STUDIES OF IMPEDANCE IN CARDIAC TISSUE USING SUCROSE GAP AND COMPUTER TECHNIQUES

I. The Influence of Sucrose and Oil as Insulating Media

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ABSTRACT Impedances of cardiac cells of an insect were determined as a function of time to test the effects of sucrose and oil as insulating media in a gap arrangement. Impedance values are shown to increase markedly with time when sucrose is used as the insulating agent. Although impedance values are steady when oil is used, it is suggested that a layer of trapped electrolyte provides a shunt pathway and seriously impairs the validity of the measurements. A quick wash with sucrose followed by oil does not alleviate the situation but leaves a layer of sucrose trapped at the tissue-medium interface into which ions diffuse. The hypotheses (*a*) that the diffusion of intracellular K^+ into the sucrose would result in an increase in tissue impedance and (*b*) that a layer of trapped electrolyte under the oil film provides a shunt pathway are examined by computer analyses of a simple model.

INTRODUCTION

Data obtained from experiments using gap techniques must be interpreted with the awareness that certain alterations in electrical behavior of excitable cells may be induced by the insulating solutions themselves. The most widely used gap technique, that introduced by Stämpfli (1954), employs isotonic sucrose as the insulating agent. This method has been applied to many types of single and multifiber preparations of excitable tissues to study various aspects of electrogenesis. By the gap method a clearly delineated and carefully restricted region of tissue is perfused with a solution of low electrical conductivity while the remaining regions are in contact with a highly conductive medium. A central area of a tissue can thereby be insulated and electrically isolated from the portions that remain in contact with electrolyte pools. If isolation is achieved, i.e. if no shunt pathway prevails across the gap, cell-to-cell communication in the gap region cannot occur by way of extracellular electrical

pathways, for a nonconducting medium now replaces the normal electrolyte in the interstitium. A distinct advantage of this technique is the circumvention of some of the technical difficulties encountered in the use of microelectrodes where cell geometry precludes the impalement of a single cell by more than one microelectrode. This feature of the sucrose gap technique has enhanced its attractiveness as a method for the application of voltage clamps. However, there are certain deficiencies inherent in the gap technique and this study focuses on the effect of the insulating media on the tissue. Although isotonic sucrose and, less frequently, mineral oil have been used as agents to achieve electrical isolation in the gap regions, there have been few studies directed to the effects of the insulating agents themselves on the tissues and the extent to which they might alter the electrical properties under investigation. This paper reports the influence of insulating media, sucrose and oil, on impedance values obtained in cardiac tissue by a gap technique.

METHOD

Cardiac tissue selected for this investigation was obtained from adult moths (*Hyalophora cecropia*) because these hearts offer several advantages for this type of experimental approach.

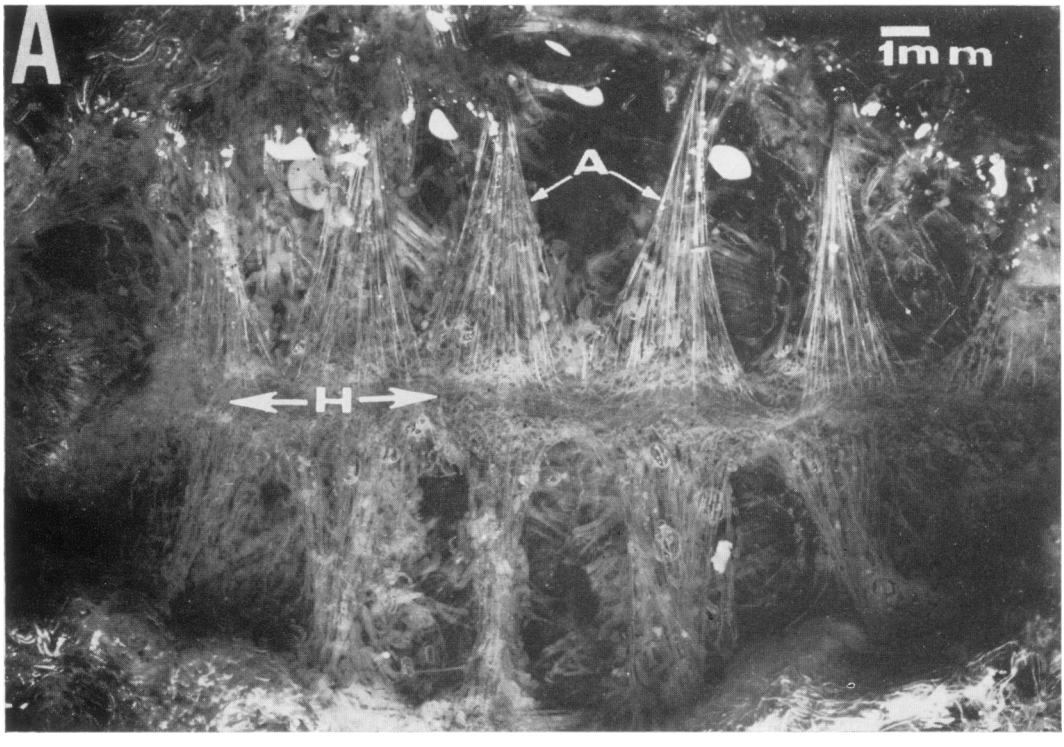


FIGURE 1 A Photograph of the exposed tubular adult moth heart (H) *in situ*. Alary muscles (A) attach directly to the heart and to the lateral body wall. (6.4×)

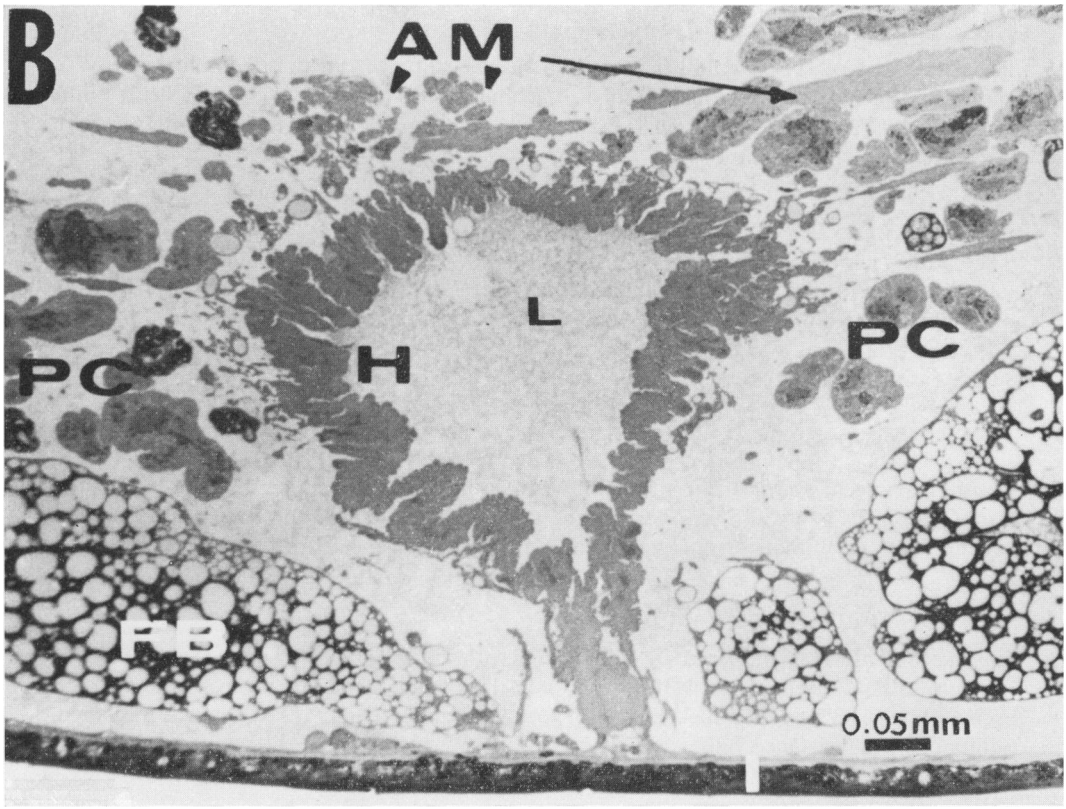


FIGURE 1 B Photomicrograph of a fixed moth heart in cross section. Letters indicate H, heart; L, lumen; PC, pericardial cells; FB, fat body; AM, alary muscles. (172 ×)

The whole heart is a simple tubular structure that extends the full length of the insect's body, about 3.5 cm. The portion of the heart suspended in the abdominal cavity by alary muscles is easily accessible as can be seen in Fig. 1 A. The whole heart is composed of only one cellular type of striated muscle; there is no evidence of "specialized" tissue. Intercalated disks have been found at the cell boundaries and other ultrastructural features of this myocardium are common to heart tissue in general (McCann and Sanger, 1969).

Animals

Moths (*H. cecropia*) were obtained in the pupal stage and retained at 5°C, 50% humidity for 3 mo or longer until diapause was artificially interrupted by placing them at room temperature and 80% relative humidity. Adults emerged within 3 wk.

Preparation

The tubular heart was exposed by a ventral midline incision extending from the caudal tip of the abdomen to the midthoracic region. Ligatures were secured around the caudal tip and the section of heart lying cephalad between the dorsal flight muscles. This provided a strip of heart muscle at least 1 cm long. Fatty tissue, tracheae, and alary muscles were carefully

removed from the myocardial surface. The cleaned, ligatured heart was then slit open along its ventral surface. The whole heart section was then lifted from the abdominal cavity by carefully excising the remaining muscular attachments that normally anchor the heart to the dorsal body wall. The isolated heart was placed in a dish of physiological saline where it remained for about 20 min.

Physiological Saline

The bathing solution contained Ca, 6 mM; K, 45 mM; Na, 1.8 mM; Mg, 24 mM; pH maintained at 6.8 with 0.5 mM Pipes buffer. Osmotic pressure was measured at 382 mosmol/kg on pooled sera from a series of five pupal moths by the method of freezing point depression (Osmette, Precision Systems, Inc., Natick, Mass.), and isotonicity was established with sucrose at this osmotic pressure.

Gap Arrangement

The gap arrangement with rubber membranes was the same as that described by Berger and Barr (1969). The gap width was 3.7 mm and the length of the portion of the heart strip that extended into each pool was measured with an ocular micrometer for each experiment.

Insulating Solutions

Isotonic sucrose was prepared by adding 382 mM/liter sucrose (Fisher Scientific Co., Pittsburgh, Pa.) to glass double-distilled water. This solution was then run through an ion exchange resin (Crystalab, Inc., Hartford, Conn., Deminite) and the resistance measured. Solutions wiith resistance values less than 2 MΩ-cm were discarded. Oil insulators tested included mineral oil, paraffin oil, and silicone oil (5, 20, 100 St viscosity).

Experimental Procedure

One end of the isolated heart, was drawn through holes in rubber membranes while the holes were widened by the application of a tension to the membranes. Each end of the heart rested in a pool of physiological saline, while the midsection was exposed to a solution of either isotonic sucrose or oil. Gold leaf electrodes were submerged in each end pool. The impedance due to the electrode-electrolyte interface was measured separately and was found to be less than 1% of the tissue impedance. One pool was also connected to the input of the bridge amplifier. A schematic diagram of the experimental arrangement is presented in Fig. 2. Since details of the bridge circuit design and operation have been published elsewhere (Stibitz et al., 1973), only a brief account need be presented here. As in all bridges, a common current flows through both a known and an unknown impedance and the complex ratio of the two impedances is determined by comparison of voltages with those in a second circuit.

The bridge used in these experiments presents several advantages over conventional bridges. It is an active bridge in which the balance point is at zero potential to avoid effects of stray impedances to ground at this point. The currents and voltages imposed upon the specimen are low (of the order of 10^{-7} A and 3×10^{-3} V or less). The conventional variable impedance circuit is replaced by a four-phase generator with circuitry for adjusting the signal phase and amplitude independently, thus avoiding the "sliding null" problem. Finally, the phase angle of the second voltage can be varied over the complete cycle rather than being restricted to $\pm 90°$ as in the conventional variable impedance bridge.

Data from the measurements are reduced to impedance values for the specimen, taking

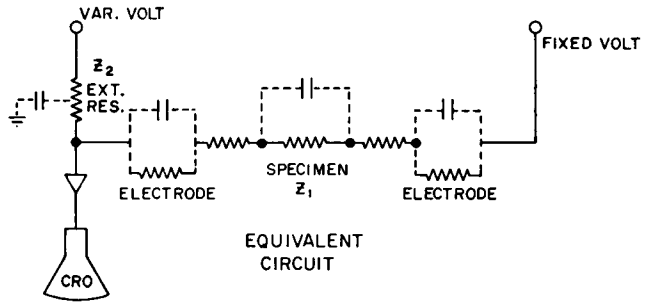


FIGURE 2 Schematic diagram of the equivalent circuit representing the moth heart (specimen) and electrodes. Z_1 represents the impedance of the whole heart, Z_2 the impedance of the external resistance. Variable voltage is adjusted until oscilloscope (CRO) shows signal is nulled. Stray capacitances are indicated.

into account all known stray impedances, many of which are measured by the bridge itself. An abstract has been published (McCann et al., 1972).

RESULTS

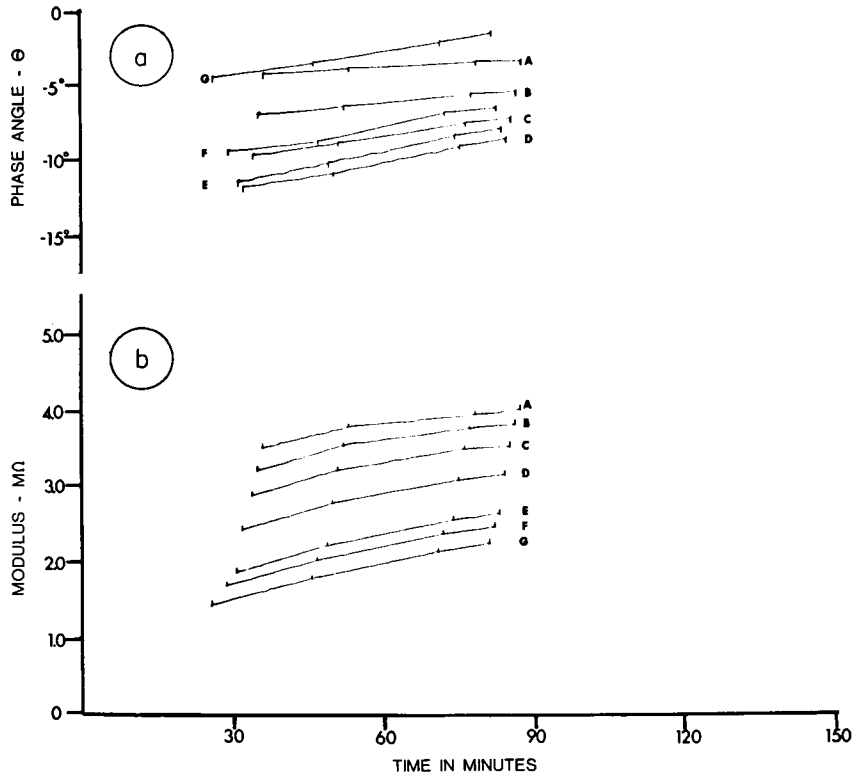
This series of experiments was designed to test the variation of membrane impedance with time as measured in a gap arrangement when several test substances were used as insulating media. The insulating substances tested were: (*a*) isotonic sucrose, (*b*) oils of varying viscosities and compositions, and (*c*) a quick wash with sucrose followed by oil.

Sucrose as the Insulator

Impedance measurements from 1 of 85 experiments carried out on moth hearts in which sucrose was used as the insulator are presented in Figs. 3 *a–d*. The phase angle (*a*) steadily declines while the modulus (*b*) increases sharply over a period of 90 min. When measurements were continued beyond this period, these values continued to vary for several hours. The complex plane curves (*c–d*) show the measured impedances: these remained about the same shape but moved toward higher resistance values, thereby suggesting that the longitudinal resistance component in the gap is affected with time.

To test whether these manifestations of impedance variation were induced by some specific biological property of the sucrose itself or were the consequence of the low ionic concentration of the solution per se, a different sugar solution, mannitol, was substituted. The results were the same as with sucrose. This evidence suggests that the variation of measured impedance with time may be attributed to ionic changes in the myoplasm such as might result from a continual loss of ions into the isotonic sugar solution.

To test the hypothesis that changes in measured impedance may be due to a redistribution of diffusate in and around the cells of a strip of tissue which lie in the



FIGURES 3 *a, b.* A computer plot of the phase angle (*a*, upper) and modulus (*b*, lower) as they vary with time when isotonic sucrose is used as the insulator in the gap. Letters identify frequencies at which impedances are measured: A = 3 Hz; B = 10 Hz; C = 30 Hz; D = 100 Hz; E = 500 Hz; F = 1,000 Hz; G = 3,000 Hz.

gap, we have calculated diffusion in a simple model, and asked whether the behavior of the model agrees in magnitude with that observed in the tissue. The lack of exact information about the physiological system is not critical since only approximate results are sought.

It is assumed that the cell itself is roughly cylindrical with a radius of about 10 μm, and that when removed from the animal it is surrounded by an adhered layer of electrolyte. The model thus takes the form shown in Fig. 4, where the inner cylindrical surface represents the cell membrane. Except for the membrane, the entire region is filled with a fluid of uniform diffusion coefficient. A coefficient value of 10^{-5} cm^2/s, approximately that for K^+, was used. The numerical value of membrane permeability used was 10^{-6} cm/s (Woodbury, 1965).

To simulate the gap experiment it was assumed that at an initial instant the cell with its adhered layer is submerged in ion-free sucrose. At this instant both the interior of the cell and its adhered layer contain ions at an arbitrary concentration

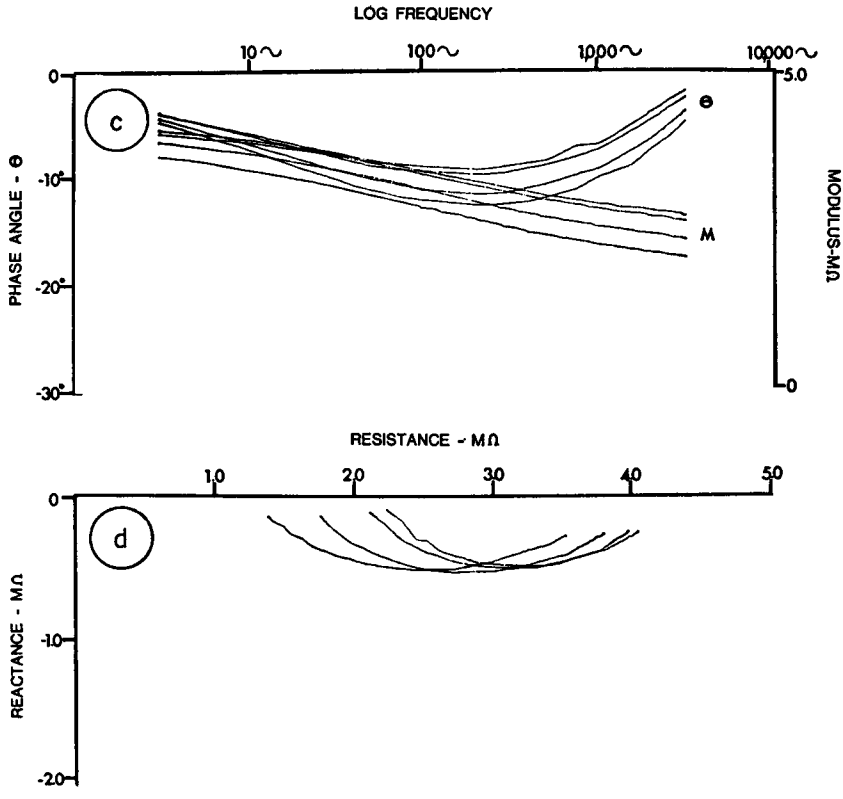


FIGURE 3 *c*. A computer plot of phase angle (θ) and modulus (M) vs. log frequency in successive runs starting at 25, 45, 75, and 85 min after exposure to flowing sucrose solution. FIGURE 3 *d*. A computer plot of impedance on the complex plane under the same conditions as in Fig. 3 *c*.

which is here defined as unity. Immediately on submersion, ions diffuse from the adhered layer into the ambient medium, from the interior of the cell into the adhered layer, and from point to point within and without the cell. Since we are concerned with electrical transmission parallel to the cell axis, it is important to know how the ions are distributed with respect to distance from the axis of the cell as time advances.

To specify the model completely it is necessary to know the thickness of the adhered layer. Thus, to obtain an approximate value for this thickness, four hearts were measured and weighed "wet" as they were removed from the animal, and "dry" after being blotted to remove excess fluid. The results were:

Wet weight (g)	0.00340	0.00325	0.00280	0.00260
Dry weight (g)	0.00195	0.00185	0.00170	0.00135
Volume cm^3	0.00145	0.00140	0.00110	0.00125

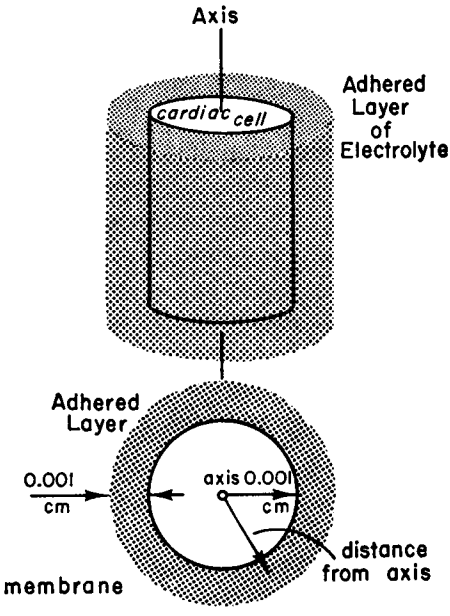


FIGURE 4 Model depicting a heart cell as a cylinder with a layer of electrolyte adhered to the exterior surface of the cell membrane. The model in cross section is shown below.

The volume was calculated on the assumption that the density of the fluid is unity.

The lengths of the hearts were less easily determined because of irregularities in shape but an average value was 1 cm. The sectional areas of the adhered fluid and of the dry tissue were thus estimated to be 0.0013 cm^2 and 0.0017 cm^2, respectively.

The perimeter of one surface of the heart was measured at 0.118 cm from a photomicrograph of a cross section such as shown in Fig. 1 B. The two surfaces of the heart are then 0.236 cm in perimeter. Using the value of 0.0013 cm^2 for the section area, the adhered layers on both surfaces are 0.005 cm thick, and each layer is then 0.0025 cm thick. It is concluded that the dimensions of the adhered layer are comparable with those of the cell itself. It will be clear that not too much reliance can be placed on the exact value of thickness thus calculated, and for simplicity the thickness of the adhered layer of the model cell is set equal to the radius of the actual cell, recognizing that it may in fact be two or even three times this value.

Preliminary calculations indicated that diffusion processes were taking place on two very different time scales. With the assumptions that diffusion from the interior to the exterior of the cell is slow compared with that in the fluid media, and that the exterior medium is initially at zero concentration, the diffusion equations were solved. However, when the membrane permeability was assigned the value 10^{-6} cm/s it was impossible to detect departures from uniformity of concentration either within or without the cell; therefore, these effects were magnified by about 100, by

increasing the permeability value to 10^{-4} cm/s. A computer program solves the diffusion equation and plots the concentration of diffusate as a function of distance from the axis, for a succession of instants of time after submersion (Fig. 5 A).

Since we are concerned with electrical currents flowing in and near the cell, the computer has been programmed to calculate and plot the total ion quantities within various distances from the axis (Fig. 5 B), as a means for estimating the shunt paths produced by ions in the extracellular layer. These calculated results show that both within and without the cell boundary the concentrations are very nearly uniform. This fact suggests that a good approximation to such diffusion is provided by a simple two-compartment system in which it is well known that the concentration inside the cell falls exponentially. When the change in concentration is exponential the system possesses a time constant (T_c) which, in the case of a cylinder, is readily shown to be equal to the radius divided by twice the permeability of the boundary membrane, or

$$\begin{aligned} T_c &= 0.001/2(10^{-6}), \\ &= 500 \text{ s}. \end{aligned}$$

In view of the fact that the actual cells are not isolated but are in contact, their time constant will certainly be greater than that calculated. While the experimentally determined changes in impedance appear to show time constants several times as great as this, they are of the same order of magnitude, and the calculated values are not believed to contradict the observations.

The next consideration is the diffusion of ions from the adhered layer into the ambient medium. Based on the tentative assumption that such diffusion is rapid compared with the diffusion of ions from the interior of the cell, the slightly permeable membrane is replaced with one that is impermeable. The computer program calculates and plots both the concentration (Fig. 5 C) and the quantity of diffusate (Fig. 5 D) as functions of distance from the axis, at specified times. Since this is not a two-compartment system, the time-course of neither concentration nor quantity is exponential, and there exists no time constant. However, it may be informative to note that the time at which the quantity of diffusate remaining within the original adhered layer falls to $1/e$ is about 0.3 s as compared with 500 s for diffusion from the interior of the cell. This observation justifies the validity of our tentative assumption that the two phenomena can be treated independently.

From these considerations, where a layer of electrolyte is visualized as adhered to the exterior of the cell membrane under initial or control conditions, K^+ appears to have diffused to great distances from the axis within 10–20 s through the layer. Therefore, it is concluded that a sucrose wash of about 0.5 min will remove electrolytes from the adhered layer and will, at the same time, allow very few ions to escape through the membrane. Contact of the cardiac cells with isotonic sucrose beyond this brief period would allow movement of ions across the membrane and thereby deplete intracellular ionic stores. The evidence presented here has demonstrated that

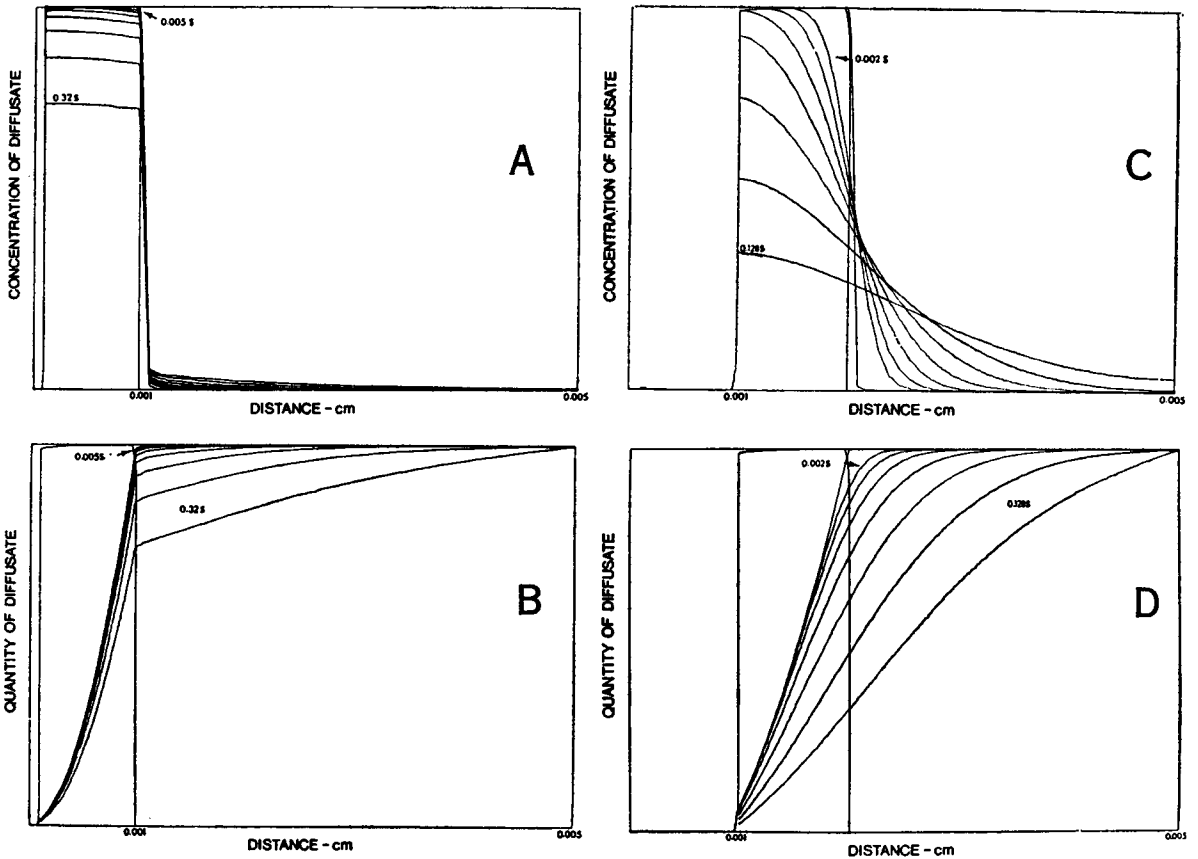


FIGURE 5 (A) Concentration of diffusate vs. distance from axis of the model cell (cf. Fig. 4) at various time intervals. Concentration at time 0 is constant within cylinder radius of 0.001 cm. Intermediate time intervals between 0.005 and 0.32 s are: 0.01, 0.02, 0.04, 0.08, 0.16 s. (B) Quantities of diffusate corresponding to the concentrations and time intervals plotted in Fig. 5 A. (C) Concentration of diffusate in adhered layer of cell model (Fig. 4) vs. distance from axis at time intervals between 0.002 and 0.28 s. Intermediate times are: 0.004, 0.008, 0.016, 0.032, 0.064 s. (D) Quantities of diffusate corresponding to the concentrations and time intervals plotted in Fig. 5 C.

the variation of impedance values with time may reflect not only the washing away of extracellular electrolytes but also the continual diffusion of ions from the intracellular stores of the myocardial cells within the gap enclosure into the sucrose solution that perfuses this region. Calculations provide a time constant of 10 s for washing K^+ out of the extracellular layers. The time constant for K^+ movement across the cell membrane from inside to outside is of the order of 1–2 h. Such estimations have lead to the conclusion that a sufficiently constant ionic state across the cell membrane would not be achieved for some 12 hours in the presence of sucrose. The physiological state of the tissue after so long an exposure to sucrose is questionable, and irreversible injury may occur.

Oil as the Insulator

Paraffin oil was introduced into the gap as an insulator in place of sucrose and the same series of impedance measurements was carried out. The substitution of oil in

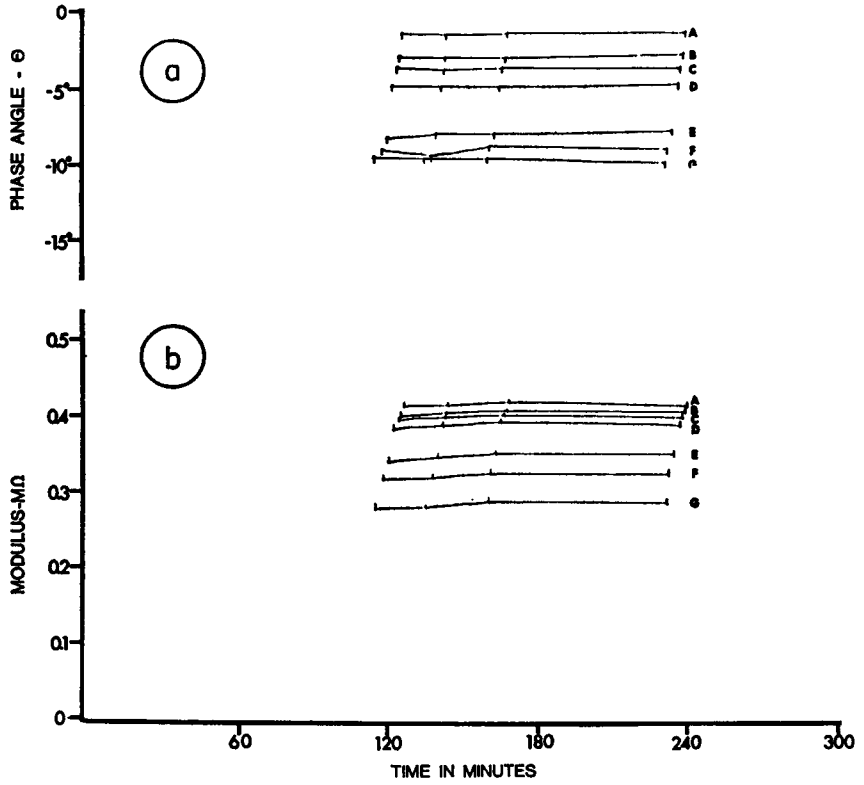


FIGURE 6 *a, b.* A computer plot of phase angle (*a*, upper) and modulus (*b*, lower) vs. time when paraffin oil is used as the insulator in the gap. Letters indicate frequencies: A = 10 Hz; B = 30 Hz; C = 50 Hz; D = 100 Hz; E = 500 Hz; F = 1,000 Hz; G = 3,000 Hz. Note different modulus scale.

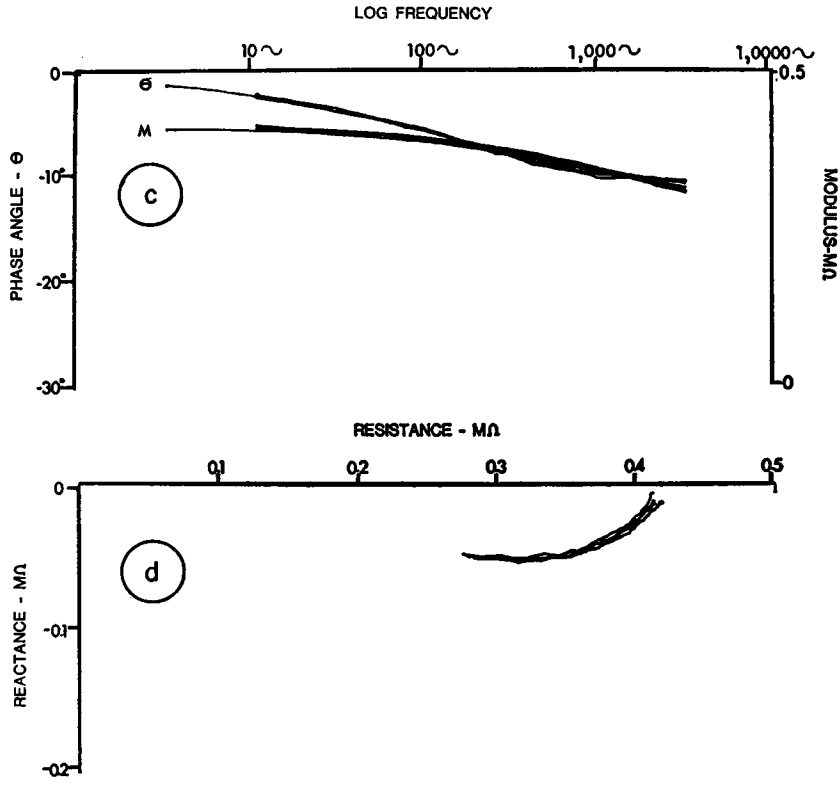


FIGURE 6 *c*. A computer plot of phase angle (θ) and modulus (M) vs. log frequency in successive runs starting at 110, 140, 160, 230 min after exposure to flowing paraffin oil. FIGURE 6 *d*. A computer plot of impedance on the complex plane under the same conditions as in Fig. 6 *c*.

the gap produced surprisingly steady values as shown in the computer plots presented in Figs. 6 *a–d*. The phase angle (*a*) and modulus (*b*) are steady over a period of several hours. However, the impedance measured under these conditions was much lower than that in the sucrose experiments.

To determine the influence of viscosity on these measurements, silicone oils in viscosities ranging from 5 to 100 cSt were used in the gap. To test for a possible influence of chemical composition, several different oils were substituted, including heavy and light mineral oil, paraffin oil, and silicone oil. These substitutions did not alter the results but special care had to be taken with the oils of higher viscosities to ensure equal pressures on the faces of the membranes so that bulging did not occur.

The stability of impedance measurements obtained when oil was used as the insulator appeared to offer a convenient solution to the problem of drifting measurements when sucrose was used to insulate the gap region. Neither the viscosity nor chemical nature of the oil influenced the measurements. However, in the presence of all of the oils tested, the measured impedances were of consistently low magnitude

when compared with those in sucrose. The possibility that a layer of electrolyte remains trapped between the oil and the tissue interface, thereby providing an electrical shunt pathway, was examined. If a layer of electrolyte remains adhered to the outer surface of the cell membrane even after washing with very low viscosity oil, the effects would be twofold: (*a*) cell-to-cell paths across the membranes would be conductive (isolation would be incomplete) and (*b*) the presence of electrolyte would be especially significant at the point where the tissue mass exits from the rubber diaphragms. In the latter case, the shunt path would not involve the cell membranes, and since only a very small amount of fluid would be present in the holes, the shunt path would be short. Thus, the impedance of these paths might be expected to be very low.

Through a series of calculations it has been possible to estimate an error factor that would influence impedance measurements when oil is used as the insulator. The leakage at the diaphragm holes is not readily measured or calculated, but the

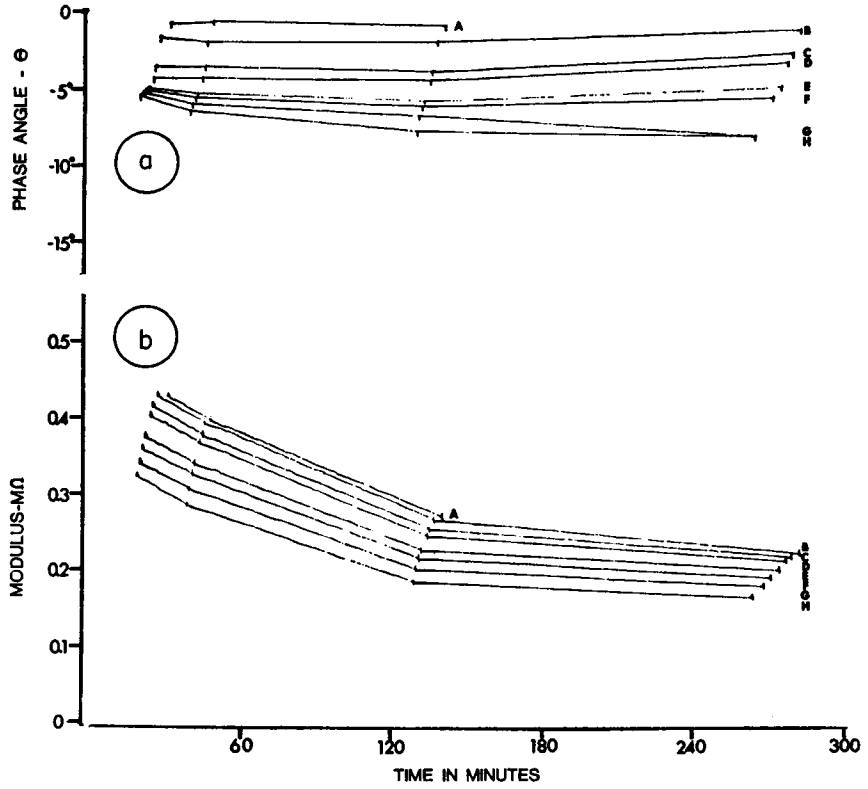


FIGURE 7 *a, b.* A computer plot of phase angle (*a*, upper) and modulus (*b*, lower) vs. time when the gap is first washed with flowing isotonic sucrose followed by paraffin oil. A = 1.70879 Hz; B = 8.3717 Hz; C = 42.9572 Hz; D = 94.1176 Hz; E = 417.868 Hz; F = 916.086 Hz; G = 2748.76 Hz; H = 13812.2 Hz.

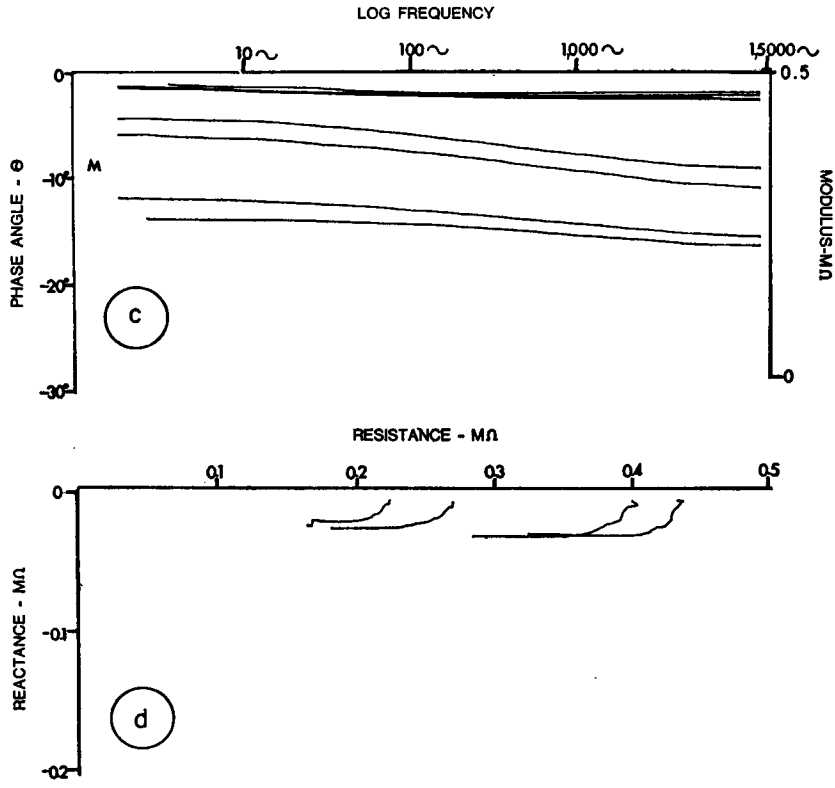


FIGURE 7 *c*. A computer plot of phase angle (θ) and modulus (M) vs. log frequency in successive runs starting at 20, 40, 130, and 270 min after oil was introduced into the gap. FIGURE 7 *d*. A computer plot of impedance on the complex plane under the same conditions as in Fig. 7 *c*.

conductivity of the shunt path itself may be approximated. If the cross-sectional area of the adhered layer is designated A, and L the distance between diaphragms (width of the gap), then the admittance of the path is K_1A/L where K_1 is the specific conductivity of the adhered fluid. The average volume of the adhered fluid, $V = LA$, obtained as described by weighing the heart wet and again after being gently blotted dry, was estimated at 0.0013 cm³; the volume of the dry tissue was 0.0017 cm³. Thus, the layers of electrolyte adhered to the two surfaces of the tissue strip are approximately 0.6 the thickness of the single cell layer that comprises the heart wall. The average length of the weighed hearts was about 1 cm, thus providing a calculated cross-sectional area of about 0.0013 cm². The resistance of the shunt path through the adhered electrolyte then becomes $1/K_1(0.0013)$. If the specific conductivity, K_1, is 0.012, then the resistance is 60,000 Ω. This is by no means a negligible value and would contribute significantly to the apparent impedance of the tissue.

Sucrose Wash Followed by Oil

In order to capitalize on the short-term efficiency of sucrose to wash away the adhered layer of electrolyte, and the long-term stability of oil, numerous attempts were made to flush sucrose quickly through the gap, then before leaching could occur, oil was perfused in place of the sucrose. The wash took less than 2 min to administer and then the oil was added. A series of readings at a frequency range of 3½ Hz to 3 KHz could be completed in 7 min. The results of one of these experiments are presented in Figs. 7 *a–d*. It is obvious that the variation of impedance with time persists and that the modulus of the impedance drops very rapidly within a few minutes. The results lead us to the conclusion that the sucrose is not completely washed out of the gap by the oil; rather, that under these conditions, instead of a layer of trapped electrolyte, a layer of trapped sucrose is provided into which ions diffuse. After several hours of diffusion, the trapped fluid becomes a highly conductive shunt pathway, electrically similar to that in the unwashed tissue. The drop in impedance that results from this condition at longer times is evident in Fig. 7 *b*.

DISCUSSION

The validity of membrane impedance values obtained in a gap arrangement must be assessed with two factors in mind, (*a*) the effects of the insulating material itself on the tissue and (*b*) the length of time of exposure of the tissue to the insulator during which the measurements are taken. Although ion-free isotonic sucrose is the most commonly used insulator, sucrose itself is known to produce certain effects on the tissue under study. These include a marked hyperpolarization of 20–60 mV in lobster giant axon (Julian et al., 1962), and a 10-fold increase in membrane time constant in guinea pig smooth muscle (Kuriyama and Tomita, 1970). An increase in membrane resistance in sheep Purkinje fibers was reported by Trautwein et al. (1965) while a decrease was noted by Freygang and Trautwein (1970). Membrane hyperpolarization has been attributed to the effects of local circuit currents driven through the membrane in the gap by the liquid junction potential occurring at the boundary of the saline and sucrose pools (Blaustein and Goldman, 1966). Resistance changes have been related to a gradual but continual leaching of intracellular K ions into the sucrose, an explanation that has been analyzed in these studies (Johnson and Lieberman, 1971). Pooler and Oxford (1972) reported a parallel leakage current in their sucrose gap and ion leakage into the sucrose could explain this observation also.

The variation of impedance values with time, as measured in the moth heart, may reflect not only the washing away of extracellular electrolytes but also the continual diffusion of ions from the intracellular stores of the myocardial cells in the gap into the sucrose solution that perfuses this region. In this investigation it has been proposed that a cardiac cell can be represented as a cylinder containing a uni-

form distribution of diffusate and a second cylindrical shell of adhered electrolyte also containing a uniformly distributed diffusate (cf. Fig. 4). With simple assumptions regarding the permeability of the cell membrane, a time constant for K^+ diffusion across the cell membrane averages approximately 1–2 h. The time for K^+ movement through the adhered layer is of the order of 10 s. It was concluded that a stable transmembrane ionic state would require more than 12 h to occur.

The substitution of oil as an insulator also introduces some special considerations, for while the measured impedances remained constant, they were of very low magnitude. That a layer of electrolyte trapped between the tissue and the oil could provide a shunt path was examined. The geometry and resistance of the entrapped layer of electrolyte and its effects on the impedance measurements were considered, and it was concluded that a relatively large error appears in such measurements where an electrical shunt pathway is provided by electrolyte at the oil-tissue interface. This factor could not be altered by substituting oils of various viscosities or chemical compositions.

Attempts were made to introduce sucrose quickly into the gap in order to wash away the ions in the extracellular space; then, before leaching could occur, oil replaced the sucrose. According to calculations, extracellular ions should be diffused away within 10 s. Therefore, a "quick wash" with sucrose followed by a variety of oils, including heavy and light mineral oil, paraffin oil, and silicone oil in viscosities of 5, 20, and 100 cSt, was applied. The drift in the measurements persisted and in view of the calculations of diffusion, it is concluded that while sucrose initially removes extracellular ions, after oil is placed in the gap, a residuum of sucrose adheres to the tissue and thus provides a layer of sucrose into which intracellular ions diffuse. A shunt pathway is thereby again created.

If it were technically feasible to execute a complete test run of impedance measurements over a range of frequencies within a period of 10 s after exposure to sucrose, measurements of impedance would have some reliable basis. The time required to carry out bridge balancing over a wide range of frequencies is far in excess of this minimum. If the variation in the measurements is due to the diffusion of intracellular ions into the sucrose, then the size of the tissue being used would alter the time constant but not the direction of the gradual diffusion process itself. Therefore, the discrepancy between decreased (Freygang and Trautwein, 1970) and increased (Trautwein et al., 1965) resistance values reported in sheep Purkinje fibers is probably not due to time. Squid axon, a larger cell, would have a somewhat longer time constant and perhaps the measurements would not change significantly within the first half hour, but the problem would be the same. The varying impedance values are not unique to the moth heart, for additional experiments were carried out on frog atrial strips and the same instability in measurements was encountered.

These results demonstrate that the duration of exposure to the insulating fluid and the time during which the measurements are carried out are of such crucial im-

portance that values reported using these techniques must be carefully scrutinized and their reliability critically weighed.

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