setting up and operation of the cryostat were mainly the contribution of Mr. Bruno Graf.

The bacteria were 2-days-old cultures of *Chromatium*, strain D, grown by Dr. Jane Gibson and, in earlier experiments, harvested by Dr. Margaret Edwards (Weiss). The assistance of Mrs. Virginia Montgomery and Mr. Jeffrey Cohlberg in supplying much of the *Chromatium* and in making the chlorophyll assays is also acknowledged. The methods[3] are described in reference (6). The bacteria were harvested by centrifugation and, in all cases reported here, except one, resuspended in fresh growth medium at a concentration 25 to 50 times greater than that before harvesting. Most of the samples used in the work reported here were also measured on a double beam spectrophotometer with steady-state actinic illumination to ensure normal responses. All the work reported here was done under the anaerobic condition resulting from the bacterial metabolism and the reducing action of the sulfide medium in the deep cuvettes.

Reaction half-times were estimated from the photographs by a combination of hand-smoothing, drawing tangents to initial slopes, noting time to intersection with final asymptotes (and multiplying by 0.693), and also by directly measuring the time taken to rise halfway to the asymptote. The uncertainties were estimated by taking extremes at the limit of what could be supported by the photographs allowing for the noise present. The estimates are believed to correspond approximately to the peak-to-peak noise and should be highly conservative as estimates of rms noise (standard deviation).

Some photographs taken earlier than the work presented here were analyed by computer to obtain a least squares fit of an exponential curve. The half-times so obtained tended to be a little smaller (for example, 0.52 μsec for the room temperature half-time) than those estimated by hand as above. This experience may indicate that the halftimes presented in this paper are a little biased toward the high side. However, the uncertainty estimates are believed to be adequate to cover this and the relative rates should be unaffected.

RESULTS

A typical room temperature response is shown in Fig. 2. It is an oscilloscope trace

[2] Purchased from Air Products and Chemicals, Inc., Allentown, Pennsylvania.

[3] The medium contained bicarbonate as the sole carbon compound, sulfide, thiosulfate, and other salts. The chlorophyll assays were made by extraction with methanol and determination of optical density of the extraction at 775 nm using an extinction coefficient of 42 $mM^{-1}cm^{-1}$ from Smith and Benitez (78) instead of 35.3 $mM^{-1}cm^{-1}$ (79) used in reference (6).

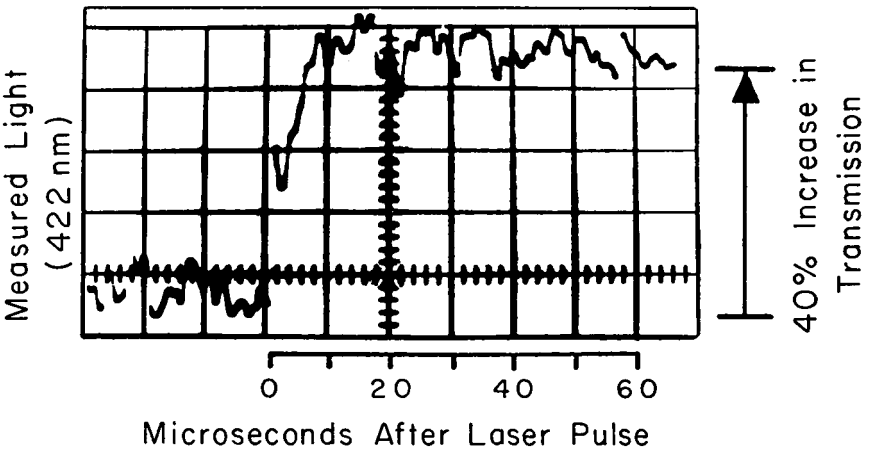

FIGURE 2 Typical *Chromatium* response at room temperature. The transmission at 422 nm increases by about 4% ($\Delta OD = -0.017$) with a half rise-time of about 3 μsec. Light path, 1.6 mm. Chlorophyll assay = 0.5 mM.

of the photomultiplier current resulting from transmission by the *Chromatium* suspension of monochromator light of wavelength 422 nm. The laser flash (Q-switched) occurred at the time marked "0." The first peak reached by the trace after this (at about half the vertical scale height) is artifact produced by the small portion of the 694 nm laser light which penetrates the guard filter to the photomultiplier tube. After recovery from the laser artifact, it is seen that an increase in transmission is still in progress and that this continues to a value 4% greater than the transmission before the laser flash. The half-time for the increase would be estimated from this trace at about 3 μsec.

Fig. 3 shows a response observed at about 35°K. In this case the laser was used in normal mode and no laser artifact shows. The half-time for the rise in transmission shown here was estimated at 3.1 (+1.7 to −1.0 uncertainty) msec. The amplitude of the change (1.3%) is less than that observed in Fig. 2 because of the difference in the concentrations of *Chromatium*. The suspension in Fig. 3 assayed 0.2 mM chlorophyll whereas that in Fig. 2 was 0.5 mM. If one takes the extinction coefficient change for cytochrome at 422 nm as 60 mM^{-1} cm^{-1} (17, 18), one calculates that both samples showed about one cytochrome oxidized per 300 molecules of chlorophyll present. The optical density decreases observed at 422 nm under strong steady actinic light are about 5 or 6 times this much—strong evidence that only one of the several cytochromes present is oxidized in the times observed immediately after the laser flashes.

Fig. 4 shows a spectrum of the optical density changes observed at room temperature as a result of Q-switched laser excitation. The disappearance of absorption observed at 554 and 422 nm and the increase at 408 nm identify the process as

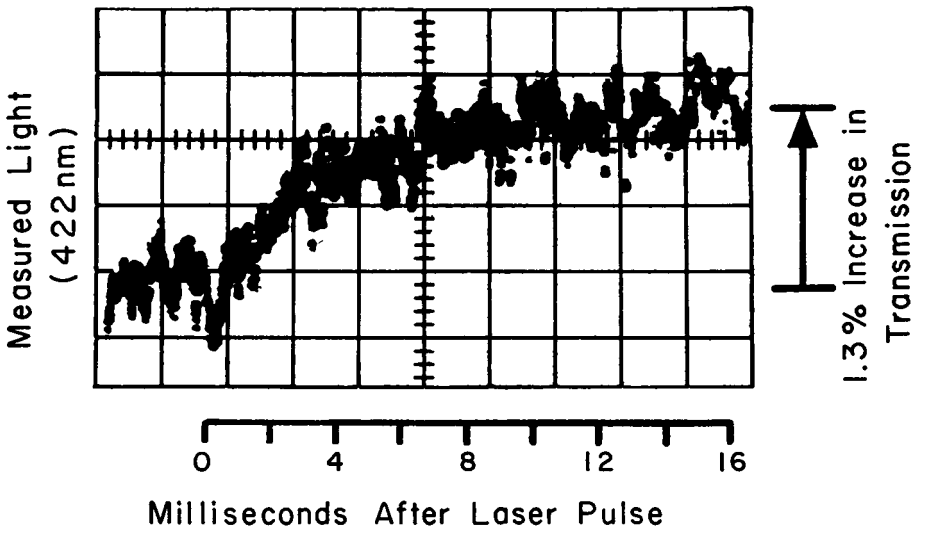

FIGURE 3 *Chromatium* response at about 35°K. A 1.3% increase in transmission (OD = −0.006) is observed at 422 nm with a half-time of about 3 msec. Light path 1.6 mm. Chlorophyll assay = 0.2 mM.

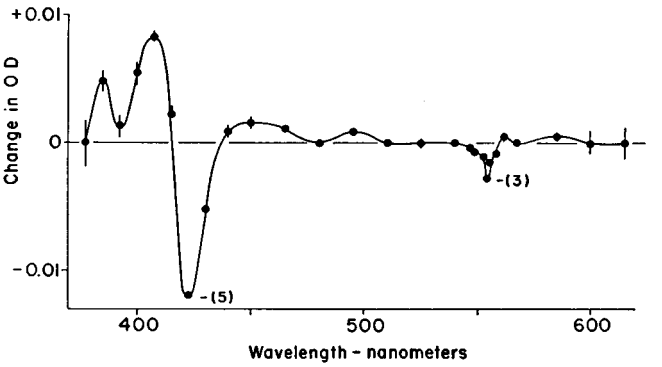

FIGURE 4 Difference spectrum produced by laser pulse on *Chromatium* at room temperature. Optical density increases plotted upward. (Opposite from Figs. 2 and 3). Alpha peak at 554 and Soret band changes from 400 to 440 nm are clear and typical of cytochrome. Other apparent features may involve a high proportion of experimental error. Numbers in parentheses beside some points indicate the number of observations averaged into the single point.

cytochrome oxidation. The resolution is probably not good enough to identify unequivocally which of the cytochromes observed in *Chromatium* by Olson and Chance (2) or by Bartsch and Kamen (17) may be active here. It could be that the cytochrome oxidized at room temperature is different from that oxidized at low temperatures. We have not run a spectrum of the laser-induced changes at low temperature, but because of the permanent nature of the changes at 422 nm, one

can expect it to be the same as that observed with steady illumination by Chance and Nishimura (1, 77). We have observed a transient optical density increase at 432 nm at about 80°K, but this will be reported more fully when the full spectrum has been run.

Fig. 5 is a plot of the optical density shifts at 422 nm observed at room temperature as a function of laser intensity. Assuming an extinction coefficient change of 60 mM^{-1} cm^{-1}, we calculated the ordinates to nmoles of cytochrome oxidized

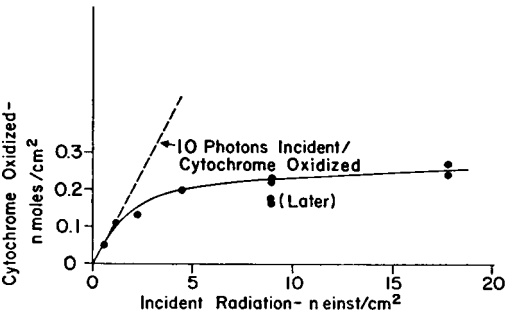

FIGURE 5 Dependence of laser-induced cytochrome oxidation on laser intensity. The calibrations indicated are rough. Most of the work reported in this paper was done with intensities in the range calculated above as 2 to 9 nanoeinsteins/cm^2.

per square centimeter of cuvette area. The limiting slope of about 10 photons incident per cytochrome oxidized must be multiplied by the absorption coefficient of the bacterial suspension for wavelength 694 nm before a quantum yield can be estimated. This absorption coefficient is quite small and a quantum yield of about 1 may be possible. The dependence on laser intensity, etc. is to be presented more fully and discussed in future papers and is presented here only to specify more precisely the conditions under which the dependence on temperature was measured.

All the pertinent data on the rate of cytochrome oxidation as a function of temperature are presented in Fig. 6. This is an Arrhenius-type plot in which the ordinates are proportional to the logarithm of the rate (presented as half-times) and the abscissas are proportional to the reciprocal of the absolute temperature. It is apparent that the data fall into two groups. The data above approximately 120°K show considerable dependence on temperature whereas that below 100°K does not. The activation energy below 100°K is estimated from the plot to lie between 0 and 80 cal per mole.

On the assumption that two parallel reactions or mechanisms are present, we next subtract the slow component (assumed to be constant at 2.3 msec half-time) from the points above 120°K and replot them in Fig. 7. This is also an Arrhenius-type plot with the reciprocal temperature scale enlarged. The activation energy estimated from it is 3.3 ± 0.6 kcal/mole.

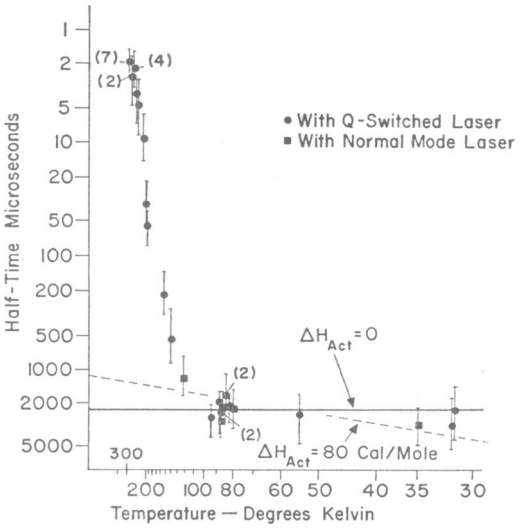

FIGURE 6 Arrhenius plot of temperature dependence of the rate of laser-induced cytochrome oxidation in *Chromatium*. The abscissas are proportional to T^{-1}, ordinates to logarithm of reaction rate. Numbers in parentheses beside some points indicate the number of observations averaged into the single point.

Minor observations made in the course of this work include the following:

The freezing point of the medium in which the bacteria were suspended was about −3°C. The rates measured just below this temperature appear slightly faster, if anything, than those measured just above, but the difference is well within experimental error. The temperature at which the bacteria themselves freeze is unknown.

At −31 ± 4°C a phase transition was observed in the medium. It consisted of a recrystallization, below this temperature, into finer, whiter, more highly scattering crystals and greatly diminished the amount of measuring light transmitted to the photomultiplier. It seems to have made no effect on the rate of cytochrome oxidation.

At −6°C both laser and steady-state double beam measurements showed a normal type of cycling (light on, cytochrome oxidized; light off, cytochrome reduced) which is similar, though a little slower, to that at room temperature. At −10° to −23°C repeated laser shots produced full amplitudes of oxidation indicating that subsequent reduction of cytochrome still occurred at this temperature range. At −70°C a second laser shot showed about ¼ the amplitude of the change produced by the first shot and a third produced practically no change. This nonrecovery was also characteristic at all lower temperatures. Considerable recovery occurs if a sample frozen at 77°K is warmed to room temperature and then after a few minutes in the dark is frozen again. However, the amplitudes of the changes

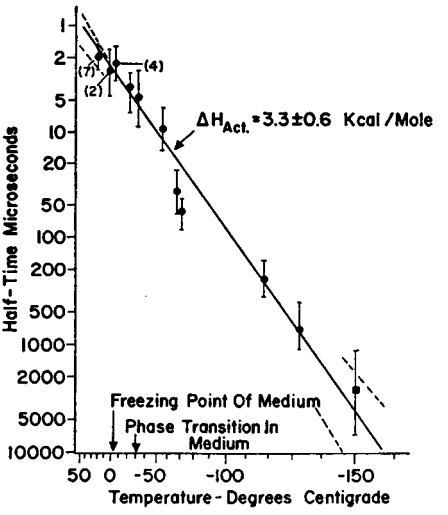

FIGURE 7 Temperature-dependent component of laser-induced cytochrome oxidation in *Chromatium*. The data of Fig. 6 are replotted with the slow component subtracted. Abscissas are proportional to T^{-1}, ordinates to logarithm of remaining reaction rate. Dotted lines show limits assumed in estimating uncertainty in activation energy. Numbers in parentheses beside some points indicate the number of observations averaged into one.

observed with laser shots after second or third freezings were less than half that observed on first freezing, and the half-times were about twice as long. The results of second freezings were not used, therefore, in Figs. 6 and 7.

DISCUSSION

The rates of light-induced cytochrome oxidation reported here made it evident that none of the previously used methods (1, 4) could have measured anything except light-limited rates. The important fact established by them was that the reaction continued at a fast rate even at liquid nitrogen temperatures and with a high quantum yield. The Q-switched laser, however, has clearly separated the cytochrome oxidation from the photoactivation of the chlorophyll. The results, therefore, give more information concerning the relationship between the cytochromes and the chlorophyll. It is now necessary to explain not only the speed of the reaction but also its slowness.

It is clear that the present data establish a temperature dependence for the reaction above 120°K.[4] However, the heat of activation, 3.3 kcal/mole (0.14 ev per molecule or 1200 cm^{-1}) is rather small. It is clear also that the reaction below 100°K, which is the reaction originally observed by Chance and Nishimura (1), is very nearly if not actually temperature independent. It is further evident through most, if not all, of the temperature range studied that the reactants must be ef-

[4] This had been contended by Vredenberg and Duysens (4), although it is difficult to see how the much slower rates reported by them could be anything but light-limited. Duysens (personal communication) now states that an instrumental artifact was responsible for the difference between their results and those of Chance and Nishimura (1).

fectively in a solid state and one must think of electron transference without transfer of molecules over distances greater than the amplitudes possible for vibration or rotation in the solid.

The literature was reviewed to determine what mechanisms for electron transfer have been proposed that might have some bearing on the process studied here. Szent-Györgyi (19) suggested that biological systems may act as semiconductors and many have followed this idea (20–31). However, the measured activation energies are above 1 or 2 ev for biological material (20, 22–27). The only organic materials with activation energies for electronic conduction in a range similar to ours are: crystalline complexes of polycyclic aromatic hydrocarbons with halogens, 0.1 ev (32); complexes of aromatic amines and halogenated quinones, 0.5 ev (33); and free radicals such as diphenyl-picryl-hydrazyl, 0.2 ev (23, 25). A proton conductivity in damp Na-DNA pellets has been observed with activation energy of 0 to 0.25 ev (34).

The data on electron transfer through collisions of mobile molecules has been reviewed by Taube (35, 36), Zwolinsky, Marcus, and Eyring (37), George and Griffith (38), and Sutin (39), among others. Activation energies found range from 0.20 ev for $Fe(CN)_6^{4-} - Fe(CN)_6^{3-}$ exchange (40) and 0.3 to 0.4 ev for $Fe^{++} - Fe^{+++}$ exchange (with complexing by OH^- and Cl^-) (41) and upwards. One group of workers implicates water as having a special role in electron transfer (42). Horne has measured the exchange between Fe^{++} and Fe^{+++} frozen into the solid aqueous solution with an average of 100 A between iron ions (43). The data appear to extend without break the rate dependences found for the liquid state (41). Although this data has been used to support a proposal that the electron is carried with a proton as a neutral hydrogen atom from water molecule to water molecule, an alternative explanation as electron (or hole) tunneling is given by Ruff (44). "Bridging" by third molecules (36, 38, 45, 46, 61) and the formation of "charge-transfer" complexes (47) have been proposed as aids to or alternatives to tunneling.

Some measurements pertinent to the ability of electrons in "π" systems to transfer through certain types of barriers are the electron paramagnetic resonance (EPR) spectra of a series of paracyclophane anions obtained by Weissman (48). These molecules consist of two benzene rings joined by two parallel saturated hydrocarbon chains, to which an extra electron has been added. The extra electron is found to transfer from one benzene ring to the other in less than 10^{-7} sec if the chains have two CH_2 groups each, but takes longer than 3×10^{-7} sec if the chains have four CH_2 groups each. A time between these two values is required if one chain has three and the other four CH_2 groups. The temperature dependence and, therefore, activation energy were not measured. Voevodskii, Solodovnikov, and Chibrikin (49) with EPR measurements on different aromatic anions confirm that the electron passes easily through two CH_2 groups in a chain. McConnell (50) has analyzed this theoretically as a tunneling process using a resonance type of calculation.

Kowalski, using nuclear magnetic resonance methods, measured the rate of exchange between ferri- and ferrocytochrome *c* in 0.02 M solution as 100 sec^{-1} (51).

Quantum mechanical "tunneling," i.e. the crossing of potential barriers by particles with less activation energy than corresponds to the height of the barrier, is well established in solid state and in nuclear physics, having been used to explain thermal- (52) and field- (53) emission of electrons, α-radioactivity (54, 55), metal-semiconductor rectifying barriers (56–58), avalanche breakdown (59), and the characteristics of narrow *p-n* junctions, "tunnel diodes" (60). In molecular chemistry it has been more difficult to establish tunnel mechanisms because usually they have been complicated by concomitant activation requirements. Libby (61) proposed to explain the slowness of the electron exchange between some simple ions like $Fe^{++} - Fe^{+++}$ compared with the rapidity of that between some coordinated ions like $Fe(CN)_6^{4-} - Fe(CN)_6^{3-}$ as a matter of electron tunneling controlled by the Franck-Condon principle which puts a premium on symmetry between donor and acceptor.[5] He argued that the equilibrium arrangement of water of hydration around the simple ions would not be the same for the ions of different charge and that the necessary rearrangement to symmetrical form required an activation energy, whereas in the complexes the arrangement of the ligands ensured symmetry at least through the first layer. The theory of the process has been extended by several others (62–64) even to electrode processes (65). Besides the other evidence already mentioned for electron tunneling in molecular processes (44, 48, 49), we may cite also the observation that electron exchange takes place between gaseous ions and their neutral gaseous parent molecules over distances of approach almost twice as large as those required for momentum transfer in collisions (66, 67). A correlation has been made between carcinogenicity of hydrocarbons and their fluorescence spectra which indicate a requirement that energy levels be matched (22, 26). Such a requirement would be expected if the process were one of electron transfer by tunneling.

The slowness of the temperature-independent part of the light-induced cytochrome oxidation in *Chromatium* observed in the present work indicates that some kind of barrier to electron transfer is present, but the lack of activation energy indicates that the electron does not jump over the barrier. Tunneling is surely the simplest explanation. The activation energy required in the previously discussed cases may be absent in *Chromatium* at low temperatures because of the solid state conditions or perhaps because the evolutionary development of *Chromatium* selected molecules with

[5] The Franck-Condon principle points out that the electron movement takes place so fast that there is no time for rearrangement of the positions of atomic nuclei. Consider the tunneling of an electron from A to B: $A^\bullet + B \rightarrow A + B^\bullet$. The nuclear arrangement of $B^\bullet$ is the same as that of B, and of A the same as $A^\bullet$. There is no radiation involved, so the process cannot take place unless the energy content of $A^\bullet + B$ is equal to that of $A + B^\bullet$, that is, unless the ionization energy of $A^\bullet$ is equal to the electron affinity of B (in some admissible electronic state). This is assured by summetry if the nuclear arrangement of B is the same as that of $A^\bullet$, but would be true only accidentally otherwise.

optimum matching of energy levels. The approximate situation, as we picture it, is shown in Fig. 8 and is described in the legend.

Alternative explanations might be: (*a*) that there is a path for the electron involving no potential barrier but which is so long and tortuous that it requires 2.3 msec to traverse it, or (*b*) that we have a combination of semiconductor mechanisms in which temperature dependences of various factors cancel out in the range 35 to 100°K (71, 72). These are more complicated than tunneling, which is shown below to occur for parameters of reasonable values.

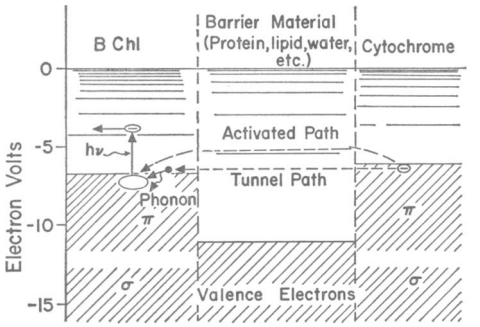

FIGURE 8 Idealization of photosynthetic cytochrome oxidation. Energy levels are plotted for bacteriochlorophyll, for cytochrome, and for an intermediate region containing typical saturated bonds. Levels occupied by valence electrons are shown shaded. The top occupied level corresponds to ionization potentials for typical similar molecules as found in the literature (68, 69). Upper empty levels as might be found by absorption spectra are indicated. Those for the prophyrins correspond to excited neutral molecules. Those for the barrier material correspond to states of the negative ion. The process envisaged consists of: (*a*) absorption of photon, $h\nu$, in *BChl*, (*b*) excitation of electron to upper level of *BChl* leaving a hole in valence band, (*c*) electron passes from cytochrome to hole in *BChl* either by tunneling or by an activated path. The latter may be as shown, through an upper conduction band or by vibration or rotation of the cytochrome or *BChl*, in such a way as to close the gap between them (70). The former may, of course, be accompanied by "phonon emission," stabilizing the transfer. It could also be that the cytochrome involved in the activated path is different from that using the nonactivated path.

If, for simplicity, we assume a rectangular barrier of sufficient height and width that the probability of penetration by tunneling is small, then the probability is given (73) by:

$$T = \frac{16}{2 + (V - E)/E + E/(V - E)} \exp\{-a[8m(V - E)]^{1/2}/\hbar\} \qquad (1)$$

where V = potential energy of electron inside barrier. Potential energy outside is taken as zero. E = kinetic energy of electron outside the barrier; a = width of barrier; m = mass of electron; and $\hbar$ = Planck's constant $\times$ $1/(2\pi)$. The probability, per

second, that an electron in a reduced cytochrome penetrates the barrier and fills the hole in the chlorophyll is equal to

$$R = 0.693/t_{1/2} = Tf \tag{2}$$

where f is the frequency with which a suitable electron in the cytochrome approaches the barrier.

Equations (1) and (2) may be rearranged to give

$$a = \frac{\hbar}{\sqrt{8m(V - E)}} \ln \frac{16ft_{1/2}}{0.693(2 + (V - E)/E + E/(V - E))} \tag{3}$$

The first five rows of Table I give two sets of parameters compatible with the observed rate of the temperature-independent reaction. They are reasonable values. They can be adjusted by varying the barrier shape and the frequency factor, and we conclude that the tunneling hypothesis is not only the simplest explanation for the temperature-independent part of the reaction but also quite plausible. A critical test of the hypothesis offered here will be the measurement of the rate at still lower

TABLE I

SOME PARAMETERS WHICH FIT THE TEMPERATURE-INDEPENDENT REACTION USING TUNNELING WITH OR WITHOUT MECHANICAL VIBRATION (EQUATION (3) OR (6))

Parameter	Notes	Value				Units
Half-reaction time, t_o	(*a*)	2.3				msec
Electron frequency, f	(*b*, *d*)	10^{15}				sec^{-1}
Electron kinetic energy, E	(*b*, *d*)	1				ev
Barrier height, $(V - E)$	(*b*)	1		0.14		ev
Barrier width, a or a_o	(*c*, *e*)	29		76		A
Vibrating mass, M	(*b*)	10^3	10^5	10^3	10^5	at. wt.
Vibrating frequency, ν	(*c*, *f*)	≥ 1	≥ 0.1	≥ 0.5	≥ 0.05	cm^{-1}
Constant in equation (6), K	(*c*)	≤ 8	≤ 0.8	≤ 2	≤ 0.02	10^{-3}
Hook's law constant (linear)	(*c*)	≥ 0.06	≥ 0.06	≥ 0.015	≥ 0.015	N m^{-1}
Room temp amplitude of vibration	(*c*, *g*)	≤ 4	≤ 4	≤ 8	≤ 8	A
$t_o - t_o$	(*c*)	≤ 20	≤ 2	≤ 6	≤ 0.6	μsec

(*a*) Experimental data.
(*b*) Assumed.
(*c*) Calculated.
(*d*) Because they appear in a logarithm, the calculation of a is quite insensitive to the values chosen for f and $(V - E)/E$. The value chosen for f is in the range of the frequencies of the lowest absorption or fluorescence band of chlorophyll, and is also the frequency of an electron in a 7 A box with 5 ev kinetic energy.
(*e*) Using equation (3). This is the same as equation (7) if the last term in the latter is negligible, as it will be if $M \times \nu > 4$ at wt. units cm^{-1}. Values taken for M and ν in later rows of the table conform to this requirement.
(*f*) To give: $t_{1/2}(100° K) \geq \frac{1}{2}t_o$.
(*g*) i.e. amplitude at which energy equals 0.03 ev.

temperature. The tunneling hypothesis predicts that the half-life of 2.3 msec will continue to absolute zero.

We explain the temperature dependent reaction (that plotted in Fig. 7) either as semiconduction via otherwise empty electronic states of (the negative ion of) the material between the cytochrome and the chlorophyll,[6] or as requiring a special contact achievable only occasionally by vibrations (linear or rotary) of the cytochrome or chlorophyll relative to each other (70). (see Fig. 9). The latter mech-

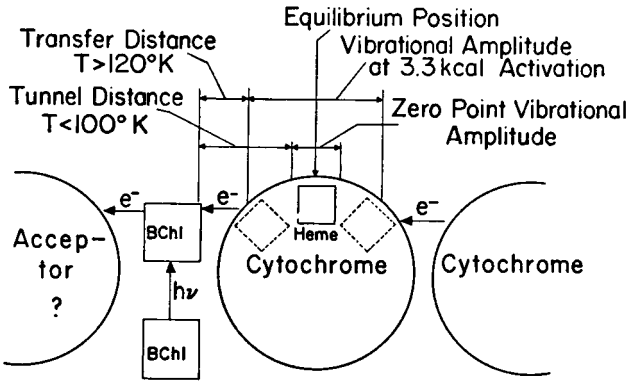

FIGURE 9 Vibrational mechanism for electron transfer from cytochrome to chlorophyll in *Chromatium*. (Adapted from Chance and Williams (70).)

anism may also be used to explain the subsequent reduction of cytochrome by other agents which must be in a location quite different from chlorophyll. That the reduction does not occur at low temperatures is explained by the freezing out of the vibrations.

In Table II we exhibit several sets of parameters which are compatible with the vibratory activation mechanism. If we imagine the cytochrome to be a spherical protein suspended from the rest of the photosynthetic apparatus by a hydrocarbon chain at each pole, then we would expect the strains in the suspension resulting from the vibrations (linear or torsional) to be divided among the links in the chain and so to give an effective Hook's law constant considerably less than those of single bonds. The values calculated in Table II may, therefore, be reasonable.

Two variations on the vibration activation come to mind. One is the possibility that the vibration is torsional but that the number of links in the suspending hydrocarbon chain (assumed saturated here) is so small that the vibratory mode is too restricted in amplitude to allow contact of the active sites and electron transfer.

[6] The semiconductor energy gap normally measured in proteins, etc., is, as mentioned earlier, above 1 or 2 ev. However, that would be with reference to the valence shell of the barrier material and would not apply to our case where the electrons start from a higher level before they enter the barrier.

TABLE II

SOME PARAMETERS WHICH FIT THE TEMPERATURE DEPENDENT REACTION USING MECHANICAL VIBRATION WITH ARRHENIUS ACTIVATION

Parameter	Notes	Value				Units
Activation energy	(*a*)			3.3		kcal/mole
Wt. of vibrating mass	(*b*)		10^3		10^5	at. wt.
Radius	(*c*, *d*)		6.4		30	A
Vibrational frequency	(*b*)	20	2	0.5	0.05	cm^{-1}
Linear amplitude for activation	(*c*, *e*)	0.4	4	1.7	17	A
Angular amplitude for activation	(*c*, *f*)	0.11	1.1	0.09	0.9	radians
Linear Hook's law constant	(*c*, *g*)	20	0.2	1.5	0.015	N m^{-1} (Kg sec^{-2})
Angular Hook's law constant	(*c*, *h*)	4	0.04	5	0.05	10^{-18} Nt m $radian^{-1}$

(*a*) Experimental data.
(*b*) Assumed.
(*c*) Calculated.
(*d*) Assumed sphere of density 1.5 g/cm^{-3}.
(*e*) If the vibration is torsional, this would be measured at the "equivalent radius" which is $\sqrt{2/5}$. of the actual radius if the shape of the body is a solid sphere.
(*f*) If vibration is torsional.
(*g*) Measured at "equivalent radius" if torsional. Note that Hook's law constant for stretching vibration of Br_2, for example, is 246 N m^{-1}.
(*h*) If torsional. Note that the angular Hook's law constant for hindered rotation about C—C single bonds may be estimated as 9/2 of the energy of the barrier height or about 1×10^{-19} N m $radian^{-1}$ for CH_3CHF_2 (74).

However, the barrier height for restricted rotation in ethane and substituted ethanes is about 3 kcal per mole (74, 75). Thus, our activation energy would just correspond to the achievement of free rotation.

The second variation is that instead of a well defined vibration about an equilibrium position, the cytochrome lies loosely in a cavity and moves by a series of random, Brownian-motion jumps.

The foregoing discussion could apply either to the case that both the temperature-dependent and the temperature-independent reactions involve the same cytochrome, or to the case that they involve different cytochromes. A later paper will consider the possibility that the room temperature reaction involves the cytochrome called C_{422} by Olson and Chance (2). The spectrum of Chance and Nishimura (1) for the temperature-independent reaction is best fitted to $C_{423.5}$.

ATTEMPT TO EXPLAIN TEMPERATURE DEPENDENCY BY TUNNELING

The foregoing discussion has explained the observed temperature dependencies by a combination of two distinct mechanisms or reactions. The mechanism, explaining the temperature dependent part, assumes no reaction can take place (except by the

other mechanism) until the system has been activated by 0.14 ev. It is tempting to see whether or not it could be explained as part of the same tunneling mechanism used to explain the temperature-independent part, the temperature dependence arising from a simple vibration of the barrier width. Fig. 9 could be used here, too, except that we allow tunneling at all BChl-to-cytochrome heme distances. At low enough temperatures, the vibration of the barrier width would be the quantum mechanical zero-point vibration and would be of constant amplitude from absolute zero to some temperature at which the first excited vibrational state would begin to have appreciable probability. Then, as the temperature rises, the excited vibrational states will have the effect of narrowing the barrier once each cycle and thus of increasing the rate. (They also *increase* the width during opposite halves of the vibratory cycle, but because of the strong exponential dependence of tunneling rate on barrier width, the increase in width is unimportant compared to the decrease in width.)

To be more specific, the average rate of tunneling would be given by:

$$R(T) = f \int_0^\infty T(a)P(a)\, da \tag{4}$$

where: $T(a)$ is given by equation (1), and $P(a)\, da$ is the probability that the barrier width is between a and $a + da$.

If the vibrations of barrier width can be described as a quantum mechanical harmonic oscillator then:

$$P(a) = \frac{\sum_{n=0}^{\infty} (\psi_n(a))^2 \exp\left(-(n + \tfrac{1}{2})h\nu/kT\right)}{\sum_{n=0}^{\infty} \exp\left(-(n + \tfrac{1}{2})h\nu/kT\right)} \tag{5}$$

where:

$$(\psi_n(a))^2 = (4\pi M\nu/h)^{1/2}(2^n n!)^{-1}[H_n(2\pi(M\nu/h)^{1/2}(a - a_0))]^2 \exp\left(-4\pi^2 M\nu(a - a_0)^2/h\right)$$

H_n = Hermite polynomial; M = effective mass of the vibrating system; ν = fundamental frequency of the vibrating system; T = absolute temperature (not to be confused with transmission probability, equations 1 to 4); k = Boltzman constant; h = Planck's constant; n = vibratory quantum number; a_0 = equilibrium barrier width.

It is shown in the Appendix that from equations (1), (4), and (5) one may derive:

$$\ln R(T) = \ln (0.693/t_{1/2}) = \ln R(0) + 2.3K \exp(-B/T)[1 - \exp(-B/T)]^{-1} \tag{6}$$

where:

ln = natural logarithm

$$\ln R(0) = \ln (0.693/t_0) = \ln R_e + 2.3K/2 \tag{7}$$

$$\ln R_c = \ln (0.693/t_c) = \ln \frac{16f}{(2 + (V - E)/E + E/(V - E))} - \frac{a_0\sqrt{8m(V - E)}}{\hbar}$$

$$K = 4m(V - E)/(2.3Mh\nu)$$

$$B = h\nu/k.$$

According to equation (6), as T approaches zero, $\ln R(T)$ approaches $\ln R(0)$. At high temperatures the function $\exp(-B/T)\,[1 - \exp(-B/T)]^{-1}$ approaches $(T/B - 1/2)$ and equation (6) approaches:

$$\ln R(T) = \ln R_c + (2.3K/B)T \tag{8}$$

R_c is the rate that would be observed if the barrier width were held at the equilibrium value. The difference between R_c and $R(0)$ is the effect of the zero-point vibration. Equations (7) and (8) are assymptotes to (6) at the extremes of temperature and may be used as a rough characterization of equation (6). The intersection of equations (7) and (8) comes at $T = \frac{1}{2}B$ at which point the logarithm of the actual rate exceeds the logarithm of $R(0)$ by 0.313 times the amount by which logarithm $R(0)$ exceeds logarithm R_c. As expected by correspondence principle, equation (8) can be derived by using for $P(a)$ the probability distribution expected on the basis of a classical harmonic oscillator.[7]

Since equation (8) predicts an approach to proportionality to the first power of the absolute temperature, the data of Fig. 6 is replotted in Fig. 10 with $\log t_{1/2}$ as ordinates but with the first power of T as abscissas. The solid curve is a plot of equation (6) fitted to the data by taking $B = 362°$, $K = 8.98$, and $\ln R(0) = -\ln (2.77 \text{ msec}/0.693)$.

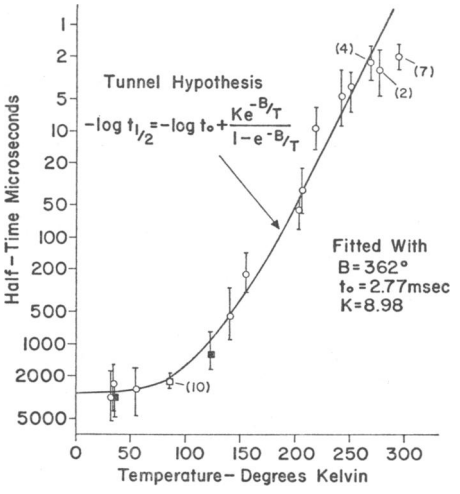

FIGURE 10 Plot of logarithm of reaction rate, same data as Fig. 6, versus the first power of absolute temperature. The solid curve is a plot of equation (6), which describes a combination of tunneling with vibration of barrier width, using the parameters shown. Numbers in parentheses beside some points indicate the number of observations averaged into one.

[7] Gaussian, with standard deviation equal to $\sqrt{kT/(4\pi^2\nu^2M)}$.

The fit turns out to be very good, but evaluation of the parameters required yields the following: the quantity, B, is rather firmly fixed at about 360° by the temperature of the "knee" in the curve (at ⅓ or ¼ of B). This fixes ν at about 250 cm^{-1}. From this and $K = 8.98$ we get $(V\text{-}E)/M$ = approximately 300 if $(V\text{-}E)$ is in electron volts and M is in atomic weight units. This means that any reasonable value (14 for a CH_2 group or more for a larger group) for M, the vibrating mass, would require an unreasonably high value (greater than 4000 ev) for $(V\text{-}E)$, the potential barrier. It would appear that the vibrational amplitudes to be expected with reasonable molecular masses, a fundamental frequency of 250 cm^{-1} and only a few quantum levels of excitation are so small that, to get a dependence on temperature as large as that observed, one needs an extremely "hard" barrier. It is not likely that other barrier shapes than the rectangular one assumed would be any "harder." Therefore, we reject this as a single explanation of the whole temperature-dependence curve.

If, on the other hand, we put reasonable parameters into equation (6), it is possible to fit the temperature-dependent and temperature-independent parts separately (as we did in the previous section by taking two different "activation energies"). In Table I are several sets of parameters (limiting values for some) which fit equation (6) to the data for the temperature independent part of the reaction. Sets of parameters which fit the temperature-dependent part are shown in

TABLE III

SOME PARAMETERS WHICH FIT THE TEMPERATURE-DEPENDENT REACTION USING TUNNELING WITH MECHANICAL VIBRATION (EQUATION (6))

Parameter	Notes	Value				Units
Half-reaction time, $t_{1/2}$, at 200° K	(*a*)	48				μsec
$-d(\log t_{1/2})/dT$ (150 to 250° K)	(*a*)	0.0194				decades deg^{-1}
Half-time at equilibrium barrier width, t_e	(*c*, *d*)	0.37				sec
Electron frequency, f	(*b*)	10^{-15}				sec^{-1}
Electron kinetic energy, E	(*b*)	1				ev
Equilibrium barrier width, a_o	(*b*)	29		76		A
Barrier height, $(V - E)$	(*c*)	1.37		0.19		ev
Vibrating mass, M	(*b*)	10^3	10^5	10^3	10^5	at. wt.
Vibrating frequency, ν	(*c*, *d*)	0.6	0.06	0.23	0.023	cm^{-1}
Hook's law constant	(*c*)	0.02	0.02	0.003	0.003	N m^{-1}
Room temp amplitude of vibration	(*c*, *e*)	7	7	18	18	A
$t_e - t_o$	(*c*)	7	0.7	3	0.3	msec

(*a*) Experimental data.
(*b*) Assumed.
(*c*) Calculated.
(*d*) Using equation (8) (differentiated). Equation (8) is an accurate approximation to equation (6) for the values of parameters given in this table at any temperature above 2° K.
(*e*) i.e. the amplitude at which the energy equals 0.03 ev.

Table III. No simple physical interpretation seems possible. If we keep the same equilibrium barrier width and the same vibrating mass for both pathways, then it is necessary that there be two vibrational frequencies present, a different one for each pathway, and also that the barrier height be slightly different for each. Furthermore, the room temperature amplitudes of vibration shown in Table III look rather large. Thus, we conclude that the tunneling-with-vibration parallel mechanisms cannot be absolutely ruled out on the basis of the parameters required, but the probability seems low.

At this time the data are not good enough to distinguish between the two-activation-energy (0 and 3.3 kcal/mole) description used in the previous section and the mechanisms discussed in the previous paragraph on the basis of the shape of the temperature-dependence curve (whether the logarithm of the rate is linear with the first power or the inverse first power of the temperature). More accurate measurements going, if possible, to higher temperatures are needed. The chief difference in principle between the two theories is that the former assumes that the reaction probability follows a step function of the energy of activation whereas the latter makes an average over a reaction probability that increases gradually with energy of activation.

APPENDIX

Derivation of equation (6) from equations (1), (4), and (5).

The denominator of the right hand member of equation (5) sums to:

$$\exp\left(-\frac{h\nu}{2kT}\right)\left(1 - \exp\left(-\frac{h\nu}{2kT}\right)\right)^{-1}.$$

The factor $\exp(-h\nu/2kT)$ cancels the same factor in the numerator. Substitution of the resulting expression into equation (4) produces integrals of the form:

$$\int_{-\infty}^{\infty} H_n^2(x)e^{-x^2-cx}\,dx$$

where

$$c = \sqrt{\frac{8m(V - E)}{Mh\nu}} \quad \text{and} \quad x = 2\pi\sqrt{\frac{M\nu}{h}}(a - a_0).$$

Setting the lower limit of integration at $-\infty$ is an approximation, since a cannot be negative, but the error involved is indeed negligible as long as the amplitudes of oscillation are small compared to a_0. These integrals may be evaluated by using the generating function for Hermite polynomials according to standard methods (76). The result of the evaluation is:

$$\int_{-\infty}^{\infty} H_n H_m e^{-x^2-cx}\,dx = n!\,m!\,\sqrt{\pi}\,e^{c^2/4}\sum_{i=0}^{n \text{ or } m}\frac{2^i(-c)^{n+m-2i}}{i!\,(n-i)!\,(m-i)!} \tag{9}$$

Substituting into equation (4) yields:

$$R(T) = \frac{16f}{\left(2 + \frac{V-E}{E} + \frac{E}{V-E}\right)} \exp\left(\frac{-a_0\sqrt{8m(V-E)}}{\hbar} + \frac{2m(V-E)}{Mhw}\right)$$

$$\cdot\left[(1 - e^{-B/T}) \sum_{n=0}^{\infty} \sum_{i=0}^{n} \frac{n!\ D^{n-i}e^{-nB/T}}{i!\ (n-i)!^2}\right] \qquad (10)$$

where D has been substituted for $\frac{1}{2}\ c^3$ and B for $h\nu/k$.

The expression in the square brackets is the temperature-dependent part. Letting $n - i = j$, it may be written:

$$1 + \sum_{n=1}^{\infty} \sum_{j=1}^{n} \frac{n!\ D^j e^{-nB/T}}{(n-j)!\ j!^2} - \sum_{n=1}^{\infty} \sum_{j=1}^{n} \frac{n!\ D^j e^{-(n+1)B/T}}{(n-j)!\ j!^2}.$$

In the first double summation, let $n = m$, and in the second, let $n + 1 = m$. Then the expression becomes:

$$1 + De^{-B/T} + \sum_{m=2}^{\infty} \sum_{j=1}^{m-1} \left(\frac{m!}{(m-j)!\ j!^2} - \frac{(m-1)!}{(m-1-j)!\ j!^2}\right) D^j e^{-mB/T} + \sum_{m=2}^{\infty} \frac{D^m}{m!} e^{-mB/T}$$

$$= 1 + \sum_{m=1}^{\infty} \sum_{j=1}^{m} \frac{(m-1)!\ D^j e^{-mB/T}}{(m-j)!\ j!\ (j-1)!}$$

$$= \quad + \sum_{j=1}^{\infty} \frac{D^j}{j!} e^{-jB/T} \sum_{m=j}^{\infty} \frac{(m-1)!\ e^{-(m-j)B/T}}{(j-1)!\ (m-j)!}$$

Letting $m - j = k$, this becomes:

$$1 + \sum_{j=1}^{\infty} \frac{D^j}{j!} e^{-jB/T} \sum_{k=0}^{\infty} \frac{(k+j-1)!}{(j-1)!\ k!} e^{-kB/T} = 1 + \sum_{j=1}^{\infty} \frac{D^j}{j!} (e^{-B/T})^j (1 - e^{-B/T})^{-j}$$

$$= \exp\left[De^{-B/T}(1 - e^{-B/T})^{-1}\right].$$

Substituting into equation (10) gives:

$$R(T) = \frac{16f}{2 + \frac{V-E}{E} + \frac{E}{V-E}}$$

$$\cdot \exp\left\{-\frac{a_0(\sqrt{8m(V-E)})}{\hbar} + \frac{2m(V-E)}{Mhw} + \frac{4m(V-E)}{Mhw} e^{-h\nu/kT}(1 - e^{-h\nu/kT})^{-1}\right\}$$

which can also be written in the form presented in equation (6).

The authors wish to acknowledge the assistance of those mentioned earlier in the paper, of Dr. William Hildreth on some of the measurements, and discussions with Dr. Heinz Schleyer and Dr. Mitsuo Nishimura.

This work was supported by a grant from the United States Public Health Service, GM 12202.

Received for publication 20 June 1966.

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