

The Effect of 2-4 Dinitrophenol on Cell to Cell Communication in the Frog Lens

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In the frog lens, the magnitude of the electrical impedance of the tissue at 796 Hz has been shown to be primarily a measure of cell to cell coupling. Using this measure, we demonstrate that the incorporation of 500 μm 2-4 dinitrophenol (DNP) into the Ringer solution bathing the lens at pH 7.4 causes uncoupling of fiber cells within 300 mm of the lens surface. This uncoupling is not total and largely reverses if the DNP Ringer is replaced by normal Ringer within 2 hr. The mechanism of action of DNP is unclear but it does not depend on Ca^{2+} influx from the bath. The results demonstrate that reversible uncoupling of lens fiber cells can occur, a point which has been uncertain in the past.

Key words: crystalline lens; 2-4 dinitrophenol; cellular coupling; calcium-free solutions; gap junctions.

1. Introduction

The crystalline lens of the eye is known to contain myriad gap junctions between its fiber cells (Cohen, 1965; Rafferty and Esson, 1974; Kuwabara, 1975; Benedetti et al., 1976; Goodenough, 1979; Kuszak, Alcalá and Maisel, 1981). Although the role of these junctions is not known with certainty, considerable evidence indicates that fiber cells in the lens are electrically coupled (Duncan, 1973; Eisenberg and Rae, 1976; Mathias, Rae and Eisenberg, 1979). In addition, Procion dyes injected into single lens fibers readily diffuse into adjacent fibers (Rae, 1974; Rae and Stacey, 1976) while cell metabolites of low molecular weight also appear to diffuse readily from one cell to another (Goodenough, Dick and Lyons, 1980). Since these types of solute movement from cell to cell flow through gap junctions in other tissues, it is reasonable to assume that lens gap junctions provide the 'pathway' for the diffusion of low molecular weight substances among lens fibers.

Evidence suggests, however, that most lens gap junctions differ from gap junctions in other tissues. The amino acid sequence of the main polypeptide of the lens gap junction is significantly different from that found in liver cells (Nicholson, Hunkapiller, Hood, Revel and Takemoto, 1980; Hertzberg, Anderson, Friedlander and Gilula, 1982). Furthermore, the particles of the lens gap junction do not assume a crystalline array upon fixation with glutaraldehyde, whereas gap junctions from most other tissues do crystallize with fixation (Goodenough, 1979). According to Peracchia, this crystalline structure corresponds to the uncoupled or non-conductive functional state (Peracchia and Dulhunty, 1976; Peracchia, 1978), while the non-crystalline structure corresponds to the coupled or conductive state.

Goodenough has speculated that the lens fiber gap junction might differ intrinsically from other gap junctions, so that it is *permanently* open in a conducting state (Goodenough, 1979) with corresponding *non-crystalline* structure. This speculation

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has produced considerable controversy since a number of workers have found conditions in which lens gap junction particles crystallize, although direct functional evidence of uncoupling in these conditions is scanty. Peracchia and Peracchia (1980a, b) reported that the bovine lens gap junctional particles could be made to crystallize in vitro by adding Ca^{2+} or H^{+} to isolated junction fragments from bovine lenses. A similar result has been reported by Alcalá, Kuszak, Katar, Bradley and Maisel (1979) from chick lenses. Bernardini and Peracchia (1981) have also been able to show that lens gap junction particles assume a crystalline array following freezing and thawing, a procedure which is known to produce a significant increase in lens calcium (Duncan and Bushell, 1976). They also showed that fibers of a mechanically damaged lens can take up Procion yellow with little or no movement into adjacent fibers (Bernardini, Peracchia and Venosa, 1981), suggesting that fibers adjacent to the damaged fibers were uncoupled.

We have explored a variety of procedures which might be thought to uncouple lens fibers, using measures of the effective internal resistivity as assays of uncoupling. Bubbling with CO_2 , incubation in a solution with elevated calcium, hypertonicity, acidic bathing solutions, superfusion with glutaraldehyde and incubation in low calcium containing solutions all fail to produce consistent substantial uncoupling, using the criteria of a more than twofold increase in effective internal resistivity. These procedures are known to produce consistent substantial uncoupling in other preparations. Of course, negative findings in exploratory experiments do not rule out the possibility that small modifications in conditions, protocols, or procedures could have produced dramatic uncoupling.

Thus there seems to be considerable substance to both sides of this controversy: morphological experiments suggest that lens junction particles can assume the crystalline array thought to reflect a non-conducting state, but physiological experiments suggest that it is difficult to make lens junctions non-conducting. The controversy has been made more difficult by a recent report (Raviola, Goodenough and Raviola, 1980) which questions the validity of using crystallinity of junctions as an indication of their conductive state. In the final analysis, it seems likely that only electrical measurements and/or dye diffusion measurements can give unequivocal evidence for the patency of junctional channels.

We have recently been successful in uncoupling lens fibers from *Rana pipiens*. Uncoupling was accomplished in a simple and partially reversible way by superfusing the lens for 0.5–8 hr with a Ringer solution containing $500\ \mu\text{M}$ –2–4 DNP. This procedure has been used successfully in *Chironomus* salivary gland (Politoff, Sclar and Loewenstein, 1969; Rose and Loewenstein, 1976) and Purkinje fibers of the sheep heart (Dahl and Isenberg, 1980). The present paper describes experiments in which frog lens fibers were at least partially uncoupled with 2–4 DNP.

2. Materials and Methods

Lenses from *R. pipiens* were prepared by removing the eye and subsequently dissecting away the posterior globe, the cornea and the iris. Four equally separated slits were then made from the remaining posterior globe to about the pars plana. The four flaps so produced were used to pin the preparation into the bottom of a chamber lined with Sylgard. In each experiment the impedance of the lens was measured using the techniques of Mathias, Rae and Eisenberg, (1981). Briefly, a microelectrode for passing current was placed in the center of the lens through the posterior capsule and cortex. Another electrode was placed some $200\ \mu\text{M}$ into the posterior side of the lens to measure the lens voltage. Both electrodes were painted with

silver conductive paint to a distance from the tip which was about $100\ \mu\text{M}$ more than the anticipated depth of insertion into the lens. The electrodes were dipped into low melting point dental wax which was allowed to harden (Sachs and McGarrigle, 1980). The wax near the tip was then removed by dipping the tip into xylene under direct microscopic observation. The wax covering the silver paint was then covered with clear fingernail polish and allowed to air dry. This method of coating the electrodes minimized the electrical artifacts discussed in Mathias et al. (1981). The voltage microelectrodes were filled with 3 M-KCl and had resistances of 3–7 megohms whereas the current passing electrodes were filled with 3 M-K⁺ acetate and had resistances of 1–4 megohms. The Ringer solution used was that described in Mathias et al. (1979). Solutions containing 2–4 DNP were made by simply dissolving the appropriate amount of DNP in the standard Ringer and adjusting the pH to 7.4. The added DNP had a negligible effect on the solution osmolality and so no correction was made for it. Low calcium Ringer was produced by omitting the calcium from the standard Ringer and substituting 2 mM-disodium EDTA in its place.

Impedance measures cell coupling in syncytia

When current is passed from a microelectrode immersed in a saline bath to a large remotely located reference electrode, the current density is greatest right at the microelectrode tip since all of the current must pass through the small opening of the microelectrode. Consider the current electrode to be surrounded by an infinite number of concentric shells of saline (Eisenberg and Johnson, 1970, pp. 6–7). The surface area of the shells increases with the distance from the electrode tip. This means that the current density, the current flowing across a unit area of each shell, decreases with the distance away from the electrode tip. Since the resistivity of the saline is uniform the voltage generated by current flow through the saline decreases progressively as one gets farther and farther away from the current electrode. This radial variation in the induced voltage can be measured with a second microelectrode by comparing the potential at its tip to the potential of the remotely located reference electrode. If measurements are made at different separations of the current and voltage microelectrode tips, the electric field can be mapped. Since the measured voltage comes from current flow through the resistance of an electrolyte solution, the time course of the measured voltage response closely mimics the time course of the current, the primary distortion being due to the finite frequency response of the voltage measuring microelectrode and associated amplifiers. Therefore, a step of current produces a step of voltage; a high frequency sinusoidal current produces a high frequency sinusoidal voltage with little phase shift from the current sinusoid. This radially varying voltage decrement away from the current electrode is referred to as the *point source effect*. It is obvious that the magnitude of the voltage signal induced by the current flow will scale with the resistance of the solution, a larger voltage being generated when the solution resistance is higher.

If the current and voltage electrodes are now placed inside a single, spherical cell, the situation is somewhat more complex since now there is a surrounding membrane which must also be charged by the current flow. The cytoplasm of the cell behaves very nearly the same as the saline in the previous case and one expects a point source effect in the cell cytoplasm. Therefore a step of current will produce a complex voltage response. When the current is turned on, there will be a jump in the voltage due to the point source effect and then a slower change in voltage as the current charges the parallel combination of the membrane resistance and the membrane capacitance. This jump is related almost entirely to the cytoplasmic resistance and not to membrane properties (see Peskoff and Eisenberg, 1975; Peskoff and Ramirez, 1975; Kevorkian and Cole, 1981, pp. 424–33). If one applies the steps at a high enough frequency so that the capacitive reactance of the membrane becomes small in comparison to the membrane resistance, there will be no discernible voltage induced across the membrane by current flow. The response to these high frequency current steps will be a reasonably pure measure of the cytoplasmic resistance of the cell. A similar result will occur, of course, with high frequency sinusoidal currents. At high frequencies, the membrane capacitance shunts these currents so that no voltage is induced across the membrane. The in-phase sinusoidal voltages which are measured are due to voltage drops within the cell cytoplasm.

If the current and voltage microelectrodes are now placed within a spherical syncytium, the situation is still more complicated, a generalization of the two previous cases. The

resistance which is analogous to the solution resistance or cytoplasmic resistance of the previous cases is now composed of two resistances in series: the cytoplasmic resistance and the resistance due to the gap junctional channels. Current would flow from cell cytoplasm 1 to gap junction 1 to cell cytoplasm 2 to gap junction 2, etc. Therefore the point source effect should also occur in the spherical syncytium and should be scaled by the combined cytoplasmic and gap junctional resistances. Previous measurements have shown the lens fiber cytoplasmic resistivity of lens fibers to be less than 5% of the total effective resistivity (Pauly and Schwann, 1964; Rae and Germer, 1974; Mathias et al., 1981) and thus we expect the point source effect in a spherical syncytium to be dominated by the gap junctional properties. An increase in gap junction resistance, produced for example by closing some of the junctional channels would result in a larger point source effect. Therefore the size of the voltage jump in step current experiments or the amplitude of the high frequency sinusoidal voltage in sinusoidal current experiments would be expected to increase as coupling between the cells decreased. Only when two adjacent cells had totally uncoupled would the induced voltage get smaller. Thus, it is clear that cell to cell coupling in a spherical syncytium can be measured either by the magnitude of the voltage jump or by the magnitude of the high frequency sinusoidal voltage response to current flow.

3. Results

2-4 DNP results in a significant increase in the high frequency magnitude of the lens impedance when the concentration of DNP in the bathing solution is at least 100 μM . Concentrations from 5 to 50 μM were tried, but they produced no unequivocal effect after 2 hr. A concentration of 500 μM was selected for the majority of experiments since this concentration produced repeatable results within a time course of a few hours.

Figure 1 shows a comparison of the impedance of a typical lens before and after superfusion with 500 μM -DNP-Ringer. It is clear that this solution affects more than the high frequency magnitude of the impedance. In fact, the magnitude and phase of the impedance are affected at essentially all frequencies. Such effects can occur only if membrane conductance properties are altered, and thus it is clear that 2-4 DNP affects much more than just cell to cell coupling. Although these 'membrane' effects of DNP are of some interest and have been described previously (Duncan and Croghan, 1970), they will not be dealt with here.

We are concerned that the large membrane conductance changes produced by 2-4 DNP might somehow produce an increase in the magnitude of the lens impedance at high frequencies via a mechanism not included in published theoretical treatments of lens electrical properties. Therefore, lens impedance was measured before and after the application of calcium-free solutions. Such solutions are known to produce large increases in the permeability of many preparations (Heilbrunn, 1952) including the lens (Thoft and Kinoshita, 1965; Delamere and Paterson, 1978). Figure 2 compares lens impedance in normal Ringer and in Ca^{2+} -free Ringer for a typical lens. It is clear that a low calcium solution has the expected severe effect on the low frequency impedance; in fact, low calcium solutions have a considerably greater effect on membrane conductance properties than does 2-4 DNP. Calcium free solutions, however, have *no* effect on the magnitude of the impedance at high frequencies. This result rules out the possibility that the action of DNP on membrane conductance produces an artifactual increase in the high frequency magnitude. The impedance recorded at high frequencies is quite independent of membrane properties, as predicted by theory. It should also be a good measure of cell to cell coupling, as predicted by the same theory.

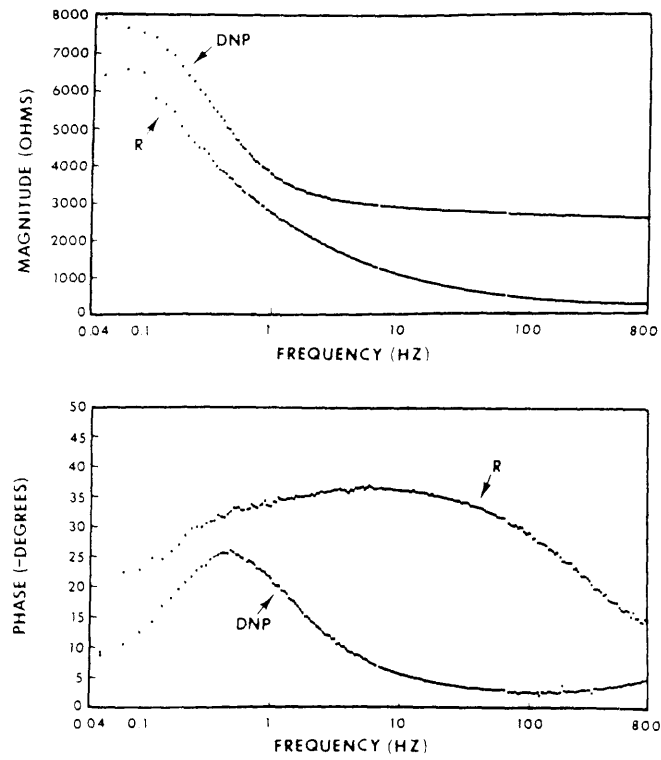


FIG. 1. Overlay of impedance plots determined from a single lens first in Ringer (R) and following a 6 hr incubation in 500 μ M-2,4-DNP-Ringer (DNP).

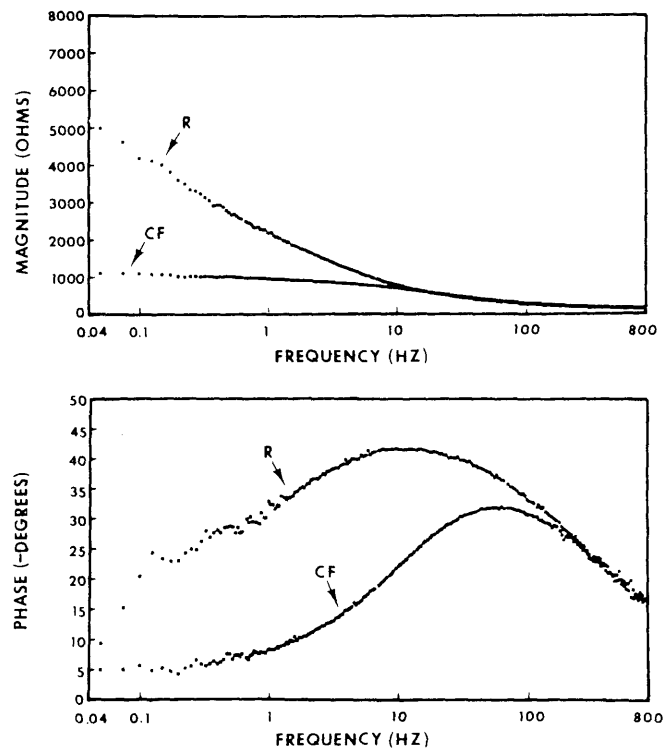


FIG. 2. Overlay of impedance plots determined from a single lens first in Ringer (R) and following a 1 hr incubation in calcium free 2 mM-EDTA Ringer (CF).

The time course of the uncoupling process can be followed by determining the magnitude of the impedance at high frequency at various times following the application of DNP. We measured the impedance at 796 Hz because it was a convenient frequency obtainable from our real-time Fourier analyzer. The estimates of impedance were averaged until further averaging produced no significant change in the magnitude at 796 Hz. With our signal to noise levels, 15–20 averages were sufficient. Figure 3 is a plot of the magnitude at 796 Hz vs. time following the superfusion of the lens with 500 μM -DNP-Ringer. It took about 30 min before any discernible change occurred; then there was a monotonic increase in the magnitude for the next 4 hr. The maximum effect was achieved after some 8 hr.

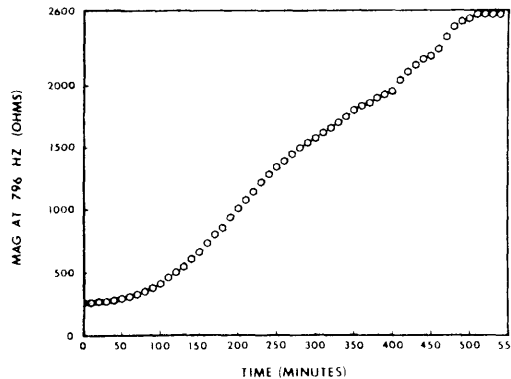


FIG. 3. Plot of the time course of cellular uncoupling for a typical lens.

The effect of DNP was largely reversed if the lens was superfused with normal Ringer within 2 hr of the original application of the DNP solution. Figure 4 compares the lens impedance after 2 hr in DNP with that measured after a 2 hr recovery in Ringer. Recovery of both the membrane conductance and the high frequency magnitude is evident. This result suggests that recoupling occurs during the recovery period.

Apparently the uncoupling of lens junctions does not occur throughout the entire lens. The spatial extent of the uncoupling was investigated by determining the magnitude of the impedance at 796 Hz at different depths within the lens. This was done by placing the voltage electrode 200 μm under the surface and measuring the impedance. The electrode was then advanced into the lens in 10 or 20 μm steps and the impedance measurement was repeated after each step. Figure 5(B), shows the result from a typical experiment started 7 hr after exposing the lens to 500 μM DNP-Ringer. The finding that the magnitude of the impedance is 1600 ohm at 200 μm and 796 Hz supports the assertion that a large degree of cell uncoupling has occurred; since before DNP treatment, the magnitude at 796 Hz was 240 ohms. There is very little change in the high frequency magnitude for an additional 100 μm of depth; beyond 300 μm there is a sharp increase. This is the result expected if the uncoupling is not total in the 'uncoupled' zone and if the uncoupling extends only to a 300 μm -depth. The gradual increase in the magnitude at further depths into the lens is that expected from the point source effect as the voltage electrode moves closer to the current electrode (Eisenberg and Rae, 1976; Mathias et al., 1981).

It is instructive to compare quantitatively the effects of DNP with the theory of Eisenberg, Barcion and Mathias (1979) which treats the internal medium of the lens as if it had a spatially uniform effective resistivity, arising from a combination of cytoplasmic and gap junction resistance. Figure 5(A) shows the magnitude of the

impedance at 796 Hz predicted by that theory for varying locations of the voltage electrode in the lens, with the current electrode in the center. The four curves shown are computed with different values of the resistivity ranging from that for Ringer (A) to the highest value we have found for surface fibers in DNP treated lenses (D). Clearly, the theory shown in Fig. 5(A) fails to describe the variation in magnitude to a depth of some 300 μm . The theoretical curves in 5(A) do fit the shape of the experimental curves at depths from 300 μm to the center of the preparation.

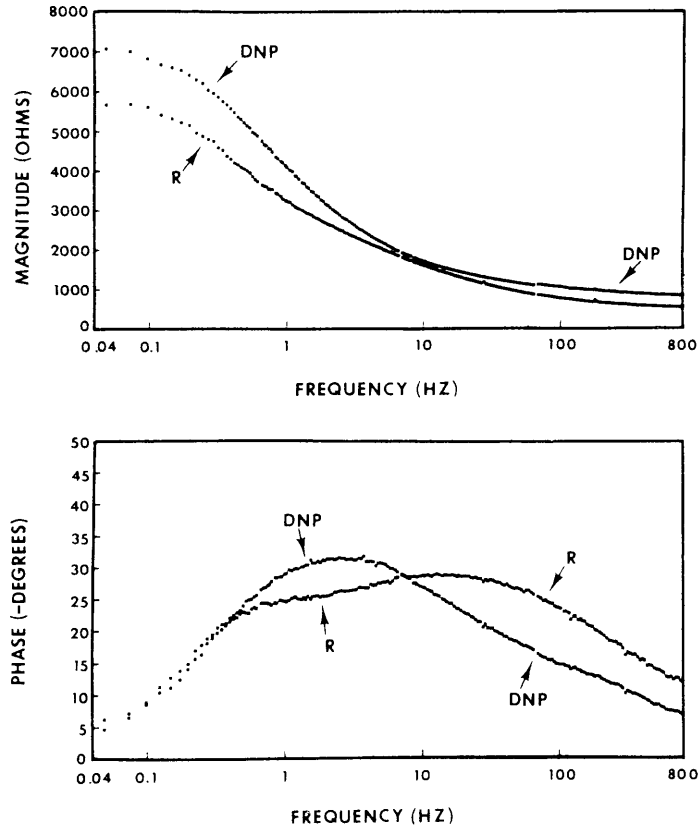


FIG. 4. Overlay of impedance plots determined from a single lens first after a 2 hr incubation in 500 μM -2-4 DNP-Ringer (DNP) and following a 2 hr recovery in Ringer solution (R).

The theory must be expected to fail, of course, if its underlying assumptions are inappropriate. For example, if DNP had its effect only in superficial fibers, the effective resistivity would be spatially non-uniform, and the theory of Eisenberg et al. (1979) would be inappropriate. Mathias et al. (1981) generalized the earlier theory to the simplest non-uniform case, in which the internal medium was supposed to have one effective resistivity in an outer shell and another effective resistivity in an inner cortex. A theoretical curve from that theory is shown as the solid line in Fig. 5(B). Specifically, the effective resistivity from the surface to a depth of 300 μm was taken to be 20 kohm . cm whereas the effective resistivity for the deeper cortex was taken as 3500 ohm . cm, the average value obtained from normal frog lenses.

The theoretical fit is reasonable, but not quantitative since it fails to match the curvature of the observed magnitude in either the superficial shell or cortex. In the cortex, the failure to fit would be expected *a priori*. Mathias et al. (1981) have shown that a non-uniform model is required to fit a normal lens; thus, the cortical region in DNP treated preparations requires a non-uniform model. Such a model would need

to include (at least) three regions of differing effective internal resistivity, one for the outer shell of fibers uncoupled by DNP, the other two for the (presumably) normal interior. In the absence of such a theory the misfit shown in the cortical region in Fig. 5(B) would be expected.

The misfit in the outer shell region illustrated in Fig. 5(B) probably has two causes. First, the action of DNP may not be uniform throughout the depth; if it is not, a theory which describes uncoupling in that region by a single effective internal resistivity would be expected to be in error. Second, the theories used to describe the

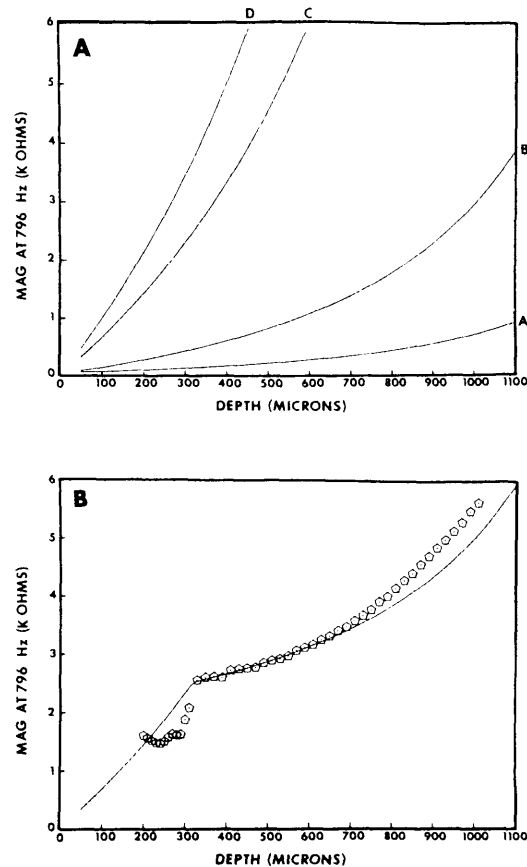


FIG. 5. Plots of the magnitude of the lens impedance at 796 Hz as a function of depth. (A) Plots of predictions from the uniform theory of Eisenberg et al., 1979 for four different internal resistivity values ($A = 80 \text{ ohm} \cdot \text{cm}$; $B = 3.5$; $C = 20$; $D = 30 \text{ Kohm} \cdot \text{cm}$). (B) Overplots data from a typical lens, incubated for 7 hr in $500 \mu\text{M}$ -2-4 DNP-Ringer and the prediction of the bidomain theory of Mathias et al (1981) where internal resistivity in the outer region is $20 \text{ Kohm} \cdot \text{cm}$ and in the inner region is $3.5 \text{ Kohm} \cdot \text{cm}$.

lens (Eisenberg et al., 1979; Mathias et al., 1981) as a spherical syncytium both use perturbation expansions that assume the effective internal resistivity is much smaller (say by a factor of 20 or greater) than the effective external resistivity. The uncoupling action of DNP may raise the effective internal resistivity enough to make this assumption invalid, requiring a new perturbation expansion of the field equations describing a spherical syncytium.

Despite the failure of the theory to fit the data in detail, the qualitative conclusion is quite clear: in an outer shell, the effective internal resistivity is raised considerably, as it would be if lens fibers were substantially but not totally uncoupled. We have also verified that the uncoupling in the outer shell was not total by intracellular injection of Procion yellow using the techniques of Rae and Stacy (1976). Observation

in the fluorescence microscope of $1\ \mu\text{m}$ thick sections (taken in the injected region of plastic embedded lenses) showed dye in several fibers. Since we have no way of dealing quantitatively with data on dye spread, we have not pursued this technique.

The uncoupling effect of DNP in the lens might not have been expected *a priori*. In other tissues where DNP is an uncoupling agent, intracellular Ca^{2+} stores associated with mitochondria or intracellular membrane systems are known to occur (Dahl and Isenberg, 1980) and DNP apparently produces Ca^{2+} release from these stores. There are, however, no mitochondria in the cells from which we record.

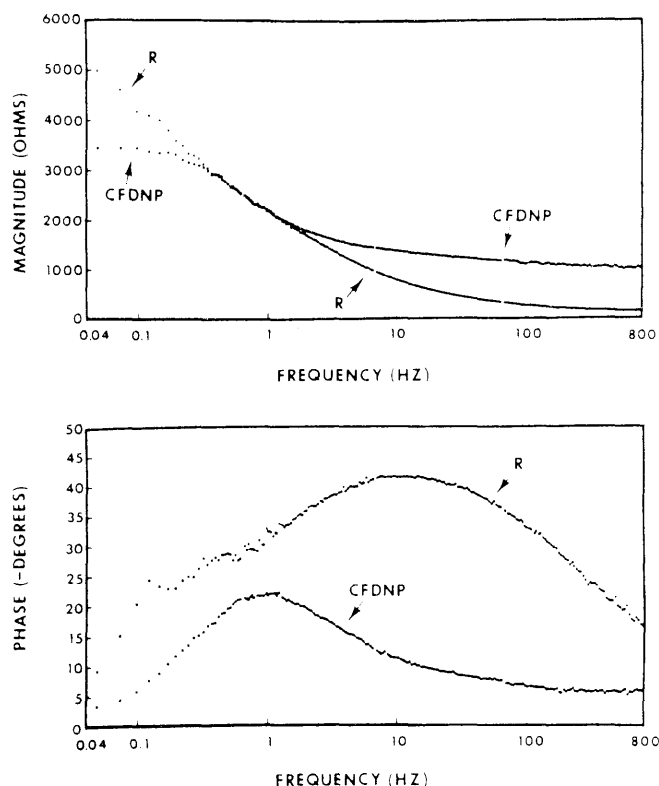


FIG. 6. Overlay of impedance plots determined from a single lens first in Ringer (R) and following a 6 hr incubation in calcium-free-EDTA DNP-Ringer (CFDNP).

Mitochondria are found in lens epithelial cells and in differentiating fiber cells near the lens equator but it is unlikely that this rather small volume of mitochondria can release sufficient calcium to have an effect on the cells from which we measure. It is therefore of some interest to investigate possible mechanisms for the action of DNP in the lens. A reasonable notion is that 2-4 DNP, because of its role in uncoupling oxidative phosphorylation, might exhaust the energy supply for the lens calcium extruding mechanism described by Hightower, Leverenz and Reddy (1980). This might result in an increase in intracellular calcium and a resulting uncoupling of gap junctions (Rose and Lowenstein, 1975; Dahl and Isenberg, 1980). This proposed mechanism can be tested by measuring the impedance in lenses following the addition of $500\ \mu\text{M}$ DNP in a Calcium-free-EDTA Ringer. If the uncoupling still occurs, then it cannot be due to influx of calcium from the bath.

Figure 6 compares the impedance in Ringer to that measured after 4 hr in $500\ \mu\text{M}$ DNP, calcium-free-EDTA Ringer. The change from Ringer to the final solution was done in two steps. Following the measurement of the lens impedance in Ringer, the

lens was superfused with calcium free-EDTA Ringer for 30 min to wash calcium from the extracellular clefts. This time is sufficient to wash out most of the extracellular space (see Appendix of Rae et al., 1982). Thereafter, it was superfused with the DNP-calcium-free Ringer. It is obvious that the increase in the high frequency magnitude of the impedance occurs without calcium in the bath. This result rules out the calcium influx mechanism described above.

4. Discussion

These results make it clear that lens fiber gap junctions, at least those within 300 μm of the lens surface, can be uncoupled. The data shows that the uncoupling is substantial, but not total. The uncoupling is largely reversible if the lens is superfused with Ringer solution within 2 hr after the onset of 500 μM DNP-Ringer incubation. A careful description of the time requirements of this reversibility has not been done. The uncoupling does not extend into the lens more than 300 μm even after 8 hr of incubation in the DNP-containing solution. Present data does not allow us to determine if all cells in this zone are equally uncoupled or if the degree of uncoupling varies radially from the surface. The problem is that the theoretical treatment used to interpret the data (Eisenberg et al., 1979) was derived with the assumption that the effective intracellular resistivity is much less than the extracellular resistivity. The validity of the theory is not known in the present circumstances, when the effective intracellular resistivity becomes comparable to the effective extracellular resistivity. Under these conditions, much of the current flows in the extracellular space rather than predominately in the intracellular space as the theory assumes. We guess that cell uncoupling would be underestimated in these circumstances because the current available to produce a voltage drop across gap junctions would be reduced; some of it is shunted into the extracellular space. If the uncoupling varied with depth and the extracellular resistivity were constant with depth, the degree of extracellular shunting would also vary with depth. Therefore, the data measured from this surface zone would show complex behavior which would require considerable effort to understand. These analytical problems in no way, however, undermine the qualitative conclusion that cell to cell uncoupling has occurred in this surface zone.

The meaning of the limiting spatial extent of DNP action will require further study. It could be that fiber cells near the surface have gap junctions which differ from those elsewhere in the lens; it may be that the penetration of DNP is limited; and many other possibilities need to be ruled out.

The mechanism of action of fiber cell uncoupling is not at all clear, although Ca^{2+} influx is clearly *not* involved. In other tissues, uncoupling seems to occur either in response to increases in the cytoplasmic H^+ or Ca^{2+} (Bennett and Goodenough, 1978). The effects of these ions may not be direct, rather they may act indirectly by activating some biochemical mechanism which controls gap junction permeability (Johnson and Ramon, 1981). Peracchia, Bernadini and Peracchia (1981) have suggested that the uncoupling action of Ca^{2+} and H^+ may involve calmodulin and that calmodulin may be the substance most directly involved in uncoupling. This intriguing possibility requires further attention, which it will no doubt presently receive. The relative importance of Ca^{2+} and H^+ in producing uncoupling is a matter of controversy (Loewenstein, 1981; Turin and Warner, 1980; Spray, Harris and Bennett, 1981; Spray, Stern, Harris and Bennett, 1982). In the lens, DNP might cause an increase in H^+ in fiber cell cytoplasm which might by itself activate the uncoupling mechanism or

cause release of some Ca^{2+} bound to lens crystallins. This free Ca^{2+} would then either directly or indirectly cause uncoupling. Investigation of these possibilities will require the measurement of free H^+ and Ca^{2+} activities in lens cytoplasm. This has not yet been done in the crystalline lens and will require the development of considerable technology.

Although the results reported here are preliminary in that they do not deal with the mechanism or spatial variation of DNP action, they clearly demonstrate that uncoupling of lens gap junctions can occur. A fraction of lens gap junctions may not be quite as static as previously suggested (Goodenough, 1979). It is obvious from previous work that quite drastic treatment is needed to produce such uncoupling, but this may not reflect a special property of the gap junctions *per se*. Rather it could be related more to an effective buffering of intracellular H^+ and Ca^{2+} by lens proteins or to differences in the mechanisms controlling gap junction permeability.

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