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FIXED CHARGE IN THE CELL MEMBRANE

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SUMMARY

1. Focal electric field was generated by passing a current of 5×10^{-7} to 1×10^{-5} A from a micropipette into the culture medium. Movement of cells at a distance of 5–50 μ from the electrode tip was observed. In case of cells embedded in the culture only local deformation of the membrane was observed.

2. The cell species explored included neurones, glia, muscle fibres, connective cells, malignant cells and erythrocytes. All cells responded in a similar manner to the electric field, and the current required was in the same range.

3. Cells were attracted to a positive micropipette and repelled from a negative one: the only exception was observed in certain malignant cells which moved in the opposite direction.

4. Movement and membrane deformation could be obtained with electrodes filled with various concentrated and isotonic solutions. The composition of the culture medium also had no qualitative influence on these effects.

5. Metabolic poisons or rupture of the cell membrane had no effect on the movement. Isolated membrane fragments showed movement similar to that of intact cells.

6. The possibility of artifacts due to proximity of the focal electrode is considered. It is shown that electro-osmosis cannot account for the present observations. Some other artifacts are also excluded.

7. It is proposed that the most satisfactory way to account for the present observations is by a membrane carrying negative fixed charge of the order of $2 \cdot 5 \times 10^{3}$ e.s.u./cm^2. Some physiological consequences of presence of negative charge in the membrane are briefly discussed.

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INTRODUCTION

Fixed electric charges in the cell membrane have often been suggested in connexion with the ionic mechanism and electrical activity of the cell. Thus, fixed charge has been invoked in hypotheses related to ionic transport across the membrane (e.g. Teorell, 1935; Ling, 1952), as well as to the ionic specificity demonstrated by synapses (Fatt, 1961); the same concept is also implicit in the treatment of the action potential due to Hodgkin & Huxley (1952, cf. pp. 507, 512). Experimental evidence has been presented for fixed charge in erythrocytes, and certain other cells, and in the bacterial coat (see reviews by Abramson, 1934; Abramson, Moyer & Gorin, 1942; James, 1957; Brinton & Lauffer, 1959; Haydon, 1964); but these electrophoretic studies did not cover neurones and muscle fibres, although the fixed charge hypothesis is perhaps most intriguing in such excitable cells.

It is often assumed that the electric charge responsible for electrophoresis must lie on the membrane surface (e.g. Bangham, Pethica & Seaman, 1958). However, in many cell species the conductance of the membrane and its permeability to water are sufficiently high to allow significant flow of ions and water through the cytoplasm when electric field is applied. In these conditions, electrokinetic force is generated also through interaction with fixed charges in the cytoplasm matrix and on the nuclear membrane (Elul & Massey, 1967). Hence interpretation of electrophoretic movement of all cell types strictly in terms of surface charge does not appear to be *a priori* justified.

For example, consider glial or connective cells in culture; the specific resistance of the membrane is about $10\,\Omega\,cm^2$ (Hild & Tasaki, 1962). Taking the radius of the cell as $20\,\mu$ and the specific resistance of the medium and cytoplasm as $20\,\Omega$ cm, the equivalent specific resistance for an homogenous sphere (Cole, 1944) would be $5000\,\Omega$ cm. Since the current density is determined by the specific resistance, the current through the cytoplasm would be 1/250 of that in the medium. The mechanical force generated is proportional on the one hand to the electric field, determined by the current density, and on the other hand to the ζ-potential, determined by the charge density (e.g. Overbeek & Lijklema, 1959); assuming uniform distribution of the fixed charge, the force is proportional to the current density and to the total amount of fixed charge. As the current through the cytoplasm is lower, it should contain 250 times as many fixed charges as those present on the membrane in order to give rise to a comparable mechanical force. The density of uncompensated fixed charge on cell membranes is about $-2{\cdot}5\times10^3$ e.s.u./cm^2 (Abramson *et al.* 1942, p. 315; Thompson & McLees, 1961; see also Discussion), yielding a total of approximately $2{\cdot}5\times10^8$ elementary charges for a spherical cell with $20\,\mu$ radius. To generate a comparable force, the cytoplasm should contain 2×10^{18} uncompensated elementary charges per cubic centimetre, corresponding to a concentration of 3·5 mM—a figure well within the realm of possibility.

The present study was undertaken to examine experimentally these problems. Employing tissue culture preparations of neurones, muscle

fibres and other cell species, and a new method of electrophoresis in a focal electric field, evidence was obtained bearing directly on the presence of fixed charges in the cell membrane.

METHODS

Focal electric field was generated by passage of current from a micropipette to a large indifferent electrode, both of which were immersed in culture medium. In such a circuit, the current passing through the micropipette orifice must equal the total current flowing through the indifferent electrode, but the current density at the indifferent electrode will be much lower, owing to the disparity in surface area of the two electrodes. The ratio of electrode surfaces in the present experiments was $1:10^6$ to 10^7, so that the current density in the vicinity of the indifferent electrode was negligible.

It is rather difficult to obtain an exact solution of the electric field generated by a micro-electrode, but, since the present experiments were not meant to provide quantitative measurements, an approximate expression derived from Poisson's equation was considered adequate:

$$E = \frac{I}{4\pi\lambda r^2}A,$$

where I denotes the current, E is the electric field, λ the specific conductance of the medium, and r the distance from the electrode tip. A correction factor A must be introduced, to compensate for the divergence of the actual field from the field due to a point source in infinite space. Three main factors contribute to distortion of the field: (i) before reaching the electrode orifice, the field is confined between nearly parallel insulating walls; in the analogous two-dimensional situation solved by Jeans (1927) the field configuration is found to be an asymmetric ellipse; (ii) the cells lie almost directly on the glass floor of the chamber; and (iii) the electrode is inserted at an angle of 20° to the floor of the chamber. As a consequence, the configuration of the field most likely is a prolate ellipsoid. Control experiments described in the Results suggest however for A a value of 20, which probably cannot be ascribed only to geometric deformation.

Electrodes. For focal electrodes, use was made of glass micropipettes, gradually tapering to a tip of 2–5μ. Pyrex glass (Corning) was employed throughout. Micropipettes prepared with an automatic pipette puller commonly have a tip smaller than 1μ, and a correspondingly high resistance, but difficulty was encountered in drawing larger pipettes with this machine, and it was therefore necessary to have the tips subsequently enlarged. Since the tip potential may be affected by the mode of preparation and past history of micropipettes (Adrian, 1956), and inasmuch as tip potentials might interfere with the experiments by altering the rate of electro-osmotic flow (see Results), electrodes were prepared by two different techniques. One method involved filling fine micropipettes with methanol under reduced pressure, transferring them to distilled water, and subsequently to a solution of 2·3 M-K-citrate. This alkaline solution gradually etches glass, and after 10–30 days the tips were found enlarged to 2–5 μ; the electrodes were then washed in distilled water, and filled with the desired solution. Alternatively, newly drawn pipettes were broken manually under the microscope, by passage over an abrasive surface. While more time-consuming, this method allowed immediate filling with the appropriate solution without preliminary exposure to other ion species, thus decreasing the likelihood of modification of surface potential.

In the majority of experiments, micropipettes were filled with either 3 M-KCl, 2·3 M-K-citrate, or Ringer solution (Paul, 1959). The indifferent electrode was prepared of a length of silver wire, approximately 500 mm^2 in area, which was shaped as a loop of 4 cm diameter and chlorided before each experiment.

Tissue cultures. Cell as well as tissue cultures were employed. The cell culture most commonly used was from a malignant cell line ('CMP'), maintained at the Pasadena Foundation for Medical Research for more than 1 year. Tissue cultures were prepared from the dorsal root ganglion of the chick, and from cerebellum and hippocampus of the 3 to 10-day rat. The explants were cultivated in Rose chambers; a plasma coagulum, or reconstituted rat-tail collagen, anchored the explant to the floor of the chamber, and it was then covered by a strip of cellophane to provide a larger gas-exchange surface (Raiborn & Massey, 1965). Cultures derived from the dorsal root ganglion often contained a few muscle strands. Muscle cultures were also prepared from the thigh and heart of the chick. All tissue cultures contained numerous connective cells. The cultures were maintained at 37° C and pH of 6·8–7·2 in modified Eagle's medium (Paul, 1959), which was replaced twice weekly.

Experimental procedures. Experiments were performed on 1–3 week cultures. After opening, by careful removal of the cellophane strip, the chamber was rigidly fixed to the stage of an inverted microscope (Unitron). Micromanipulators were mounted on opposite sides of the stage. One of these served to place the focal electrode; a micro-dissection knife was mounted on the other. A special long working-distance phase-contrast condenser (Wild) provided 5 mm clearance from the top of the culture chamber. A 40× phase-contrast objective was used, still photography and cinematography being made through a light-deviating prism built into the microscope. With this arrangement, the micropipette could rapidly be manoeuvred to the desired distance from any given cell in the visual field. The microscope was also equipped with a heated stage regulated at $38 \cdot 0 \pm 0 \cdot 2°$ C, the optimal temperature for these tissue cultures employed. The pH of the culture did not generally remain constant after the chamber was opened, and all experiments were terminated within 30 min.

Electrical circuit. Preliminary experiments have demonstrated near identity of the effects of direct and pulsed current (Elul & Massey, 1967). To minimize the total charge passed through the solution, square wave trains at 30/sec repetition rate, and 2–5 msec pulse duration, were normally used.

As a rule, the indifferent electrode was earthed. A 10 MΩ resistor between the current source and the micropipette was always included to ensure constant current with electrodes of different resistance.

RESULTS

The effect of a focal electric field on free cells

When a focal electric field was set up in the medium, cells suspended in the vicinity of the focal electrode were observed to move (Elul, 1965). Movement commenced as soon as current was applied, and lasted for the duration of current flow. Upon interrupting the current, the cell came to a halt without any noticeable rebound. At this point, it could readily be made to move in the opposite direction by reversing the current flow. In the entire series of experiments, there was practically no exception to the rule that inversion of current polarity was associated with reversal of cell movement.

All cell types investigated showed movement in focal electric field. Suspensions of malignant cells, which were readily available, were often utilized. Human erythrocytes were also employed. Movement was also demonstrated for neurones isolated from a freshly excised slab of cerebellar

tissue by free-hand dissection (Elul, 1965), as well as cultured neurones, muscle fibres and connective cells.

Current polarity and direction of cell movement

With the tip of the micropipette positive with respect to earth, cells within the focal field generally moved towards this electrode. Only rarely have malignant cells been encountered which were attracted to a negative focal electrode; the morphological correlates of affinity to the negative pole were not explored. However, it is worth noting that although in electrophoresis experiments most cell species also show movement to the positive pole (Brinton & Lauffer, 1959), there exists one report describing migration of malignant cells to the negative pole of the field (Péterfi & Kapel, 1928). The polar affinity of normal and malignant cells was not dependent on their well-being, and persisted even following appearance of irreversible cytoplasmic granulation from exposure or to mechanical damage to the cell.

Deformation of immobilized cells

In contrast to cell suspensions, tissue cultures typically present a mesh-like appearance. Most cells are attached to the glass slide forming the floor of the culture chamber, or else are interlocked with processes of neighbouring cells. Cell movement could not be observed in these circumstances, and flow of current elicited a more complex effect, with deformation of the cell membrane at the region closest to the focal electrode. The membrane was either attracted or repelled, depending on the polarity of the current, but as soon as current was terminated the cell resumed its original shape. The polar affinity of this effect was similar to that of movement of free cells; as a rule, the cell membrane was attracted to a positive focal electrode (Fig. 1), and repelled from a negative one. No difference in the reaction of various cellular regions, or of different cell types, could be found, although more prominent responses were obtained with cells that had not spread out too thinly on the glass surface.

After initial exploration while immobilized, cells could often be gradually freed from the main body of the culture by gentle probing with a fine glass rod. It was then noted that membrane deformation disappeared once the cell was free to move. Such evidence suggested that deformation of immobilized cells and movement of free cells were in reality expressions of the same basic process. Some possible neurophysiological consequences of deformation of the membrane are discussed elsewhere (Elul, 1966*a*).

Current requirements

There was no fundamental difference in the action of pulsed and uninterrupted current. Trains of square waves were only slightly less effective

than continuous current of the same peak intensity (Elul & Massey, 1967); it is possible that the relative inefficiency of continuous current was due to the viscosity of the medium, which caused increased friction as the velocity of the cell increased. To avoid local heating, most experiments were conducted with pulsed direct current. The peak intensity did not exceed 10^{-5} A in any circumstances; more commonly, current of 0·5–5 μA was adequate to induce movement. For example, movement of erythrocytes was obtained with 1·6–2·7 μA. There were considerable differences in the responsiveness of cultures prepared at different times, but no clear correlation between cell movement and the age of the culture, nor of the donor animal. Significantly, current requirements for different cell

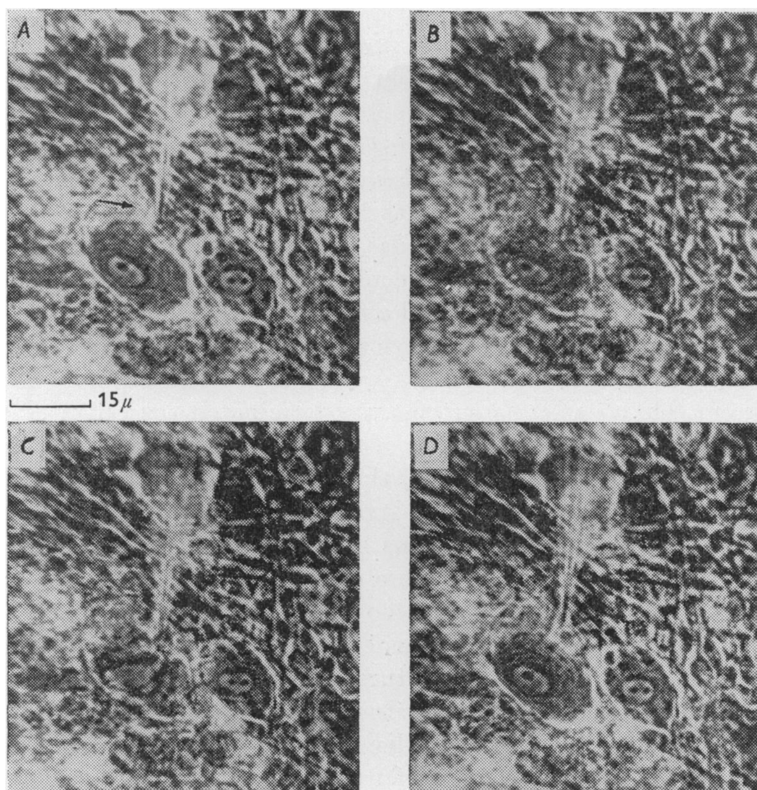

Fig. 1. Deformation and recovery of neurone. *A*—control, location of electrode tip indicated by arrow. *B*—current flowing out of micropipette, adjacent neurone is displaced and rotated towards the pipette. *C*—taken 30 sec after *B*, without interrupting the current. *D*—2 min following interruption of the current. Note the almost complete recovery of neurone. New-born rat, dorsal root ganglion, 2-week culture. Normal culture medium, pulsed current, 0·8 μA.

species were nearly identical, the maximal variation being less than one order of magnitude. To elicit movement of free cells, it was generally sufficient to place the micropipette at a distance of 10–50 μ; deformation appeared only at a separation of 5–10 μ. With smaller separation additional cytological changes were observed (Fig. 1 and Elul & Massey, 1967).

A comparison of current requirements for movement in focal electric field and for electrophoresis in a uniform field could be made with human erythrocytes. The electrophoretic mobility of red blood cells in saline and other solutions of similar ionic strength has been previously found as 1–2 μ/sec per V/cm (Furchgott & Ponder, 1941; Heard & Seaman, 1960). With focal electric field, three sets of measurements for erythrocytes (obtained with the same electrode, whose tip of which was broken down each time to minimize the likelihood of a tip potential) averaged 2 μA, and the velocity was estimated at 0·5 μ/sec. Employing the modified Poisson equation given in the Methods with $r = 5\ \mu$, and taking for the saline-phosphate medium $\lambda = 0{\cdot}017$ mho/cm (Furchgott & Ponder, 1941, p. 448), the field intensity is found 15–30 times lower than that used in electrophoresis. Such a discrepancy cannot be attributed only to non-uniform distribution of the focal electric field (see Methods).

One possible explanation may be that the diminished efficiency of uniform field is due to increased viscous drag. The field intensity employed in practical electrophoresis is around 1 mV/μ, so that actually cell migration is 20–50 times faster than in the present experiments. However, this interpretation requires the drag to be proportionate to the second power of the velocity (i.e. Newtonian flow), whereas calculation of the Reynolds number, for 10 μ sphere moving through water at 20 μ/sec, yields a figure far below 0·5. Hence, the hydrodynamic equation of Stokes, which involves only the first power of the velocity, is more appropriate. Although elevated viscosity at the boundary layer, and particularly surface roughness (cf. Brinton & Lauffer, 1959, p. 435) might increase the Reynolds number, the contribution of this effect is not clear. A more likely explanation (suggested by Professor K. J. Mysels, of the Chemistry Department, University of Southern California), may be that in the present experiments, and especially when pulsed current is employed, cells will move in 'jumps' without ever attaining terminal velocity, and the drag therefore always remains below that at uniform velocity.

Is movement in a focal electric field active or passive?

There have already been some reports of cell movement in tissue culture; spontaneous movement (Pomerat, 1951), as well as movement with focal electric stimulation (Chang & Hild, 1959) have been previously described, but only for glial cells, and the time course was in the order of minutes. In the present experiments, on the other hand, movement was recorded with cells of various types, and became apparent within a second or two. Nevertheless, it was desirable to establish whether this reaction to focal stimulation involved active participation of the cell.

As a rule, movement persisted in deteriorated cultures; there was no

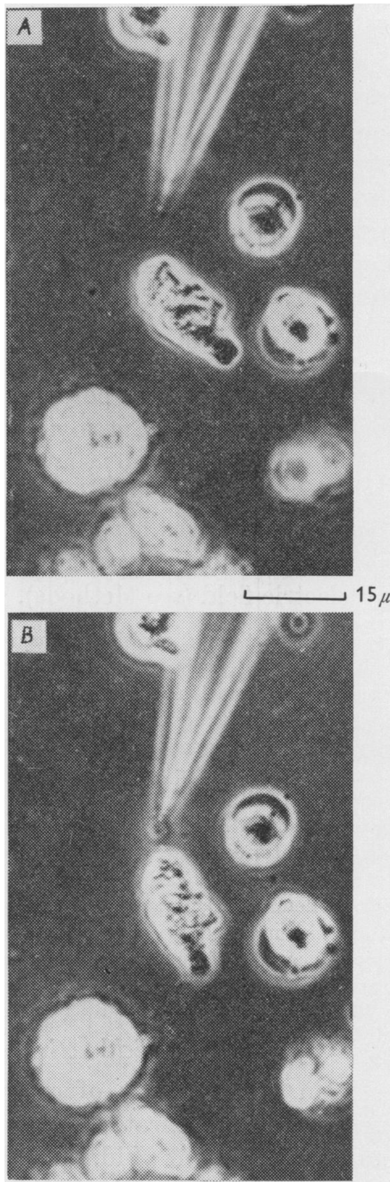

Fig. 2. Displacement of a 'dead' malignant cell. The control photograph *A* shows a cell with obvious signs of irreversible degeneration. In *B* the electrode tip is positive with respect to the medium. 'CMP' malignant cell line, Ringer solution, pulsed current 0·8 μA.

significant difference between the reaction of cells showing irreversible cytoplasmic changes and normal ones. Figure 2 illustrates the effect of focal field on a 'dead' malignant cell. Deteriorated cells were often found in tumour cell cultures, and their polar affinity was identical to that of apparently healthy cells in the same culture.

The influence of metabolic poisons on viable cells was also tested. After the effect of the field was induced, and while maintaining the relationship of the pipette to the cell under study, potassium cyanide solution was added to achieve a final concentration in the medium of about 1 %. No effect on movement and membrane deformation could be detected, so that these phenomena do not appear to depend on the integrity of cellular metabolism. However, it might be argued that certain cell activities—e.g. spike firing in nerve fibres (Hodgkin, 1964)—are impervious to metabolic poisons. Additional experiments were performed in order to reach an unequivocal conclusion.

Cell integrity and movement in focal electric field

The question of dependence of the effect of the electric field on cell integrity was raised by the preceding observations. Using micro-dissection, it was possible to rupture the cell, and isolate some membrane fragments, which were then incubated in the medium at 37° C for 10–20 min to remove adherent cytoplasm. Upon application of a focal electric field in the vicinity of such fragments, movement with the same polar affinity as the intact cell was always observed (Fig. 3). Similar observations on membrane fragments have been made previously in electrophoretic studies; there, too, the specific polar affinity was not modified by fragmentation of the cell (Ponder & Ponder, 1960).

The 'cell membrane' mentioned here is visible in light microscopy; hence it must represent, in addition to the 100 Å plasma membrane of electron microscopy, adhering layers of macromolecules on one or both sides. Although the electric charge may reside in the adhering cytoplasm, observations on liquified cells suggest this factor may not be of great importance. In these cells, present in most cultures, the cytoplasm had undergone degeneration at some earlier time, turning into aqueous fluid bounded by the membrane. Nevertheless, membrane fragments from these cells showed movement similar to that of normal cells (Elul, 1965).

Artifacts associated with a focal electric field

Electro-osmosis. Passage of current through a glass tube filled with ionic solution induces electro-osmotic flow in the solution, which may well lead to displacement of solid particles about the orifice. The observations reported above are, however, difficult to reconcile with an electro-osmotic mechanism: (i) While using current that was effective in attracting the majority of the culture population, cells moving in the opposite

direction have also been occasionally encountered (p. 355). Interpretation by electro-osmosis leaves these observations unexplained. (ii) Glass surfaces assume a negative electric charge in solutions of alkaline metal salts (Bikerman, 1958); in order to preserve electroneutrality of the entire system, the charge acquired by solution inside the pipette must be positive (cf. e.g. Rutgers, 1940). Positive potential applied to one end of the pipette would therefore cause the solution to flow out of the other end.

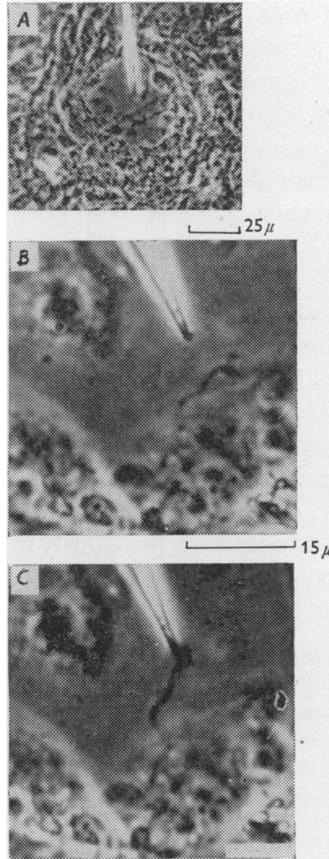

Fig. 3. Attraction of membrane fragments to a positive focal electrode. The general experimental situation is indicated in *A*, taken at lower magnification. With the aid of an additional micro-tool, an artificial cell-free space is created in the culture. One of the neurones lying on the border of this space is dissected, the fragments of membrane are then pulled into the central space and left there for 20 min at 37° C. *B* and *C* illustrate movement of membrane fragments when current is passed out of the pipette. *B*—control, *C*—taken immediately following termination of current flow. Culture derived from dorsal root ganglion of 3-day rat. Normal culture medium. Pulsed current, 1·5 μA.

Accordingly, electro-osmosis should repel solid particles from the tip of a positive micropipette, whereas the movement obtained in the great majority of the experiments was directed towards the pipette.

Considering that the charge depends on nature and concentration of the solution, it may be questioned whether in the present situation the glass was indeed charged negatively. Experimental data are available for 0·05 M potassium chloride; with this solution the surface potential of glass is about −50 mV (Rutgers, 1940; Rutgers & De Smet, 1945). Experiments were therefore undertaken with micro-pipettes containing 0·05 M-KCl solution; this solution was also used as medium in order to eliminate diffusion gradients. In these circumstances, cells were attracted to a positive focal electrode, and movement must therefore have been against the electro-osmotic flow. As the surface potential is not reversed by increased concentration of alkaline metal salts (McBain, Peaker & King, 1929) and the potential due to Na^+ is close to that generated by K^+ (Bikerman, 1958), it is rather unlikely that the concentrated solutions used in most experiments significantly interfered with the results.

'*Electrophoretic effect.*' As anionic and cationic transport numbers often are quite different, and ionic volumes also differ, current passage may cause net flow of solution (Robinson & Stokes, 1959, p. 133). For a filling solution identical with the medium, the volume moved is given as product of the transport number and volume of hydrated ion. Employing the hydrated radii calculated by Gorin (Abramson *et al.* 1942, p. 125) and the transport numbers of Harned & Owen (1958, p. 699), the ratio of cationic to anionic mole fraction with 0·15 M-NaCl was found as 1·46, leading to net fluid motion in the direction of current passage. With KCl the ratio is 1·04, and solution movement would be negligible, whereas with K-acetate the ratio is 0·65, giving rise to fluid flow opposite to the electric current. The effects of 1·5 M-KCl, NaCl and K-acetate solutions on cell movement were therefore explored, but the composition of solution did not have any significant effect; in all solutions, erythrocytes were attracted to a positive focal electrode and repelled from a negative one, indicating that the movement was not caused by an electrophoretic effect.

DISCUSSION

The difficulty of explaining the present observations as an artifact of electro-osmosis became apparent in the preceding section; interpretation in terms of effects specific to the focal electric field can also be demonstrated to be inadequate (see Appendix). Rejecting these explanations, the only feasible mechanism appears to be that involving the presence in the cell of net electric charge. Cell movement must then be governed by Coulomb's law, the attraction to a positive focal electrode, and repulsion from a negative one implying that the charge carried by the cell is predominantly negative.

This conclusion is in agreement with previous investigations of the electrophoretic behaviour of cells (Abramson, 1934; Abramson *et al.* 1942; James, 1957; Brinton & Lauffer, 1959; Haydon, 1964). However, as pointed out in the Introduction, there is an inherent difficulty in defining the site of charge in the cell from electrophoresis. This difficulty can be resolved with the observations described in the present paper. Movement in focal electric field was displayed not only by cells with comparatively

low membrane resistance, but also by neurones, in which the current flowing through the cytoplasm is negligible. The observations on movement of isolated membrane fragments, in particular, indicate that the fixed charges must be located on the cell surface.

Implications for membrane function

There were no significant differences in the experiments reported here in the reaction of different cell species to focal electric field. This finding implies that the charge density in the membrane of cells of different types is nearly the same; it also allows utilizing data available on erythrocytes to estimate the charge density. Electrophoresis at normal pH of red blood cells indicates that these cells carry free charge of approximately $-2{\cdot}5\times10^3$ e.s.u./cm^2 (Abramson *et al.* 1942, p. 315; Thompson & McLees, 1961). It is evident from the present results that other cells species carry similar charge and, assuming uniform distribution, the charge density would amount to one elementary negative charge for every 2000 Å^2 membrane area.

Since the charge density on neurones and muscle fibres is similar to that on excitable cells, it appears rather unlikely that fixed charge, *per se*, may directly be related to the specialized function of excitable cells. Nevertheless, there are some physiological implications of the presence of excess negative charge in the membrane. In analogy to the mechanism already discussed with relation to electro-osmosis, electroneutrality dictates an elevated concentration of cations in the solution adjacent to the sites of fixed negative charge in the membrane. In considering the physiological significance of this effect, the possibility cannot be neglected that the increase in cation concentration relative to the concentration in the bulk of the solution may affect ion activities and diffusion rates across the cell membrane.

APPENDIX

Force on dielectric. It is well know that when a body of a given dielectric constant is placed in a medium of differing dielectric constant, an imposed electric field will give rise to mechanical force. Since the dielectric constants of the cell membrane and the extracellular fluid are quite different, this phenomenon may be of some significance. A figure often mentioned for the membrane constant is 3 (Cole, 1947). The dielectric constant of physiological ionic solution may be shown, using the empirical relationship defined by Hasted, Ritson & Collie (1948) to be quite close to that of pure water (78). There is thus a significant difference in dielectric constant between the cell membrane and the medium.

The work (W) done by electric field on a body with dielectric constant ϵ_2 in a medium with dielectric constant ϵ_1 is given by Smythe (1950):

$$W = \tfrac{1}{2}\int_v \epsilon_0(\epsilon_1-\epsilon_2)\,\mathbf{E}_1.\mathbf{E}_2\mathrm{d}v;$$

where ϵ_0 is the permittivity of free space, and $\mathbf{E}_1$ and $\mathbf{E}_2$ denote the electric field vector before introduction of the body, and inside the body, respectively (integration is performed over the entire volume of the body v). From Gauss's theorem

$$\epsilon_1\mathbf{n}_1.\mathbf{E}_1 = \epsilon_2\mathbf{n}_2.\mathbf{E}_2,$$

where $\mathbf{n}_1$, $\mathbf{n}_2$, are unit directional vectors of the imaginary surfaces of integration outside and inside the body. At the solid–liquid boundary $\mathbf{n}_1 = \mathbf{n}_2$, so that

$$\mathbf{E}_2 = (\epsilon_1/\epsilon_2)\,\mathbf{E}_1.$$

Since in the present experiments the imposed field $\mathbf{E}_1$ is a monotonous function in the half-space limited by a plane normal to the long axis of the pipette, it will suffice to consider the work done on a single volume element Δv;

$$\Delta W = \tfrac{1}{2}\epsilon_0(\epsilon_1-\epsilon_2)\frac{\epsilon_1}{\epsilon_2}\,\mathbf{E}_1^2\Delta v.$$

In some circumstances the force exerted by the field may be quite significant. Consider, for example, a sphere of radius $a = 1\,\mu$, with dielectric constant close to that of the membrane (i.e. 3), suspended in saline (specific conductivity $\kappa = 5$ mho/m, dielectric constant 72·8). The force F is the gradient of ΔW; for a point source, the field has only a radial component, found from $|\mathbf{E}| = I/4\pi\kappa r^2$, and hence the force is given as the complete differential

$$|\mathbf{F}| = \frac{\mathrm{d}}{\mathrm{d}r}\Delta W = \tfrac{1}{2}\epsilon_0(\epsilon_1-\epsilon_2)\frac{\epsilon_1}{\epsilon_2}\frac{\mathrm{d}}{\mathrm{d}r}(\mathbf{E}_1^2\Delta v).$$

For small particles this simplifies to

$$F = 2\epsilon_0(\epsilon_1-\epsilon_2)\frac{\epsilon_1}{\epsilon_2}\,v\,\frac{|\mathbf{E}_1|^2}{r}.$$

Under conditions of the present experiments, typically $I = 10^{-6}$ A. Taking the distance from the source $r = 5\,\mu$, and employing the rationalized MKS unit system ($\epsilon_0 = 10^{-9}/36\pi$), the force $F \approx 5\times10^{-15}$ kg.m/sec^2. Since a particle of these dimensions will attain terminal velocity within one radius length (R. Elul, unpublished), the hydrodynamic equation of Stokes ($F = 6\pi\eta aV$) may be employed to find the velocity V. Putting the viscosity of water $\eta = 4\times10^{-6}$ kg.sec/m^2, the velocity of movement of the particle is found approximately 64 μ/sec. This figure is exaggerated

for several reasons, the most important of which is the reduced conductivity commonly associated with low dielectric constants, which will markedly attenuate the radial component of the field in vicinity of the particle (Elul, 1966*b*) and inside it (R. Elul, unpublished). The calculation does demonstrate, however, that for certain specimens, such as myelin particles, this property of focal electric field need be taken into account.

The product $\mathbf{E}_1^2 = \mathbf{E}_1 . \mathbf{E}_1$ is a scalar. Hence the work is independent of the sense of the imposed electric field. For steady current, $\mathbf{E}_1^2$ is therefore always positive, and as $\epsilon_2 < \epsilon_1$, ΔW should also be positive. The cells should thus migrate to the locus of minimal field intensity, and from the present line of reasoning they may be expected to be displaced away from the focal electrode regardless of the current polarity. However, the present experiments were characterized by cellular movement towards, as well as away from the electrode; hence it is clearly not possible to explain the results in terms of dielectric differences.

I am deeply indebted to the late Dr C. M. Pomerat for extending his hospitality and for instruction in tissue culture methodology. I am also grateful to Dr W. R. Adey for constant encouragement and for many stimulating discussions in the course of this work. Thanks are due to Mr J. F. Massey for participation in the early experiments and to Mr J. Booher and Mr C. G. Lefeber for preparation of tissue cultures and for assistance in photography.

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