RELAXATION PHENOMENA IN HUMAN ERYTHROCYTE SUSPENSIONS

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ABSTRACT Previous work has shown that the application of the Joule heating temperature jump technique of Eigen and de Maeyer to an isotonic suspension of human erythrocytes induced an interiorization of [^{3}H]glucose and a hemolysis of the red cells (Tsong, T.Y., and E. Kingsley, *J. Biol. Chem.* **250**:786 [1975]). The result was interpreted as due to the thermal osmosis effect. Further considerations of the various effects of the Joule heating technique indicate that the hemolysis of the red cells may also be caused by the rapid dielectric perturbation of the cell membranes. By means of turbidity measurements of the suspensions we have detected at least four relaxation times. Two of the faster ones ($\tau_1 \sim 20\ \mu$s and $\tau_2 \sim 5$ ms) are tentatively attributed to water relaxations in the membrane structures. The other two are attributed to membrane ruptures ($t_{lag} \sim 0.1$ s) and the hemolysis reaction ($\tau_3 \sim 0.5$ s). Studies with the erythrocytes from different hematological disorders indicate that whereas the two slower relaxations are sensitive to the overall physical property of the red cell membranes the two faster relaxations are not. These observations are consistent with the above assignment of the relaxation processes. The apparent activation energies are, respectively, 8.4, 12.0, and 11.8 kcal/mol for the τ_1, τ_2, and τ_3 reactions. Experiments with erythrocyte ghosts indicate a single relaxation for the water permeation, and biphasic kinetics for the membrane rupture and resealing reactions. The phenomena reported here may contribute to our understanding of water transport and molecular release in cellular systems.

INTRODUCTION

The Joule heating temperature jump technique of Eigen and de Maeyer (1963) has been used to probe the bilayer structures of synthetic and biological membranes (Hammes and Tallman, 1970; Owen et al., 1970*a*,*b*; Träuble, 1971; Tsong, 1974; Tsong and Kingsley, 1975). The technique when applied to erythrocyte suspensions induced an interiorization of [^{3}H]glucose and a hemolysis of the red cells (Tsong and Kingsley, 1975). Light microscopic examination of the erythrocyte sample treated with the temperature jump indicated ruptures of cell membranes. Membrane rupture generated by a rapid Joule heating of the solution has also been observed in synthetic phospholipid suspensions (Tsong, 1974). Although experimental evidence clearly demonstrates ruptures of membrane bilayers, quantitative analysis of these relaxation processes has not

been done, since detailed mechanism leading to these membrane ruptures has not yet been worked out. In an earlier publication (Tsong and Kingsley, 1975) we suggested some possibilities, including the electric dichroic effect (Dourlent et al., 1974). The thermal osmosis effect (Katchalsky and Curran, 1965) was one of the possible causes. Further considerations of the various effects induced by the rapid Joule heating of membrane suspensions will be given here.

It will be shown that passage of direct current through the erythrocyte suspension could generate a transmembrane potential in the red cells. Such a rapid creation of transmembrane potential perturbs the membrane structures, and at a critical point causes dielectric breakdowns of the membranes. We will also present a systematic study of the four relaxation processes reported previously (Tsong and Kingsley, 1975) for the erythrocytes from normal human blood.

It should be mentioned that similar kinds of experiments have been done in several laboratories (Owen et al., 1970*a*,*b*; Sale and Hamilton, 1967, 1968; Coster and Zimmerman, 1974, 1975). However, different authors have focused on the different aspects of Joule heating and the electric field pulsation of membrane suspensions. The relaxation phenomena induced by these effects have not been fully explored.

EXPERIMENTAL

Erythrocyte Samples

Human blood from healthy adults was obtained by venipuncture in the presence of heparin and was washed three times with an isotonic saline containing 5 mM phosphate buffer at pH 7. The erythrocytes were collected by centrifuging the suspension for 15 min at 5,000 rpm in a refrigerated Sorvall RC2-B centrifuge (DuPont Instruments, Sorvall Operations, Newtown, Conn.). In all experiments the red cell suspensions were 50–200 times diluted from the normal blood. The freshly drawn blood samples from leukemia, sickle cell anemia, and myotonic dystrophy patients were obtained from the clinical departments of the Johns Hopkins Hospital. The erythrocyte ghosts were prepared as described by Burka et al. (1967).

Joule Heating Temperature Jump

The temperature jump experiments were performed with an Eigen-deMaeyer temperature jump apparatus (Eigen and de Maeyer, 1963). Several different capacitors ranging from 0.001 to 0.05 μF were used. In the Joule heating-induced hemolysis experiments the fraction of hemoglobin release was monitored by the absorbance of the supernate at 410 nm in a Zeiss spectrophotometer (Carl Zeiss, Inc., New York). In the relaxation measurements turbidity changes of the suspensions were recorded with a Tektronix 5103N storage oscilloscope (Tektronix, Inc., Beaverton, Ore.). The turbidity changes were followed at 300 nm but were checked at various wavelengths to ensure that the changes were due to light scattering changes not absorbance change of the suspensions. Unless otherwise specified all temperature jump experiments were done in the isotonic conditions.

The temperature jump cell was slightly modified from its original design to eliminate the dead volume. The dimension of the cell is as follows: Two platinum electrodes spaced at 1.02 cm apart are enclosed by a square Teflon compartment of 0.70 cm × 0.70 cm. On two opposite sides of the cell walls are quartz windows which allow measurements of absorption changes of the red cell suspensions. The effective volume of the cell, as calibrated by a solution of cresol red in Tris buffer, is 0.47 ml, as compared with 0.50 ml of the solution added. The magnitude of the temperature jump depends linearly on the power imput in the range of 0.3–5°C. In small temperature jump experiments ($\Delta T < 0.3$°C) the jump size (ΔT) was calculated by the relation $\Delta T = CV^2/8.36 \cdot C_p \rho v$, in which C, V, C_p, ρ, and v denote, respectively, the heat capacity, density, and the effective volume (= 0.47 ml) of the solution. C_p and ρ were taken as unity. We recognize a large uncertainty in the estimation of the temperature jump for $\Delta T <$ 0.2°C. However, this uncertainty does not significantly affect our interpretation of the temperature jump result.

To double check ΔT in small temperature jump experiments, capacitors of different capacitances were used to perform similar measurements. Within experimental error they agreed.

The temperature stability of the cell was also one of our main concerns and was, likewise, checked by the cresol red-Tris solution. The cooling of the solution can be monitored by the return of the signal after the temperature jump. In a 3°C jump the signal dropped back to 90% of the maximal level in 5 s, indicating that the cell temperature is stable for at least a few seconds. For a reaction with a relaxation time greater than 2 s we used only that portion of the kinetic curve which occurred within 2 s in our analysis of data.

Electric Field Pulse Experiment

A high power electric pulse generator, model 605P of Cober Electronics, Inc. (Stamford, Conn.), was employed. The pulse generator can produce up to 2-kV pulses of variable pulse length. Two copper capacitor plates spaced at 2 mm were connected to the generator. A 200 Ω resistor is used for line termination to maintain the shape of the square wave pulses. The rise time of pulse is about 50 ns. The erythrocyte suspensions in 0.15 N NaCl were filled in glass capilaries and were placed in between the two copper plates. The application of a 2 kV pulse in effect exposed the erythrocytes to a 10 kV/cm field strength. After the pulse experiment the erythrocyte samples were removed and examined with a light microscope to check the extent of damage to the cell membrane. Hemolysis of the samples after the pulse experiment were estimated by colorimetric analysis of the supernates.

RESULTS AND DISCUSSION

Electric Field Pulse Experiment

Early work reported by Riemann et al. (1975) and Tsong and Kingsley (1975) involved a simultaneous exposure of the erythrocytes to a rapid temperature jump and an elec-

tric voltage pulse, and the cause of the membrane rupture is difficult to determine. For this reason we have employed a Cober 605P high power pulse generator (Cober Electronics, Inc., Stamford, Conn.) to examine the effect of high electric field on the red cell membrane. The procedure as given in the experimental section exposes the erythrocyte suspensions to a 10 kV/cm field strength without passage of electric current, and hence no change in the temperature of the solutions.

Of the four samples, one was exposed to 20 1-μs pulses, one to 10 50-μs pulses, one to 10 200-μs pulses, and the last one to 1,000 1-μs pulses with 10 ms off-duty between two pulses. None of the four samples showed any change in membrane morphology and none showed appreciable hemolysis. In our temperature jump experiment the application of a 10 kV/cm field strength in 10 μs caused a total hemolysis of the erythrocytes.

This experiment indicates that mere electric field pulsation to the suspension at this magnitude is not enough to induce rupture of erythrocyte membranes. In fact a 10 kV/cm field strength corresponds to a 10 mV transmembrane potential. Rupture of lipid bilayers requires 10–100 times that magnitude (Tien and Diana, 1968; Coster and Zimmermann, 1974). The present experimental setup does not permit us to reach that high field. It will be shown later that the passage of the direct current through the suspension poses a totally different situation.

Effects of Temperature Jump

Owen et al. (1970*a*,*b*) suggested that the temperature jump-induced permeation of water in the red cells was due to a colligative osmotic effect. Tsong and Kingsley (1975) pointed out that if a thermal differential could be established between the two sides of a membrane a much greater effect, namely the thermal osmosis effect (Katchalsky and Curran, 1965), should dominate. In both cases the crucial point is how fast the temperature can equilibrate between the interior and the exterior of a red cell.

If we assume that the temperature jump is very fast, i.e., when a jump is applied to the suspension the temperature of the solution is instantaneously raised from its initial value T_1 to T_2. If the current does not penetrate through the red cell membrane, the cell temperature will remain at T_1. Assuming that there is no mass transfer the thermal equilibration of a red cell (including the cell contents) with its surrounding can be described by the equation (see Appendix I)

$$T_2 - T(t) = (T_2 - T_1)e^{-Kt}, \tag{1}$$

where $K = 3\sigma/RC_p\rho l$. In the equation, σ, R, ρ, C_p, and l denote, respectively, the thermal conductivity of membrane, the inside diameter of red cell, the density and the heat capacity of membrane material, and the thickness of membrane.

A precise value of the time constant of thermal equilibrium, K, can not be obtained since the thermal conductivity of membrane bilayers, σ, is not yet known. A rough estimate of K, by assuming that $R = 5 \times 10^{-4}$ cm, $\rho = 0.9$ g/cm^3, $C_p = 0.4$ cal g^{-1} deg^{-1}, $l = 10^{-6}$ cm, and $\sigma = 4 \times 10^{-4}$ cal $\cdot$ cm^{-1} $\cdot$ s^{-1} $\cdot$ deg^{-1} (for wax), gives a value of 6.7×10^6 s^{-1}. This means that the thermal equilibration could be achieved in the

microsecond time range. On the other hand the thermal conductivity of phospholipid in the bilayer arrangement could differ by a few orders of magnitude from that of wax. Moreover, the thermal equilibration may be limited by other factors inside the cells.

As mentioned earlier, circumstantial evidence does exist which suggests that it may take several hundredths of a second for the thermal equilibrium to reach between the two sides of the membrane (Tsong and Kingsley, 1975; Tsong, 1974).

Granting that the above argument is correct, the theory of thermal osmosis (Spanner, 1954; Katchalsky and Curran, 1965) would predict a movement of solvent water into the erythrocytes when a rapid temperature jump is applied to the suspension. In fact the turbidity changes of the suspension after a rapid temperature jump of less than 2 μs are consistent with a swelling of the red cells (Tsong and Kingsley, 1975). Owen et al. (1970*a*,*b*) have studied the effects of D_2O and the viscosity of the solution on the relaxation time detected by the 90° light scattering measurement. They concluded that the relaxation was due to the water permeation in the red cell membrane.

Water transport after a rapid Joule heating of the erythrocyte suspension has also been implicitly shown in the [^{3}H]glucose trapping (Tsong and Kingsley, 1975) and $Na^+ - K^+$ permeation experiments (Riemann et al., 1975). The transport of glucose and sodium and potassium ions may be facilitated by the flow of water. If the transport is due to a leakage of the membranes any jump exceeding a threshold should completely homogenize the concentrations of these ions and molecule in the suspensions. This is not what was found in these experiments.

The magnitude of the thermal osmosis depends on the energy of transfer (Q^*) of water molecules according to the relation

$$\delta P = -(Q^*/\bar{V}T)\delta T, \tag{2}$$

in which $\bar{V}$ is the molal volume of water. Spanner (1954) suggested that Q^* may be obtained by measuring the temperature dependence of the permeability of the membrane. The equivalent of Q^* in our experiment would be the activation energy of the relaxation time. The activation energies of water relaxations are around 10 kcal/mol. When suitable numerical values are substituted in Eq. 2 a 0.01°C of temperature differential, δT, would give rise to a stationary pressure difference, δP, of 0.8 atm (the upper limit). This δP would be the driving force of water movement in the membrane.

Effects of the Electric Current

The exposure of the erythrocytes in an intensive direct current, as is required in the Joule heating of the suspension, can generate a transmembrane potential in the red cells. Calculation of this transmembrane potential can be done by solving the Laplace equation with given boundary conditions. For a spherical shell with an inside radius of R_i and an outside radius of R_o, exposed in an electric field E_o (Fig. 1), the induced transmembrane potential, $\Delta\Phi$, can be expressed by the equation (see Appendix II)

$$\Delta\Phi = 1.5\left(1 - \frac{3\mu R_o^2/R_i^2}{(R_o^3/R_i^3) - 1}\right)R_o E_o \cos\theta. \tag{3}$$

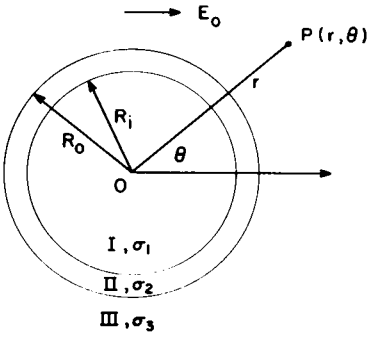

FIGURE 1

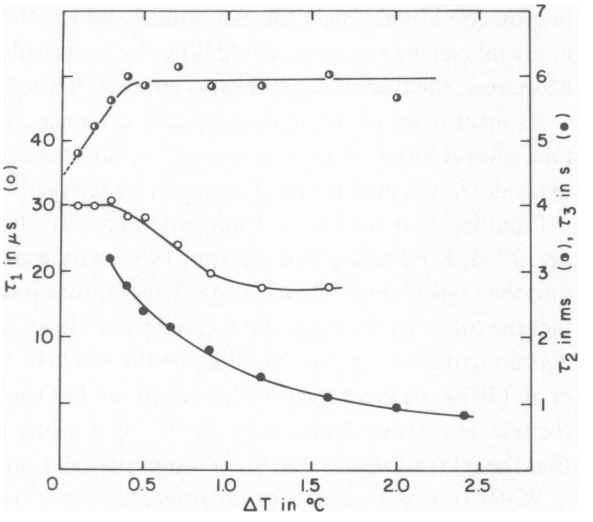

FIGURE 2

FIGURE 1 A schematic representation of a red blood cell exposed to an intensive electric current density. The electric field vector very far away from a cell, indicated by the arrow, has a strength of E_o. R_i and R_o denote the inside and the outside radii of the cell. The system is divided into three regions, I, II, and III, with the conductivity σ_1, σ_2, and σ_3 corresponding to each region. 0 is the geometric center of the cell. The coordinate of the point of observation P is expressed by a radial vector, r, and the angle between the field vector and the radial vector, θ. See text for details.

FIGURE 2 Dependence of the erythrocyte relaxations on the magnitude of the temperature jump. A 0.05 μF capacitor was used in the experiment. The initial field strength of each temperature jump can be expressed by $E = (1.03 \times 10^8 \Delta T)^{1/2}$. The final temperature was kept constant at 17°C. The experiment was done at the isotonic conditions.

In the equation, θ denotes the angle between the current vector and a point of interest in the membrane shell (Fig. 1). $\mu = \sigma_2/\sigma_1$, which is the ratio of the conductivity of the membrane and the conductivity of the medium. Given that $R_o/R_i \sim 1.1$, and $\mu \ll 1$, Eq. 3 can be simplified to

$$\Delta\Phi = 1.5\, R_o E_o \cos\theta \tag{4}$$

The same expression has been given in the treatment of the dielectric breakdown of cell membrane by Sale and Hamilton (1968) and Zimmerman et al. (1974). It should be mentioned that under the isotonic conditions the red cell is biconcave and is far from spherical in shape. Correction of this shape factor is under investigation. However, according to Zimmerman et al. (1974) the correction factor lies between 1 to 2 with a greater transmembrane potential, as predicted by Eq. 4, for the biconcave ellipsoid.

Dielectric breakdown of bilayer lipid membrane occurs around 200 mV transmembrane potential (Tien and Diana, 1968). Coster and Zimmerman (1974) have directly measured the breakpoint of *Valonia utricularis* membrane to be at 850 mV. According to Eq. 4 an electric pulse of less than 10 kV/cm strength in the solution would generate

a transmembrane potential of this magnitude in the red cell. The hemolysis of the red cell in the Joule heating temperature jump experiment may also be caused by the dielectric breakdown of the membranes.

One of the rather puzzling observations in Joule heating type of experiments is that the extent of the red cell hemolysis depends more on the single pulse energy input rather than on the initial field strength of the electric pulse (Tsong and Kingsley, 1975). Another example in the Fig. 1 of Riemann et al. (1975) shows that the 50% hemolysis of the human erythrocytes occurs around the 2.5°C temperature jump although the field strength of the pulses shows a greater variation. It appears that both the effects of temperature and the electric current may be contributing to the hemolysis of the red cells.

Water Relaxations

As discussed in previous sections water relaxations in erythrocyte suspension induced by a temperature jump may be due to a thermal osmosis effect. Water relaxations may also be induced by a dielectric perturbation on an aqueous solution (Eigen and de Maeyer, 1963). A rapid Joule heating of an isotonic suspension of human erythrocytes has detected complex transient signals (Fig. 4 of Tsong and Kingsley, 1975). The fast biphasic decrease in turbidity ($\tau_1 \sim 20$ μs and $\tau_2 \sim 5$ ms) is consistent with a swelling of the red cells (Seufert, 1970; Yi and MacDonald, 1973). There is much experimental evidence to suggest that these signals arise from the water relaxations in the membrane structures (Tsong and Kingsley, 1975; Owen et al., 1970*a*,*b*). Since membrane rupture occurs when the perturbation exceeds a certain critical point, extrapolation of the relaxation time to a zero temperature jump (which is also a zero electric field perturbation) is required to obtain the relaxation times of intact erythrocyte membrane. This is shown in Fig. 2. It appears that some changes in the shape of curves occur at the point where erythrocyte hemolysis takes place ($T \sim 0.5$°C, Tsong and Kingsley, 1975).

The temperature dependence of τ_1 and τ_2 are given in Fig. 3. The apparent activation energies are, respectively, 8.4 kcal/mol and 12.0 kcal/mol for τ_1 and τ_2 reactions. These values are in apparent agreement with those reported in the literature for the water transport in erythrocyte membranes (Solomon, 1972; Foster and Finch, 1975; Sphorer and Civan, 1975). The complexity of the water relaxations in erythrocyte membranes has also been reported by the NMR studies (Foster and Finch, 1975; Sphorer and Civan, 1975).

One of the rather interesting observations shown in Fig. 4 is that τ_2 seems to be a marked function of the osmolarity of the suspension. τ_2 exhibits a minimum in the isotonic solution. The minimum in the relaxation means a maximum in the rate of the water transport. We recently observed that the maximum rate of dye transport in synthetic phospholipid vesicles occurs at the midpoint of the lipid phase transition (Tsong, 1975). The erythrocyte membrane may also assume a unique physical state for the solvent transport in the isotonic conditions. Similar experiments have not been done for τ_1 because of the poorer signal to noise ratio for this reaction.

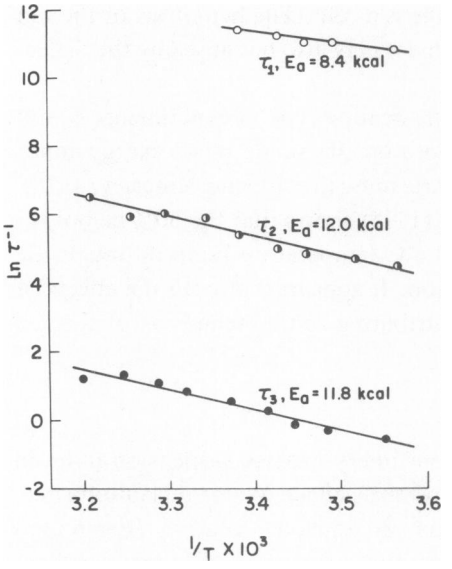

FIGURE 3

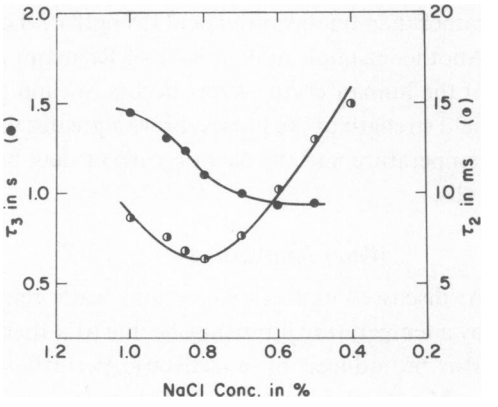

FIGURE 4

FIGURE 3 Temperature dependence of the erythrocyte relaxations. The experimental conditions were the same as those in Fig. 2 except that τ_1 and τ_2 were measured with a 0.5° temperature jump, and τ_3 was measured with a 1.5° temperature jump.

FIGURE 4 Dependence of the erythrocyte relaxations on the osmolarity of the suspension mediums. The experimental details are the same as those in Fig. 3.

Relaxations Due to the Membrane Ruptures

The membrane ruptures accompanied by the release of cell content into the solution are reflected in the slower return of the turbidity of the suspension to nearly its initial value (Fig. 4 of Tsong and Kingsley, 1975). These signals have been found to be directly proportional to the extent of hemolysis induced by the rapid Joule heating of the cell suspensions. In the presence of millimolar concentrations of Triton X-100 the signal completely disappeared. Sonicated erythrocyte suspensions also gave no hemolysis reaction.

The signals are complex and are composed of a time lag, t_{lag}, of about 0.1 s and a reaction τ_3 of about 0.5 s. The lag has been interpreted as due to the membrane ruptures and τ_3 is assigned to the hemolysis reaction (Tsong and Kingsley, 1975).

Both reactions are extremely sensitive to the magnitude of the temperature jump (or the dielectric perturbation). Fig. 2 indicates that τ_3 becomes very slow when the perturbations are small. The temperature dependence of τ_3 is given in Fig. 3. The apparent activation energy is 11.8 kcal/mol in this case. These signals are also sensitive to the sources and the history of erythrocyte samples. Aged samples gave faster relaxations. As will be mentioned later the erythrocyte samples from sickle cell anemia patients gave very different signals in this time range.

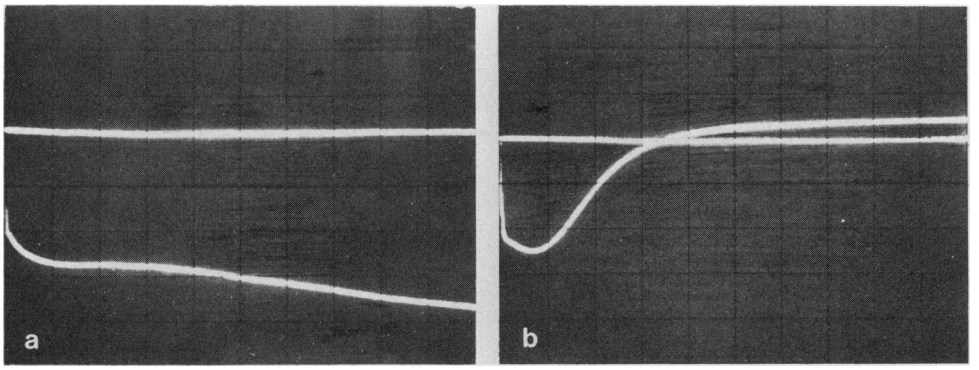

FIGURE 5 Comparison of the hemolysis patterns of a sickle cell sample and a normal erythrocyte sample at 0.1 V/div, 0.2 s/div. Oscillograph (*a*) records the hemolysis pattern of an erythrocyte sample from a homozygous sickle cell anemia patient. (*a*) is to be compared with the hemolysis pattern of a normal erythrocyte suspension in oscillograph (*b*). The temperature jump was from 15 to 17°C.

Relaxations of Erythrocytes from Different Sources

Experiments with the erythrocyte samples from at least 25 healthy young adults gave consistent results, with very little variation, in the four relaxation times discussed in previous sections. To see whether the relaxation times reported here are sensitive to minor changes in the membrane structures we have done the same temperature jump experiment for the erythrocyte samples from leukemia, dystrophy, and sickle cell anemia patients. In all samples tested the two faster relaxations, τ_1 and τ_2, showed only a slight variation. However, a much larger change was frequently observed for the two slower relaxations, t_{lag} and τ_3.

Fig. 5 *a* gives an oscillograph of the hemolysis reactions of sickle cells to be compared with the same reactions in normal cells in Fig. 5 *b*. Incubation of the sickle cell sample with cyanate restored the hemolysis reactions to resemble the normal curve. Systematic studies of the hemolysis pattern of erythrocytes from different sources remain to be done. Nevertheless, the experiment described here supports our assessment that the slow relaxations result from the membrane ruptures and the hemolysis reactions.

Experiment with Erythrocyte Ghost

The relaxation pattern of the erythrocyte ghost suspension is grossly different from that of intact erythrocytes. Fig. 6 gives an example of such experiments to be compared with Fig. 4 of Tsong and Kingsley (1975). As can be seen from the figure the rapid decrease in the turbidity of the ghost suspension occurs in the 10 μs time range and is single exponential (τ_1). The return of the turbidity, on the other hand, follows a complex kinetics and can be separated into two phases: $\tau_2 \sim 0.8$ ms and $\tau_3 \sim 10$ s (τ_3 not shown in Fig. 5). We have tentatively assigned τ_1 to the water relaxation, τ_2 to the membrane rupture, and τ_3 to the annealing reaction of ruptured membranes.

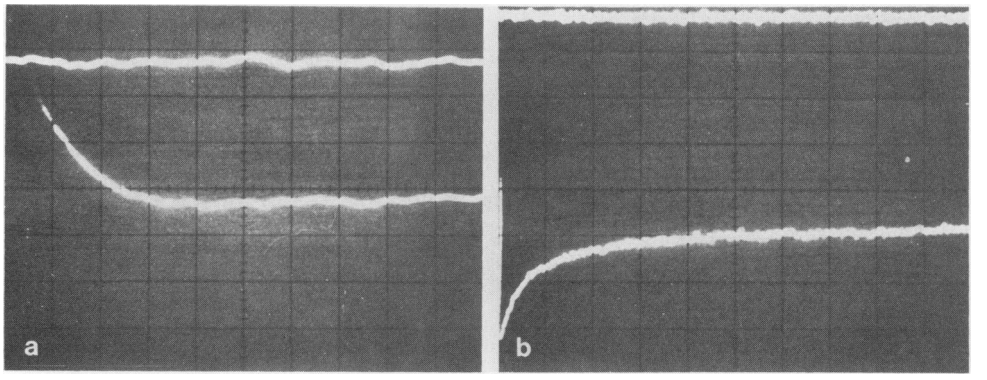

FIGURE 6 Temperature jump relaxations of human erythrocyte ghost suspension. Oscillograph (*a*) records a rapid decrease in the turbidity of the suspension following a 2°C temperature jump, at 50 mV/div, 5 μs/div. The drop in turbidity is followed by a biphasic return of the turbidity to its initial value. Only the faster phase of this return is shown in oscillograph (*b*), at 20 mV/div, 1 ms/div. For experimental details and assignment of the relaxation times see Table I.

In fact the relaxation pattern of the erythrocyte ghost resembles that of synthetic liposomal suspension. The relaxation times of the liposomal suspensions have for the most part been worked out (T. Y. Tsong, manuscript in preparation). Table I gives some numerical values of the erythrocyte and the ghost experiments.

TABLE I

SOME NUMERICAL VALUES FOR THE RELAXATIONS OF HUMAN ERYTHROCYTE AND ITS GHOST SUSPENSIONS

Reaction	Relaxation time at 25°C	Apparent activation energy	Turbidity change	Tentative assignment
		kcal		
Erythrocyte				
τ_1	15 μs	8.4	Decrease	Water relaxation
τ_2	2.8 ms	12.0	Decrease	Water relaxation
τ_{lag}	0.2 s	13.5	No change	Membrane ruptures
τ_3	0.40 s	11.8	Increase	Hemolysis reaction
Ghost				
τ_1	9.5 μs	8.0	Decrease	Water relaxation
τ_2	0.6 ms	13.0	Increase	Membrane ruptures
τ_3	9 s	12.5	Increase	Membrane annealing

(*a*) A capacitor with a capacitance of 0.05 μF was used in these measurements. The temperature jump was set at 2.0°C. The initial field strength was 14 kV/cm with a decay constant of about 4 μs. (*b*) Experimental conditions: 0.85% NaCl, 5 mM phosphate buffer at pH 7.0. Erythrocyte concentrations: 60–150 fold dilution of the blood concentration. (*c*) Turbidity measured at 300 nm. Turbidity measured at different wavelengths gave the same result. However, wavelengths in the vicinity of the Soret band of hemoglobin are not suitable for turbidity measurement because of the strong background absorptions. (*d*) The apparent activation energies given here are only meant to denote the temperature dependence of the reactions. They do not correspond to Arrhenius activation energy in strict sense.

Conclusion

The Joule heating temperature jump technique when applied to complex biological systems may induce relaxation phenomena which are not easily interpretable by the ΔH effect of the reactions. In the case of the erythrocyte suspensions the relaxations detected include membrane ruptures, hemolysis, and perhaps solvent permeations. Both the thermal osmosis effect and the dielectric perturbation may contribute to the observed phenomena. It appears that more experiments are needed for the separation of the two effects. Application of laser heating technique (Beitz et al., 1970; Baldo et al., 1975) may not solve the problem as no thermal gradient across the membrane can be established by the laser heating. Since trapping of glucose without causing hemolysis of red cells have already been observed (Tsong and Kingsley, 1975) further development of the experimental method described here may eventually provide a means for the interiorization of clinically active molecules into the red cells. The experiment reported here may also help us understand the mechanisms of molecular release in cellular systems.

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APPENDIX I

Temperature Equilibration of Erythrocytes in the Suspension Medium After a Rapid Temperature Jump

The following calculations are based on a simplified model where no mass transfer between the red cells and the suspension medium is assumed. Further, the red cells are treated as spherical shells. It is also assumed that: (*a*) Prior to the temperature jump the entire system is at equilibrium with a homogeneous temperature of T_1. (*b*) The temperature jump is very rapid compared with other processes of interest, so that after the jump the medium reaches a higher temperature T_2 at $t = 0$. However, because the membrane is a poor conductor for electricity the cell temperature remains at T_1. (*c*) The membrane is a much poorer heat conductor than the cytoplasmic medium, so that membrane alone sustains the thermal differential between the red cells and the solution.

Under these conditions the heat flow across the membrane is expressed by

$$dQ/dt = \sigma(4\pi R^2/l)[T_2 - T(t)] = \tfrac{4}{3}\pi R^3 \rho C_p(dT/dt), \tag{4}$$

or

$$dT/dt = K[T_2 - T(t)], \tag{5}$$

in which

$$K = 3\sigma/R\rho C_p l. \tag{6}$$

In the equations, σ, ρ, C_p, and l represent, respectively, the thermal conductivity, density, heat capacity, and the thickness of the membrane.

Solving the differential Eq. 5 with the initial condition that at $t = 0$, $T = T_1$ one obtains

$$T_2 - T(t) = (T_2 - T_1)^{-Kt}, \tag{7}$$

which is given in the main test as Eq. 1. In real situations mass transfer accompanying the thermal conduction may speed up the temperature equilibration, and the time constant K may be greater than that predicted by Eq. 6.

APPENDIX II

Transmembrane Potential Produced by an Intense Electric Current

As shown in Fig. 1 we separate the space into three regions represented by I, II, and III. Their electronic conductivities are, respectively, σ_1, σ_2, and σ_3. As a first approximation, let us assume that the dielectric constants in all three regions are the same, the current density $\mathbf{J}$ satisfies

$$\nabla \cdot \mathbf{J} + (\partial\rho/\partial t) = 0. \tag{8}$$

Since there is no charge generated in all three regions $\partial\rho/\partial t = 0$, and Eq. 8 becomes

$$\nabla \cdot \mathbf{J} = 0. \tag{9}$$

From Ohm's law $\mathbf{J} = \sigma\mathbf{E}$. Since $\mathbf{E} = -\nabla\Phi$, where Φ denotes the electric potential, we have

$$\nabla^2\Phi = 0, \tag{10}$$

which is the Laplace equation.

Take the point center of a red blood cell 0 as the reference potential of our system. As $r \to \infty$, $\Phi_{III} = -E_o r\cos\theta$, where E_o is the electric field far away from the cell. The general solutions satisfying these conditions are

$$\begin{aligned} \Phi_I &= \delta r\cos\theta, \\ \Phi_{II} &= (\beta r + \gamma/r^2)\cos\theta, \\ \Phi_{III} &= -E_o r\cos\theta + (\alpha/r^2)\cos\theta, \end{aligned} \tag{11}$$

where δ, β, γ, and α are constants to be determined by the following boundary conditions.

$$\begin{aligned} \Phi_I(R_i) &= \Phi_{II}(R_i), \\ \Phi_{II}(R_o) &= \Phi_{III}(R_o), \\ \sigma_1(d\Phi_I/dr)_{r=R_i} &= \sigma_2(d\Phi_{II}/dr)_{r=R_i}, \\ \sigma_2(d\Phi_{II}/dr)_{r=R_o} &= \sigma_3(d\Phi_{III}/dr)_{r=R_o}. \end{aligned} \tag{12}$$

These conditions manifest the fact that the potential and the normal component of $\mathbf{J}$ are continuous in the space. Assuming that $\sigma_1 \sim \sigma_3$, and let $\sigma_2/\sigma_1 = \sigma_2/\sigma_3 = \mu$, one obtains

$$\alpha = (2\mu + 1)(\mu - 1)(R_o^3 - R_i^3)E_o/$$
$$[(2\mu + 1)(\mu + 2) - 2(R_i/R_o)^3(\mu - 1)^2], \quad (13)$$

and

$$\delta = 9\mu E_o/[(2\mu + 1)(\mu + 2) - 2(R_i/R_o)^3(\mu - 1)^2]. \quad (14)$$

In the case of red blood cells, $\sigma_2 \ll \sigma_1 \sim \sigma_3$, i.e. $\mu \ll 1$, and Eq. 13 and Eq. 14 become

$$\alpha = -\tfrac{1}{2}R_o^3 E_o, \quad (15)$$

$$\delta = -4.5[\mu R_o^3/(R_o^3 - R_i^3)]E_o. \quad (16)$$

Substitute these equations into Eq. 11 and take a differential yield

$$\Delta\Phi = \Phi_I(R_i) - \Phi_{III}(R_o)$$
$$= \{1 - (3\mu[R_o/R_i]^2/[(R_o/R_i)^3 - 1]\} \times 1.5\, R_o E_o \cos\theta. \quad (17)$$

As $R_o/R_i \sim 1.1$ and $\mu \ll 1$ it can be simplified to

$$\Delta\Phi \sim 1.5 R_o E_o \cos\theta. \quad (18)$$

$\Delta\Phi$ is the transmembrane potential produced by the current density **J**.

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