THE ELECTRICAL IMPEDANCE OF MUSCLE DURING THE ACTION OF NARCOTICS AND OTHER AGENTS

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INTRODUCTION

Since Bernstein first put forward the membrane hypothesis in 1900, the peculiar importance of the membrane in nerve and muscle function has been recognized. Because of their extreme thinness nerve and muscle fiber membranes have proved difficult to measure and to study directly.

With the electrical impedance method it is possible to analyze the electrical properties of these delicate structures under various conditions without injury to them. Höber (1912) in studying the conductivity of a suspension of erythrocytes, was the first to use high frequency impedance measurements on biological material. Fricke developed the theory of electrical impedance measurements and was the first to suggest an equivalent circuit for the living cell. More recently Cole has extended the measurements to marine eggs, muscle, nerve, and various other tissues. The success of the method as used by Cole and Curtis in determining changes in the electrical properties of Nitella and the squid giant axon during activity (Cole and Curtis, 1938, 1939) suggested that electrical impedance measurements of muscle might throw light upon the membrane changes which accompany the action of certain agents, especially narcotics.

Lillie (1922) reasoned that on the theory that normal excitability and conductivity of the irritable living system is determined by the properties of the surface film, one should expect that in narcosis the membrane would be rendered less alterable than before; i.e., stabilized. Höber (1907) held a similar view, and both agree that diminution of

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permeability accompanies narcosis. However, more recently, an initial increase in permeability has been found to accompany narcosis in certain organisms under certain conditions (e.g. Seifriz, 1923; Höfler and Weber, 1926).

Inasmuch as electrical impedance measurements can be used as an indication of permeability of membranes to ions, it was decided to reinvestigate the question with this method.

**Material and Methods**

The sartorius muscle of *Rana pipiens* was used throughout.

**Muscle Chamber.**—The construction of the muscle chamber can be noted in Fig. 1. Actually, three variations of this chamber were used in the course of the experiments but they did not differ materially except for the size and shape of electrodes used.

The chamber consists of two plates of bakelite separated by celluloid spacers of various thicknesses. The four brass screws which unite the two plates are insulated by bakelite cups in one model. In another model (in which there are round electrodes 1 mm. in diameter) they are smeared with vaseline and situated quite a distance away from the electrodes.

The electrodes, which are of platinum black upon platinum, are flush with the surface of the bakelite plates. Copper wires are soldered to them and pass out of the cell through holes drilled in the bakelite. Where they emerge the copper wires are coated with picein cement to preclude the possibility of moisture seeping in to the copper-platinum junction.

The electrodes are replatinized for each experiment and are always kept moist. The excellent reversibility of the reactions studied shows that no significant amount of the agents used upon the muscle is adsorbed by the platinum black in such a way that it cannot easily be washed away.
As has been mentioned three types of electrodes were employed (a) small round electrodes 1 mm. in diameter, (b) larger discs 15 mm. in diameter, and (c) long strip electrodes 20 mm. in length and 1 mm. wide. The first type was found to be unsatisfactory inasmuch as its small size does not permit parallel lines of current flow. The long electrodes (c) were most satisfactory from the point of view of parallel current flow. As the length of the electrodes is increased the central component of the current flow, which is perpendicular to the fiber axes of the muscle, becomes increasingly important and the end components, where the current flows out into the tissue beyond the ends of the electrodes and the lines of current flow are not parallel, become less important. It is much easier to analyze transverse impedance measurements where the lines of current flow are mostly parallel and perpendicular to the muscle, and for this reason the muscle chamber containing the strip electrodes 20 mm. long and 1 mm. wide was the one finally selected for these studies although the others were used in preliminary work.

To give an example of the type of difficulty which was encountered when using the small electrodes (1 mm. in diameter): it was found impossible to determine with these electrodes the resistance of the moist strips of filter paper which were used for circulation of solutions past the muscle. To be specific, with the small electrodes the resistance of a muscle covered with one strip of filter paper seemed to be greater than the resistance of a muscle covered with two strips of filter paper (one on each surface). This was obviously a problem to be investigated. The difficulty disappears when larger electrodes are used. In other words, when longer electrodes are used, the total resistance of muscle plus filter papers increases as filter papers are added, as is to be expected.

The reason for the anomalous results with small electrodes is that with small electrodes, most of the lines of current flow are not parallel. The electrical set-up is a complicated one which may be expressed by saying that it results in a situation in which the resistances of muscle and filter papers are not in series with one another.

Solutions Used.—The composition of the Ringer's solution used in these experiments was as follows: 7 gm. NaCl, 0.3 gm. KCl, 0.25 gm. CaCl₂ in a liter of solution. The solution was buffered to pH 7.4 with sodium bicarbonate.

All salt solutions and sugar solutions used were isosmotic with frog blood as calculated from depression of freezing point data.

Ringer's solution to which a small quantity of narcotic had been added was used in some experiments. These solutions are not strictly isosmotic with frog blood, but the amount of narcotic added was in every case quite small and the departure from the isosmotic condition was not considerable.

In practically all cases the pH of the solutions was adjusted to 7.4 (the pink of phenol red) with sodium bicarbonate. It was not found necessary to control the temperature.

Method of Circulation.—Solutions are circulated past the muscle by means of two moist filter paper strips completely covering the muscle and placed one on
each side between the muscle and each electrode (Fig. 1). The dimensions of the filter paper strips are approximately 5 mm. by 10 cm., their width being approximately the same as the width of the muscle.

Solutions are conducted through them to the muscle very rapidly. If a second solution of a different conductivity from the one which has been circulating is introduced, an impedance change is noted almost immediately in the headphones. Also, solutions seem to be washed out from filter paper strips as quickly as they enter.

The rate of drip of the solutions was kept fairly constant from experiment to experiment, 1 cc. of solution being delivered in about 35 seconds.

Whether the filter papers run along the muscle (parallel to the long axis of the muscle) or across the muscle (at right angles to the long axis of the muscle) seems to have no measurable effect upon either (1) the final equilibrium resistance value when a new solution is circulated or (2) the time constant (the term “time constant” will be used throughout to mean the time interval elapsing between the beginning of the reaction and the time when half the effect has taken place).

When it is realized that the muscle volume is only about 0.084 cc. and the “sugar space” (which, as will be proven later, is intercellular space) is only about 0.021 cc. and that the volume of solution delivered in a few seconds is much greater than the sugar space, it is understandable that it should make practically no difference whether the filter papers are placed along or across a muscle. Also, diffusion into the muscle from the point of contact with the solution is probably very rapid.

This filter paper method of changing solutions is much to be preferred to one method used by Osterhout (1922) in measuring the conductivity of Laminaria. When changing solutions Osterhout took his measuring cell apart, removed the tissue, washed the tissue in the new solution, and replaced it in the measuring chamber. In the experiments here reported, the handling of the tissue and variations in pressure attendant upon the reassembling of the cell were eliminated. Also, continuous uninterrupted reading during diffusion of the new solution into the tissue was possible.

The electrical properties of the filter papers are not altered by the solutions which are circulated. It was found that the ratio of the resistance per filter paper to the specific resistance of the solution circulated is practically the same if Ringer’s solution or a solution of half isosmotic sugar and half Ringer’s solution (50 per cent sugar) is used:

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<tr>
<th></th>
<th>Ringer’s solution</th>
<th>50 per cent sugar</th>
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<tr>
<td>Resistance per filter paper</td>
<td>0.092</td>
<td>0.088</td>
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<tr>
<td>Specific resistance of solution</td>
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In many cases the muscle was tested at the end of the experiment and was found to be excitable.
**Apparatus**

The alternating current Wheatstone bridge used was devised by Cole and Curtis (1937) and has been described by them. The current sent through the measuring cell is so small that the impedance was independent of it. Transverse impedance was measured at all times. It was measured as parallel resistance, $R_p$, and capacity $C_p$.

With the substitution method used in these experiments, an accuracy of 0.5 per cent was obtained.

The equivalent series resistance $R_s$ and the series reactance, $X_s$, were calculated from the formulae:

$$R_s = \frac{R_p}{1 + (R_pC_p\omega)^2}; \quad X_s = \frac{R_p^2C_p\omega}{1 + (R_pC_p\omega)^2}$$

![Figure 2](image_url)

**Fig. 2.** Frequency impedance locus, i.e. series resistance, $R_s$, vs. series reactance, $X_s$, for transverse measurements of frog sartorius muscle in Ringer's solution. Frequencies indicated are in kilocycles per second.

In the earlier experiments complete frequency runs were made from 200 cycles to 1000 kc. (0.2 kc., 0.5 kc., 1 kc., 2 kc., 5 kc., 10 kc., etc.). When it was noted however, that the experimental points on the resulting impedance locus gave so good a fit (Fig. 2), it was decided for the sake of speed and simplicity to determine but three frequency points experimentally, i.e. 1 kc., 10 kc., and 100 kc., since three points determine a circular arc. Inasmuch as speed was of considerable importance in some of the experiments where impedance measurements were made during the action of a chemical agent, this procedure was found to be a great convenience.

In some experiments dealing with the effect of chemical agents upon electrical impedance over long times, impedance measurements were made at 1000 cycles only. It might at first seem preferable to make D.C. measurements in order to
follow changes of membrane resistance. However, d.c. measurements cannot give any indication of capacity variations which may be taking place.

**Theory and Calculations**

The theoretical muscle may be considered to be a uniform random distribution of parallel, circular, cylindrical fibers in a medium (Bozler and Cole, 1935; Cole and Curtis, 1936). Then the specific resistance of the muscle perpendicular to the fiber, \( r \); the resistance of the medium, \( r_1 \); the resistance of the fibers, \( r_2 \); and the volume concentration of the fibers in the medium, \( \rho \), bear the following relationship to one another:

\[
\frac{r}{r_1} - 1 = \left( \frac{r_2}{r_1} - 1 \right) \frac{r_2}{r_1} + 1
\]

The above equation is analogous to the Maxwell equation for the conductivity of a suspension of spheres.

It has been shown that the muscle is the electrical equivalent of a circuit containing at least two resistances and one variable impedance element of constant phase angle (Bozler and Cole, 1935). If the series resistance of such a circuit, \( R_s \), is plotted on the abscissa and the series reactance, \( X_s \), is plotted on the ordinate, for different frequencies, the circle diagram or impedance locus, which has long been used in communication engineering and which was introduced by Gildemeister and by Cole to express biological data, is obtained. Theoretically, it would be expected that the center of the circular arc representing the impedance locus of such a circuit would lie on the resistance axis; i.e., that the phase angle would be 90°. It is found experimentally for muscle that the center of the circle is below the resistance axis and that the phase angle is less than 90° (Fig. 2). Cole interprets this to mean that the single variable impedance element in the circuit has dielectric loss.

On the assumption that the membrane is non-conducting (as will later be shown to be the case) it is possible to solve for \( \rho \) in the following manner:

\[
1 - \frac{r_2}{r_0} = \rho \frac{r_1}{r_0}
\]

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where \( r_0 \) is the extrapolated zero frequency resistance. Then

\[
\frac{r_2 - r_1}{r_0 - r_\infty} = \frac{r_2}{r_0} - \frac{r_1}{r_\infty}
\]

where \( r_\infty \) is the extrapolated infinite frequency resistance.

**Discussion of Results**

It was decided to investigate first the effect of simple inorganic cations upon the electrical impedance of muscle. When the 1000 cycle resistance of a muscle treated with certain cations, *i.e.* Ba and Ca, was plotted against time it was found that the curve describing the resistance changes consisted of two parts (Fig. 3). It was thought that perhaps the first part of the curve represented merely penetration of the cation into the intercellular spaces of the muscle and the second, interaction of the cation with the muscle tissue. In order to investigate this possibility, the effect upon electrical impedance of the diffusion into the intercellular spaces of the muscle of a substance which was known not to react with the muscle substance and to be, physiologically speaking, rather inert was studied. Mixtures of Ringer's solution and isosmotic sugar solutions were used for this purpose. When the effect of diffusion into the intercellular spaces alone upon the electrical impedance of muscle had been quantitatively determined it was then possible to continue the studies and to investigate the effect of complex organic narcotics.

**Effect of Inorganic Cations upon Electrical Resistance of Muscle**

The effect of inorganic cations upon the 1000 cycle resistance of frog sartorius was studied in the following manner. All solutions used were isosmotic with frog Ringer's solution and were circulated past the muscle by means of strips of filter paper as described above. Chlorides of sodium and potassium and of the alkali earths: barium, calcium, and magnesium were used. It is important that all solutions used be strictly isosmotic with frog blood inasmuch as varying osmotic pressure seems to have a profound influence upon the electrical resistance of muscle as can be seen from an experiment where 50 per cent, 120 per cent, and 200 per cent Ringer's solution was employed. By a 50 per cent Ringer's solution is meant a solution containing one part of Ringer's solution to one part of distilled water, while by a
Fig. 3. Thousand cycle resistance of muscle, expressed as percentage of equilibrium value in Ringer's solution, vs. time in minutes after substitution of test solutions for Ringer's solution. Typical experiments. All solutions isosmotic with Ringer's solution. Resistance of isosmotic chloride solutions compared with resistance of Ringer's solution: NaCl, 101 per cent; KCl, 96 per cent; BaCl₂, 81 per cent; CaCl₂, 87 per cent; MgCl₂, 91 per cent.

Fig. 4. Influence of Ringer's solutions of various osmotic pressures upon resistance of muscle. Thousand cycle resistance of muscle, expressed as percentage of equilibrium value in pure Ringer's solution, vs. time in minutes after substitution of test solution for Ringer's solution. Test solutions are: 50 per cent Ringer (○), 120 per cent Ringer (●), and 200 per cent Ringer (●).
200 per cent Ringer's solution is meant a solution in which the salts are dissolved in one-half of the usual amount of distilled water. In Fig. 4, percentage resistance change is plotted against time. It will be noted that a hypotonic solution causes a marked rise in resistance while hypertonic ones cause a drop. This is, of course, to be expected since the conductivities of these solutions differ from that of Ringer's solution. It should also be noted, however, that the muscle has been found to follow Boyle's law and act as a simple osmometer when treated with Ringer's solutions of different osmotic pressures (Chao, Chiao, and Chi, 1938).

The effect of inorganic ions upon 1000 cycle resistance of muscle can be seen in Fig. 3, where the percentage change in resistance (the resistance in Ringer's solution being taken as 100 per cent) on addition of the solutions is plotted against time. Addition of the isosmotic chloride solution was not begun until the muscle had reached a steady resistance value in Ringer's solution. If not treated, this equilibrium value may be maintained for many hours. Each curve represents a typical experiment. In all, fourteen experiments of this type were done.

In every case the difference in conductivity between Ringer's solution and the isosmotic chloride solution should be taken into consideration in estimating the percentage change. The resistances of the solutions are listed under Fig. 3. (The differences in conductivity could not be corrected for in the graph since the rate of penetration of the solutions was not known.)

The resistance of a solution of MgCl₂ isosmotic with frog Ringer's solution was found to be 91 per cent of that of pure Ringer's solution. Thus the 10 per cent drop in resistance of the muscle after treatment with isosmotic MgCl₂ is not significant, and we may say that isosmotic MgCl₂ has no effect upon the 1000 cycle resistance of sartorius muscle. Also, addition of NaCl and KCl, respectively, seems to have little effect upon the resistance of muscle. Isosmotic KCl raises the resistance 1 or 2 per cent during the course of about 2 hours and NaCl raises it a little more (about 10 per cent). The small effect of NaCl and KCl is surprising inasmuch as these substances cause marked contracture in muscle (Gasser, 1930). Isosmotic solutions of BaCl₂ and CaCl₂, on the other hand, markedly affect muscle resistance, causing it to drop.
A slight degree of recovery (as measured by increase in resistance in the direction of the normal value) was obtained in these experiments after the muscle had been allowed to remain in the isosmotic BaCl₂ and CaCl₂ solutions for as long as 2 or 3 hours. If solutions containing one part of isosmotic alkali or alkali earth chloride solution and four parts of Ringer's solution are used, the normal resistance is maintained for hours.

The relative lack of effect of pure isosmotic solutions of NaCl, KCl, and MgCl₂ upon the muscle, insofar, at least, as these effects are mirrored in impedance changes is of interest. Since the work of Sidney Ringer, the importance of balanced salt solutions and the toxicity of solutions of single pure isosmotic salts have been stressed again and again. Since impedance measurements are usually (cf. experiments with narcotics reported in this paper, for instance) a delicate means of determining physiological membrane changes, and toxicity has been associated with breakdown of membrane resistance, it might be supposed that pure solutions of Na, K, and Mg would affect muscle resistance markedly, yet this is not the case.

In this connection it might be interesting to refer to observations which will be published shortly on injury potentials of the non-myelinated nerve of the spider crab, where it was found that pure isosmotic solutions of the chlorides of the earth alkalis: Ba, Ca, Sr, and Mg have no effect upon the magnitude of the injury potential when applied to the uninjured portion of the nerve. Inasmuch as substances which are ordinarily considered toxic cause the injury potential to fall or, if strong enough, completely disappear, when applied to the uninjured part of a nerve, one would expect pure isosmotic solutions of the earth alkalis to have the same effect if they were truly toxic.

In general then, it may be said that while changes in osmotic pressure of solutions affect muscle impedance greatly, the use of certain salt solutions which are not physiologically balanced does not seem to do so. Some cause other than the fact that they are "pure solutions" and "unbalanced" must be found to account for the strong action of isosmotic CaCl₂ and isosmotic BaCl₂.

It is dangerous to generalize concerning the action of ions upon electrical resistance of tissues and membrane permeability. Osterhout
found in most cases, from observations on *Laminaria* and other similar forms, that monovalent ions cause electrical resistance to fall and that bivalent ions cause it to rise and then fall (Osterhout, 1922). However, the work of others on different types of tissue (e.g. Hogben and Gordon, 1930) as well as the results of these experiments make a generalization inadvisable.

It will be noted that there is a distinct break in the curves representing the influence of isosmotic BaCl₂ and CaCl₂ upon the resistance of muscle (Fig. 3). The break was encountered in every experiment where these solutions were used. It was thought that possibly the first part of the curve represented penetration of the substance into the muscle and the second interaction between the substance and the muscle. As has been stated above, it was in order to investigate this question, as well as for other reasons that it was decided to study the question of penetration in connection with some substance which was believed to be relatively inert as far as interaction with living tissues is concerned.

**Experiments with Sugar**

*Why Sugar Solutions Were Used.*—It has been established (Overton, 1904) that although isosmotic sugar solutions cause loss of irritability, the sugar itself is not toxic, but the effect is produced as a result of the absence of intercellular electrolytes. If one part of Ringer's solution or isosmotic NaCl is added to as much as three or four parts of isosmotic sugar solution, no loss of irritability results. Such a solution may be said to be physiologically inert, and yet it has a conductivity very different from that of pure Ringer's solution. It seemed to be an ideal type of solution to use for penetration studies, inasmuch as if the resistance of the muscle in Ringer's solution is first determined and the specific resistance of the sugar solution is known, the electrical resistance of a muscle treated with the sugar solution will throw some light on the penetration of sugar or exit of electrolytes from the intercellular spaces. By observing changes in electrical resistance we should be able to study the time course of diffusion, reversibility of diffusion, and similar problems. Penetration of the substance into the interior of the muscle fibers, if any, the effect of the substance upon the integrity of the fiber membranes, and like problems may
be investigated if resistance and capacity to an alternating current are measured over a wide frequency range.

Sugar-Ringer Mixtures Do Not Alter the Tissue.—In order to be certain that mixtures of Ringer's solution and isosmotic sugar solutions are truly inert and do not alter the tissue in any way, i.e. that they merely penetrate the intercellular spaces and do nothing else, the following experiment was performed.

![Graph](image)

Fig. 5. Thousand cycle resistance of muscle in various concentrations of isosmotic dextrose-Ringer mixtures vs. time in minutes. Percentages indicate concentration of dextrose in dextrose-Ringer mixtures. ○, muscle in Ringer's solution; ●, muscle in Ringer-dextrose mixture. Typical experiment. All data obtained on same muscle. Note almost perfect reversibility.

A muscle which had attained an equilibrium resistance value in Ringer's solution was treated successively with various concentrations of Ringer's solution-isosmotic sugar solution mixtures, and recovery in pure Ringer's solution was permitted after treatment with each concentration of sugar (Fig. 5). Reversibility of resistance changes due to treatment with sugar was nearly perfect. This makes it difficult to believe that the sugar solutions cause serious leakage of salts from the muscle fiber interiors.
Besides, it was found that the ratio:

\[
\frac{1000 \text{ cycle resistance of muscle}}{\text{Specific resistance of solution}}
\]

for the muscle in Ringer's solution and in each concentration of sugar remains remarkably constant. In the experiment represented in Fig. 5, the mean is 19.8 with a variation of only \(\pm0.33\) from the mean. This indicates that at 1000 cycles the fiber membranes are practically non-conducting, at least as far as can be detected by these measurements, and that the sugar space is the intercellular space. By entirely different experimental procedure, Urano and Fahr have shown that the electrolytes replaced by sugar are intercellular electrolytes (Urano, 1908; Fahr, 1909).

When the percentage of Ringer's solution in the Ringer-sugar mixtures used is plotted against the reciprocal of the resistance of the muscle in that mixture, the data fit a straight line passing through the zero point fairly well (Fig. 6). This would seem to indicate that what we are measuring is the amount of Ringer's solution present in the intercellular spaces, and, again, that the fiber membranes are non-conducting at 1000 cycles.

**Sugar-Ringer Mixtures Do Not Cause Swelling or Shrinking.**—When the volume concentration, \(\rho\), is calculated from the data furnished by such an experiment, it is found to remain constant when varying concentrations of sugar are used to bathe the muscle, indicating that sugar of these concentrations (10 to 50 per cent) causes no swelling nor shrinking of the fibers. If sugar solutions of these concentrations did cause swelling or shrinking, their effect would increase with increasing concentration, and \(\rho\) would not be similar for all of them.

**Rapidity of Diffusion.**—Diffusion of sugar into muscle (or exit of electrolyte from the intercellular spaces) is quite rapid, half the effect being over, when two filter paper strips are used, in about three-quarters of a minute. If sugar were able to penetrate the fiber membranes, the rapidity of diffusion would, of course, be greater. Inasmuch as narcotics, as will later be shown, do penetrate fiber membranes, the value obtained with sugar sets an upper limit to the time required for penetration of narcotics through muscle.

In Fig. 7, 1000 cycle resistance is plotted against time for two paired muscles treated with 40 per cent sugar (isomotic sugar solution:
Ringer's solution as 4:6), in one case the muscle being in contact with two filter papers (one on each side) and in the other, with only

![Graph](image)

**Fig. 6.** Reciprocal of equilibrium value of thousand cycle resistance of muscle in various concentrations of isosmotic dextrose-Ringer mixtures vs. percentage of Ringer in dextrose-Ringer mixture. Data from same experiment as is represented in Fig. 5.

![Graph](image)

**Fig. 7.** Thousand cycle resistance of muscle in Ringer's solution (○) and in 40 per cent dextrose, i.e. isosmotic dextrose solution: Ringer's solution as 4:6, (●) vs. time in minutes. On left, solutions circulated past muscle by means of one filter paper; on right, solutions circulated past muscle by means of two filter papers, one on each side of muscle. Same muscle in both experiments. On left, equilibrium (1 per cent change in 3 minutes) attained in 23.5 minutes; on right, in 6.5 minutes.
one filter paper. It will be noted that the total rise in resistance after treatment with sugar is about the same for both muscles, but that the time necessary for this resistance change to be brought about is four times as long when only one filter paper is in contact with the muscle as it is when two filter papers are in contact with the muscle, one on each side. Theoretically of course, this result is to be expected since when there are two filter papers, not only need a substance diffuse only half as far into the muscle, but it is diffusing in from both surfaces simultaneously.

The ratio of the times of rise in the two cases, 1:4, is to be expected if the process follows the well known diffusion equation which states that the amount of substance diffusing in is proportional to the square root of the time. This equation has been shown to hold for the diffusion of lactic acid and oxygen through muscle (Eggleton, Eggleton, and Hill, 1928) and for the diffusion of inorganic phosphate through muscle (Stella, 1928).

Additional Data Obtained from Sugar Experiments.—Values for \( \rho \), the volume concentration of fibers; \( r_s \), the resistance of the interior of the fibers; and \( \theta \), the phase angle of the impedance locus, were calculated for the muscle in Ringer's solution and in the sugar mixtures according to the equations quoted above (cf. Theory and calculations).

The volume concentrations in Ringer's solution vary from 75 per cent to 81 per cent with an average of 77.7 per cent. The \( r_s \) values in Ringer's solution vary from 176 to 310 with an average of 231 ohms specific resistance, which is about 3.2 times that of Ringer's solution. The phase angle, \( \theta \), was found to average 71.0°.

These values of \( \rho \), \( r_s \), and \( \theta \) compare favorably with those obtained for frog sartorius by Bozler and Cole (1935). They obtained volume concentrations of 77.3 per cent and 76.2 per cent and \( r_s \) values of 264 and 253 for two muscles, and found the phase angle to average 71°. Schulze (1927), using a somewhat different method, obtained a volume concentration of 86.1 per cent.

It will be noted that with low concentrations of sugar, e.g. 25 per cent, the volume concentration remains constant, as does the fiber interior resistance value. In other words, it seems that dilute sugar solutions do not cause swelling or shrinking or penetrate into the
fibers, so far as can be detected by our methods. When higher concentrations of sugar are used, however, we find a different situation. In 50 per cent sugar (one experiment) the volume concentration seems to remain practically the same as in Ringer's solution, but the resistance of the interior of the fibers increases about twofold. Two experiments were done with a 75 per cent sugar solution. It is difficult to tell whether this solution causes membrane permeability to change or shrinking of fibers to take place. At any rate the resistance of the fiber interiors increases about two and one-half times.

The findings just enumerated, however, although interesting, are by-products of the sugar experiments. The rapidity of the resistance changes on treatment of the muscle with sugar solutions indicates that penetration alone cannot account for the small break in the resistance curve which was always encountered when the muscle was treated with Ba or Ca, and suggests that intercellular penetration need not be taken into consideration when studying the effect of organic narcotics.

Once the troublesome question of the order of magnitude of impedance changes due to penetration between the fibers alone had been dealt with, it was possible to begin the study of the effect of the more complex substances.

Effect of Narcotics upon Muscle Impedance

The following group of substances, each of which has some narcotic action, surface active behavior, or lipid solubility was studied: saponin, chloroform, sodium taurocholate, butyl and amyl alcohol, iso-amyl carbamate, chloral hydrate, and sodium salicylate. All of these were found to depress 1000 cycle resistance of muscle when used in sufficient quantity.

Similar results were obtained for Laminaria and for frog skin with ether, alcohol, chloroform, and chloral hydrate by Osterhout (Osterhout, 1922). Gildemeister (1920) studied, by measuring the inductance necessary to neutralize it at 1000 cycles, the capacity of frog skin and muscle during the action of various agents.

An analysis of the effect upon 1000 cycle resistance of muscle of increasing doses of iso-amyl carbamate is presented in Fig. 8, where resistance is plotted against time. It will be noted that minute changes in concentration alter the character of the influence of the
drug upon resistance. As the concentration of the drug is increased, the rise in resistance is followed by a gentle fall in resistance. On increasing the concentration still further, the rise in resistance is momentary and the depression of resistance more marked. It has been known for some time that dilute solutions of narcotics have an effect different from that of higher concentrations. This is brought out quantitatively by the electrical resistance experiment just described. Besides it is possible in such an experiment to follow the effect of the narcotic with time and to establish that the action of a dilute narcotic solution over a long time resembles that of a higher concentration acting for a shorter time.

Since dilute solutions of narcotics result in anesthesia, while higher concentrations are toxic, these results suggest a correlation of resistance increase with anesthesia, and resistance decrease with toxicity.

On a somewhat different basis, Osterhout (1922) working with
alcohol and ether on *Laminaria agardhii*, was led to a similar conclusion. He found that the initial rise in resistance encountered when using these agents was reversible, while the subsequent drop in resistance below normal was not. This led him to believe that anesthesia, which is by nature inherently reversible, is associated with permeability decrease, while the subsequent irreversible toxicity on continued action of the narcotic is associated with permeability increase.

It was felt that perhaps information from impedance data over a wide frequency range and long times could throw light upon which aspects of the electrical characteristics of the cell; *i.e.*, resistance of the medium, of the fiber interior, resistance, and capacity of the membranes were affected by narcotics.

Cole and Curtis (1938) have just found for *Nitella* that on excitation there is a 200-fold decrease in membrane resistance and a 15 per cent decrease in membrane capacity, while in the giant fiber of the squid (Cole and Curtis, 1939) there is a 30-fold decrease in membrane resistance and a 2 per cent decrease in membrane capacity. It was hoped that an analysis similar to theirs might throw some light upon the mechanism of narcosis.

Cole and Curtis have shown that on theoretical grounds, if the membrane resistance alone were to change, the impedance locus at a single frequency would describe a circular arc which is tangent to \( r_0 \) and has its center on a line perpendicular to the resistance axis and running through \( r_0 \) if \( \theta \) is unchanged. If, on the other hand, the membrane capacity alone were to change, the impedance values at any one frequency would follow along the familiar frequency impedance locus.

It was found that during the action of narcotics the impedance follows almost perfectly the path predicted for a change in membrane resistance alone (Figs. 9 and 10). With weaker drugs, *e.g.* 0.34 isopropyl carbamate (Fig. 10) the circular arc describing the impedance change with time does not actually pass through the \( r_0 \) point, but if extrapolated, it would. With stronger solutions, *e.g.* a solution of Ringer's solution saturated with chloroform (Fig. 9), the arc actually does pass through the \( r_0 \) point.

In other words, narcotics break down the membrane resistance progressively and, if strong enough, and permitted to continue their
action long enough, completely. Membrane capacity is only very slightly affected by narcotics.

In every case when a drug was first applied there was a short initial increase in membrane resistance. This must not be overlooked since it may be associated with the initial narcotizing action of the anesthetic, while the decrease in membrane resistance on longer action may be linked to toxicity. This is all the more probable since the initial increase in membrane resistance is more pronounced when a lower concentration of narcotic is used than when a strong dose is employed (Fig. 10).
In connection with the results here obtained with narcotics, it would be interesting to ascertain the effect of possible swelling or shrinking of the muscle fibers upon the impedance of muscle. Secondly, proof that the changes which have been ascribed to the membrane are, indeed, due to changes in the fiber membranes (plasma membranes) and not to changes in the fibrillae, the mitochondria, or other cell inclusions, is also necessary.

Swelling and Shrinking.—As to the first point, if swelling or shrinking takes place on application of a drug, and swelling or shrinking affects impedance, the circle diagram should be shifted to the right or to the left, and the extrapolated infinite frequency resistance should be different when the muscle is in Ringer’s solution or in the drug. This, however, is not the case (Fig. 11); the extrapolated infinite frequency resistance during drug action coincides with the extrapolated infinite frequency resistance during treatment with Ringer’s solution.

It was thought that this question might be further studied by determining in the following manner whether the sugar space, i.e. the intercellular space, is altered during the action of a drug.

A muscle which had first attained equilibrium in Ringer’s solution was treated first (a) with a dilute solution of a drug dissolved in Ringer’s solution, secondly, after equilibrium had again been attained (b) with a solution of one part of a concentration twice as great of the drug dissolved in Ringer’s solution plus one part of isosmotic sugar solution and then thirdly (c) with the dilute solution of drug dissolved in Ringer’s solution again. Since it had already been established that 50 per cent sugar does not cause appreciable swelling nor shrinking of the fibers, it was felt that the sugar space might remain unaltered if the drug itself causes no swelling or shrinking and a dilute concentration of drug is used.

However, this method was not very satisfactory inasmuch as the action of the drug often continued when solution (b) was used and may have prevented the resistance from reaching the value it would ordinarily have reached with 50 per cent isosmotic sugar alone.

In some cases the ratio:

\[ \frac{R_0}{R_1} \]

where

- \( R_0 \) is the resistance of muscle in medium
- \( R_1 \) is the resistance of medium
was practically constant for solutions (a) and (b) but this constancy seems to be the result of a delicate balance of just the correct con-

Fig. 11. Frequency impedance loci, i.e. series resistance, $R_s$, vs. series reactance, $X_s$, for muscle in Ringer's solution (○) and in Ringer's solution saturated with chloroform (●). Frequencies are given in kilocycles per second.

Fig. 12. Thousand cycle resistance of muscle in Ringer's solution (○), in 1-400 Na salicylate dissolved in Ringer's solution (●), and in one part 1-200 Na salicylate dissolved in Ringer's solution plus one part isosmotic sucrose solution (●) vs. time in minutes.
centrations. In the cases where the ratio: \( R_o \div R_1 \) did not remain constant for solution (a) and solution (b), it was always noted that after maximum resistance was attained in solution (b) the curve representing the resistance sloped downward (Fig. 12). This would suggest that the action of the drug continues in solution (b) inasmuch as no decline was ever encountered when sugar and Ringer's solution alone were used.

It is possible that some swelling or shrinking takes place when the various narcotics mentioned above are used. However, it is highly improbable that swelling or shrinking can account for the entire effect of narcotics upon muscle impedance.

Is the Impedance of the Fiber Membranes Being Measured?—It has been assumed all along that the complex impedance locus is the locus of the impedance of the plasma membrane and that the changes in the overall muscle impedance are due to changes in membrane impedance. It might be well to examine now the arguments which support this assumption.

Höber's experiments on the conductivity of erythrocytes (Höber, 1912), which have already been mentioned, suggest strongly that it is the cell membrane which offers impedance to alternating current. He found that the conductivity of erythrocytes to alternating currents of nine million cycles per second is about the same as that of a solution of disintegrated cells at any frequency. Living cells have a lower conductivity than dead ones. That this is due to the presence of cell surfaces rather than to the cell as a whole is shown by the above type of experiment.

The simplicity of the complex impedance locus of muscle (Fig. 2) favors the view that there is only one type of structure which is offering resistance to the measuring current. That this single structure is the plasma membrane is probable since Cole has found that a circle diagram describes the complex impedance locus for a great variety of cells and tissues.

An argument which favors the view that it is the cell membrane rather than the membranes of the fibrillae which has been measured in these experiments on muscle, is the following. If it is the fiber membranes which are the structures offering impedance to the electric current, the calculated fiber membrane capacity is of the order of 1.0 \( \mu \text{f./cm}^2 \) (Cole and Curtis, 1936); if it is the membranes of the
fibrillae, their membrane capacity would have to be about 20 μf./cm.²
Inasmuch as practically every type of living material measured has
been found to have a membrane capacity in the neighborhood of
1 μf./cm.², it is highly probable that it is the fiber membrane which
is being measured here.

CONCLUSIONS

Cole and Curtis (1938) consider that the membrane capacity
represents the ion-impermeable aspect of the membrane and the
membrane resistance, the ion-permeable aspect. In the case of
narcotics, we have an example of the independence of these two postu-
lated aspects of the membrane. Here one, the ion-permeable, alone
is affected. This same aspect of the membrane is affected in excita-
tion in Nitella and the squid giant nerve fiber (Cole and Curtis, 1938,
1939). In fertilization of marine eggs, on the other hand, the non-
conducting, ion-impermeable part of the membrane appears to be
changed (Cole and Spencer, 1938). It would seem reasonable to
suppose that narcosis and injury, on the one hand, would have a
greater resemblance to excitation, on the other, than either would
have to the mechanism behind fertilization. This supposition seems
to be supported by impedance data.

That, in general, membrane resistance is a less stable aspect of
the membrane than is membrane capacity, also seems to be brought
out by these experiments on the effect of narcotics upon muscle
impedance.

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SUMMARY

1. The effect of certain inorganic cations upon the electrical im-
pedance of the sartorius muscle of the frog was investigated. While
Na, K, and Mg have little effect upon the resistance of muscle, Ba
and Ca cause it to fall. The use of physiologically “unbalanced”
salt solution does not in itself seem to affect muscle impedance.

2. The time course of the effect upon muscle impedance of the
penetration of substances into the intercellular spaces was studied by treating the muscle with sugar solutions. Half of the effect is over in three-quarters of a minute when the sugar solution is permitted to circulate past both sides of the muscle. This sets an upper limit for the time necessary for inorganic cations and organic narcotics to reach the cell surfaces. The action of inorganic cations and organic narcotics upon muscle is slow compared to the time necessary for them to reach the scene of action.

The penetration of the sugar solutions into the intercellular spaces of muscle was found to follow the well known diffusion law, the amount diffusing in being proportional to the square root of the time.

Average values of 77.7 per cent for \( \rho \), the volume concentration of fibers; 231 ohms specific resistance for \( r_I \), the resistance of the interior of the fibers; and 71.0° for \( \theta \), the phase angle of the impedance locus, were obtained for the muscle in Ringer's solution. How these values change when the muscle is placed in various concentrations of sugar was also studied.

3. The action of a number of organic narcotics upon muscle was studied. All decrease 1000 cycle resistance if the concentration is sufficiently high. A detailed analysis of the action of the narcotic, iso-amyl carbamate, was made, and it was noted that low concentrations increase resistance while higher concentrations decrease it.

By investigating the effect of narcotics upon muscle impedance over a wide frequency range, it was found that during narcosis the resistance of the fiber membranes first increases and then decreases, and, if the drug is present in sufficiently great concentration, membrane resistance may completely disappear. Membrane capacity is only very slightly affected.

BIBLIOGRAPHY


