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7. Green light was produced by electroluminescent Sylvania panelescent night lights (one per box, approximately 0.1 lux). White light (approximately 500 lux) was produced by Ken Rad 4-watt fluorescent bulbs (cool white F4T5/CW).
8. Students *t*-tests were performed and yielded the following P values: A and D, $P < .001$; B and D, $P < .001$; C and D, $P < .005$. The small differences between A and B and between A and C may reflect either chance variation or a very slight effect of the dim green light. Final body weights were very similar in all groups and showed no correlation with testis weights.
9. With the onset of activity taken as hour 0, the birds in group B received the white light beginning at hour 1.5 ± 46 minutes (mean and standard deviation of the 11 birds), whereas those in group D received the white light beginning at hour 11.6 ± 46 minutes (mean and standard deviation of the 22 birds).
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Electrical Recordings from Meningioma Cells during Cytolytic Action of Antibody and Complement

Abstract. *Resting membrane potential and total cell resistance of human meningioma cells in tissue culture have been measured with fine microelectrodes. Addition of either antiserum inactivated with heat or control serum from normal rabbits produced small depolarizations (2 to 4 millivolts) with no discernible (< 5 percent) change in resistance. Addition of antiserums with complement, however, produced larger depolarizations and decreases in resistance before any changes in cell morphology were visible with light microscopy; as cytoplasmic swelling progressed, membrane potential dropped close to zero, and resistance decreased five- to tenfold. The electrical recording technique may be useful in the study of sublethal as well as lethal damage to immune cells and, in particular, may permit temporal resolution of damaging events and repair mechanisms in a single cell.*

A method which could assess the initiation and time course of cell damage and subsequent repair mechanisms in individual isolated living cells would be an important addition to present methods of evaluating cell membrane pathology and might be of particular application to a number of immunological problems involving sublethal as well as lethal types of cell membrane damage. We have used the intracellular microelectrode recording technique to study the cytolytic action of antibody and complement.

Human meningioma cells were directly explanted after surgical removal and were then maintained in tissue culture through six subcultures for use as antigen. The cells were cultivated in monolayer on a glass surface in a fluid consisting of 80 percent F_{10} medium (a modified Eagle's medium) (*1*) and 20 percent fetal calf serum, and containing penicillin (100 mg/ml) and streptomycin (100 μg/ml).

After treatment with trypsin and centrifugation in the cold, ghost cells were prepared by Haughton's technique (*2*) and stored in Earle's balanced salt solution at −70°C until ready for use as antigen. We prepared rabbit antibody against these cell membranes by injecting subcutaneously in the left mid-dorsal area 60,000 meningioma ghost cells in 0.7 ml of balanced salt solution with 0.3 ml of complete Freund's adjuvant. A second injection was made in 10 days, and serum was collected at 20 days.

Preliminary reactions were observed on cultured cells of the same line on cover slips (50 by 10.5 mm) in Leighton tubes (small deep-well chambers for holding the coverslip and media). The antiserum to meningioma cells produced a cytolytic reaction in meningioma cells, characterized by swelling and bleb formation. For purposes of comparison human glioblastoma multiforme cells (grade IV astrocytoma) were tested with antiserum to meningioma under identical conditions. No morphological changes were noted even after 24 hours, and growth continued unchanged. As controls, serums were also collected and pooled from rabbits before inoculation. Portions of antiserum to meningioma were inactivated with heat at 56°C for 30 minutes. Control serums, those inactivated with heat and unheated serums, were stored at −70°C. Experiments were performed in a room kept at 30°C.

Cells that appeared viable and which had distinct cytoplasmic boundaries were selected for microelectrode study 3 days after subculture. Selected cover slips were transferred to a small chamber on a microscope substage and immersed in 1 to 2 ml of fresh F_{10} media. Isolated (nonsyncytial) cells were viewed through a microscope (× 320) and impaled with micromanipulator-directed fine glass micropipettes filled with 3*M* KCl [direct-coupled (DC) resistances 15 to 30 megohm] (Fig. 1). Cells which adhered well to glass were easily impaled. Electrical activity was led to a PICO-metric amplifier (Instrumentation Laboratories, Inc.) by a short chlorided silver wire and then to a DC input of a Tektronix-502 oscilloscope, a similar wire immersed in the bath being used as reference. A Wheatstone bridge permitted simultaneous stimulation through and recording from the electrode. Rectangular constant current pulses, 30 to 100 msec in duration, were delivered through a 10^9-ohm resistor in series with the electrode, and current intensity was calculated as the voltage drop across the resistor. The microelectrode techniques have been described fully (*3*), and our method for measurements of membrane properties of Betz cells has been further described elsewhere (*4*).

In all, 28 meningioma cells were satisfactorily impaled; in ten cases membrane properties alone were studied; in 18 cases studies were made before and then after addition of control, heat-inactivated, or unheated serum. Resting membrane potentials ranged from −10 to −20 mv and were remarkably stable (< 1 mv change) during the control period of 5 to 10 minutes. Total cell or "input" resistances ranged from 10 to 40 megohm, the higher values being obtained in the smaller cells. Time constants [times for voltage to reach $(1 - e^{-1})$ or 63.2 percent of the final values] ranged from 5 to 30 msec; the six largest values (20 to 30 msec) were obtained in rounded meningioma cells with large nuclei. The steady-state changes of the voltage with applied current were linear over a range of ± 40 mv from the resting value (Fig. 2, A and B).

Addition of one to two drops of inactivated antiserum caused a 2- to 4-

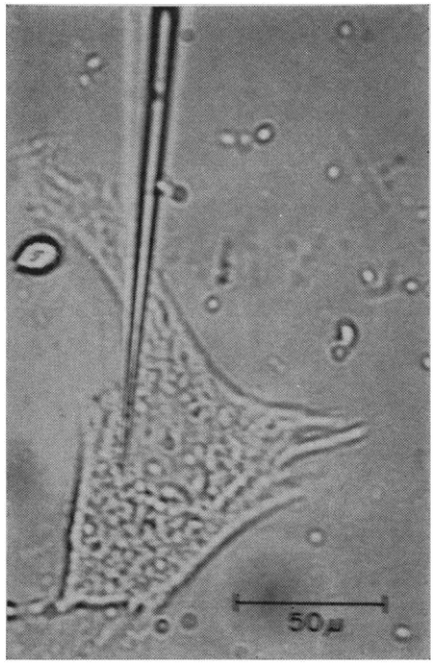

Fig. 1. Unstained meningioma cell in tissue culture, impaled by fine microelectrode. Focus adjusted to permit observation of both cell outlines and electrode. Terminal portion of electrode tip dips into the cell interior and is not seen in this focal plane.

mv depolarization occurring within 5 to 20 seconds, but there was no measurable (< 5 percent) change in resistance. Similar levels of depolarization without change in resistance resulted from addition of one to two drops of control serum. We do not yet know whether the small effect is caused by antiserum to meningioma or heterophile antibody or by some other factor in rabbit serum.

After addition of one to two drops of antiserum, an early 2- to 4-mv depolarization also occurred with no other change for 2 to 3 minutes, after which time the resistance began to decrease. The slight decrease in membrane resting potential (Fig. 2C, trace c) compared to the control trace (Fig. 2C, trace d) is typical of the small depolarizations that occurred after addition of control serum, heat-inactivated serum, or in the early period after addition of unheated antiserum to meningioma. However, in the first two cases voltage response to applied current would remain unchanged, whereas in Fig. 2C, trace c the first decrease in the voltage response to the applied current pulse about 3 minutes after addition of serum with antibody and complement is also seen. The subsequent decreases in resistance are plotted in Fig. 2D, and a trace taken about 5 minutes after the addition of antibody and complement demonstrating the marked decreases in both membrane potential and resistance is shown in Fig. 2C, trace b.

The resistance had usually decreased by 15 to 20 percent before any microscopic signs of the reaction were present. The initial resistance decrease preceded the first microscopic signs of cell swelling or bleb formation by 10 to 30 seconds. Whereas resistance decreased progressively in all cells so studied, resting potentials fell in a less regular manner, in part depending upon the speed of the cytolytic reaction; sometimes transient returns toward resting level were seen in the early period of cell damage.

Changes of steady-state voltage with applied current were also linear when tested for brief intervals during the course of the cytolytic reaction. These data and the linear relationship between current and voltage found before addition of antibody and complement prove that the decreased membrane resistance during cytolysis is not a secondary passive consequence of membrane depolarization.

The early decrease in resistance is probably the electrical counterpart of the increased permeability to electrolytes and small molecules resulting from the cytolytic action of antibody and complement. Such a change has been demonstrated in chemical studies on Krebs ascites tumor cells by Green and co-workers (*5*), who also observed surface membrane alterations in 20 percent of the cells after addition of antibody alone and structural damage of cytoplasmic matrix, mitochondria, and endoplasmic reticulum in virtually all cells treated with antibody and complement. In further studies to explain the increased permeability, Green, Barrow, and Goldberg (*6*) postulated functional "holes" in the membranes of cytolyzed cells. These "holes" would permit the cellular escape of electrolytes, amino acids, and ribonucleotides even when antibody and complement were added to cells in a medium with sufficient albumin to balance the colloid osmotic pressure of the cells and prevent cell swelling. Later, negative staining defects (88 Å average diameter) in electron micrographs of red

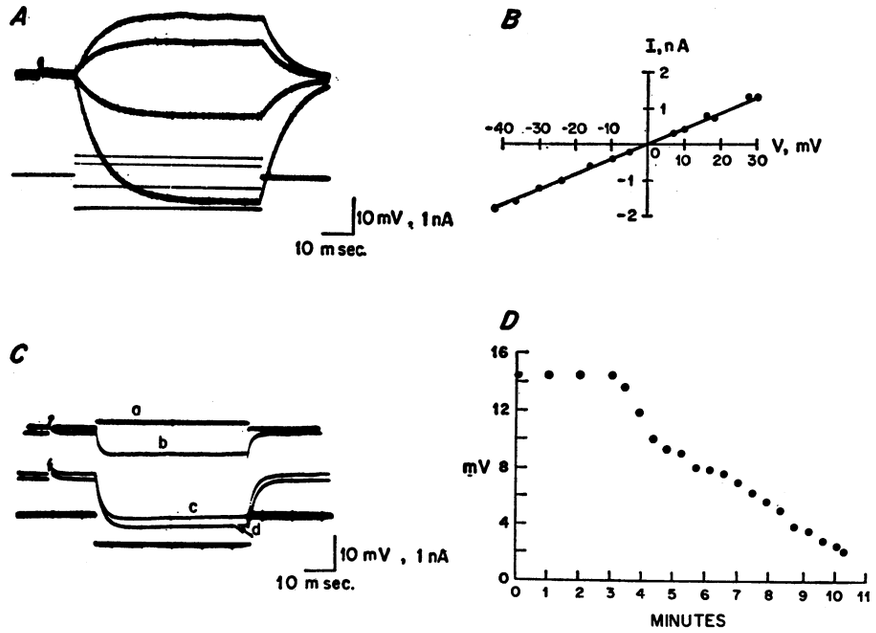

Fig. 2. (A) Upper set of traces represents voltage responses to current steps (lower traces). Positive polarity here and in (C) represented by upgoing deflection. (B) Linear plot of current-voltage relationship. (C) (Trace d) Response to constant current pulse before addition of serum; (trace c) first trace to show decrease in resistance (as decrease in voltage for a constant current pulse) about 3 minutes after addition of serum with antibody and complement; (trace b) resting potential now close to zero and resistance about one-third initial level about 5 minutes after trace d; (trace a) control extracellular trace after electrode withdrawn from cell at about 8 minutes after trace c. All traces were recorded by DC. (D) Plot of voltage drop for constant current pulse for same cell shown in (C). Time zero represents last trace before addition of antiserum.

cells after immune hemolysis with guinea pig complement were demonstrated, and it was noted that similar defects were seen in cytolyzed tumor cells (*7*). See also a recent review of steps in immune hemolysis (*8*).

We have estimated that an 88-Å defect, if completely through an 80-Å unit membrane, would have a resistance of 100 megohm in parallel with the remaining cell resistance, with the resistivity of intracellular fluid taken as 75 ohm cm. Hence, one "hole" could produce a 9 percent decrease in resistance in a cell with a 10-megohm input resistance and a 17 percent decrease in a 20-megohm cell. Such initial decreases were seen (frames taken every 1.3 seconds), but the quantity of antibody used and the present uncertainty of the rate at which a "hole" develops prevent resolution of whether the earliest observable changes represent full development of one hole or gradual development of many.

We believe that the electrode recording technique may be of increasing value in the study of sublethal or lethal cell damage, providing an opportunity for temporal resolution of damaging events and possible repair mechanisms in single cells.

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Prostaglandins: Localization in Subcellular Particles of Rat Cerebral Cortex

Abstract. *Homogenates of rat cerebral cortex contain material corresponding to prostaglandins E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$ which are concentrated mainly in the light microsomal and mitochondrial fractions. Only the former fraction exhibits significant ability to synthesize prostaglandins E_1 and $F_{1\alpha}$ from bis-homo-γ-linolenic acid. After subfractionation of the crude mitochondrial fraction, prostaglandin E and F material is found mainly in the cholinergic and noncholinergic nerve endings. We conclude that the nerve endings are a storage site, whereas the light microsomes are the site of synthesis.*

Prostaglandins are hydroxycarboxylic acids, derived by cyclization of ω6 polyunsaturated C_{20} fatty acids; they form a unique class of potent, pharmacologically active compounds (*1*). A mixture of prostaglandins E_2 and $F_{2\alpha}$ (PGE_2 and $PGF_{2\alpha}$) is released from the superfused somatosensory cortex of cats, and increased release is detected following direct, as well as transcallosal and contralateral stimulation of peripheral nerves (*2*). An evoked release of $PGF_{1\alpha}$ from the frog spinal cord is also observed after electrical stimulation of the hind limbs (*3*). In addition, an efflux of material similar to prostaglandins from the cat cerebellum and ventricles has been described, and $PGF_{2\alpha}$ and other prostaglandins have been identified (*4*) in bovine, feline, and fowl brains. The conditions for release of prostaglandins are similar to those governing the release of the transmitter acetylcholine from the cerebral cortex after stimulation of presynaptic nerves (*5*), and thus these substances may be regarded as potential neurohumoral transmitters (*6*). A second criterion for a neurohumor is that application to the postsynaptic membrane should mimic the effects of stimulation of presynaptic nerves. In fact, iontophoretic application of prostaglandins modifies the firing of neurones in the brain stem (*7*). In addition, PGE_1 in chicks has a potent action similar to that of strychnine (*8*).

The successful application, by Whittaker (*9*) and De Robertis (*10*), of the subcellular fractionation technique to brain tissue has made possible the direct testing of a third criterion, namely, that the putative transmitter should be present in the nerve endings. Therefore, prostaglandins were sought in freeze-dried fractions of nerve endings from rat cerebral cortex sent from Argentina by Dr. E. De Robertis. Material similar to prostaglandins was located in the fraction containing light microsomes (*6*). We now report a more detailed study of the prostaglandins contained in fresh subcellular fractions of rat cortices.

Eight to fifteen male rates (Sprague-Dawley, 200 g) were decapitated in each experiment. After removal of the brains, the cortices were homogenized with sucrose (0.32*M*) to a final concentration of 10 percent before differential centrifugation by the procedure of Kataoka and De Robertis (*11*). The *Mic*-20 fraction, which contains the small nerve endings (fraction B) contained little prostaglandin-like material, and therefore only the crude mitochondrial fraction (*Mit*) was further fractionated, either on a sucrose density gradient or by osmotic shock followed by differential centrifugation (*11*). These operations were carried out at + 4°C.

The prostaglandins were extracted from the fractions with ethanol (95 percent) overnight. The supernatant was evaporated, and the residue was dissolved in buffered saline, which was washed three times with an equal volume of light petroleum (boiling point, 30° to 60°C), at *p*H 7.0 and then at *p*H 3.0 to remove other lipids, such as phospholipids, glycerides, cholesterol, esters, and free fatty acids. The prostaglandins were finally extracted into diethyl ether at *p*H 3.0. Recovery of labeled PGE_1 was greater than 90 percent. The prostaglandin E series was separated from the prostaglandin F compounds by thin-layer chromatography on silica gel G in the unequilibrated AI solvent system (benzene, dioxane, acetic acid, 20:20:1 by volume) of Gréen and Samuelsson (*12*), with authentic prostaglandins used as reference compounds. The method used for elution of the silica-gel plates with localization of the prostaglandins by bioassay has been described previously (*2*).

A yield equivalent to 231 ± 25 and 218 ± 36 mμg (eight experiments) of PGE_1 and $PGF_{1\alpha}$, respectively, per gram of active material was localized in