AN EXPERIMENTAL STUDY OF DIATHERMY.

VI. Conduction of High Frequency Currents through the Living Cell.

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(Received for publication, May 24, 1928.)

In this paper an attempt will be made to correlate the investigations of biophysicists with those questions which are of fundamental importance in a study of diathermy. A survey of the literature shows that the intimate relationship of these fields has not been appreciated. Biophysicists on the one hand have paid little attention to the phenomenon of heat production with the passage of high frequency currents, while those studying diathermy have neglected the problem of cellular conduction and penetration. It is obviously of great importance to know whether the diathermy current passes through the living cell, and if so, what are the effects produced.

The only constant effect which is known to be produced by high frequency alternating currents is that of heat production. The chemical effects of electrolysis disappear as the alternations exceed a frequency of 5,000 to 10,000 per second (1). The explanation for this fact lies in the rapidity of the reversal of the current which does not allow the chemical action of one phase to manifest itself before it is neutralized by the opposite phase (2). The absence of any exciting influence on the tissues through which the current passes is probably analogous to this absence of chemical effect. If the high frequency current is rectified so that it is not alternating in character, electrolysis and muscle excitation will be produced, even if the frequency of interruptions be as high as 500,000 per second (3).

The complexity of living tissue makes determinations extremely difficult of the actual course and means of transportation of the current through it. Philippson (4), Fricke and Morse (5), and McClendon (6), in their work on muscle, liver, and blood cells, have shown that as the frequency of alternations is increased the impedance of the living cell decreases, meaning by impedance the sum of all the various hindrances to the passage of the current. At a frequency of 10^5 to 10^7 cycles per second a point is reached where the impedance of living and dead cells is in close agreement. Beyond this frequency, according to these authors, the

current passes in part at least through the cell, and not wholly through the extracellular fluid, as was formerly supposed. The red blood cell is surrounded by a very efficient dielectric layer, equal in thickness to a layer composed of approximately 20 to 30 carbon atoms, and allowing little or no penetration of ions. The interior of the cell has a comparatively low resistance, equivalent to a 0.17 per cent NaCl solution. The cell, therefore, acts as a condenser, which transmits the current by its capacitance. In the living body the various fascial layers also act as dielectrics (7) of varying degrees of efficiency.

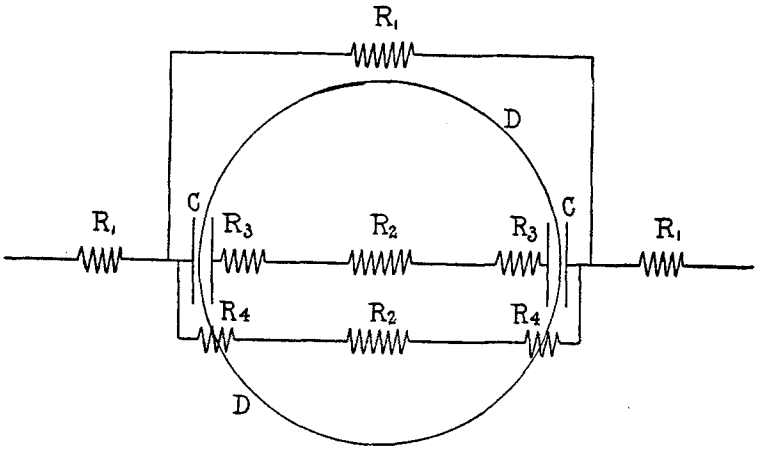

FIG. 1. Diagrammatic representation of the passage of high frequency currents through the living cell.

R_1—Resistance of extracellular fluid.
R_2—Resistance of cell interior.
R_3—Dielectric loss.
R_4—Condenser leak.
C —Capacitance of cell.
D —Dielectric layer surrounding cell.

It will be realized that the passage of high frequency currents through the living body is an extremely complex phenomenon. The whole system consists of resistances and capacitances, both in parallel and in series, some of which are stationary and some in motion. Fig. 1 represents in diagram the passage of the current through a single unit of this system—namely, the living cell. Even this representation is, of course, too simple, since the various phases in the colloidal system we call protoplasm must influence the passage of the current within

the cell (8, 9) and the protoplasm itself is constantly changing in conductivity (10).

One would expect these general principles to be applicable to the diathermy current. However, there are several differences between the currents used by the investigators mentioned and those used in diathermy which might render such an application fallacious. The diathermy current consists of pulses of impressed discontinuous oscillatory trains which probably contain many harmonics. This type of current is not necessarily comparable to a high frequency current of the pure sine wave form. Also, impedance measurements with currents of low and high voltage are not necessarily comparable (11).

There are two characteristics of the passage through living cells of low voltage high frequency alternating currents of the pure sine wave form. These, as described by the authors referred to, manifest themselves in the fact that the impedance is not changed when the cell is broken down by lysis, and in the fact that with increase of cellular concentration the impedance increment is less than that found with continuous currents.

The experiments described in this paper were planned for the purpose of finding out whether these two characteristics hold true with the currents used in diathermy.

EXPERIMENTAL.

Details of the apparatus used to produce the high frequency alternating current have been described elsewhere (12). The oscillatory current generated by the discharge of the condenser across the spark gap is of high voltage but low amperage. Owing to the high decrement of the circuit the oscillations are in the form of a series of discontinuous trains, each train being damped down before the next train commences. The frequency is 1.25×10^6 but the oscillations are not of the pure sine wave form, as harmonics are probably present.

Since it is impossible to make any accurate impedance measurements by the bridge method with this type of current, we were forced to approach the subject from a somewhat different angle from that which has been adopted by the investigators just mentioned. The experiments about to be described naturally fall into two groups, more or less independent of each other. They will, therefore, be described separately.

I. Direct Measurement of Heat Production in Blood and Serum.

The heat produced in blood and serum by the passage of a known quantity of current was measured by placing the blood or serum in a specially constructed glass cylinder, so arranged that temperature measurements could be made while the current was passing through it.

The material used was defibrinated sheep blood, obtained fresh from the slaughter house. The cell volume of the blood was varied by centrifugalization. Hematocrit readings were made at 2,000 revolutions per minute for 45 minutes. The cylinder was 20 cm. long and 6.5 cm. in diameter, with a capacity of 640 cc. It was closed by two cork stoppers equipped with circular lead-tin electrodes, the leads passing through the corks. Three openings on the long axis of the cylinder allowed the introduction of thermometers and the fluids to be examined. The cylinder was insulated against heat loss by wrapping it with thick felt. With this apparatus it was possible to measure the temperature changes in blood during the passage of high frequency currents through it.

To correct for heat loss during the current flow, measurements were made on the rate of cooling of water contained in the cylinder, with various differences between the temperature of the water and room temperature. When this difference amounted to 10°C. the rate of cooling was 0.04°C. per minute, showing that insulation against heat loss was fairly efficient. In the experiments to follow, however, corrections for this heat loss were made.

The strength of the current in all the experiments was 400 milliamperes, as recorded by the hot wire milliammeter and the time of exposure to the current was 10 minutes. By altering the spark gap, the milliammeter reading was kept constant throughout each experiment, so that, although the true amperage was probably changing owing to the changes of temperature in the various circuits (13), the amount of current which passed through each individual sample was approximately the same. To standardize as far as possible these changes in temperature, both the apparatus and the sample to be examined were at room temperature when each experiment began. In spite of these precautions, preliminary experiments with salt solutions of various concentrations showed that the range of error was considerable, being in the neighborhood of $\pm$ 10 per cent.

The heat production in blood and serum was measured by this method. As might be anticipated, it was found that whereas the heat production in various samples of serum was the same, that in blood increased as the cell volume was increased. For example, the ratio $\frac{H\ \text{Blood}}{H\ \text{Serum}}$ (where H represents heat production per unit of time) was found to increase from 1.48 to 3.13 when the cell volume was increased from 36 per cent to 72 per cent (Table I). At the same cell

volumes McClendon found the ratio $\frac{Z \text{ Blood}}{Z \text{ Serum}}$ at $F = 10^6$ to increase from 1.8 to 3.5 and $\frac{Z \text{ Blood}}{Z \text{ Serum}}$ at $F = 10^3$ to increase from 1.8 to 4.7 (Table I) (where Z represents impedance measured by the Wheatstone bridge). It is evident from these figures that as the cell volume is increased the increase of the ratio $\frac{H \text{ Blood}}{H \text{ Serum}}$ resembles the increase in $\frac{Z \text{ Blood}}{Z \text{ Serum}}$ at $F = 10^6$ rather than at $F = 10^3$. This is especially evident at the higher cell volumes, where there is a really significant difference

TABLE I.

Impedance of Blood and Serum.

Cell vol.	H_s	H_b	$\frac{H_b}{H_s}$ $F = 10^6 \times 1.25$	$\frac{Z_b}{Z_s}$ $F = 10^6$	$\frac{Z_b}{Z_s}$ $F = 10^3$
per cent					
36	2.0	2.95	1.48	1.8	1.8
40	1.98	4.07	2.06	1.9	2.1
46.5	2.0	3.33	1.67	2.2	2.4
70	2.0	6.78	3.36	3.4	4.4
72	1.98	6.19	3.13	3.5	4.7

H = Heat produced by 400 milliamperes in 10 minutes.
Z = Impedance (data from McClendon (6)).

between $\frac{Z \text{ Blood}}{Z \text{ Serum}}$ at $F = 10^6$ and $F = 10^3$. That the ratio $\frac{H \text{ Blood}}{H \text{ Serum}}$ is as an average slightly lower than $\frac{Z \text{ Blood}}{Z \text{ Serum}}$ at $F = 10^6$ is probably of some significance, although the difference is within the limits of experimental error. Should the difference be real, it would indicate that some portion of the impedance of blood cells does not lead to heat production.

Measurements were also made on blood and blood laked with saponin but we have not published these figures, as subsequent observations showed that saponin itself produces a marked increase in conductivity.

II. Relative Impedance and Resistance Measurements on Blood, Laked Blood, and Serum.

A circuit was constructed whereby the relative impedance and resistance of two unknowns could be ascertained simultaneously. This was done to obviate the errors arising from current measurements by means of the hot wire milliammeter and from varying atmospheric conditions.

Four identical cells were constructed, each of 200 cc. capacity, and equipped with brass electrodes, the dimensions of which were 7 cm. × 4 cm. The cells were then placed in a circuit as represented in Fig. 2, so that Cell I was in series with Cell III, and Cell II in series with Cell IV. Cells I and III together were in parallel with Cells II and IV. With the same electrolyte in each of the cells, it is obvious that the amount of current, and therefore the rate of heat production in each, should be the same. To calibrate the cells, all four were filled with 0.05 M NaCl and the heat increment measured after the passage of 1,300 milliamperes for 10 minutes. This was repeated 14 times. A slight but constant discrepancy in the heat production in the respective cells was found. The average figures for the 14 observations were:

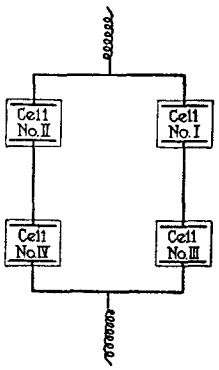

FIG. 2. Circuit used to determine the relative impedance and rate of heat production in two unknowns. Details of the apparatus are described in the text.

Cell I	8.38°C.
Cell II	8.69°C.
Cell III	8.33°C.
Cell IV	8.57°C.

This discrepancy can only be due to some differences in the electrical constants of the cells, or differences in the calibration of the thermometers. Cell I was arbitrarily chosen as the standard and the others corrected on the basis of the above observations. The correction factors used were therefore:

1.000 for Cell I.
0.964 for Cell II.
1.006 for Cell III.
0.977 for Cell IV.

With these corrections the temperature increments in all 4 cells corresponded to within ± 1.7 per cent. These correction factors were used in the experiments about to be described. To correct for heat loss in the cells cooling curves were

constructed with saline and blood. The appropriate correction was applied to the observed temperature increase. All experiments were started with the contents of the cells 0.5–1.5°C. below room temperature. An effort was made to keep the environmental temperature about the cells constant.

The two unknowns, the impedances of which were to be measured, were placed in Cells I and II, respectively, while Cells III and IV were filled with 0.05 M NaCl. The current was then turned on so that the milliammeter registered 1,300, and after 10 minutes the temperature increment in all four cells was measured. (This temperature increment will be referred to as H.) Since the circuit was constructed so that, apart from the contents of the cells, its impedance was very small, we can say that the expression $\frac{Z_1 + Z_3}{Z_2 + Z_4}$ represents the inverse ratio of the currents passing through the two parallel arms of the circuit (where Z_1 represents the impedance of the contents of Cell I, Z_2 the impedance of the contents of Cell II, etc.). The amount of current passing through Cell I is the same as that passing through Cell III, and the amount of current passing through Cell II is the same as that passing through Cell IV. Hence we can write:

$$\frac{Z_1 + Z_3}{Z_2 + Z_4} = \frac{I_2}{I_1} = \frac{I_4}{I_3}$$

Since Cells III and IV contain an electrolyte of the same conductivity, we can write $\frac{I_4}{I_3} = \frac{\sqrt{H_4}}{\sqrt{H_3}}$.
It follows that:

$$\frac{Z_1 + Z_3}{Z_2 + Z_4} = \frac{\sqrt{H_4}}{\sqrt{H_3}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Since Z_3 and Z_4 are identical, the figure $\frac{\sqrt{H_4}}{\sqrt{H_3}}$ will tell us whether $\frac{Z_1}{Z_2}$ is greater or less than unity.

If we assume that the contents of the four cells have a similar dielectric constant, an assumption which seems reasonable since they consist of electrolytes with no great divergence of conductivity, we can obtain from these heat measurements a figure which will represent quantitatively the ratio $\frac{R_1}{R_2}$, where R represents that part of the impedance which leads to heat production. Since the amount of current flowing through Cells I and III is the same and that flowing through II and IV is the same, we can write $\frac{R_1}{R_3} = \frac{H_1}{H_3}$ and $\frac{R_2}{R_4} = \frac{H_2}{H_4}$. It follows that

$\frac{R_1}{R_3} \Big/ \frac{R_2}{R_4} = \frac{H_1}{H_3} \Big/ \frac{H_2}{H_4}$, which can be written $\frac{R_1}{R_3} \times \frac{R_4}{R_2} = \frac{H_1}{H_3} \times \frac{H_4}{H_2}$. Since Cells III and IV both contain 0.05 M NaCl, R_4 and R_3 can be cancelled out so that:

$$\frac{R_1}{R_2} = \frac{H_1}{H_3} \times \frac{H_4}{H_2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

The accuracy of this method was checked by a series of observations on various electrolyte solutions, of which the conductivity had been measured by a Wheatstone bridge at a frequency of 10^3. There is no reason to suppose that conductivity measurements by the low frequency Wheatstone bridge should not be applic-

TABLE II.

Saline and Saponin Controls.

Contents of cells			$\frac{R_1}{R_2}$		$\frac{Z_1 + Z_3}{Z_2 + Z_4}$	
I	II	III and IV	$F = 10^6 \times 1.25$	$F = 10^3$	$F = 10^6 \times 1.25$	$F = 10^3$
0.04 M NaCl	0.06 M NaCl	0.05 M NaCl	1.474	1.439	1.171	1.195
			1.429	1.439	1.198	1.195
			1.427	1.439	1.201	1.195
			1.473	1.439	1.211	1.195
0.55 M NaCl	0.05 M NaCl	0.05 M NaCl	0.915	0.916	0.957	0.955
			0.930	0.916	0.971	0.955
0.05 M NaCl	0.05 M NaCl + 1 per cent saponin	0.05 M NaCl	1.317	1.302	1.130	1.132
			1.278	1.302	1.139	1.132

Z = Impedance of contents of cell.
R = Impedance represented by heat production.

able to the diathermy current when simple electrolytes are used. In all the experiments the unknowns were placed in Cells I and II while Cells III and IV were filled with 0.05 M NaCl. With 0.04 M NaCl and 0.06 M NaCl as the unknowns, the average values for $\frac{R_1}{R_2}$ and $\frac{Z_1 + Z_3}{Z_2 + Z_4}$ were 1.451 and 1.195 with the diathermy current, 1.439 and 1.195 with the Wheatstone bridge. With 0.055 M NaCl and 0.050 M NaCl the figures were 0.922 and 0.964 with diathermy and 0.916 and 0.955 with the Wheatstone bridge. With 0.05 M NaCl and 0.05 M NaCl + 1 per cent saponin, the figures were 1.297 and 1.134 with diathermy and 1.302 and 1.132 with the Wheatstone bridge (Table II). These controls clearly show that even slight differences in impedances can be measured by this method with a considerable degree of accuracy.

Similar experiments were carried out with blood cells and laked blood cells, and also with blood cells and serum, as the unknowns. The material used was defibrinated ox blood, obtained fresh from the slaughter house. The serum was separated and the cell volume varied by centrifugalization. The blood cells were laked by freezing and thawing. As in the control experiments, the unknowns were placed in Cells I and II, while Cells III and IV were filled with 0.05 M NaCl. It was found that whereas laking produced no appreciable change in

TABLE III.

Impedance of Blood and Laked Blood.

Hematocrit reading	$F = 10^6 \times 1.25$	$F = 10^6$	$F = 10^6 \times 1.25$
	$\frac{R \text{ Blood}}{R \text{ Laked blood}}$	$\frac{Z \text{ Blood}}{Z \text{ Laked blood}}$	$\frac{Z \text{ Blood} - Z \text{ 0.05 M NaCl}}{Z \text{ Laked blood} - Z \text{ 0.05 M NaCl}}$
73	0.976	1.31	0.986
	0.985	1.31	0.990
50	0.925		0.959
	0.945		0.969
47	0.987	1.092	1.013
	0.997		1.006
	0.990		1.013
39	0.965		0.969
	0.990		0.996
	0.963		0.975
Average.....	0.973		0.997

Z = Impedance.

R = Impedance represented by heat production.

the impedance of blood, the rate of heat production was increased by about 3 per cent. The average of the 10 observations made was 0.997 for the value of $\frac{Z \text{ Cells} + Z \text{ 0.05 M NaCl}}{Z \text{ Cells laked} + Z \text{ 0.05 M NaCl}}$ and 0.973 for the value of $\frac{R \text{ Cells}}{R \text{ Cells laked}}$ (Table III). Since presumably all the impedance of laked blood is ohmic in character, this can only mean that about 3 per cent of the impedance of intact cells is inductive, and therefore does not lead to the production of heat.

With blood of 72 per cent cell volume and serum as the unknowns, the ratio $\frac{R\,\text{Blood}}{R\,\text{Serum}}$ was found to average 2.5. McClendon found that at the same cell volume $\frac{Z\,\text{Blood}}{Z\,\text{Serum}}$ was 3.5 at $F = 10^6$ and 4.7 at $F = 10^3$. Thus we again have $\frac{R\,\text{Blood}}{R\,\text{Serum}}$ somewhat lower than $\frac{Z\,\text{Blood}}{Z\,\text{Serum}}$ at the same frequency and very much lower than $\frac{Z\,\text{Blood}}{Z\,\text{Serum}}$ at low frequencies (Table IV). The discrepancy between $\frac{R\,\text{Blood}}{R\,\text{Serum}}$ and $\frac{Z\,\text{Blood}}{Z\,\text{Serum}}$ at

TABLE IV.

Impedance of Blood and Serum.

Cell vol.	$\frac{R\,\text{Blood}}{R\,\text{Serum}}$	$\frac{Z\,\text{Blood}}{Z\,\text{Serum}}$	$\frac{Z\,\text{Blood}^*}{Z\,\text{Serum}}$	$\frac{Z\,\text{Blood}^*}{Z\,\text{Serum}}$
	$F = 10^6 \times 1.25$	$F = 10^3$	$F = 10^6$	$F = 10^3$
per cent				
72	2.60	4.36	3.5	4.7
72	2.42	4.41	3.5	4.7

Z = Impedance.

R = Impedance represented by heat production.

* Data from McClendon (6).

the same frequency confirms the previous observation that part of the impedance of blood cells does not lead to the production of heat. The difference, however, is more than one would expect from the results obtained on whole and laked blood. Some of this difference can no doubt be explained by differences in the technic employed for making hematocrit readings by McClendon and ourselves. However, if we make use of the impedance measurements at $F = 10^3$ (Table IV), as a standard of comparison, a considerable discrepancy still exists $\left(\frac{R\,\text{Blood}}{R\,\text{Serum}} = 2.5 \text{ and } \frac{Z\,\text{Blood}}{Z\,\text{Serum}} = 3.3\right)$.

DISCUSSION.

The results of these experiments seem to show pretty conclusively that, with regard to its passage through biological media, the dia-

thermy current behaves in the same manner as low voltage high frequency currents of the pure sine wave form. With both, the laking of cells produces no change in impedance, and with both, an increase in cellular concentration of blood produces an impedance change which is characteristically lower than that found with low frequency currents. The passage of the diathermy current through the living cell can then be represented by Fig. 1. The cell membrane is a very efficient condenser which transmits the current by its capacitance with little or no dielectric loss and consequently little or no stress and strain on the "cell wall." Cellular massage is a term commonly met with in the literature on diathermy. There is no evidence to show that either this, or the electromechanical vibration which is said to occur with currents of a higher frequency, exists.

From theoretical considerations we should not expect that the addition of a glucoside such as saponin to an electrolyte would produce any appreciable change in the conductivity. To our surprise, however, we found that a very definite increase in conductivity occurred (Table II). This increase appears to be independent of voltage and frequency, so it must be a true conductivity change. No attempts were made to determine the purity of the saponin used, but it was obtained from C. A. F. Kahlbaum of Berlin and classified as purified saponin. Others working on the conductivity of biological media under various conditions seem to be unaware of this phenomenon (6) and we, ourselves, were for some time led astray by it.

SUMMARY.

1. A method is described for measuring the relative impedance of living cells to diathermy currents.

2. The diathermy current penetrates the living cell, and heat production is intracellular as well as extracellular.

3. A small proportion of the impedance of living cells to the diathermy current seems not to lead to the production of heat.

4. Evidence is given that the addition of saponin produces an appreciable increase in the conductivity of an electrolyte. Its use is therefore contraindicated when electrical measurements are being made on biological material.

5. The currents used in diathermy behave as do high frequency

currents of the pure sine wave form in respect to their passage through biological material.

BIBLIOGRAPHY.

1. d'Arsonval, *Arch. phys. norm. et path.*, 1893, v, 401.
2. Turrell, W. J., *Proc. Roy. Soc. Med.*, 1926, xx, 103.
3. Cluzet, J., and Chevalier, J., *Arch. elect. méd.*, 1926, xxxiv, 65 (see Turrell).
4. Philippson, M., *Bull. Acad. roy. méd. Belgiques, Cl. Sc.*, 1921, vii, series 5, 387; *Compt. rend. Soc. biol.*, 1920, lxxxiii, 1399.
5. Fricke, H., and Morse, S., *J. Gen. Physiol.*, 1925–26, ix, 153.
6. McClendon, J. F., *J. Biol. Chem.*, 1925, lxiii, p. xiv; *Am. J. Physiol.*, 1927, lxxxii, 525; *J. Biol. Chem.*, 1926, lxix, 733.
7. Gicklhorn, J., *Protoplasma*, 1926, i, 124.
8. Lillie, R. S., Protoplasmic action and nervous action, Chicago, 1923.
9. Chambers, R., *Am. J. Physiol.*, 1917, xliii, 1; *J. Gen. Physiol.*, 1921, iv, 41.
10. McClendon, J. F., *Am. J. Physiol.*, 1912, xxix, 302.
11. Blinks, L. R., personal communication
12. Binger, C. A. L., and Christie, R. V., *J. Exp. Med.*, 1927, xlvi, 571.
13. Christie, R. V., and Binger, C. A. L., *J. Exp. Med.*, 1927, xlvi, 715.