Some Electrical Properties of a Nuclear Membrane Examined with a Microelectrode

WERNER R. LOEWENSTEIN and YOSHINOBU KANNO

From the Department of Physiology, Columbia University, College of Physicians and Surgeons, New York. Dr. Kanno is on leave of absence from the Department of Physiology, Tokyo Medical and Dental University, Tokyo, Japan

ABSTRACT Electrical potential and resistance were measured with microelectrodes in *in situ* and isolated nuclei of gland cells of *Drosophila flavorepleta*. The nucleus-cytoplasm boundary was found to be rather impermeable to ion diffusion. It presents a resistance of the order of 1 Ω cm^2 and sustains a "resting" potential, the nucleoplasm being about 15 mv negative with respect to the cytoplasm. Both the resistance and potential appear to be associated with the nuclear membrane: the potential declines to zero and the resistance to a fraction of its original value, when the membrane is perforated experimentally.

INTRODUCTION

A considerable amount of information on biological membranes has been gathered during the past fifteen years. On the one hand, the development of appropriate electron microscope techniques led to the discovery of a number of structural aspects of living membranes, while on the other, the introduction of the microelectrode disclosed some of their electrochemical properties. But while electron microscope studies have dealt in detail with a wide variety of membranes, plasma membranes as well as a number of intracellular ones, electrochemical studies have been limited to the plasma membrane alone. Membranes of cellular organelles, such as the nuclear membrane, although they are the site of passage of materials of particular biological importance, have not yet been examined with electrophysiological techniques. The main reason for this neglect is the smallness of most nuclei, which places them beyond the reach of the microelectrode. There are, however, a few animal cells with nuclei large enough for electrophysiological work. An example is the salivary gland cell of *Drosophila* larvae. In a large gland cell, the nucleus measures 30 to 40 μ, and is readily viewed without staining aids through the transparent cell walls under an ordinary microscope. Under the electron micro-

scope, its surface membrane, like that of other nuclei, differs in at least two structural aspects from the cell membrane; it is double layered and presents numerous gaps in the unit membrane structure (Gay, 1956). The present paper deals with some electrical properties of this membrane. It will be shown that the nuclear membrane has a high electrical resistance and that there is a potential across it which is of about the same magnitude as that across the cell membrane. A preliminary account of the results has appeared (Loewenstein and Kanno, 1962).

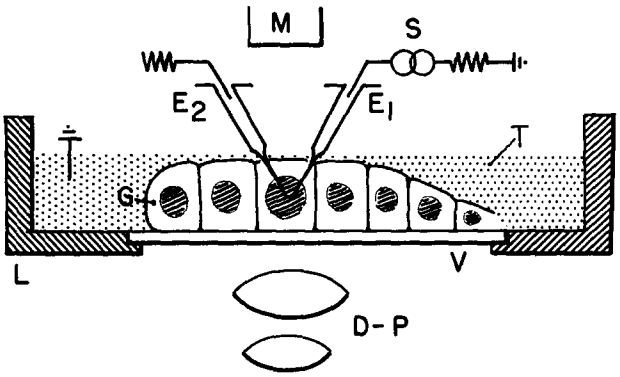

FIGURE 1. Diagram of set-up. *L*, lucite box. *V*, optical glass. *T*, Shen's solution. *G*, isolated gland. A gland contains about 200 cells, a few of which are drawn out of proportion. E_1 and E_2, Ling-Gerard microelectrodes driven by micromanipulators. *M*, microscope with darkfield and phase contrast condensers (*D-P*).

METHODS

Salivary gland cells of larvae of *Drosophila flavorepleta* were used throughout the investigation. The larvae were in the early or midstages of the third instar period of development. During this period the salivary gland cells have distinct boundaries, the cells and nuclei reach their largest size and do not divide. The glands were isolated and mounted in a bath of Shen's solution for micromanipulation under a compound microscope equipped with a Heine condenser with dark field and phase contrast optics (Fig. 1).

Membrane Potential Measurements Electrical potentials were recorded between an external Ag-AgCl Ringer electrode which was always kept in the external solution and a microelectrode of the Ling-Gerard type filled with 3 M KCl (Ling and Gerard, 1949). The following electrode systems were used:

1. Ag-AgCl | 3 M KCl microelectrode | test object | Shen solution | Ag-AgCl
2. Ag-AgCl | Shen-agar | 3 M KCl microelectrode | test object | Shen solution | Shen-agar | Ag-AgCl

Electrode tip potentials, namely the potentials of the systems in absence of test object, minus the potentials in absence of both test object and microelectrode, were measured as a matter of routine before each experiment (Adrian, 1956). Special arrangements

for measuring electrode tip potentials in cytoplasm and nucleoplasm are described in the text. Microelectrodes of tip potentials below 2.5 mv and of resistances of 10 to 35 megΩ were selected for the experiments. The electrodes were drawn out from capillaries to a 12 to 18 μ long end segment, tapering from a diameter of less than 1 μ in diameter to a final tip diameter less than 0.5 μ. The nuclear membrane seemed to seal well around such tips; in successful electrode penetrations, there were no signs of current leakage. There were also no signs of nuclear deterioration, such as have been observed in other types of nuclei upon puncture with instruments of larger tips (Chambers and Fell, 1931; Kopac and Mateyko, 1958). The object of the long taper was to prevent local damage and eventual depolarization of the cell membrane in the experiments with nuclei *in situ*, since the electrode had to slide then for considerable length through the cell membrane before it reached the nucleus. The membrane potentials of cell and nucleus were measured as a standard procedure at the beginning and at the end of an experiment. Besides, in most experiments the continuous recording of the nucleus or cell membrane potentials provided in itself an adequate check of the preparation. Preparations that presented changes in membrane potential greater than 10 per cent in the course of an experiment were discarded.

Membrane Resistance Measurements Nucleus membrane resistances were measured with two microelectrodes inserted in the nucleus, one to pass current and the other to record membrane potential. The current was supplied by a square pulse generator and monitored on one of the beams of an oscilloscope. Potentials were fed into the second beam through a DC amplifier. The input stage of the amplifier was a cathode-follower circuit with negative-capacity feedback to compensate for stray capacities in the recording system. The grid current was less than 10^{-13} A. The general procedure was to insert first one microelectrode into the nucleus and to pass repetitive square pulses of current through its membrane, and then to insert the recording electrode. The penetration of the latter through the nuclear membrane coincided with the sudden appearance of voltage pulses which provided the most reliable check that the nuclear membrane had been penetrated. The current was then varied over a wide range in both inward and outward directions, and the membrane current and voltage displayed simultaneously on separate beams of the oscilloscope.

The simplest case for analysis is that of the isolated nucleus. The nucleus is nearly spherical and lies suspended in a relatively large volume of fluid (including some cytoplasm of about the same conductivity). It is reasonable, therefore, to assume that under these conditions the membrane current is of uniform density. Since the nucleus surface is readily measured, its membrane resistance of unit area, hereinafter referred to as transverse membrane resistance, can be calculated with a high degree of accuracy.

The resistance of the cell membrane was measured by passing current pulses through it with an internal electrode with a fixed placement in the cytoplasm of the cylindrical portion of the gland, and by recording the membrane potentials with a roving internal electrode at various distances along this gland portion. As has been shown in earlier experiments, the (total) cell membrane resistance at the contact surface between gland cells is negligible compared with that at the external surface; the potentials observed in response to current pulses did not decrement measurably

as the roving electrode was displaced across the boundary between two cells (Kanno and Loewenstein, 1963 *b*). The gland, with its 200 cells, behaves as a cable-like structure with a continuous core and an estimate of the outer cell membrane resistance in the cylindrical portion of the gland was obtained from the expression

$$V = \frac{I}{2}\sqrt{(r_m \cdot r_i)} \cdot e^{-x/\lambda}$$

(Fatt and Katz, 1951; Hodgkin and Rushton, 1946), where I is the current passed through the fixed electrode; V, the steady-state potential recorded by the roving electrode; r_m, the membrane resistance times unit length; x, the distance between electrodes; r_i, the resistance per unit length of the cytoplasm; and λ is $\sqrt{(r_m/r_i)}$, the length constant. The term $(1/2)\sqrt{(r_m \cdot r_i)}$ is the "effective" resistance between the inside and outside, obtained from V/I at $x = 0$ at the "cylindrical" portion of the gland. The application of the theory of a linear cable to the present case requires that the diameter of the gland be small in relation to its length constant. This condition was not always fulfilled and it was necessary to restrict this kind of measurement to glands of small diameter, in which a fairly good agreement between the results and cable theory was found.

Nucleus Preparation Experiments were done on nuclei *in situ* and in isolation. In the former, the electrodes were advanced through the cell walls and the nucleus impaled inside the cells. The cells were easily penetrated. They are large (about 100μ in diameter) and their walls are thin and contain little connective tissue. The depth of the nucleus within the cell was estimated from its focal plane under the microscope. In these experiments the nucleus had to be reached at the first, or at most, the second run of the electrode through the cell in order to avoid damage to the cell membrane. This required some practice and was only successful if the nucleus, which was often pushed for some distance in front of the electrode before actual penetration occurred, was not displaced out ot the line of electrode movement. The insertion of a second electrode into the nucleus was generally easier, because the nucleus was then fixed and its depth exactly known. In good preparations, the cell walls remained free from opacity, and the cell and nucleus membrane potentials and resistances were constant for about half an hour. It was then usually possible to study more than one nucleus of the same gland preparation. All the experiments were done within one-half hour after isolation of the gland.

For the experiments with isolated nuclei the following procedure was adopted. First, the nucleus was impaled with one electrode inside the cell. Then another electrode was introduced across the cell wall and moved away from the first along the gland axis, tearing a hole of several microns in the cell wall, and, thereby, irreversibly short-circuiting its resistance and capacitance. The nucleus was then either left in place, or taken out of the cell with the first electrode. The first condition will be referred to in the text as the semiisolated nucleus and the second, as the isolated nucleus. In either case the nucleus was kept surrounded by a mass of viscous cytoplasm and impaled with a second electrode. All experiments on isolated and semiisolated nuclei were done within 1 to 3 min. after rupturing the cell membrane. Fig. 2 illustrates a few examples of nuclei impaled *in situ* and in isolation.

Perforation of Nuclear Membrane In some tests one or more holes were drilled into the nuclear membrane. Unless stated otherwise, this was done by driving an empty micropipette (of the type and dimensions described above for microelectrodes) repeatedly across the nuclear membrane in excursions of 1 μ or so. The hydraulically coupled drive of our micromanipulators was rather free of vibrations; sizable membrane perforations could be made without dislodging the measuring microelectrodes from the nucleus.

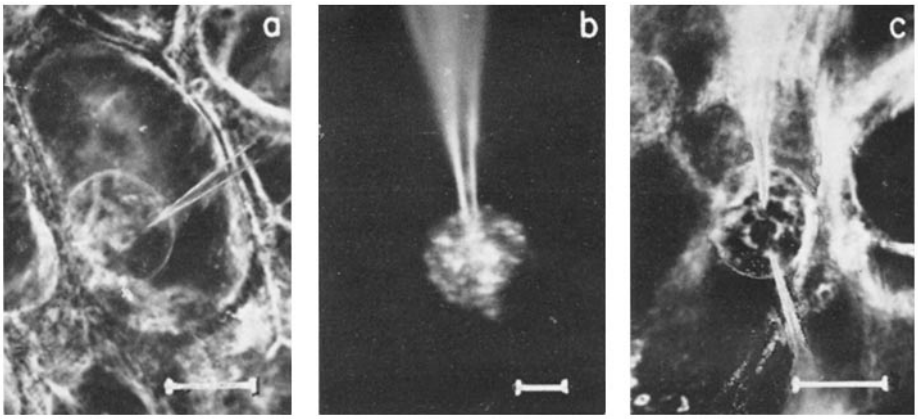

FIGURE 2. Darkfield photomicrographs of unstained, fresh salivary gland cells. Views of nuclei impaled *in situ* with one (left) or two microelectrodes (right), or in isolation after destroying the cell membrane (center, the portion of cytoplasm that surrounds the nucleus is not visible in this photomicrograph). Calibration 25 μ.

Solutions The composition of Shen's solution was: NaCl, 154 mM; KCl, 5.6 mM; $CaCl_2$, 2.25 mM. All experiments were done at room temperature ranging from 20 to 24°C. The animals were bred and kept at the constant temperature of 22°C.

RESULTS

Nucleus Membrane Potential Fig. 3 illustrates an experiment in which a cell and its nucleus were successively impaled with a recording microelectrode. Salivary gland cells are quite transparent during the third instar state. The nucleus, its outline, and some aspects of its interior are clearly visible in the living cell in phase contrast or in a dark field. It is thus possible to follow the progress of a microelectrode through the cell with some detail (Fig. 2). As the microelectrode is advanced from the cell exterior towards the nucleus, one finds a sudden change in potential when the electrode penetrates the cell membrane, and another sudden change when it enters the nucleus. No change in potential is detected as the electrode moves through the cytoplasm or nucleoplasm.

The first potential is the well known cell membrane potential and needs no further description. The average of 25 cases was 13.3 mv with a standard error of ±0.8 mv (cytoplasm negative). This is about 10 mv less than the cell membrane potential of mammalian salivary glands, type I (Lundberg, 1955).

The second potential occurs clearly at the surface of the nucleus. It is associated with the penetration of the electrode tip into the nucleus and is usually preceded by a visible dimpling of the nuclear surface. No change in potential is seen as the electrode tip advances through the interior of the nucleus, until it pierces the nuclear membrane again on its way out.

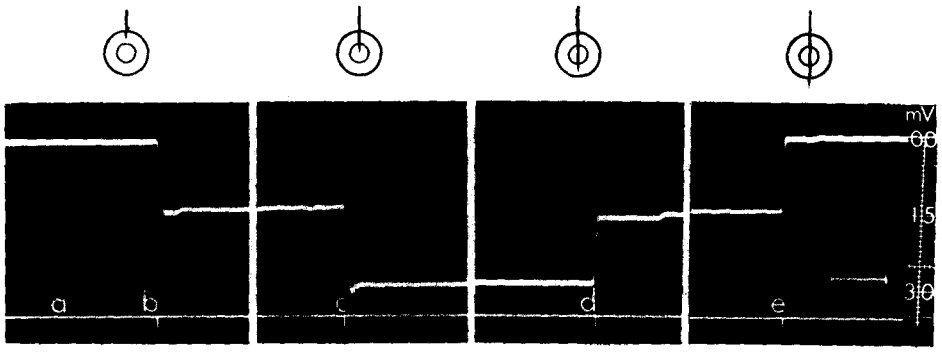

FIGURE 3. Potentials across cell and nucleus membranes. A microelectrode is advanced progressively in the direction cell exterior–nucleus. Upper beam records the potential (downward negative) as the electrode tip is *a*, outside the cell; *b*, entering the cell membrane; *c*, entering the nucleus; *d*, leaving the nucleus; *e*, emerging into the cell exterior. Reference electrode is in the cell exterior. Time calibration 0.05 sec.; film interrupted for about 1 sec. in between photographs.

The potential between the nucleoplasm (negative) and cytoplasm appears thus to develop at the nucleoplasm-cytoplasm boundary, presumably across the membrane of the nucleus. It will hereinafter be referred to as the nucleus membrane potential. The potential has the following characteristics: it arises abruptly as the electrode crosses the nucleus-cytoplasm boundary; it disappears abruptly as the electrode displacement is reversed; its magnitude is nearly reproducible upon repeated probing; and it is associated with a high resistance in the nuclear membrane. When this resistance is short-circuited by rupturing the nuclear membrane, the potential disappears (see below).

The mean potential of 18 nuclei was 14.5 mv with a standard error of ±1.2 mv.

Tests In the present experiments the danger from changes in electrode tip potential, due to differences in ionic composition between the extracellular and intracellular fluids, was minimized by selecting electrodes which had small tip potentials in the bathing Shen's solution (0.1 to 2.5 mv). The tip poten-

tials of such electrodes were constant within 1 to 1.8 mv when the concentrations of KCl and NaCl of the bathing solution were varied from 0 to 400 mM, the total concentration remaining constant (see also Adrian, 1956). This covers the range of concentrations of the corresponding ions in extracellular fluid, cytoplasm, and almost certainly also in nucleoplasm.

But the possibility of another source of change in tip potential remained which might have vitiated our measurements of membrane potentials. It was conceivable that in the process of electrode insertion, the tip became plugged with a nuclear material of high electrical resistance, a protein, for example, which through the presence of fixed charges or other mechanisms magnified the difference in ion mobility in the tip, changing thereby its potential (*cf.* Adrian, 1956; Sollner *et al.*, 1955). In fact, occasionally unsuccessful attempts at nucleus penetration, in which it was clear from microscopic observation that the electrode had not entered the nucleoplasm, gave potentials which may have been caused by electrode plugging. Such potentials had characteristics quite different from membrane potentials. They were never abruptly established, were of very variable magnitude in repeated attempts at penetration, and did not entirely vanish when the electrode displacement was reversed. These potentials were thus easily distinguished from membrane potentials, and were rejected.

It was still conceivable, however, that in successful electrode penetrations a reversible shift in tip potential occurred due to some form of tip plugging, reversible on electrode withdrawal. The ideal test would have been to measure the tip potentials directly in nucleoplasm. But since it was not feasible to collect the bulk of nucleoplasm which this required, the following tests were made.

i. The tip potentials of a series of microelectrodes were measured in Shen's solution. Two electrodes, *A* and *B*, were then connected to the recording system at a time, and their potential difference recorded and photographed during the following sequence: (1) Both electrodes in contact with the cytoplasm of an intact cell. (2) Electrode *A* in the nucleoplasm; electrode *B* in cytoplasm. (3) Electrodes *A* and *B* in nucleoplasm. (4) Electrode *A* in cytoplasm; *B* in nucleoplasm. (5) Electrodes *A* and *B* in cytoplasm.

Four nuclei were tested in this manner. Each time a different electrode pair was used. Three nuclei showed no difference in potential between (1), (3), and (5); one case, a difference of 1.0 mv between (1) and (3); and in all four the potential shifts were equal in (2) and (4). The tip potentials were then checked again in Shen's solution and were found unchanged in all four cases. Fig. 4 illustrates a typical test.

The same measuring sequence was performed also across the cell membrane. The cytoplasm instead of the nucleoplasm was then the measuring medium and the Shen's solution around the cell the reference medium. In all seven cases so tested, the potential differences were equal between the electrode

positions across the cell membrane corresponding to (1), (3), and (5); and the potential shifts were equal in those corresponding to (2) and (4).

This test shows clearly the reversibility of the measurement of the nucleus potential and its independence of the original tip potential. (In the example of Fig. 4 the electrodes *A* and *B* had tip potentials of −0.5 and −2.2 mv respectively.) It seems extremely unlikely that the two electrodes could have been blocked and unblocked symmetrically and to the same extent in each movement across the membrane, and it seems safe to conclude that no change in tip potential due to such cause took place.

ii. Besides, another test was available on this point. The nuclear membrane could be ruptured without causing a recording microelectrode to be dislodged

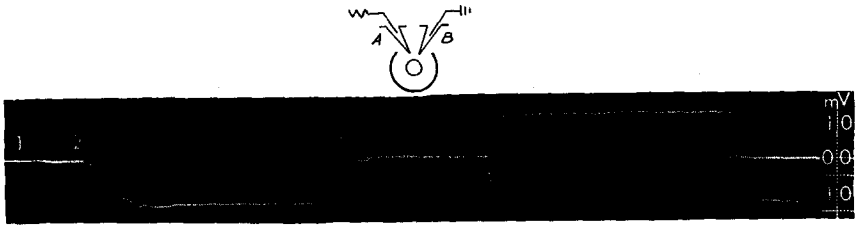

FIGURE 4. Reversibility test. Continuous records of potential difference between a pair of microelectrodes *A* and *B*, as *A* and *B* are in cytoplasm (1); *A* enters nucleus (2); *B* enters nucleus (3); *A* leaves nucleus entering cytoplasm (4); *B* leaves nucleus (5). In this experiment the nucleus was pushed in front of electrode *A* for some distance through the cytoplasm before definite entry occurred; this caused the slow onset of the potential in (2). Time calibration 1 sec.

from the nucleus. The procedure was to destroy first the cell membrane and to allow the cytoplasm to exchange freely with the bathing Shen's solution. A microelectrode was then inserted into the nucleus and the nucleus membrane potential recorded continuously with respect to a reference electrode in Shen's solution, while a sizable hole was drilled in the nuclear membrane with a second micropipette (see Methods). Invariably, with delays ranging from 10 to 40 sec., the nucleus membrane potential declined to zero. Similar results were obtained when the cytoplasm was not contaminated with Shen's solution. In this case the gland was placed in mineral oil, and the reference electrode inserted in the cytoplasm. Examples are illustrated in Fig. 5.

It appears from these results that the recordings of nucleus potentials are free from electrode junction artifacts and that the potentials develop at the nucleoplasm-cytoplasm boundary, presumably across the nuclear membrane. Results obtained in another kind of nucleus, that of certain amphibian oocytes, are interesting in this connection. Unlike the present membrane, that of the amphibian nucleus is a rather permeable structure; its resistance is indistinguishable from that of cytoplasm and nucleoplasm (Loewenstein and Kanno,

1963). The physiological situation in this nucleus resembles thus that of the ruptured nucleus of the experiment above; and, indeed, no nucleus potential is detectable (Kanno and Loewenstein, 1963 *a*).

Nucleus Membrane Resistance There is a high electrical resistance associated with the nuclear surface. This is conveniently shown by passing repetitive pulses of current from the nucleus interior to the cell exterior with an electrode placed inside the nucleus, and by recording continuously the resulting voltage drops with a second electrode advanced progressively from the cell exterior to

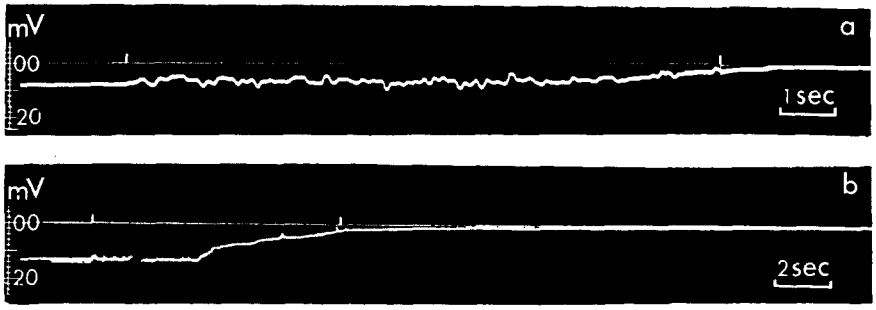

FIGURE 5. The nucleus potential after destruction of the nuclear membrane. A microelectrode inside the nucleus records continuously the nucleus membrane potential while a hole is drilled into the nuclear membrane. Beginning and end of drilling period are marked on upper beam. The small deflections in the potential record (lower beam) of *a* are movement artifacts caused by the drilling; in *b* the deflections are partly eliminated by a filter. The recording electrode stays inside the nucleus throughout the experiments.

In experiment *a*, the cell lies in Shen's solution with which the cytoplasm is freely exchanging; and the reference electrode, a Ag-AgCl wire is in the Shen's solution. In *b*, the cell lies in oil, and the reference electrode, a micropipette of 15 μ tip diameter filled with 3 M KCl-agar gel, is inserted in the cytoplasm.

the nucleus. Two sharp changes in resistance are then observed; one, as the electrode penetrates the cell membrane, the cell membrane resistance; and another as it enters the nucleus (Fig. 6). The latter coincides with the appearance of the nucleus membrane potential and is clearly associated with the nuclear surface; as the electrode moves through the cytoplasm or nucleoplasm, changes in resistance are undetectably small.

That the high resistance is confined to the nuclear surface is also shown by other evidence. When the nuclear membrane is damaged by repeated insertions of micropipettes or by strong electrical currents, or torn in a manner similar to that described in the methods for the cell membrane, the high resistance drops and the nucleus membrane potential declines to zero. Fig. 7 illustrates this for a nucleus through which currents of constant strength were pulsed between a microelectrode inside the nucleus and a large Ag-AgCl electrode in common with the recording circuit, placed in the Shen fluid around the cell.

(The cell membrane had been destroyed previously, and the cytoplasm was in direct contact with the Shen fluid.) Upon perforation of the nuclear membrane, the recorded voltage lost its capacitative component and dropped to a

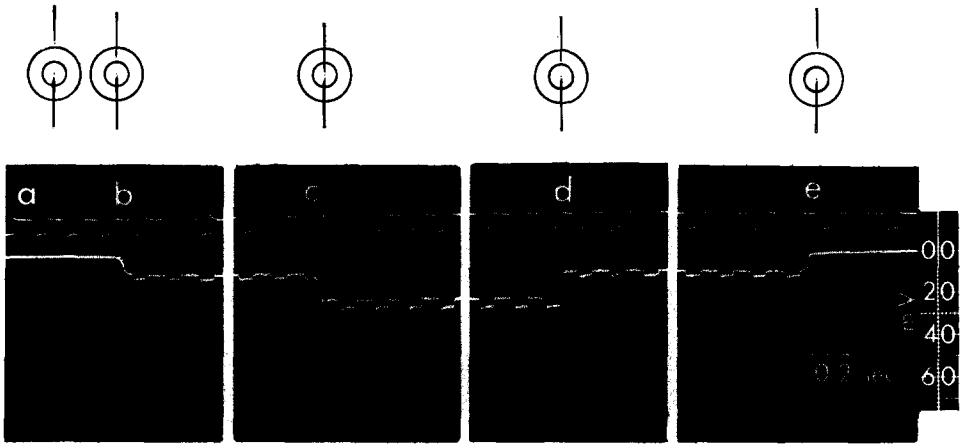

FIGURE 6. Nucleus and cell membrane resistance. Current pulses of constant strength (upper beam) are passed through the nucleus and cell membranes between a microelectrode placed inside the nucleus and an electrode in the extracellular fluid. The membrane potential (lower beam) is recorded continuously between a reference electrode in the extracellular fluid and a second microelectrode, as pictured in the diagram of Fig. 1, moving in the direction extra-cellular fluid–nucleus and back. Recording electrodes *a*, *e*, in extracellular fluid; *b*, *d*, entering cytoplasm; *c*, entering nucleoplasm.

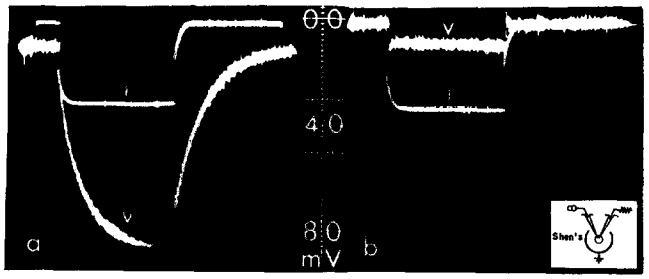

FIGURE 7. Nucleus resistance after destruction of the nuclear membrane. Square pulses of 1.5×10^{-6} A (*i*) are passed into a semiisolated nucleus from a large ground electrode in the Shen's solution in contact with the open cytoplasm (current density = 39.5×10^{-3} A/cm^2); the resulting voltage drops (*v*) are recorded before (*a*) and after (*b*) drilling a hole into the nuclear membrane. Time calibration 1 msec.

fraction of its original value. The residual resistance corresponds approximately to the resistance one observes between the recording electrodes in this system in absence of a membrane.

A more quantitative experiment is illustrated in Fig. 8. Here, the gland was

placed in oil before rupturing the cell membrane to retard changes in the composition of cytoplasm; and the common ground electrode, a Ag-AgCl wire sharpened to a tip of less than 1 μ diameter, was inserted into the cytoplasm. The resistance of this electrode in cytoplasm was 31.9 K Ω. The ground electrode, as well as the microelectrodes, was kept in fixed position throughout the measurements of nucleus resistance. Current voltage relations were obtained before and after perforation of the nuclear membrane; the ordinates of

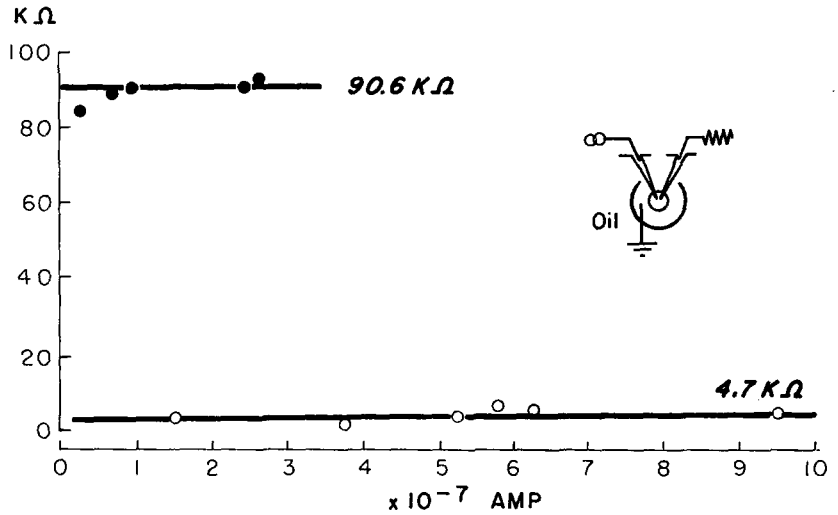

FIGURE 8. The membrane resistance of the nucleus. The resistances between a microelectrode in the nucleus and a fine Ag-AgCl electrode in the cytoplasm with intact (●) and perforated (○) nuclear membrane at various current values (nuclear membrane area = 1.5×10^{-5} cm^2).

Fig. 8 give the corresponding resistances after subtraction of the ground electrode—cytoplasm resistances. The resistance in this nucleus dropped from 90.6 K Ω to 4.7 K Ω on perforation.

The resistance associated with the nuclear surface will be referred to as nucleus membrane resistance. Measures of nucleus membrane resistance were obtained from membrane current—"steady-state" voltage relations in which the current was varied over a wide range. Representative relations are illustrated in Figs. 9 and 10. Typically, the nuclear membrane shows little or no rectification. (This is also true for the present cell membrane (Kanno and Loewenstein, 1963 *b*).) Moreover, the nuclear membrane gives no sign of excitation over the entire range of current that can be used without damage to the membrane.

Table I gives values obtained in a series of experiments in which resistance was measured in each nucleus under two conditions, *in situ* and in semiisola-

tion. A pair of electrodes was first inserted into the nucleoplasm to measure the total nucleus membrane resistance in series with the total cell membrane resistance; and then into the cytoplasm to measure the latter alone. The nu-

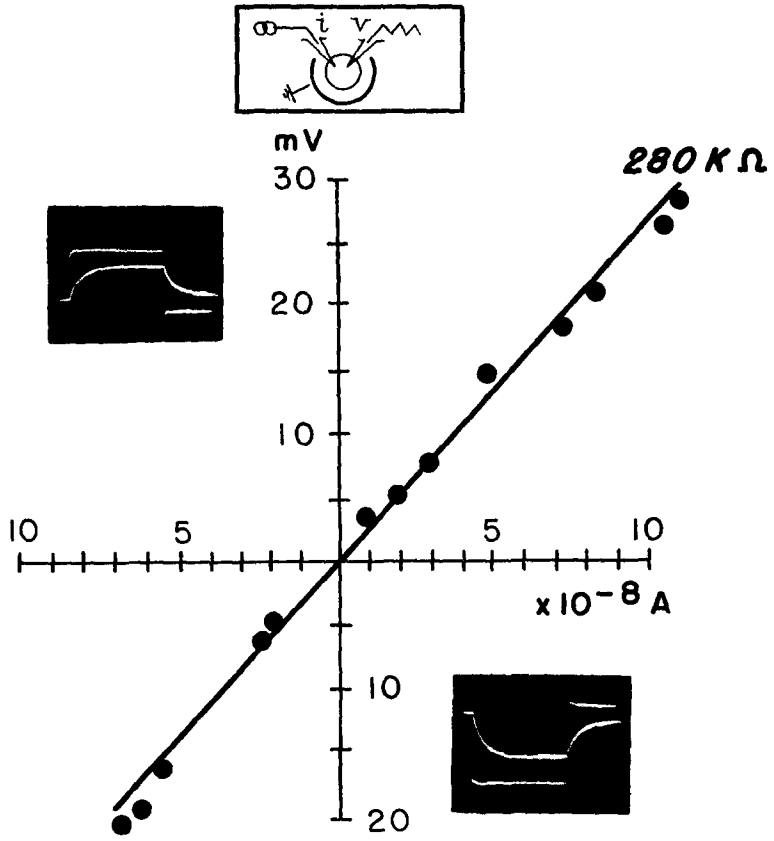

FIGURE 9. Current-voltage relation in a nuclear membrane (semiisolated nucleus). Square pulses of current of varying intensity (24 msec. duration) are passed inward and outward through the membrane of a semisiolated nucleus with one microelectrode (i) and the corresponding electrotonic potentials are recorded with a second microelectrode (v). Insets are samples of membrane current and voltage at 6.3×10^{-8} A inward and 4.6×10^{-8} A outward currents. Abscissae, total nucleus membrane current, inward current left. Ordinates, "steady-state" electrotonic potential, hyperpolarization downwards. Nuclear membrane area = 1.6×10^{-5} cm^2.

cleus membrane resistance was obtained by subtraction. The cell membrane was then destroyed and the nucleus membrane resistance was measured directly in the semiisolated nucleus within 1 min. As is seen in Table I, the resistances are somewhat smaller after isolation. This is probably due to mixing of cytoplasm with Shen's solution which is not a good medium for nuclei. But the main point here is that the order of magnitude of the resistance and

the non-rectifying properties of the membrane in *in situ* nuclei are the same as in semiisolated nuclei, where the conditions of current flow and analysis are simpler (Fig. 10). The mean transverse membrane resistance of 4 *in situ* nuclei was 3.9 with a standard error of ± 1.4 Ω cm^2 and of 12 semiisolated nuclei 1.5 ± 0.3. The transverse resistance of the cell membrane was on the order of 6×10^2 Ω cm^2.

Membrane Time Constants The rising and falling phases of the cell membrane voltage resulting from a square pulse of current are exponential. The time constants are of the order of 1 msec. From this an apparent membrane

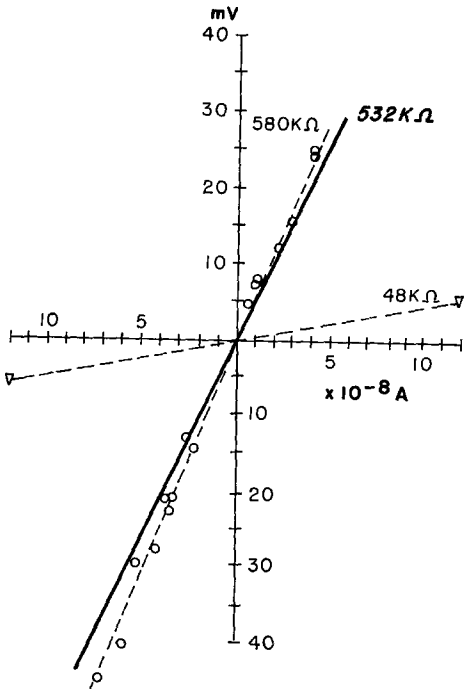

FIGURE 10. Current-voltage relation in a nuclear membrane (nucleus *in situ*). △, membrane current-voltage relation of cell membrane and ○, of cell membrane and nuclear membrane in series. Heavy line gives the calculated relation of the nuclear membrane alone (nuclear membrane area = 1.6×10^{-5} cm^2) (case A-35 of Table I).

capacitance of the order of 1 μf/cm^2 is calculated. Capacitances of this sort are found in a wide variety of cells (*cf.* Cole, 1940; Hodgkin and Rushton, 1946; *cf.* Eccles, 1953).

The rising and falling phases of the nuclear membrane voltage are also exponential. The time constants range from 0.2 to 2.8 msec. in the semiisolated nucleus. With the prevailing nuclear membrane resistances, one obtains apparent capacitances on the order of 100 μf/cm^2 (Loewenstein and Kanno, 1962). This is far outside the range of capacitances found in many cell membranes. It seems unlikely that so high a value corresponds to the capacitance of a classic dielectric made up of polarizable dipoles. It is difficult to imagine, for example, that a thin lipid layer between two aqueous phases can exhibit a

dielectric constant of 1000, as the above capacitance would require. However, this result will be considered preliminary until reactance measurements can be performed over a wide range of frequency (*cf.* Cole, 1949).

A possible explanation of the high capacitance is that the surface over which the capacitance is distributed is larger than that of the nuclear membrane itself. The electron microscope gives no evidence of large infoldings of the nuclear membrane that might have caused gross errors in our estimate of area of the nuclear membrane itself. However, a considerable extension of the relevant surface could conceivably result from connections between the nuclear membrane and the endoplasmic reticulum. Connections of this sort have, in fact, been described in electron micrographs of some other nuclei (Watson, 1955). There is no electron micrographic evidence showing such connections in the present nucleus. But it should be kept in mind that the chance for obtaining positive evidence is small in the present case, in which the area occupied by the endoplasmic reticulum is small compared to that of the cytoplasmic matrix. Positive evidence is available only for a few nuclei, such as of pancreas acinar cells and reticular cells, which offer the favorable situation of an extensive and well developed endoplasmic reticulum (*cf.* Watson, 1955). In pursuing this line of speculation, it is interesting that an increase in area by a factor of 100 would bring both the capacitance and the resistance of the nuclear membrane in line with those of the cell membrane.

TABLE I

TRANSVERSE NUCLEAR MEMBRANE RESISTANCE

Case No.	In situ	Semiisolated
	$\Omega\ cm^2$	$\Omega\ cm^2$
A-14	0.5	0.45
A-32	3.3	2.0
A-34	3.4	2.1
A-35	8.5	4.5

DISCUSSION

The high electrical resistance and potential are both clearly associated with the nuclear surface, presumably with the structure that appears as a double layered membrane under the electron microscope. In a wide variety of nuclei, this structure appears to consist of two unit membranes (*cf.* Robertson, 1959) with numerous circular gaps at which the two membranes are fused together. The gaps have diameters of the order of 0.1 μ and are spaced rather regularly with distances of a similar order of magnitude over the unit membrane surfaces (Callan and Tomlin, 1950; Bahr and Beermann, 1954; Gall, 1954; Afze-

lius, 1955; Palay and Palade, 1955; Watson, 1955; Pappas, 1956). An important question concerning the exchange of material between nucleus and cytoplasm is whether these gaps are freely communicating membrane pores (*cf.* Watson, 1955, 1959; *cf.* Mirsky and Osawa, 1961). The present results shed some light on this question. They provide a measure of membrane conductance against which the calculated conductance of a "porous" membrane may be checked. The measured resistance of the nuclear membrane here is of the order of 1 Ω cm^2. Although this resistance is smaller than that of the cell membrane, it is still large enough to represent a formidable barrier for ion diffusion. A membrane with free pores, of the size and frequency shown in the electron micrographs, should offer a much lower resistance. On the basis of the "pore" diameter and distribution given by electron micrographs of the present nucleus and a specific cytoplasm and nucleoplasm resistance of 100 Ω cm, it is calculated that such a sieve-like membrane would have a transverse resistance of the order of 10^{-3} Ω cm^2, a value three orders of magnitude smaller than the observed one (see Appendix). One must conclude, therefore, that the discontinuities in the nuclear membrane of *Drosophila* gland cells are not freely communicating fenestrations.[1]

What structural elements of the nuclear surface account for the high resistance? There are no reasons to doubt that the two unit membranes are actually interrupted at the regions where they fuse together; transverse sections through the nuclear envelope of a wide variety of cells give evidence for this (*cf.* Watson, 1955) and the present nucleus is no exception (Fig. 11). But the gap may not necessarily be bridged by nucleoplasm or cytoplasm, as has often been assumed. Electron microscope examination of thin sections of the gap space of the *Drosophila* gland cell nucleus reveals, in fact, the presence of electron-dense material, more diffuse than the unit membranes, filling the gap and often projecting beyond it into the cytoplasm and nucleoplasm (Wiener *et al.*, 1963). Formations of this and other kinds have also been seen in other nuclei (Palade, personal communication; Afzelius, 1955; Watson, 1955; Wischnitzer, 1958; Merriam, 1961). It is tempting to speculate that these formations are the additional diffusion barriers which confer upon the nuclear envelope its high electrical resistance. It will be interesting to see in what way the nuclei of amphibian oocytes, which have a very low membrane resistance and no membrane potential (Kanno and Loewenstein, 1963 *a*), differ in gap constitution or other aspects of membrane structure from the present nucleus.

[1] It is interesting in this connection that recent work with labeled elements also reveals the nuclear membrane as a strong diffusion barrier; Allfrey *et al.* (1961), for example, have shown that the entry of amino acids into the nucleus is not a simple diffusion process, but involves a mechanism of specific transport.

APPENDIX

The resistance of a membrane pore treated as a cylindrical volume conductor with a pore diameter of 500 A, a pore length of 200 A including the two unit membranes, as given by electron micrographs (Gay, 1956; Wiener *et al.*, 1963), and a specific cytoplasm and nucleoplasm resistance of 100 Ω cm, is 10^7 Ω per pore. A similar resistance is obtained if the pore is treated as a thin disc buried in a volume conductor (*cf.* Mason and Weaver, 1929; *cf.* Kanno and Loewenstein, 1963 *a*).

The pore distribution given by electron micrographs is roughly hexagonal. But since the possibility of membrane distortion introduces uncertainty, it will be best to calculate the transverse resistance of a porous membrane on the basis of two extremes

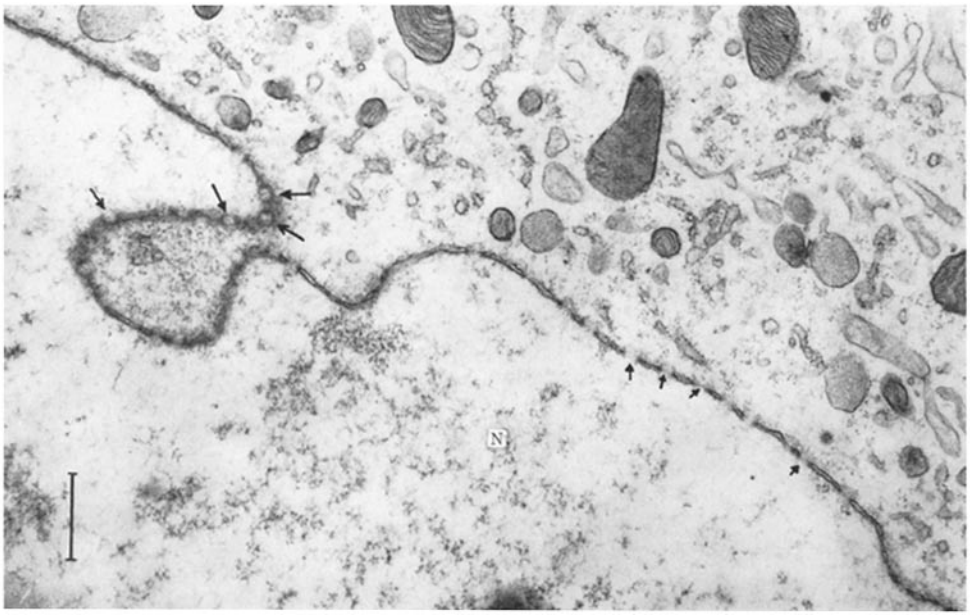

FIGURE 11. Electron micrograph of nuclear membrane of *Drosophila flavorepleta* salivary gland cell. Membrane "gaps" in transverse (short arrows) and oblique sections (long arrows). The nuclear membrane is fairly regular over most of its surface; the relatively rare infolding here was chosen to illustrate an oblique section. Material fixed in OsO_4. *N*, nucleoplasm. Calibration, 0.5 μ (courtesy of Dr. J. Wiener and Dr. D. Spiro).

of plausible pore distributions, one in which the pores form triangular arrays and one in which they form hexagonal arrays, with a center-to-center distance b of 1000 A between nearest neighbors. In the former, the most efficient sieve with regular one-parameter spacing, there are $2/(\sqrt{3}b^2)$ pores per unit area of membrane, and the transverse membrane resistance is 0.8×10^{-3} Ω cm². In the hexagonal distribution, the least efficient, there are $4/(3\sqrt{3}b^2)$ pores per unit area, and the transverse membrane resistance is 1.3×10^{-3} Ω cm².

The possible additional resistance introduced by the interaction of potential fields

between neighboring pores has been estimated. Dr. K. S. Cole has kindly provided us with analog computations of this resistance based on the assumption that the potential field is similar to that of a boundary tube of a diameter equal to the pore interval and with a pore on its axis. The resulting increase in resistance changes the value of transverse membrane resistance given above by less than a factor of 2.

We take pleasure in thanking Dr. K. S. Cole for much valuable discussion and for providing us with a solution to the problem of resistance due to pore interaction. We are indebted to Dr. T. Dobzhansky for a gift of specimens of *Drosophila flavorepleta* that started out our fly colony, and to Dr. S. J. Socolar for valuable discussion and help in the control experiments.
This work was supported by a research grant from the National Science Foundation.
Received for publication, January 14, 1963.

REFERENCES

ADRIAN, R. H., The effect of internal and external potassium concentration on the membrane potential of frog muscle, *J. Physiol.*, 1956, **133**, 631.

AFZELIUS, A., The ultrastructure of the nuclear membrane of the sea urchin oocyte as studied with the electron microscope, *Exp. Cell Research*, 1955, **8**, 147.

ALLFREY, V. G., MEUDT, R., HOPKINS, J. W., and MIRSKY, A. E., Sodium-dependent "transport" reactions in the cell nucleus and their role in protein and nucleic acid synthesis, *Proc. Nat. Acad. Sc.*, 1961, **47**, 907.

BAHR, G. F., and BEERMANN, W., The fine structure of the nuclear membrane in the larval salivary gland and midgut of *Chironomus*, Exp. Cell Research, 1954, **6**, 519.

CALLAN, H. G., and TOMLIN, S. G., Experimental studies on amphibian oocyte nuclei. I. Investigation of the structure of the nucleus membrane by means of the electron microscope, *Proc. Roy. Soc. London Series B*, 1950, **137**, 367.

CHAMBERS, R., and FELL, H. B., Micro-operation on cells in tissue culture, *Proc. Roy. Soc. London, Series B*, 1931, **109**, 380.

COLE, K. S., Permeability and impermeability of cell membranes for ions, *Cold Spring Harbor Symp. Quant. Biol.*, 1940, **8**, 110.

COLE, K. S., Some physical aspects of bioelectric phenomena, *Proc. Nat. Acad. Sc.*, 1949, **35**, 558.

ECCLES, J. G., Neurophysiological Basis of Mind, Oxford University Press, 1953.

FATT, P., and KATZ, B., The end-plate potential recorded with an intracellular electrode, *J. Physiol.*, 1951, **115**, 320.

GALL, J. G., Observations on the nuclear membrane with the electron microscope, *Exp. Cell Research*, 1954, **7**, 197.

GAY, H., Chromosome–nuclear membrane–cytoplasmic interrelations in *Drosophila*, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, (suppl.), 407.

HODGKIN, A. L., AND RUSHTON, W. A. H., The electrical constants of a crustacean nerve fibre, *Proc. Roy. Soc. London, Series B*, 1946, **133**, 444.

KANNO, Y., and LOEWENSTEIN, W. R., A study of the nucleus and cell membranes of oocytes with an intracellular electrode, *Exp. Cell Research*, 1963 *a*, **31**, 149.

KANNO, Y., and LOEWENSTEIN, W. R., A gland syncitium: low resistance connections between cells of a salivary gland, *Nature*, 1963 *b*, in press.

KOPAC, M. J., and MATEYKO, G. M., Malignant nucleoli in cytological studies and perspectives, *Ann. New York Acad. Sc.*, 1958, **73**, 237.

LING, G., and GERARD, R. W., The normal membrane potential of frog sartorius fibers, *J. Cell. and Comp. Physiol.*, 1949, **34**, 382.

LOEWENSTEIN, W. R., and KANNO, Y., Some electrical properties of the membrane of a cell nucleus, *Nature*, 1962, **195**, 462.

LOEWENSTEIN, W. R., and KANNO, Y., The electrical conductance and potential across the membrane of some cell nuclei, *J. Cell Biol.*, 1963, **16**, 421.

LUNDBERG, A., The electrophysiology of the submaxillary gland of the cat, *Acta Physiol. Scand.*, 1955, **35**, 1.

MASON, M., and WEAVER, W., The Electromagnetic Field, University of Chicago Press, 1929.

MERRIAM, R. W., On the fine structure and composition of the nuclear envelope, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 559.

MIRSKY, A. E., and OSAWA, S., The interphase nucleus, *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press Inc., 1961, **2**, 677.

PALADE, G., Personal communication.

PALAY, S. L., and PALADE, G. E., The fine structure of neurons, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 69.

PAPPAS, G. D., Fine structure of the nuclear envelope of *Amoeba proteus*, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 431.

ROBERTSON, J. D., Ultrastructure of cell membranes and their derivatives, *Biochem. Soc. Symp.*, 1959, **16**, 3.

SOLLNER, K., DRAY, E., GRIM, E., and NEIHOF, R., Membranes of high electrochemical activity in studies of biological interest, *in* Electrochemistry in Biology and Medicine, (T. Shedlovsky, editor), New York, John Wiley and Sons Inc., 1955.

WATSON, M. L., The nuclear envelope, its structure and relation to cytoplasmic membranes, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 257.

WATSON, M. L., Further observations on the nuclear envelope of the animal cell, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 147.

WIENER, J., SPIRO, D., and LOEWENSTEIN, W. R., 1963, Ultrastructure and impedance of the nuclear membrane, data to be published.

WISCHNITZER, J., Electron microscope study of the nucleus envelope of amphibian oocytes, *J. Ultrastruct. Research*, 1958, **1**, 251.