FACTORS INFLUENCING ELECTRICAL PROPERTIES OF WHOLE SMALL ANIMAL BODIES

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There are several indications that the measurement of electrical impedances of whole animal bodies might provide useful information concerning the physiological state of the animal, particularly with regard to the water and electrolyte content of the tissues. Such measurements have been used as a diagnostic tool in the study of thyroid disease, (Brazier, 1933; Barnett, 1937; Krepsky, 1950, for example.

Unfortunately, most of the studies to date have been made on the human body, and there has been very little opportunity for study of the physical and physiological factors contributing to variations in these properties. The work to be described herein was done preliminary to making a series of measurements for the determination of the influence of endocrine factors on the electrical properties of the body.

It is apparent that the use of small animals for these studies would make possible studies on ablation of endocrine organs, injection of hormones, etc. Such studies are difficult to perform on humans. Also, such experiments can be performed in much larger numbers on small animals, so that statistical variations can be dealt with. For several reasons, it was decided to make these measurements on white rats.

In order to achieve useful analyses of data obtained in subsequent experimental procedures, it was nessessary to know the effect of the following on the measurements:

Physical factors:
1. Type and size of contact electrode.
2. Salt concentration of contact material.
3. Temperature of the contact electrode.
4. Frequency of alternation of the applied electrical voltages.
5. Magnitude of the applied voltages.

Physiological factors:
1. Weight and age of the animal.
2. Effects of repeated measurements.
3. Effects of Adrenaline.
4. Effects of anesthesia.

METHODS AND PROCEDURES

Measurement techniques.—In order to make the measurements as uniform as possible, it was necessary to immobilize the animal on a special rat board constructed for the purpose. On either side of the board were two stainless steel plates so arranged that the paws of the animal could be conveniently placed in contact with the plates. The paws of the animal were scrubbed thoroughly and were bound to the contact plates by gauze strips soaked in saline solution. The salt concentration of the solutions used were varied as one of the experimental procedures to be described later.

In our early experiments, the measurements were made with a General Radio Co. impedance bridge, type 650A, using a microphone hummer at 1000 cps. as the source of current and a telephone receiver as the detector. This method was in accordance with that used in much of the earlier literature.

Since this setup was very much limited in its variability, the system was modified to the use of a Schering bridge (modified Wheatstone) using an audio oscillator as the source of current and a cathode ray oscilloscope as the detector. Such an arrangement does provide more flexibility than the other setup but still requires considerable effort to compute the final values. Finally, we assembled and tested a system using an ammeter-voltmeter arrangement and measuring the phase angle between voltage and current with a commercial phasemeter. This last arrangement was found to be quite as accurate as the other methods, and was much easier to use.

The scheme thus finally settled on is shown in figure 1. The current source is a Hewlett-Packard Model 200B audio oscillator. The resistances used were two Leeds and Northrup decade resistance boxes (1 to 1000 ohms), and the capacitance was a decade capacitor, Leeds and Northrup, 0.001 to 10 microfarad. The voltmeters used were two Ballantine Model 310 electronic AC voltmeters. The phase angle measurements were made with a Technology Instrument Co. Type 320AB phase angle meter. This latter meter provides for direct readings of phase angle without ambiguity on a meter which has full scale ranges of 360°, 180°, 90°, and 36°. In addition, 180° phase reversing switches for each channel are provided, making possible "expanded scale" operation for measurement of small changes in any of four quadrants.

In use, the output of the audio oscillator was adjusted to 10 volts. The system was then switched into the measuring condition, and the resistance was varied until the two voltmeters read identical values (*i.e.*, 5 volts each). The value of the resistance is recorded as the impedance of the tissue. The phase angle is read directly from the meter. A check on the phasemeter operation was made with each series of measurements, by switching to the check condition and putting in known values of capacitance and resistance. The theoretical phase angles were computed from the relation:

$$\tan \emptyset = \frac{I_R}{I_C} = \frac{R_1}{X}$$

where $\emptyset$ is the impedance angle of the physical model, I_R is the current through the pure resistance, I_R is the current through the resistance-reactance combination, R_1 is the value of the pure resistance at balance, and X is the reactance of the physical model. Actually, the phasemeter measures θ, which is the supplement of $\emptyset$.

Experimental procedures.—Our first item of concern in this series of experiments was the determination of variation of impedance and phase angle with frequency of alternation of applied voltage. For this study, 8 normal, adult rats were anesthetized with Evipal sodium (10 mg./100 gm. body weight, intraperitoneally) and placed on the board. The saline used was 1 percent NaCl. Impedance and phase angle values were obtained at 10 cps. intervals to 100 cps., at 100 cps. intervals to 1000 cps., and at 1000 cps. intervals to 20,000 cps. A few measurements at higher frequencies were made.

On the same series of animals, measurements were made at frequencies of 1000 cps., 5000 cps., and 15,000 cps. with voltages such that the applied voltages were 1, 2, 4, 6, 8, and 10 volts.

A series of measurements were made on 5 animals, in which the electrodes used were 60, 120, and 240 cm² in area. Full frequency spectra were determined in each case.

Another series of 5 animals were measured with the 120 cm² electrodes (used in all other experiments, incidentally) in which the saline concentration was varied to give 0.5%, 1%, 5%, and 10% NaCl. Again, full frequency spectra were determined in each case.

The measurements were repeated on these same animals with variation of the temperature of the saline solution applied to the electrodes such that the temperatures were 18°, 23°, and 28° C.

Determinations of the effects of anesthesia on the values obtained were made on three normal animals. These animals were used to being handled, and were tied down to the rat board and their electrical impedance characteristics measured without anesthesia. Each rat then was injected intraperitoneally with Evipal and as soon as anesthesia was surgically complete (using the ordinary signs) the measurements were repeated.

To determine the effect of age and weight of the animals on the findings, a group of 11 male and 12 female rats were selected at random from our rat colony, anesthetized, and full frequency spectra were determined on each.

In a group of 4 animals, complete frequency spectra were determined in the usual way. Then, each animal was given a dose of Epinephrine and rapid determinations made at 100, 1000, and 10,000 cps. at 1 min., 5 min,. and 20 min. intervals. A second group of animals was subjected to similar experimentation,

TABLE 1

Variation of Impedance (Z) and Phase Angle (θ) with Anesthesia

		Before Anesthesia	After Anesthesia	Difference
Z (ohms)	10KC	2967	2600	−367±32 (−12.4%)
	15KC	2617	2430	−187±23 (− 7.1%)
	20KC	2533	2390	−143±14 (− 5.6%)
	10KC	14.3	15.2	+0. 9±.04
θ (degrees)	15KC	13.0	13.75	+0.75±.04
	20KC	11.75	12.42	+0.67±.02

TABLE 2

Variation of Impedance (Z) and Phase Angle (θ) with Weight of Animals

Weight (gms)	Z (ohms)	θ (degrees)
120–129	2543±16	16.67±1.04
130–139	2140±17	16.25± .04
140–149	2253±135	16.1 ±1.3
150–159	2377±76	13.08± .12
160–169	2440±54	12.9±0.6
170–179	2085±87	12.0 ±0.4

except that the Epinephrine concentration was higher. This was repeated until four groups were studied, with epinephrine concentrations running from physiological doses to massive pharmacological doses.

Lastly, in a group of 8 animals (3 female and 5 male) measurements were made on successive days for a two week period. These measurements were compared to determine the effect of repeated measurement.

RESULTS

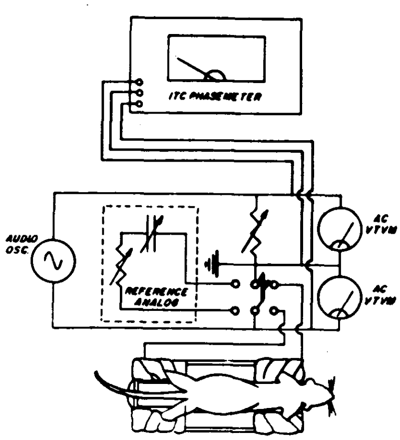
The individual data obtained in these experiments comprise a voluminous mass of information bits too extensive for rapid comprehension. Therefore, the data to follow are averaged for each group of animals and for each experimental procedure. Where such information is available and applicable, the average value will be followed by the standard error of the mean.

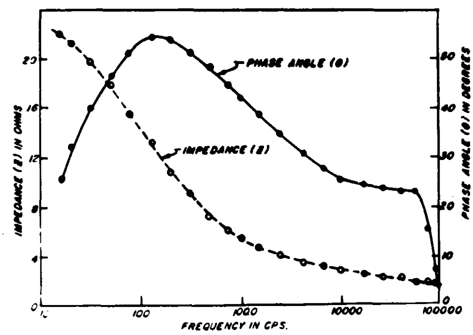
The average data on the first group of animals (the determination of the effects of frequency) are presented graphically in figure 2. Impedance is plotted on the vertical scale at the left of the figure, and phase angle on the right. It will be noted that the impedance is maximal at low frequencies (22,000 ohms at cps or less), and is reduced in the familiar X_c manner with increase in frequency. The impedance is minimal for all practical purposes above about 10,000 cps.

The phase angle plot indicates a maximum phase angle of about 53° at 200 cps. The phase angle drops off at higher frequencies, but not so rapidly as does the impedance. Above 50,000 cycles, the phase angle drops off precipitously, but it should be noted that these frequencies are greater than the frequency response rating of the phase meter, and therefore may not be of significance.

The spread of values in these frequency measurements was relatively large, but every individual spectral curve followed closely the contours of the average curve.

In the determinations of the effects of voltage applied on the measurements, it was found that impedance values were the same at all applied voltages. How-





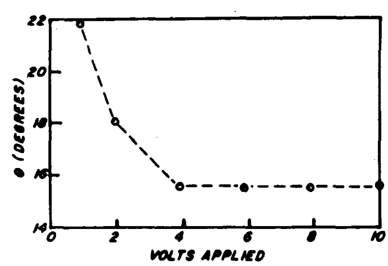


FIGURE 1 (left). Apparatus arrangement for measurement of electrical impedance properties of white rats.

FIGURE 2 (upper right). Variation of electrical impedance characteristics of white rats with frequency.

FIGURE 3 (lower right). Variation of impedance phase angle with applied voltage.

ever, the phase angle indicated on the phase meter varied as shown in figure 3. For reasons to be listed in the discussion, it is believed that this variation is a function of the phase meter, and not of tissue properties. It is apparent that at voltages of 5 volts or greater, no variation is to be expected.

There were no differences noted in the impedance and phase angle values obtained when the electrode areas were 60, 120, and 240 cm². Since the 120 cm² electrodes were of a convenient size, they were used in all subsequent measurements.

Also there were no significant differences noted when the solutions used to soak the gauze wrappings were changed in concentration from 0.5 to 10 percent

NaCl. Thus, it is indicated that the contact resistance so obtained is minimal, and that our measurements are actually those of electrical properties of tissues. The same lack of effect was noted when the gauze packs were saturated with saline solutions of different temperatures.

The data showing the effect of anesthesia on the values obtained is given in table 1. Since differences at very low frequencies were negligible, only those values at 10,000 cps and higher are given. The difference was maximal at 10,000 cps. The impedance differences noted are definitely greater than the error of the measuring instruments, and the phase angle measurements are such that the differences are considered significant. The accuracy of the phase angle meter is generally considered to be 0.5° in the range in which these measurements fall.

The measurements of variations of impedance and phase angle with size of the animal are shown in table 2. It is seen that there is no consistent pattern of variation of the impedance (measured at 10,000 cps) with size, but the phase angle showed a progressive diminution from 16.7° to 12° as the size and age of the animal increases. The number of animals falling in each size group is relatively small, so that the standard errors are large. However, the diminution of phase angle is so consistent that there is not overlap in any of the groups.

In passing, it was noted that by far the greater variability in phase angle and impedance was seen in the female rats. This later was traced to a cyclic variation with the estrous cycle (to be described in a subsequent publication).

In the last group of animals, no significant differences were noted with several successive daily determinations of impedance and phase angle in the male rats. In the females, the cyclic variations did not appear because the estrous cycle was stopped completely after the first two or three days, and the females behaved in the same manner as the males, showing little or no variability from day to day.

DISCUSSION

The use of small animals for impedance studies.—These experiments demonstrate beyond doubt that significant studies of factors varying the electrical impedance properties of the whole body can indeed be performed on small animals. This should make it possible to devise experimentation with biological procedures which are impossible of accomplishment with man or larger mammals. An example of this will be given in the paper to follow.

In this case, the impedance one measures is that of the deep tissues plus two layers of skin. In view of the success various workers have experienced in making measurements of deep tissue impedance by using several electrodes (Horton and Ravenswaag, 1935) there is no reason why such an arrangement cannot be used with rats. It may be that these isolating arrangements may provide more useful data biologically.

The method used in measurement of impedance properties.—It is apparent from this work and that of others that the impedance alone is not enough for intelligent analysis of the electrical properties of the whole body; the impedance phase angle is more constant than the impedance magnitude and seems to show more variation with physiological manipulation. The method used in these studies (*i.e.*, the use of a phase meter with the voltmeter-ammeter combination) is very much easier to use than the methods formerly applied. The errors involved are perhaps a little larger than when one computes the phase angles by the long method. However, these errors are small compared with the usual biological variation. Therefore, it would seem that such errors are not significant enough to warrant eliminating the method.

Physical factors influencing the measurements.—Both the impedance magnitude and the phase angle are more susceptible to physiological changes in the middle of the frequency range covered in these experiments. Therefore, it would seem that approximately 10,000 cycles per second is the proper frequency for making physiological studies. In some cases, one might wish to determine the entire frequency spectrum, since this can be done with little difficulty by the voltmeter-ammeter method. If, on the other hand, one wishes to follow changes which occur within seconds or a very few minutes, one can make repeated measurements at a single frequency and obtain meaningful information.

It is fortunate that the measurements are not appreciably affected by electrode size greater than our minimum, the concentration of salt in the soaking solution, or the temperature of the soaking solution. All of these observations mean in the long run that with the preparations and solutions and contact method used, the skin to electrode impedance is minimal and is not greatly influencing the results.

The constancy of the impedance values with magnitude of applied voltage is entirely to be expected in the range of voltages used here, for within this range the tissues are linear components for all practical purposes. At very high applied voltages, it is likely that such linearity would not hold, and the impedance values would be different. The phase angle values do change up to about 4 volts in our system, and we believe this is due to voltage dependence of the phasemeter response at low applied voltages. The manufacturer's specifications indicate that the phasemeter should work properly at 1 volt input, but this has not been true in our experience. At the frequencies which are apparently useful to us, the voltage magnitude is not too important from the standpoint of its biological effects, since stimulation of the biological tissues will not occur at such frequencies. In the anesthetized animals, even the low frequency currents seemed to produce no undesirable effects.

The influence of biological factors.—The biological factors influencing our measurements are probably much more important than any others in designing reproducible and interpretable experiments. The fact that even large doses of epinephrine do not produce significant changes in the impedance phenomena is a fortunate one, since such possible changes would complicate the interpretation of data annoyingly. The handling of animals preparatory to making the measurements or even to anesthesia certainly produces significant quantities of epinephrine in the circulation, and if this had significant effects, one would have to design his experiments much more carefully.

The comparatively small changes associated with anesthetization are nevertheless statistically significant and meaningful. It is most likely that the decrease in impedance magnitude which occurs when the animals are anesthetized is caused by a change in the volume of blood contained in the tissues contributing to the impedance. The blood impedance is less than that of the other tissues. Again, however, the changes associated with anesthetization are small, and so long as one makes sure that all the animals in an experiment are treated similarly in this respect, there is no reason that this would be a complicating factor.

The relative constancy of readings in male rats and in females after the first two or three days of a series of daily measurements also makes the interpretation of changes associated with physiological manipulation easier. From data to be described in subsequent experiments, it has been established that most of the variations noted with female rats measured repeatedly are associated with changes of physiological condition of the tissues during the estrous cycle. Repeated measurements on the same animal invariably bring about a complete cessation of the estrous cycle, as evidenced by vaginal smear studies, and this disappearance adequately accounts for the changes noted in impedance and phase angle values on daily repetition of measurement.

Lastly, the physical significance of phase angle measurements lies in the relationship of resistance to capacitive reactance, in the absence of inductive properties. Most analyses of the basic mechanisms involved in the production of a capacitive reactance in living material bear strongly on the rôle of dipole orientation (Debye, 1929; Fröhlich, 1949). In view of the major water shifts which are known to occur in a number of physiological conditions, there is every reason to believe that electrical property measurements may prove of value in making physiological studies.

SUMMARY

Studies have been made of the physical and physiological factors which determine the impedance and phase angle of the whole bodies of laboratory rats. These measurements have been made with a voltmeter-ammeter method, determining the phase angle with a commercial phase angle meter.

The technique used has proved to be sufficiently accurate and much easier to use than previous bridge techniques.

It is demonstrated that it is quite feasible to make such measurements on small laboratory animals, and the advantages of being able to make major physiological changes and work in large numbers renders such measurement valuable.

Within the practical ranges used, electrode size and contact solution salt concentration do not effect the results. Epinephrine also does not have an appreciable effect, so that excitement would not influence the results. Anesthetization produces a small, quite reproducible reduction in impedance and a similar increase in phase angle. It was found that there is not consistent impedance variation with animal size, but that the phase angle was reduced progressively with increased size of the animal.

Daily determinations on a single group of rats indicates that female rats show a variability associated with the estrous cycle. Repeated measurements bring about a cessation of the estrous cycle, and these females as well as males give consistent results on subsequent measurements.

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