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RELEASE AND UPTAKE OF HAEMOGLOBIN AND IONS IN RED BLOOD CELLS INDUCED BY DIELECTRIC BREAKDOWN

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SUMMARY

External electric field strengths of the order of 10^3–10^4 V · cm^{-1} induce potassium release and concomitant sodium uptake in human and bovine red blood cells, as demonstrated in an electrolytic discharge chamber. The reversible increase of the membrane permeability once the critical membrane potential is reached is caused by dielectric breakdown of the membrane. The values of the critical membrane potential differences calculated from the potassium release and sodium uptake curves are close to those which were calculated from dielectric breakdown measurements in a hydrodynamic focussing Coulter Counter using the Laplace equation. With bovine red blood cells, the potassium release and the concomitant sodium uptake is coupled with haemoglobin release from the cells, while with human red blood cells much higher external electric field strengths are required for haemoglobin release.

The external electric field strength required for solute release and uptake in bovine and human red blood cells depends on the pulse length, particularly below a value of about 10 μs, when a strong increase in the field strength occurs with decreasing pulse lengths. At 50–100 μs pulse lengths an asymptotic value of the critical electrical field strength of 2.6 kV · cm^{-1} for the modal volume of human red blood cells and 2.8 kV · cm^{-1} for the modal volume of bovine red blood cells is reached, corresponding to a critical membrane potential difference of about 1.1 V for both species. This value is close to that measured directly for dielectric breakdown of the membranes of *Valonia utricularis* (0.85 V, 20 °C).

The increase in electric field strength with decreasing pulse length can be explained by the capacitance of the membrane, which becomes the rate limiting step for the temporal build-up of the electric potential across the membrane. The time constant of this process was determined to be approx. 10 μs. The critical membrane potential difference for breakdown is therefore pulse-length independent.

The breakdown of the membrane can be interpreted by an electromechanical collapse of the membrane material. Numerical considerations of the dynamics of this membrane collapse predict that the breakdown time is a very rapid process.

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INTRODUCTION

When high electric fields are applied to cell membranes a dramatic increase in the membrane conductance is observed at a critical membrane potential difference (PD). This effect was demonstrated for the cell membranes of red blood cells, *Escherichia coli* and *Ochromonas malhamensis* [1–4] using a Coulter Counter and an electrolytic discharge cell and also more directly for the membranes of *Valonia utricularis* using intra- and extracellular electrodes [5–7]. The dramatic increase in membrane conductance could be accounted for by dielectric breakdown of the membranes.

Punch through [8] and local heating [7] as possible mechanisms for the conductance increase could be definitely excluded. It was shown that the results, particularly the strong non-linear I-V characteristic obtained with *Valonia utricularis* could be interpreted in terms of an electromechanical model [2, 6]. In this model, a membrane collapse is predicted when the membrane potential reaches a critical value. On the basis of this model, the elastic modulus for deformation normal to the plane of the cell membrane could be calculated for the first time. The value of $5 \cdot 10^6$ $N \cdot m^{-2}$ (at 20 °C) deduced from the experiments with *Valonia utricularis* is close to those reported recently for "solvent-free" lipid bilayers [9].

Furthermore, dielectric breakdown measurements using bacteria [2] and red blood cells (Pilwat, G., Zimmermann, U. and Riemann, F, manuscript in preparation) have indicated that changes in membrane structure are reflected very sensitively in changes of the critical breakdown voltage. Therefore, this bioelectric technique offers a possible new tool for the investigation of membrane structure and the influence of chemical agents and physical parameters on the structure.

Dielectric breakdown also has a possible clinical application in enzyme therapy for certain diseases caused by a deficiency of enzymes [10]. Performing dielectric breakdown of red blood cell membranes in an electrolytic discharge chamber haemoglobin release from the erythrocytes was observed. After about 1 h, the electrically lysed erythrocytes were resealed and the resulting ghosts were more uniform than those obtained by osmotic lysis. As shown previously, proteins (and enzymes) can be trapped into the ghost cells during the resealing process in larger amounts [4, 11]. As suggested by Zimmermann [12–14] and independently by Ihler et al. [15], enzyme-loaded ghost cells can be used as bioactive capsules in the blood circulation for treating diseases without an unfavorable immune response.

Because of the relevance of dielectric breakdown to membrane research, to the preparation of ghost cells and to the injection of foreign material into the cells, we investigated some of the parameters involved in dielectric breakdown and in the processes resulting in the haemoglobin release. For bovine erythrocytes, the membrane PD at dielectric breakdown, determined with the Coulter Counter, was comparable to the voltage at which haemoglobin release was observed in the electrolytic discharge cell. However, in these experiments with human erythrocytes, a large discrepancy occurred between the breakdown voltage measured by the Coulter Counter and the critical voltage for haemoglobin release in the discharge cell. In particular, therefore, we studied the influence of pulse length, at different electric field strengths, on haemoglobin release and on potassium release and sodium uptake in bovine and human erythrocytes.

The experiments were performed in an electrolytic discharge cell. The results are discussed in relation to measurements of dielectric breakdown using a Coulter Counter and intra- and extracellular electrodes.

MATERIALS

Human and bovine blood was withdrawn from apparently healthy donors and was stored in acid/citrate/dextrose buffer for no longer than 1 day before an experiment. The erythrocytes were centrifuged and cleaned in the usual way [16] and suspended in a solution, pH 7.2, of the following composition (in mM): Na_2HPO_4, 7.6; NaH_2PO_4, 2.4; MgCl, 4.0; NaCl, 145.

METHODS

Electrolytic discharge cell

For measuring the haemoglobin and ion release and uptake in the erythrocytes, the following set-up was used. A high voltage generator was used to charge a capacitor. When a given voltage was reached, the capacitor was discharged through the chamber containing the cell suspension. Electrical connection between the capacitor and the discharge cell was made with a spark gap switch. The discharge chamber was formed by two platinum electrodes each 3.0×3.0 cm, separated by 1 cm with plexiglass spacers. The voltage pulse which was applied to the cell suspension had the characteristic shape of that of a capacitor discharging through a resistance. The time constant $\tau = RC$, where R is the resistance of the discharge cell containing the cell suspension and C the capacitance of the storage capacitor, was taken as a measure of the pulse lenght. The resistance R in the experiments was 10 Ω. The pulse length could then be varied by varying the storage capacitance.

Haemoglobin determination

Voltage was applied to cell suspensions of 1 part erythrocytes to 100 parts of 0.9 % NaCl solution. Following this, aliquots of this suspension were diluted further with 0.9 % NaCl solution and, after 10–15 min, centrifuged at $10\,000 \times g$ for 10 min. The haemoglobin in the supernatant was determined at 398 nm using a Zeiss spectrophotometer [16].

Potassium and sodium determination

For determining the potassium release and the concomitant sodium uptake in erythrocytes due to dielectric breakdown, the suspension density was increased to obtain better accuracy for the ion determination in the sediment; in general, 1 part of sediment was given to 10 parts of 0.9 % NaCl solution. 5 min after applying the voltage, the suspension was centrifuged at $10\,000 \times g$ for 10 min. Aliquots of the sediments were taken, the freshweight determined and the organic material ashed using atomic oxygen. In the inorganic residue, the potassium and sodium content was determined flame-photometrically using a Zeiss flame photometer.

For the simultaneous determination of haemoglobin release and ion release and uptake, the suspension was divided, after applying the voltage, into two equal parts: one part was used for the potassium and sodium determination, the other part,

after 1:200 dilution with 0.9 % NaCl solution, for the haemoglobin determination.

The Coulter Counter of AEG-Telefunken, Ulm, W. Germany was used to determine the volume distributions of the erythrocytes.

RESULTS

Figs 1 and 2 show the haemoglobin release from bovine and human erythrocytes as a function of external electric field strength for various pulse lengths (that is different time constants τ for the discharge of the capacitor). The values of haemoglobin released refer to the amount relative to that induced by osmotic lysis using distilled water with the same ratio of erythrocytes sediment to supernatant. This measure of haemoglobin release is thus independent of the dilution, i.e. of the ratio of erythrocyte sediment to supernatant. As can be seen from Figs 1 and 2, typical sigmoid curves were obtained. The slope of the curves at the inflection point was not changed when the suspension was stirred immediately after dielectric breakdown (Fig. 1, Curve 30 μs and Fig. 2, Curve 40 μs). The electric field strength which leads to a given percentage of haemoglobin release increased slowly with decreasing pulse length (from 50 μs to about 10 μs). However, below a pulse length of 5 μs, the electric field strength required increased rapidly for bovine erythrocytes. In the experiments with human erythrocytes, routinely only pulse lengths between 50 μs and 10 μs could be used since, for a given pulse length, much higher electric

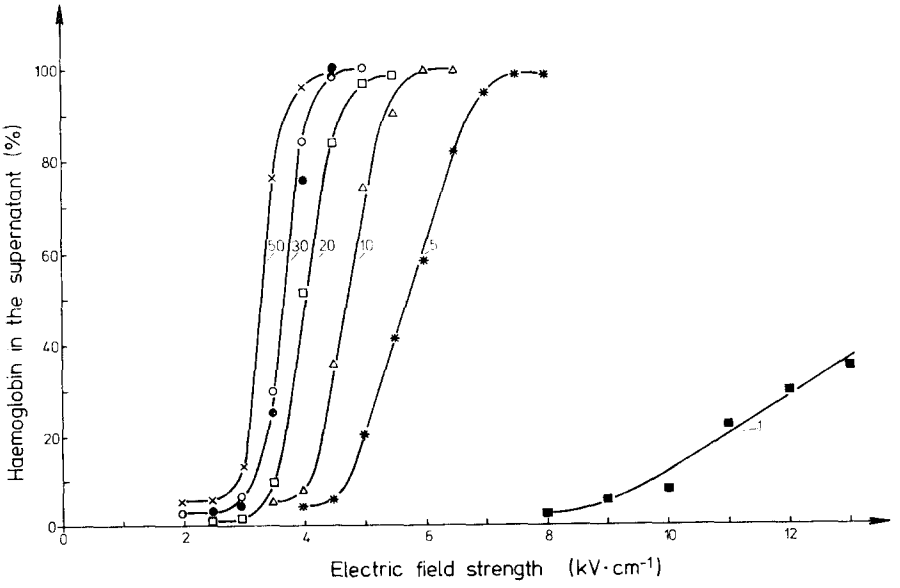

Fig. 1. Haemoglobin release from bovine red blood cells as a function of the electric field strength for various pulse lengths (τ). The haemoglobin released was measured in the supernatant after centrifugation. The values of haemoglobin released by the electric field strength in the electrolytic discharge cell refer to the amount relative to that induced by osmotic lysis by distilled water. The suspension density was 1 part red blood cells to 100 parts 0.9 % NaCl solution. The pulse length was varied between 1 and 50 μs by changing the storage capacitor of the discharge cell. 50 μs (×–×), 30 μs (○–○), 30 μs, stirred (●–●), 20 μs (□–□), 10 μs (△–△) 5 μs (*–*), 1 μs (■–■).

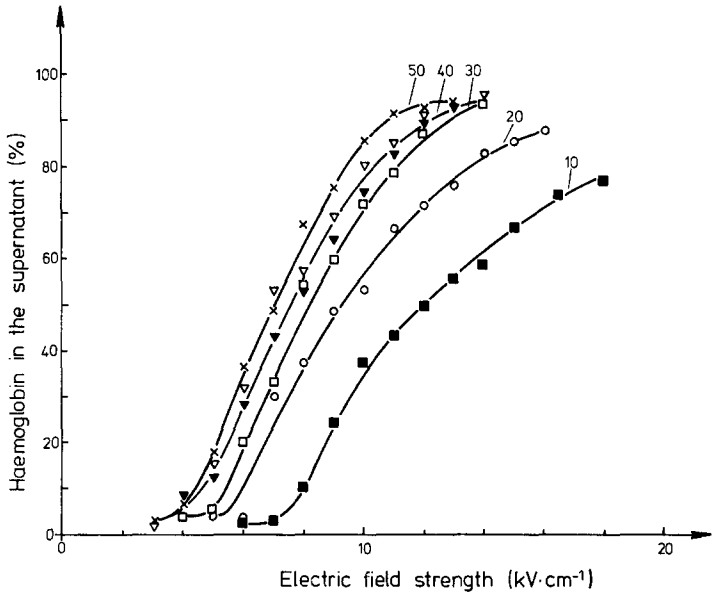

Fig. 2. The same experiment as in Fig. 1 for human red blood cells. The pulse lengths are varied between 50 and 10 μs. Note the different scale of the abscissa. 50 μs (×–×), 40 μs (▽–▽). 40 μs, stirred (▼–▼), 30 μs (□–□), 20 μs (○–○), 10 μs (■–■).

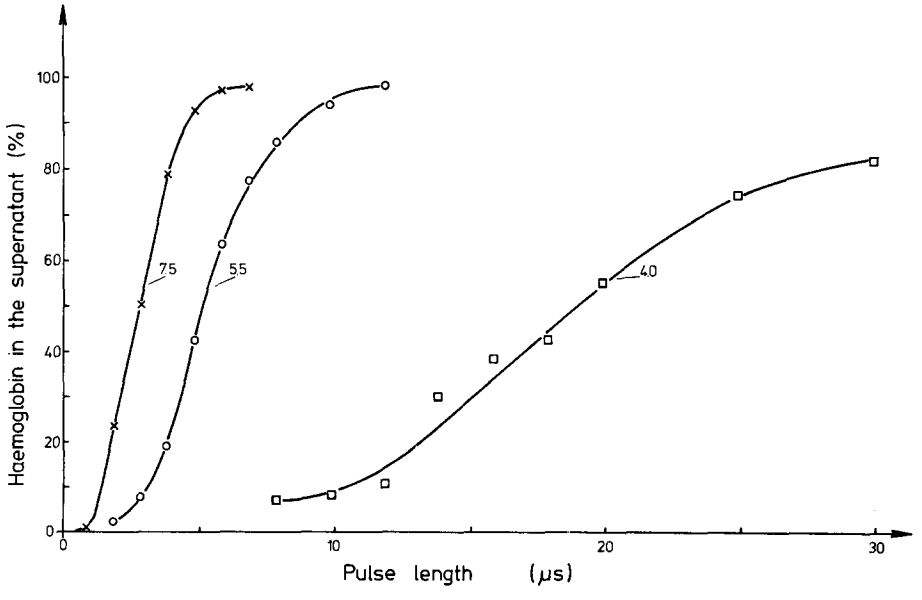

Fig. 3. Percentage of haemoglobin released from bovine red blood cells as a function of increasing pulse length (τ) at different electric field strengths. For experimental conditions, see Fig. 1. 7.5 kV · cm^{-1} (×–×), 5.5 kV · cm^{-1} (○–○), 4.0 kV · cm^{-1} (□–□).

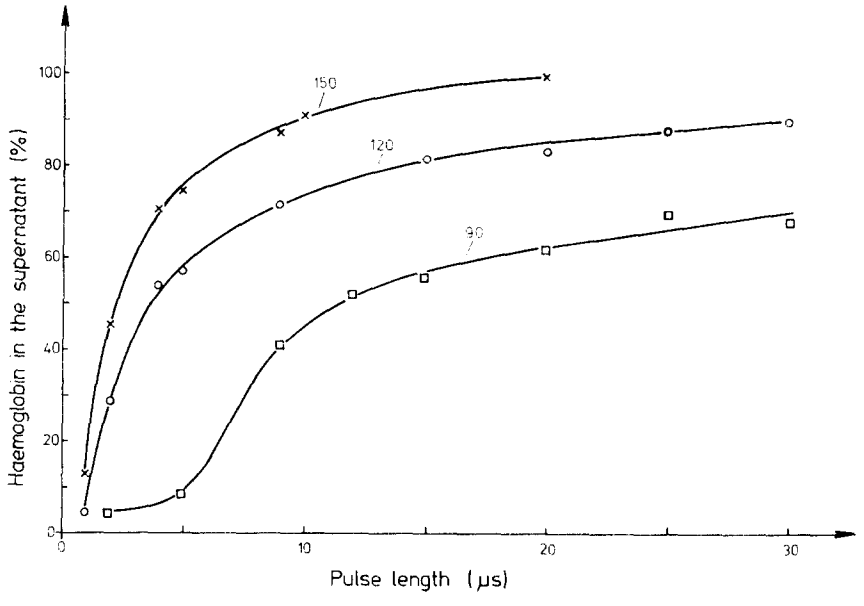

Fig. 4. Percentage of haemoglobin released from human red blood cells as a function of increasing pulse length (τ) at different electric field strengths. For experimental conditions, see Fig. 1. 15.0 $kV \cdot cm^{-1}$ (· · ·), 12.0 $kV \cdot cm^{-1}$ (○-○), 9 $kV \cdot cm^{-1}$ (□-□).

field strengths were necessary to obtain the same percentage of haemoglobin release in comparison with bovine erythrocytes*. It was not possible to work with electric field strengths greater than 18 $kV \cdot cm^{-1}$ due to spark discharge between the electrodes. Hence we were not able to verify whether the field strength required for haemoglobin release in human erythrocytes continues to increase with decreasing pulse length for very short pulses.

The dependence of electric field strength on pulse length is also evident for bovine erythrocytes from Fig. 3 and for human erythrocytes from Fig. 4. In these figures, the percentage of haemoglobin released from bovine and human erythrocytes is plotted against increasing pulse length, at different electric field strengths. At a given electric field strength, increasing the pulse length increased the haemoglobin release.

The relationship between external electric field strength required and the pulse length can be more clearly seen in Fig. 5A, where the electric field strength required for a 50 % haemoglobin release is plotted against the pulse length for human and bovine erythrocytes. From this figure, it is evident that for bovine erythrocytes, and probably also for human erythrocytes, the electric field strength required for haemoglobin release increased rapidly at very short pulse lengths. At longer pulse lengths an asymptotic value of the electric field strength seemed to be reached. A linear relationship was found between the electric field strength required and $1/\tau$.

* Some measurements were made for pulse lengths as short as 1 μs. However, the complete curve of haemoglobin release as a function of the field strength for such a short pulse could then not be obtained for technical reasons.

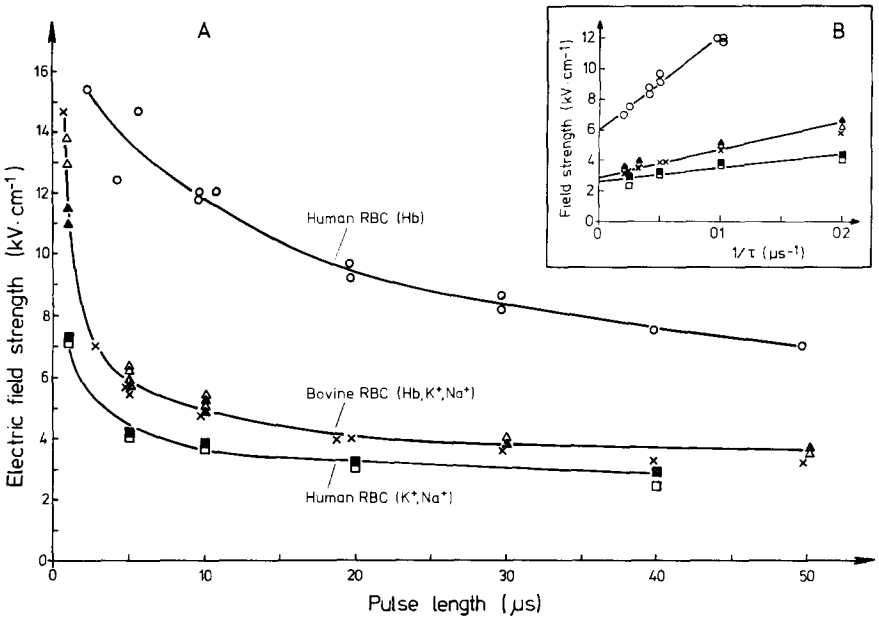

Fig. 5. (A) Electric field strength required for a 50 % haemoglobin (human, ○–○; bovine, ×–×) and potassium (human, ■–■; bovine, ▲–▲) release and sodium (human, □–□; bovine, △–△) uptake as a function of increasing pulse length (τ) for human and bovine red blood cells. Haemoglobin was measured in the supernatant, potassium and sodium in the sediment (see Figs 6 and 7). (B) Plot of the electric field strength versus the reciprocal pulse length ($1/\tau$) for heamoglobin release, potassium release and sodium uptake for both human and bovine red blood cells. The data are taken from Fig. 5A. For experimental conditions, see Fig. 1. Symbols as in Fig. 5A.

For $\tau \to \infty$, the electric field strength, which, for example, leads to a 50 % haemoglobin release, was found to be 2.8 kV · cm^{-1} for bovine and about 6 kV · cm^{-1} for human erythrocytes (Fig. 5B). It can also be seen from Fig. 5A that, for a given pulse length, a much higher electric field strength is necessary for haemoglobin release in human erythrocytes than in bovine erythrocytes. This result is surprising since the solution of the Laplace equation for the critical breakdown voltage of the membrane measured in the Coulter Counter predicts a decrease of the critical external electric field strength with increasing volume* [2]. The modal volume of the human erythrocytes, however, was 84 μm^3. This is 1.9 times the modal volume of the bovine red blood cells which was 45 μm^3. However, using potassium and sodium as indicators of breakdown in the electrolytic discharge cell experiments, it can be clearly demonstrated that for human erythrocytes the dielectric breakdown occurs at much lower external electric field strengths than reflected by the haemoglobin release.

The results of such experiments with human and bovine erythrocytes are

* Solution of the Laplace equation yields the following expression for the membrane potential difference V for a given external field strength:

$V = f_i \cdot a_i \cdot E$

where f_i is the shape factor and a_i is the semi-axis parallel to the electric field E.

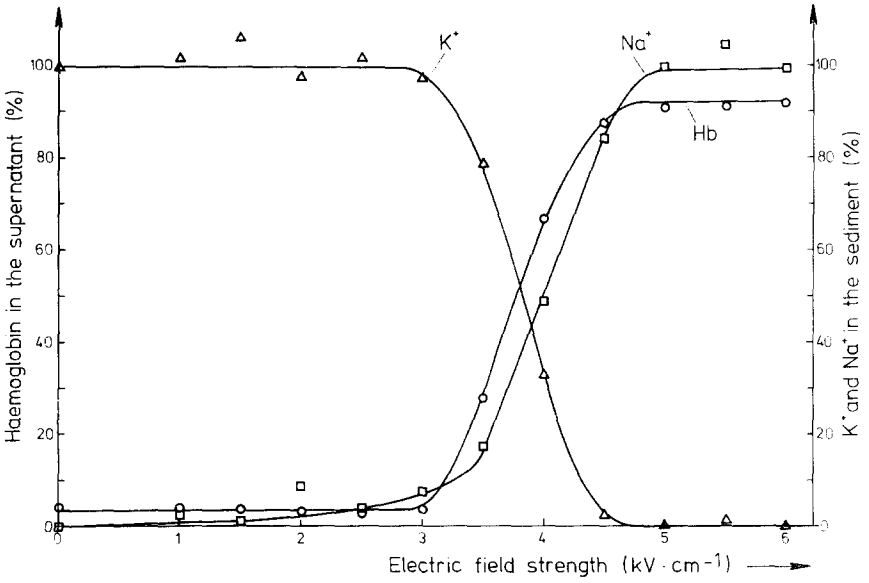

Fig. 6. Potassium release (△–△), haemoglobin release (○–○) and sodium uptake (□–□) as a function of increasing external electric field strength for bovine red blood cells. The pulse length was 30 μs. K^+ and Na^+ were determined in the red blood cell sediment after centrifugation. The values of K^+ and Na^+ plotted are relative to the potassium and sodium contents of the red blood cell sediment before electric field application.

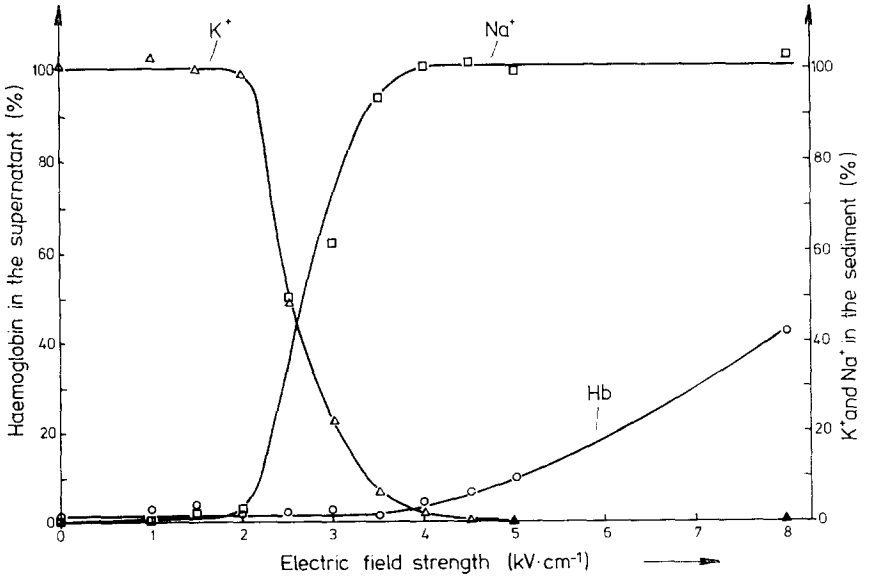

Fig. 7. The same experiment as in Fig. 6, for human red blood cells. The pulse length was 40 μs.

shown in Figs 6 and 7. The values of potassium and sodium plotted are relative to the potassium and sodium content of the erythrocyte sediment to which no electric field was applied. It is not possible to present absolute values since the data refer to a mixture of intact and depleted cells. The pulse length used in the experiments to which Fig. 6 refers was 30 μs and for Fig. 7, 40 μs, that is in the range where the electric field strength was almost independent of the pulse length (see Fig. 5A).

For bovine erythrocytes, haemoglobin and potassium release and the concomitant uptake of sodium are observed at the same critical electric field strength. The inflection points of the three curves occur at 3.9 $kV \cdot cm^{-1}$. For human erythrocytes the situation is quite different. The potassium release and the uptake of sodium both occur at a external electric field strength of 2.7 $kV \cdot cm^{-1}$. The haemoglobin release, however, is observed at about 8 $kV \cdot cm^{-1}$ (not shown in Fig. 7). These differences between human and bovine erythrocytes were also apparent in experiments on the dependence of ion and haemoglobin release on the pulse length (see Fig. 5A). For bovine erythrocytes the potassium, sodium and haemoglobin curves are coincident,while for human erythrocytes the haemoglobin release curve is displaced.

DISCUSSION

The external electric field strength required for the breakdown of erythrocyte membranes depends on the pulse length, particularly below a value of about 10 μs, where a strong increase in the field strength required for breakdown occurs with decreasing pulse length. This is suggested from the pulse-length dependence of the release of haemoglobin and potassium from bovine and human erythrocytes in the discharge cell. The dependence of the critical field strength on pulse length for the release of haemoglobin and potassium and uptake of sodium from bovine and human erythrocytes can be approximately represented by hyperbolic functions. The asymptotic value of the critical external field strength calculated for 50 % release of haemoglobin or potassium and uptake of sodium, respectively, is about 2.8 $kV \cdot cm^{-1}$ for bovine erythrocytes. In comparison, for human erythrocytes the value for the release of haemoglobin was approx. 6 $kV \cdot cm^{-1}$ and for the release and uptake of potassium and sodium, respectively, approx 2.6 $kV \cdot cm^{-1}$.

These asymptotic field strengths will in practice be reached at a pulse length of about 50–100 μs, as seen from the curves in Fig. 5. Inserting the value of 2.6 $kV \cdot cm^{-1}$ for human erythrocytes and 2.8 $kV \cdot cm^{-1}$ for bovine erythrocytes into the Laplace equation, the critical breakdown PD for human and bovine erythrocytes is calculated to be approximately 1.1 V. With *Valonia utricularis* at room temperature, a value of 0.85 V was measured directly. We believe that the similar values for the breakdown voltages of membranes of different species are a hint that a common property is involved in the dielectric breakdown.

The two critical breakdown voltages of human and bovine erythrocytes do not seem to be significantly different. Comparison of the dielectric breakdown experiments in the discharge cell with those obtained in the Coulter Counter [2] also support this assumption. Cells whose dielectric breakdown PD was measured in the Coulter Counter pass the orifice length of 40 μm in approx. 25 μs, i.e. corre-

sponding to a pulse length of 25 μs*. This pulse length is an intrinsic property of the commercially available Coulter Counter and can be varied only in a very limited range. It seems reasonable, therefore, to take a pulse length of 25 μs as a point of reference, in order to compare breakdown voltages that have been measured in the Coulter Counter with those critical field strengths that produce a certain release of haemoglobin or potassium in the discharge cell.

Assuming that the inflection point in the release curves in discharge cell experiments reflects the dielectric breakdown of the modal cell sizes, the critical membrane breakdown voltage (taken for a pulse length of 25 μs) is calculated from the haemoglobin and potassium release and sodium uptake curves of bovine erythrocytes to 1.5 V using the Laplace equation. For human erythrocytes, the curves for potassium release and sodium uptake yield a value of 1.4 V for the breakdown PD. These values are nearly equal and in good agreement with those calculated from the Coulter Counter measurements (bovine erythrocytes 1.8 V, human erythrocytes 1.7 V, mean values), since one must take into account the rough assumptions that need to be made to solve the Laplace equation. On the other hand, the breakdown voltage of membranes of human erythrocytes calculated from the release curves of haemoglobin in the discharge chamber yields a value of 3.8 V assuming, again, that the inflection point of those curves can be identified with the breakdown of the membranes of the modal cell sizes. Therefore, one can conclude that in general breakdown does not include implicitly the release of larger molecules. For bovine erythrocytes, however, the release of haemoglobin is directly connected with the occurrence of the dielectric breakdown. This confirms the assumption made previously that, for bovine erythrocytes, the haemoglobin release is an indicator, in the discharge cell experiments, for dielectric breakdown [2].

This aspect of the dielectric breakdown phenomenon is not understood. It seems likely that the different behaviour of human and bovine erythrocytes in high external electric fields is related to differences in membrane composition [17] and with the well-known less cohesive structure of the bovine erythrocyte membrane compared with the structure of the human erythrocyte membrane [18]. The possibility cannot be excluded, at the present state of information, that differences of the intracellular cation content between human and bovine erythrocytes may be partly responsible for the observed effects [19].

The pulse-length dependence of the haemoglobin and solute release induced by external electric fields, however, can be explained either by postulating that the critical membrane breakdown voltage is pulse-length dependent or by assuming that the charging of the membrane capacity becomes the rate limiting step for the temporal build-up of the electrical potential across the membrane below a pulse length of 10 μs. A decision between these two hypotheses requires a discussion of the electrical breakdown mechanism.

As shown previously [2, 7] the dielectric breakdown can be explained by an electro-mechanical collapse of the membrane material. An electrical field in a membrane causes stresses that lead to a mechanical compression of the membrane.

* The "pulse shape" produced by the Coulter Counter is different from the pulse shape of the capacitor discharge process in the discharge cell [2]. Therefore, a comparison of values measured with the Coulter Counter and the discharge cell is only very rough.

Assuming that the electrical field in the membrane is independent of the position, the electric compressive force P_e per unit area is given by

$$P_e = \frac{\varepsilon\varepsilon_0 V^2}{2\delta^2} \tag{1}$$

where ε is the dielectric constant or relative electric permittivity, ε_0 is the electric permittivity of the free space, V is the total PD appearing across the membrane, δ is the thickness of the membrane.

The compression of the membrane creates elastic strain forces. Following Crowley [20], if we assume that the membrane is ideally elastic the mechanical restoring forces P_m per unit area is given by

$$P_m = Y \ln \frac{\delta}{\delta_0} \tag{2}$$

where Y is the elastic modulus referring to deformations normal to the surface of the membrane, δ_0 is the original, unstrained thickness (i.e. at zero PD). For dimensional equilibrium the following equation must be valid:

$$P_e - P_m = 0 \tag{3}$$

and therefore

$$\frac{\varepsilon\varepsilon_0 V^2}{2\delta^2} = -Y \ln \frac{\delta}{\delta_0} \tag{4}$$

It is easily seen from the equations that a mechanical instability, i.e. a catastrophic collapse of the material, must occur with increasing electric field strength, since when δ is sufficiently small the electric compressive force will increase more rapidly with decreasing δ than the elastic restoring force. This occurs at a critical voltage V_c given by the following equation:

$$V_c = \left[\frac{0.3679\ Y \cdot \delta_0^2}{\varepsilon\varepsilon_0}\right]^{\frac{1}{2}} \tag{5}$$

Equation 5 indicates that the critical voltage depends on the ratio of Y/ε, i.e. on the intrinsic elastic and dielectric properties of the membrane. As shown previously [2] for erythrocytes, the breakdown PD calculated from Eqn 5 using the physiologically reasonable values of $\varepsilon = 3$–4 and $Y = 7 \cdot 10^7$ dyne $\cdot$ cm^{-2} [21–23] is in very good agreement with those obtained experimentally. In particular, Coster and Zimmermann [6] have shown with *Valonia utricularis* that the pulsed I-V curves measured directly with intra- and extracellular electrodes are fitted well by this model without assuming adjustable parameters. Eqns 3 to 5 assume, however, that the compression of the material does not involve inertial forces, so that the critical breakdown PD should be independent of the pulse length. The results reported here seem to contradict this. It seems worthwhile, therefore, to investigate whether considerations of the dynamics of the electro-mechanical collapse could shed light on the pulse length dependence of the electrical field strength required. To do this, we must add two terms to Eqns 3 and 4. The mass that has to be accelerated during the compression consists of the mass of the various layers within the membrane, as well as the adjacent space charge containing layers of the external solutions. The thickness of this layer,

determined from the Debye screening length, is about 0.4 nm. Further viscous forces both in the external solution and due to energy losses by structural changes in the membrane etc. must also be taken into account. This then leads to the following differential equation for the compression:

$$m \frac{d^2\delta(t)}{dt^2} = \left[\frac{\varepsilon\varepsilon_0 V^2(t)}{2\delta^2(t)} + Y \ln \frac{\delta(t)}{\delta_0} + K \frac{d\delta(t)}{dt} \right] \tag{6}$$

where m is the mass and k is the damping constant.

For all values of m in the range of $m = 10^{-7}\ g \cdot cm^{-2}$ to $5 \cdot 10^{-6}\ g \cdot cm^{-2}$ and $k = 10^{-8}\ g \cdot s^{-1} \cdot cm^{-2}$ to $1\ g \cdot s^{-1} \cdot cm^{-2}$ and $\varepsilon = 2$ to 10, which may include all possible physiological values of these constants, the breakdown time was found to be in the ns range by solving Eqn 6 numerically using the DIFSY1-FORTRAN Program of Bulirsch [24], assuming the critical PD is reached.

Once the critical PD is reached, the breakdown dynamics predicted from this calculation are so fast that the pulse length dependence of the critical electric field strength that was found experimentally cannot be explained by it. The experimental results, however, can be explained much more readily by assuming that for such short pulse lengths the capacity of the membrane becomes the rate-limiting step for the temporal build-up of the electrical potential across the membrane. The time constant of this process can be easily calculated from the following considerations of the equivalent electrical circuit of the erythrocyte suspension in the discharge cell.

We assume that a red blood cell is placed into the middle of the chamber. The whole chamber has a width of 1 cm and a resistance of 10 Ω when filled with the

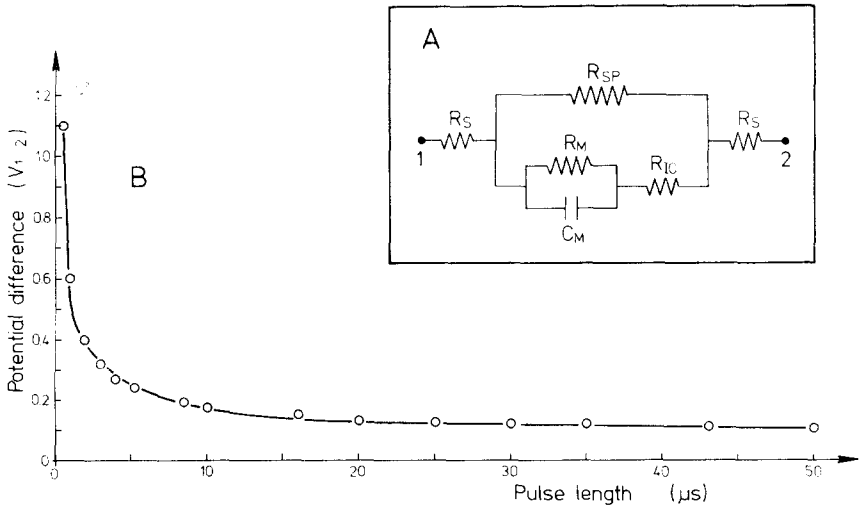

Fig. 8. (A) Simplified equivalent circuit for the electrolytic discharge cell containing a diluted red blood cell suspension. R_S, resistance of the electrolyte; R_{SP}, resistance of the electrolyte shunting current around the cell externally; R_{IC}, resistance of the cell interior; R_M, resistance of the red blood cell membrane; C_M, capacitance of the red blood cell membrane. (B) Plot of the heights of the pulses applied to points 1 and 2 of Fig. 8A required to produce the same peak value for the PD across the membrane elements R_M and C_M is shown as a function of the pulse length (τ). The pulses have the characteristic shapes of a capacitor discharge in a resistor.

solution. The solution is represented, therefore, by the two resistances R_S equal to 5 Ω each. If we suppose that the red blood cell is orientated with its longitudinal axis of 7 μm parallel to the electrical field, the resistance of the solution shunting the cell externally can be represented by a resistor R_{SP} which will have a value $7 \cdot 10^{-3}$ Ω. The red blood cell itself is represented by a parallel combination of the membrane resistance. R_M, which has an area-specific resistance of $10\ \Omega \cdot cm^2$ [25] in parallel with the membrane capacitance C_M which has a specific capacitance of $1\ \mu F \cdot cm^{-2}$ [26]. These elements are in series with the resistance R_{IC} of the internal medium of the red blood cell, which has a resistivity of $193\ \Omega \cdot cm$[27]. It follows readily that the time constant for the rise of the membrane potential for an externally applied PD to the discharge cell as a whole is then approx. 10 μs. The simplest way to obtain the exact value of this time constant is to construct an analogue of the circuit shown in Fig. 8A, using resistors and capacitors. The PD across the membrane elements C_M and R_M following a voltage pulse with the typical capacitor discharge shape across the points 1 and 2 can then be followed on a cathode ray oscilloscope. Voltage pulses with different time constants were then applied to the points 1 and 2. The heights of the pulses were arranged so as to produce always the same peak value for the PD across the membrane elements. A plot of the heights of the pulses applied to points (1 and 2) so required as a function of pulse length is shown in Fig. 8B. In the experiments reported on dielectric breakdown in *Valonia utricularis* using intra- and extracellular electrodes, it was also evident that the electric rise time of the membrane was involved in determining the minimum pulse length required to take the membrane to the breakdown PD (for example, see Fig. 2 in ref. 6). We can conclude, therefore, that the observed pulse length dependence for breakdown is due to the time required to charge the membrane capacitance: the membrane PD for breakdown is constant. At the same time, this means that it is impossible to detect experimentally the mechanical dynamics of the breakdown process in the membrane since this 'occurs in ns once the critical PD is reached.

From this discussion, we can conclude that the dielectric constant of the membrane material and its thickness determines the rise time of the breakdown PD across the membrane. The value of the breakdown PD, however, depends on the dielectric constant, the thickness and the elastic constants.

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