as judged from spectral data, the catalytic pathway might involve binding of O_2 to singly reduced laccase.

It is widely accepted that the type 1 Cu is inaccessible to external ligands, and that there exist intramolecular redox equilibria between the different copper sites. Based on this and on (*a*), we propose that O_2 reacts with a solvent accessible redox site (type 2 Cu(I) or half-reduced type 3 site), in an electron exchange equilibrium with the type 1 site:[1]

$$\left\{\begin{matrix}\text{type 1 Cu(I)}\\ \text{X(ox)}\end{matrix}\right\} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \left\{\begin{matrix}\text{type 1 Cu(II)}\\ \text{X(red)}\end{matrix}\right\} + O_2 \xrightarrow{k_2} \left\{\begin{matrix}\text{type 1 Cu(II)}\\ \text{X(red)}:O_2\end{matrix}\right\} \downarrow$$

$$K_1 = k_1/k_{-1} \ll 0.1$$

$$\text{X} = \text{type 2 or type 3 site.}$$

The rate-determining step is probably the intramolecular electron transfer from the type 1 site to the site of O_2 interaction ($k_{ox} = k_1 \ll k_2[O_2]$). The fluoride effect is consistent with the type 2 site being involved directly or indirectly, e.g. via allosteric modulation of the type 3 electron acceptor properties.

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[1] A complex and as yet unresolved transient pattern was observed around 330 nm (type 3 band).

VOLTAGE-INDUCED CHANGES IN THE CONDUCTIVITY OF ERYTHROCYTE MEMBRANES

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Previous reports (1–3) have shown that exposure of an isotonic suspension of erythrocytes to an electric field of a few kilovolts per centimeter for a duration in microseconds dramatically increases the permeability of the cell membranes. As a result, the erythrocytes undergo hemolysis through colloid osmotic swelling. Since the enhanced permeability is limited to small ions or molecules, the effect has been attributed to the formation of aqueous pores in the membranes. These pores are formed when the transmembrane potential induced by the externally applied field exceeds 1 V. The effective radius of the pores is several Ångstroms and can be varied by the adjustment of field intensity, field duration, or the ionic strength of the medium. Here we report a study of the kinetics of the pore formation, where the increase in permeability was detected by conductivity measurements.

Erythrocytes suspended either in isotonic NaCl or in a 1:9 mixture of isotonic NaCl

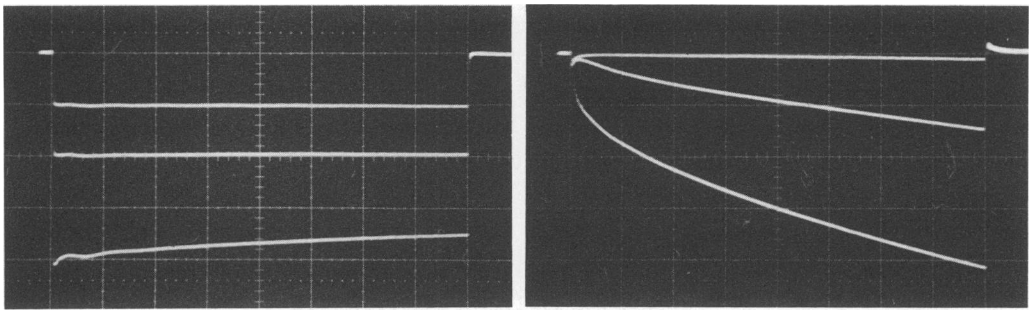

FIGURE 1 Increase in the conductivity of an erythrocyte suspension (20% vol/vol in isotonic NaCl) during voltage pulsation. Left traces, electric field (1 kV/cm per major div.); right traces, relative conductivity increment (5% per div.); time scale, 10 μs/div. Both traces are positive downward. Similar changes in conductivity, but with approximately three times greater amplitudes, were observed for the NaCl-sucrose medium. Part of the conductivity increase in NaCl is due to the Joule heating.

and sucrose solutions were exposed to a square-wave voltage-pulse, and change in the conductivity (current density divided by field intensity) of the total suspension was measured with a differential circuit. As shown in Fig. 1, time-dependent increases in the conductivity were observed when field intensity exceeded about 1.2 kV/cm. This value coincides with the intensity at which the most susceptible cells in the suspension are perforated with the pores under an 80-μs pulse (3). Thus, the observed increase in conductivity is due to the formation of pores through which current can penetrate the cell interior. At field intensities where most of the cell population undergoes the perforation ($\gtrsim$3 kV/cm), the time-course of the conductivity change consists of a rapid ($\approx$1 μs) and a slow ($\approx$100 μs) phase, as seen in the bottom traces of Fig. 1. The rapid phase corresponds to initial perforation, while the slow phase probably detects the expansion of the pores (3). Although an increase in conductivity might be expected if the cells reoriented or underwent a shape change, erythrocytes made spherical by an external agent or by hypotonic conditions gave essentially similar results. When a linearly increasing field was applied to the suspension, the conductivity started to increase at a field intensity of about 1.2 kV/cm, indicating that the cell membranes respond to the field intensity itself and not to its time derivatives.

Conductivity of the suspension after the removal of the applied field was estimated either by applying a second pulse or by a conventional measurement using a small-amplitude alternating voltage. In the NaCl-sucrose medium, the results are summarized as follows: (*a*) under an 80-μs pulse of 3.1 kV/cm, conductivity increased by 60%. (*b*) After the field was removed, the conductivity returned toward the original value until about 100 ms. (*c*) It then sharply rose again to an almost 100% increase at 1.5 min. (*d*) Thereafter it gradually decreased over a period of 1 h. Comparison with the previous results (1, 3) indicates that phase *c* is due to the leakage of intracellular ions into the low-ionic-strength external medium, and that the decrease in phase *d* is due to the swelling of the cells, which reduced the volume of extracellular space. Phase *b* is tentatively attributed to the shrinkage of widely opened pores.

Results in isotonic NaCl were qualitatively similar, except that the increase in phase *c* was very small, or almost absent.

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FAST BIOCHEMICAL REACTIONS IN THIN FILMS INDUCED BY NUCLEAR FISSION FRAGMENTS

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Fission track dynamics in thin films. The passage of nuclear fission fragments through thin solid films produces a fission track characterized by a high power density ($\sim 10^{13}$ W/cm^2), a diameter of ~100 Å, and a length of ~10 μm (1). Measurement of the kinetic energy and angular distribution of ion (2) emitted from the track give evidence for the formation of a superradiant state containing a high density of molecules simultaneously excited by the intense electromagnetic field associated with the fission fragment (3). The radiation emitted in two narrow cones at 0° and 180° to the fission fragment direction develops strong hypersonic pulses by stimulated Brillouin scattering (4). These nonlinear processes have two effects: molecules in the fission track are electronically and vibronically excited and can undergo fast chemical reactions in the excited state, and reaction products formed on the surface have a high probability of being emitted when the hypersonic pulse reaches the surface. If the reaction products are charged, they can be characterized by mass spectrometry.

Electron transfer reactions. This reaction has been studied with chlorophyll-*a* as a model compound. Molecular aggregates of chlorophyll produce a singlet exciton state characterized by an electron exchange current within the aggregate (5). We have verified this directly by observing the breakup of this state into $(\text{Chl-a})^+$ and $(\text{Chl-a})^-$ ions. We also have detected dimers, trimers, and tetramers of chlorophyll and have shown that the presence of Mg is essential for the stability of oligomer formation.

Proton transfer reactions. Molecules that form aggregates in the solid state, mediated by hydrogen bonding where the H^+ is weakly bound (acidic), give evidence of proton charge transfer in the singlet exciton states. Reaction products emitted from those states included $(M + H)^+$ and $(M - H)^-$ ions. Amino acids, peptides, and small oligonucleotides exhibit this reaction.

Cationization. Molecules forming aggregates that do not produce a charge delo-