CORRELATION OF THE PROPAGATION-VELOCITY OF THE CONTRACTION-WAVE IN MUSCLE WITH THE ELECTRICAL CONDUCTIVITY OF THE SURROUNDING MEDIUM.

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(Received for publication, May 20, 1921.)

An attempt was made at Clark University in 1916 and 1917 to test experimentally the thesis that the rate of physiological conduction in irritable tissues is a direct function of the electrical conductivity of the medium in which the tissue acts. The investigation was a series of experiments with reference to the propagation-velocity of the contraction-wave in the heart muscle of the river terrapin under artificial conditions. Measurements of the velocity of the wave in a series of isotonic balanced salt solutions of graded activity (mixtures of Ringer's solution with isotonic sugar solution) were made by means of special apparatus. These preliminary results indicated a direct correlation between the propagation-velocity and the electrical conductivity, and that the ratio of velocity with respect to conductivity was nearly a constant until the concentration of the salts of the Ringer's solution was reduced more than half. The original apparatus has been modified and the investigation has been extended during 1919 and 1921 to include the heart of the king-crab, *Limulus*, and the sartorius of the leopard-frog, *Rana pipiens*. Observations have been made in the above solutions over an extended range of electrical conductivity and under nearly constant conditions of oxygen tension, hydrogen ion concentration, and temperature.[1]

Comparative observations upon the spreading of an excitation state in irritable tissues indicate that the speed of conduction is

[1] Pond, S. E., Contraction wave in heart-muscle of River Terrapin, Thesis Worcester, 1917.

dependent principally upon the specific constitution of the tissue, the temperature, and the composition of the surrounding medium. What part the medium plays in the transmission of the contraction wave is not fully known.

Mayer[2] found that the rate of nerve conduction in the marine medusa *Cassiopea* was closely proportional to the total concentration of the cations Na, Ca, and K in the medium. When the sea water was diluted with distilled water a decline in the rate of nerve conduction was observed with increasing dilution. He attributed these results to the change in the adsorption of the above cations by the tissue; but it may also indicate, as Lillie pointed out[3], a direct correlation of the rate of physiological conduction with the electrical conductivity of the medium. In fact, the decline in propagation-rate runs closely parallel with the decline in the electrical conductivity of the sea water when similarly diluted. Mayer, however, found that under some conditions the propagation-rate may be independent of the electrical conductivity; thus a dilution of sea water with 0.4 *molar* magnesium chloride solution causes a decrease in the velocity of the nerve conduction to a degree closely proportional to the degree of dilution, although the electrical conductivity remains essentially unchanged.[4] The addition of magnesium however, must disturb the balance of the salts in the medium and introduce other factors of a special kind; and the possibility remains that in physiologically balanced media the rate of transmission of the excitation state may be determined, other conditions being equal, by the electrical conductivity of the solution.

In the light of these and other facts and of general theoretical considerations, Lillie advocates the theory "that the transmission of the excitation-state from the immediate site of activity to the adjoining resting areas is dependent on an *electrical local action* of the same essential nature as that which is responsible for the etching or corrosion of non-homogeneous metallic surfaces (*e.g.*, of iron) in con-

[2] Mayer, A. G., *Am. J. Physiol.*, 1915–16, xxxix, 375; 1916–17, xlii, 469; 1917 xliv, 591.

[3] Lillie, R. S., *Am. J. Physiol.*, 1916, xli, 133.

[4] Mayer, A. G., *Am. J. Physiol.*, 1915–16, xxxix, 381.

tact with an electrolyte solution."[5] This hypothesis "assumes that the electromotor properties of the protoplasmic surface-film are determined by conditions which are fundamentally similar to those governing the electromotor phenomena at metallic surfaces."[6] With irritable tissues, just as with metallic elements under certain conditions, *e.g.*, passive iron in nitric acid solution, local alteration gives rise to a characterisitic spreading effect, which has a rate dependent partly upon the composition of the medium, and partly upon the peculiarities of the tissue or metal.[7] The effects of local stimulation in living tissues spreads to other parts at different rates, very rapidly in some tissues, *e.g.*, nerve, while in others, *e.g.*, smooth muscle, the rate of transmission is slow. Similarly there is a wide varation in the rate of electrolytic changes in metals under different conditions.[8, 9] The slow extension of a rust spot in iron in the presence of an electrolyte is an instance of a gradual spreading effect; while the change from the passive to the active state, in iron and other metals, spreads from a region of local alteration, under certain conditions, with great rapidity.[10, 11]

The general view that the bioelectric variation, as such, is the essential change on which conduction of excitation in irritable tissues depends is by no means a new one and was favored by du Bois-Reymond, Hermann, Kühne and other early students of the bioelectric phenomena.[12]

The present investigation relates to the rôle of the composition and electrical conductivity of the medium in the transmission of the contraction-wave in muscle.

[5] Lillie, R. S., *Am. J. Physiol.*, 1916, xli, 126.

[6] Lillie,[5] p. 129.

[7] Lillie, R. S. *Am. J. Physiol.*, 1914, xxxiv, 414; 1915, xxxvii, 348.

[8] Lillie, R. S., *Scient. Monthly*, 1919, viii, 456, 552. *Science*, N. S., 1919, l, 259, 416.

[9] Lillie, R. S., and Johnston, E. N., *Biol. Bull.* 1919, xxxvi, 225.

[10] Bennett, C. W. and Burnham, W. S., *J. Phys. Chem.* 1917, xxi, 107.

[11] Lillie, R. S., *Am. J. Physiol.*, 1915, xxxvii, 348; 1916, xli, 126; *J. Gen. Physiol.*, 1920, iii, 107.

[12] du Bois-Reymond, E., *Ges. Abhandl. allg. Muskel- und Nervenphysik*, 1888, ii, 698, 733; Kühne, W., *Proc. Roy. Soc. London*, 1888, xliv, 446; *Z. Biol.*, 1888, xxiv, 383.

Method.

The velocity of the contraction-wave in muscle, *i.e.*, of the transmission of the excitation state, is measured by recording the time of the bulging of a strip of tissue at two or more points along its length as it undergoes contraction. In the present investigation the local bulging is made to operate (by a simple lever system) minute mirrors, which bring to focus upon a moving photographic film mirror-images of an illuminated slit. The muscle is either stimulated at one end by a minimal "break" induction-shock, or (as in the *Limulus* heart) it is rhythmically beating and the contraction-wave moving along the tissue operates the mirrors as it passes certain points. Each organ or strip of muscle while fixed in place for experimentation is arranged so that without otherwise changing the conditions it may be exposed to a succession of solutions of graded electrical conductivity; *e.g.*, mixtures of sea water or Ringer's solution with isotonic sugar solution in varying proportions (1:9, 2:8, 3:7, *etc.*). An electrically actuated tuning-fork of suitable frequency, with an attached mirror, is so arranged that a time curve is recorded upon the moving film above the muscle record. The solutions used with turtle and frog tissues have been corrected to, or kept at, constant temperature, about 20°C., and the hydrogen ion concentration of the solutions has been adjusted before experimentation to that of the Ringer's solution employed in the same series; *i.e.*, about pH = 6.8. Measurements of the hydrogen ion concentrations have been made by the colorimetric method of Clark and Lubs with standardized buffer solutions.[13] The electrical conductivity of the solutions in all the work has been measured at 25°C. by the method of Kohlrausch, determinations being made immediately before and after taking the muscle record.

The apparatus which has been used in this study for recording the passage of the contraction-wave is specially constructed so as to allow the external medium to be changed at will without otherwise disturbing the tissue. It is described in detail by the author elsewhere. The supporting part of the apparatus coming into direct contact with the frog and turtle tissues is entirely composed of vulcanized

[13] Clark, W. M., The determination of hydrogen ions, Baltimore, 1920, 38ff.

fiber impregnated with Bakelite; this substance is insoluble in the solutions used, and does not affect the tissues. In the experiments with the heart of *Limulus* the organ was fixed in a trough composed of paraffin.

The tissues were prepared as follows: Frogs were first curarized, with about 2 cc. of a 1 per cent solution of curare (in Ringer's solution), and after a period of 20 minutes both sartorii were removed. One of the muscles was mounted on the support and the other kept in cold Ringer's solution. The companion muscle was used in a few cases in duplicate experiments. The ventricle of the turtle was removed, cut into rings, and two of these opened up as strips of thick muscular tissue. One was placed in position on the apparatus and the other kept reserve for later use. The heart of the female *Limulus* (in preference to the male because of its greater length) was removed through a dorsal opening in the carapace and placed in the paraffin trough with the ventral side up, so that the ganglion was completely immersed in the solution. On one occasion, the muscle tissue was cut away from the ganglion between the third and sixth segments, leaving the anterior and posterior segments connected by the ganglion.

Complete records of one or more muscle contraction waves were taken upon a strip of photographic film wrapped about a kymograph drum. The procedure adopted was as follows: The tissue was placed in position for a record, washed in at least two changes of the test solution over a period of 15 or 20 minutes, and then exposed to a final bath in which the records were taken. *Limulus* hearts in the troughs were so arranged that the test solution flowed slowly over and about the tissue; while the turtle and frog tissues were exposed in tumblers holding about 250 cc. of solution. The arc-lamp was lighted and adjusted to uniform illumination of the mirrors; the tuning-fork was then started and the kymograph brought up to speed. The latter was so arranged (inside of a light-proof box) that a shutter on the arc-lamp remained open during one complete revolution of the drum. An inductorium circuit was closed (in response to a signal attached to the kymograph) shortly before the shutter was opened and broken just afterward. Thus during one revolution of the kymograph drum the tissue was stimulated and one complete muscle record was made, together with the time curve.

Thre curves, those of the tuning-fork and of the contraction-waves at two points of the muscle were thus described above one another. Experiments with the rhythmically beating heart of *Limulus* required opening the shutters by hand just before a contraction of the organ.

Determinations were made to ascertain the time which should elapse after a change of solution in order to enable the tissue to reach a uniform condition and permit duplicate records to be secured. This period, with frequent changes and stirring of the solutions in the tumblers, was rarely less than 20 minutes in the smallest frog muscle. During this time the records indicated gradual changes in the speed of the contraction-wave. After a lapse of this initial period records were taken at intervals duringan hour or more. These related to the behavior of the tissue (*a*) in the normal medium, Ringer's solution or sea water, (*b*) in a medium made by diluting the normal medium with isotonic sugar solution, and (*c*) again in the nor mal medium. The change in the behavior of the tissue on passing from the normal medium of high conductivity to a medium of lower conductivity could thus be compared with the change following the reverse transfer. The rate of recovery of propagation-velocity on returning from the medium of low conductivity to the normal medium could also be determined.

RESULTS.

Limulus.—The experiments upon the heart of the king-crab were designed to show (*a*) changes of irritability in pure isotonic sugar solutions, (*b*) the behavior in mixtures of sea water with isotonic sugar solution in various proportions, and (*c*) the behavior in sea water under the same experimental conditions over a period equivalent to that covered by the tests. The determinations of the propagation-velocity have been made over different distances and with respect to different portions of the hearts. The most consistent records are from the anterior third; while those from the posterior and anterior region, *i.e.*, covering the length of the whole organ, are of doubtful value. In all cases the temperature of the solutions employed has been adjusted to that of the sea water on the day of the experiment.

In running sea water the rate of beat of the excised heart may differ from that observed in the body before the operation. After transfer to the paraffin trough used in the tests the rate of beat at first decreases, or the heart may stop beating altogether. In the latter case hearts usually resume beating in a few minutes after slight pinching or tapping. The rate of beat was always found to increase during the next 10 minutes, and, after this interval it usually remained essentially unchanged. In some cases a decrease in the rate of beat was observed, and the amplitude of the contraction decreased, although the excised heart will frequently beat for 2 days or more. If now, after 30 minutes in sea water, the heart is placed in pure isotonic sugar solution, the beat at first slightly increases in rate and then diminishes through a period of 70 to 80 minutes and finally ceases altogether. In six hearts observed under these conditions the average time required for a complete loss of rhythmic beating was 76 minutes. Artificial stimulation, pinching or turning the heart about in the trough did not induce any regular beating. Rhythmical beating was resumed, however, as soon as sea water was allowed to flow about the heart for a few moments; the rate of beat was in some instances somewhat slow at first but soon became about the same as before exposure to the sugar solution. Two of the above hearts which had been exposed to sugar solution for a little over 2 hours recovered in sea water within 3 minutes; one heart exposed to sugar solution for $3\frac{1}{2}$ hours; recovered in a little less that 4 minutes. The three remaining hearts were returned to sea water within a few minutes after the rhythmic beating had stopped and recovered in between $2\frac{3}{4}$ and 3 minutes. Duplicate records of the speed of the contraction-wave were not secured in these experiments because of the variability of the beating in sugar. In solutions in which three-quarters of the sea water is replaced by sugar solution, *i.e.*, 25 per cent sea water or less, there is a constant decrement in the rhythmic beating and a final loss of irritability; the time required for this loss is greater than in pure sugar solution, varying from 2 to $3\frac{1}{2}$ hours. Attempts to get duplicate records of the propagation-velocity in these solutions were not successful. Some observations were made in 30 per cent sea water, but the velocity of the contraction-wave over two or three segments proved exceedingly slow and the observations showed poor agreement.

Most determinations of the propagation-velocities have accordingly been made in mixtures of sea water and sugar solution containing 40 per cent or more of the normal electrolyte content; *i.e.*, 0.4 or more sea water.

Estimates of the velocity of propagation of the contraction-wave in the *Limulus* heart based upon records taken from the two opposite ends of the organ are variable and inconsistent.. Records taken from the second and eighth segments also appear in no cases to have given consistent results. If recording devices are attached to the second,

TABLE I.

Contraction-Wave in Limulus Heart. Experiment 30. Sea Water, 21°C.

Distance.	Velocity per second.	Segments.	Time.
cm.	*cm.*		*hrs.*
3.0	75	4→2	1
4.4	62	4→8	1
7.4		8→2	1
3.0	69	4→2	3
4.4	65	4→8	3
7.4		8→2	3
3.0	75	4→2	6
4.4	65	4→8	6
7.4		2→8	6
3.0	68	4→2	8
4.4	64	4→8	8
7.4		2→8	8
3.0	75	4→2	10
4.4	65	4→8	10
7.4		2→8	10

fourth and eighth segments as recorded in Table I, the records show that at times the contraction of the second segment may actually precede that of the eighth, while the intermediate segment may contract before either of the others. Hence the time elapsing between the contractions at opposite ends of the heart may be so brief as to show that the case is not one of simple transmission of a single contraction-wave from end to end of the organ. Transmission, however appears to be uniform over a short region near the middle of the heart. Records from this middle region, *i.e.*, fourth, fifth, and sixth segments, indicate that these segments contract almost simultaneously.

The majority of the records have been taken from two points situated a short distance apart in the middle region. The results are not combined in a single set of figures because the same conditions do not appear to prevail in all regions. Each experiment by itself however, indicates that a lowered electrical conductivity of the medium is always associated with a reduction in the propagation-velocity of the contraction-wave over the region under observation. It will be noted that in all of the experiments the ratios of velocity with respect to conductivity are of the same order.

Another variable feature has been encountered in the irregular behavior of the hearts of animals which were kept in captivity for more than two months at Woods Hole. After being exposed in the trough for a few hours such hearts would often reverse their direction of beat, or would become arhythmical. In the case of animals fresh from the shore and others which had been artificially fed with small fish the hearts showed regular behavior and have provided consistent records, from which Table II has been compiled.

In running sea water the propagation-velocity of a healthy heart may be regularly so high as 80 cm. per second, but this velocity is not always found if the same regions are compared. In the eight experiments reported the velocity varies from 63 to 81 cm. per second. The average velocity is in the neighborhood of 70 cm. per second. The concentrations given in the second column of Table II are expressed as the fractional part of sea water present in the mixed solutions, *i.e.*, a mixture of 8 parts sea water and 2 parts isotonic sugar solution is designated as "0.8 *Sw.*" The conductivity of the sea water is taken as unity, and the proportional conductivity of the mixtures if calculated from the experimental determinations of resistance. The velocity of the propagated disturbance is expressed in centimeters per second. These two sets of determinations are combined in the last column as the ratio of velocity v with respect to conductivity c. All of the calculations are subject to an error introduced in reading the records, varying from 5 per cent at the speed of 80 cm. per second to 2 per cent at 20 cm. per second.

A number of observations were made with reference to the aeration of the solution. *Limulus* hearts examined in mixed solutions made with sea water which had been shipped to Worcester from

TABLE II.

Contraction-Wave in Limulus Heart.

Record.	Experiment.	Solution.	Conductivity. (Sw 1).	Temperature.	Distance.	Velocity per second.	$\frac{v}{c} = k.$	Segments.
				°C.	cm.	cm.		
C	32	*Sw*	1.00	22.0	4.0	81	81	5→2
D	32	0.7*Sw*	0.66	22.0	4.0	55	83	5→2
E	32	*Sw*	1.00	22.0	4.0	83	83	5→2
H	32	0.5*Sw*	0.55	22.0	4.0	43	78	5→2
K	32	*Sw*	1.00	22.0	4.0	76	76	5→2
B	35	*Sw*	1.00	22.0	3.5	78	78	5→2
F	35	0.8*Sw*	0.77	22.0	3.5	57	74	5→2
J	35	*Sw*	1.00	22.0	3.5	73	73	5→2
B	36	*Sw*	1.00	22.0	5.0	78	78	6→2
F	36	0.5*Sw*	0.55	22.0	5.0	42	61	6→2
H	36	*Sw*	1.00	22.0	5.0	74	74	6→2
C	44	*Sw*	1.00	22.0	3.5	73	73	5→2
E	44	0.7*Sw*	0.66	22.0	3.5	42	64	5→2
G	44	*Sw*	1.00	22.0	3.5	73	73	5→2
I	44	0.7*Sw*	0.66	22.0	3.5	42	64	5→2
K	44	*Sw*	1.00	22.0	3.5	67	67	5→2
B	52	*Sw*	1.00	21.5	3.1	70	70	4→2
D	52	0.5*Sw*	0.55	21.5	3.1	35	64	4→2
F	52	*Sw*	1.00	21.5	3.1	60	60	4→2
I	52	0.7*Sw*	0.66	21.5	3.1	49	73	4→2
L	52	*Sw*	1.00	21.5	3.1	60	60	4→2
B	53	*Sw*	1.00	21.5	4.3	63	63	4→8
D	53	0.9*Sw*	0.92	21.5	4.3	60	65	4→8
F	53	*Sw*	1.00	21.5	4.3	63	63	4→8
I	53	0.7*Sw*	0.66	21.5	4.3	46	69	4→8
K	53	*Sw*	1.00	21.5	4.3	63	63	4→8
N	53	0.5*Sw*	0.55	21.5	4.3	36	65	4→8
P	53	*Sw*	1.00	21.5	4.3	60	60	4→8
B	54	*Sw*	1.00	21.5	4.0	63	63	5→2
D	54	0.8*Sw*	0.77	21.5	4.0	55	72	5→2
G	54	*Sw*	1.00	21.5	4.0	63	63	5→2
J	54	0.4*Sw*	0.42	21.5	4.0	24	58	5→2
L	54	*Sw*	1.00	21.5	4.0	63	63	5→2
O	54	0.6*Sw*	0.62	21.5	4.0	38	62	5→2
R	54	*Sw*	1.00	21.5	4.0	59	59	5→2
C	55	*Sw*	1.00	21.5	3.0	68	68	4→2
E	55	0.7*Sw*	0.66	21.5	3.0	47	71	4→2
G	55	*Sw*	1.00	21.5	3.0	68	68	4→2
I	55	0.4*Sw*	0.42	21.5	3.0	29	69	4→2
K	55	*Sw*	1.00	21.5	3.0	63	63	4→2
M	55	0.6*Sw*	0.62	21.5	3.0	44	71	4→2
P	55	*Sw*	1.00	21.5	3.0	63	63	4→2

Woods Hole, exhibited a smaller decrement in the rate of beat when air had been bubbled through the solutions than when this procedure was omitted. At Woods Hole the records showed better agreement when the behavior of the hearts was studied in freshly made mixtures or in aerated mixtures than when similar solutions were used which had stood in bottles for 2 days or more.

Frog.—More extensive studies of the relationship between the electrical conductivity of the surrounding medium and the propagation-velocity of the contraction-wave have been made with the sartorius muscle of the leopard-frog. Each muscle strip is mounted vertically on a supporting apparatus; the muscle is stimulated electrically at one end by minimal single shocks, and the contraction-wave travels thence to the other end of the tissue along the parallel fibers. The distances between the two points at which the wave has been recorded vary between 1.5 cm. and 2.4 cm. Break-shocks are sent into the tendinous end of the muscle through a platinum stimulating electrode. The temperature during the experiments has been adjusted to 20°C. In some cases reported below the oxygen tension has been increased by bubbling oxygen through the solutions. The hydrogen ion concentration of all the mixtures is nearly 6.8.

Tests made over long periods of time indicate that the sartorius muscle of the frog is very resistant to the artificial conditions imposed in the experiments. Observations on muscles immersed in Ringer's solution have been made at intervals during periods so long as 96 hours, and toward the end of this time only a slight loss in the propagation-velocity of the contraction-wave has been recorded. The amplitude of the contraction-wave appears to decrease very gradually during the first 2 days, and more rapidly during the third and fourth days. In solutions of cane-sugar the irritability of the tissue is soon lost completely; the time required with several changes of solution varies from 20 to 30 minutes. Upon return to Ringer's solution the irritability is restored within approximately 1 minute. At 20°C. the normal rate of conduction of the tissue in Ringer's solution is about 3 m. per second. In the Ringer-sugar mixtures this rate decreases in close proportionality with the reduction of the electrical conductivity until the concentration of the salts has been reduced to about one-half the normal. In the range between the

dilution with one-half reduction of salts to three-fourths reduction, the propagation-rate falls off more rapidly than the electrical conductivity; while if more of the Ringer salts be replaced by sugar the irritability is soon decreased to zero. In the solutions of low electrical conductivity the tissue becomes non-irritable in the course of 2 hours or more, but recovers immediately upon being replaced in Ringer's solution. It is remarkable that increase of oxygen tension may largely compensate for the reduction in salts. If oxygen be bubbled through the solutions the decrease of propagation-velocity may remain almost proportional to the decrease of electrical conductivity until only one-twentieth of the salts of Ringer's solution remain (*cf.* Table V).

Seventy-four series of experiments have been conducted upon propagation-velocities in varying mixtures of Ringer's solution and isotonic sugar solution. The order of the solutions used has been frequently altered to offset errors; while the time between the transfer of the muscle to a given solution and the taking of the record has always been long enough to allow the tissue to reach an equilibrium with the medium. Experiments 141, 142, and 143 (*cf.* Table III) are typical of the observations, and are selected with reference to the mixtures ranging from 30 per cent Ringer's solution to normal Ringer. The velocities and the conductivities are averaged and the ratios calculated in Table IV. In these experiments the mixtures were agitated continuously with a slow stream of air.

Experiments have been made on the velocity of the contraction-wave in solutions of still lower electrical conductivity, in which an increase of oxygen tension enables transmission to occur. Nine experiments in which oxygen was bubbled through the mixtures are recorded in Tables V and VI. In three of these experiments a mixture was employed containing only 5 per cent of the normal salt content of Ringer's solution. The muscle contracted and transmitted the contraction wave under these conditions; although in the same solutions containing oxygen at air tension no such behavior appears possible. In pure M/4 sugar solution saturated with oxygen these muscles lost irritability in slightly less than a half hour. Two of the experiments in the 0.05 Ringer's solution show a transmission rate very close to that calculated from the electrical conductivity.

TABLE III.

Contraction-Wave in Sartorius of Frog.

Record.	Solution.	Conductivity.	Velocity.	$\frac{v}{c} = k.$
Experiment 141; Wave Distance = 1.6 cm.; 20° C.				
B	Ringer	1.00	319	319
D	0.6R	0.58	174	300
G	0.4R	0.38	104	276
J	Ringer	1.00	319	319
Experiment 142; Wave Distance = 1.6 cm.; 20° C.				
A	Ringer	1.00	320	320
C	0.8R	0.81	258	319
D	0.5R	0.48	140	291
G	Ringer	1.00	320	320
J	0.3R	0.30	79	262
L	0.7R	0.69	215	312
N	Ringer	1.00	320	320
P	0.9R	0.92	288	313
R	Ringer	1.00	320	320
Experiment 143; Wave Distance = 1.7 cm.; 0 20° C.				
A	Ringer	1.00	306	306
G	0.3R	0.30	90	300
J	Ringer	1.00	306	306
M	0.9R	0.92	278	302
R	0.5R	0.48	127	265
S	Ringer	1.00	306	306
X	0.7R	0.69	213	309
Z	Ringer	1.00	306	306

TABLE IV.

Contraction-Wave in Sartorius of Frog.

(Average of Experiments 141, 142, 143.)

Solution.	Conductivity.	Velocity.	$\frac{v}{c} = k.$
Ringer	1.00	315	315
0.9R	0.92	283	308
0.8R	0.81	258	318
0.7R	0.69	214	310
0.6R	0.58	174	300
0.5R	0.48	134	279
0.4R	0.38	104	274
0.3R	0.30	85	283

In all of the solutions having a conductivity less than seven-tenths that of Ringer's solution the average ratio of velocity with respect to conductivity is higher than in Ringer's solution (*cf.* Table VI).

TABLE V.

Contraction-Wave in Sartorius of Frog.

(With Constant Stream of Oxygen.)

Solution.	Conductivity. (Ringer=1.0)	Velocity per second.								
		Experiment No.								
		94	95	96	97	98	99	101	103	107
		cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
Ringer	1.00	295	310	315	312	300	320	300	306	315
0.9R	0.92	—	—	—	—	—	—	—	271	280
0.8R	0.81	—	246	—	250	—	—	245	—	—
0.7R	0.69	—	—	—	—	—	—	—	220	214
0.5R	0.48	150	—	160	—	144	—	147	—	155
0.4R	0.38	—	125	—	122	—	130	—	119	126
0.3R	0.30	100	—	—	—	—	—	95	—	—
0.2R	0.20	—	70	73	—	110	60	—	75	70
0.1R	0.11	40	—	45	—	—	35	—	—	—
0.05R	0.06	—	20	22	37	—	—	—	—	—
0.00R	0.0001	0	0	0	0	—	—	—	—	—

TABLE VI.

Contraction-Wave in Sartorius of Frog.

(Average Velocities of Table V.)

Solution.	Conductivity. (Ringer = 1.0)	Velocity per second.	$\frac{v}{c} = k.$
		cm.	
Ringer	1.00	308	308
0.9R	0.92	276	300
0.8R	0.81	247	304
0.7R	0.69	217	315
0.5R	0.48	151	314
0.4R	0.38	124	326
0.3R	0.30	97	323
0.2R	0.20	76	380
0.1R	0.11	40	458
0.05R	0.06	26	433

TABLE VII.

Contraction-Wave in Ventricle of Turtle.

Record.	Solution.	Conductivity (Ringer = 1.00).	Velocity per second.	$\frac{v}{c} = k$.
Experiment 152; Wave Distance = 2.0 cm.; 20°C.				
			cm.	
A	Ringer	1.00	7.6	7.6
D	0.5R	0.48	5.9	12.3
F	0.7R	0.69	6.4	9.2
H	Ringer	1.00	7.8	7.8
Experiment 154; Wave Distance = 1.6 cm.; 20°C.				
B	Ringer	1.00	8.8	8.8
E	0.8R	0.81	6.7	8.3
H	0.5R	0.48	4.0	8.3
L	0.4R	0.38	2.8	7.4
N	0.6R	0.58	4.8	8.3
P	Ringer	1.00	7.2	7.2
Experiment 155; Wave Distance = 3.0 cm.; 20°C.				
C	Ringer	1.00	9.1	9.1
E	0.4R	0.38	3.4	8.9
F	Ringer	1.00	7.5	7.5
Experiment 156; Wave Distance = 3.0 cm.; 20°C.				
C	Ringer	1.00	9.9	9.9
E	0.7R	0.69	6.0	8.7
H	0.9R	0.92	6.8	9.8
J	Ringer	1.00	8.8	8.8
M	0.5R	0.48	4.6	9.6
R	Ringer	1.00	6.5	6.5
Experiment 158; Wave Distance = 2.4 cm.; 20°C.				
B	Ringer	1.00	7.4	7.4
F	0.8R	0.81	7.1	8.7
G	0.6R	0.58	5.2	8.9
I	Ringer	1.00	6.9	6.9

Turtle.—Strips cut from the ventricle of the river terrapin were used in 1917 and 1921 with two different recording devices. The earlier observations were recorded by means of levers writing on smoked paper; in the later experiments the optical recording device

described above was used. In both sets of observations the initial speed is practically the same at about 20°C.; *viz.* about 7 cm. per second. Both the old and the newer readings agree also in respect to the rate and character of the changes undergone by the tissue under the experimental conditions. After a period of 6 hours in Ringer's solution the transmission-velocity begins to be irregular and the minimal stimulus in Ringer may fail to produce contraction. Upon changing the tissue end for end, or cutting off a little of the tissue on both ends, the regularity of response to the original minimal stimulus is restored and the transmission-velocity recovers. The strips of tissue selected for use have been from 3 to 5 mm. wide and from 3.5 to 4.5 cm. long. The observation points were between 0.6 and 3 cm. apart.

In 0.3 Ringer's solution strips of ventricle lose their irritability in the course of about 2 hours, and in pure isotonic sugar solution in about 50 minutes. Recovery of irritability upon replacement in normal Ringer's solution occurs in about 2 minutes. Little difference has been observed between the propagation-velocities in freshly prepared Ringer's solution and in older solutions through which air was bubbled.

The velocity of the contraction-wave is reduced in mixtures of Ringer with sugar solution; but in solutions of less than 0.6 Ringer the reduction of transmission-velocity is not proportional to the reduction in conductivity. Five experiments of the last series are recorded in Table VII.

DISCUSSION.

The data presented in this paper lend further support to the thesis that the electrical conductivity of the medium surrounding an activated protoplasmic system is in direct correlation with the rate of spread of the state of activation over the system. In skeletal muscle, the contraction-wave travels along the fibers at a velocity which is closely proportional to the electrical conductivity of the fluid in contact with it. A reduction, by means of dilution with an indifferent non-electrolyte solution, in the electrical conductivity of the normal balanced medium is followed by a corresponding reduction in the propagation-velocity of the contraction-wave. This parallelism

is closer in solutions of higher than in those of lower electrical conductivity. The ratio of velocity with respect to conductivity is nearly a constant with frog's muscle, and less constant with muscles of the turtle and *Limulus*.

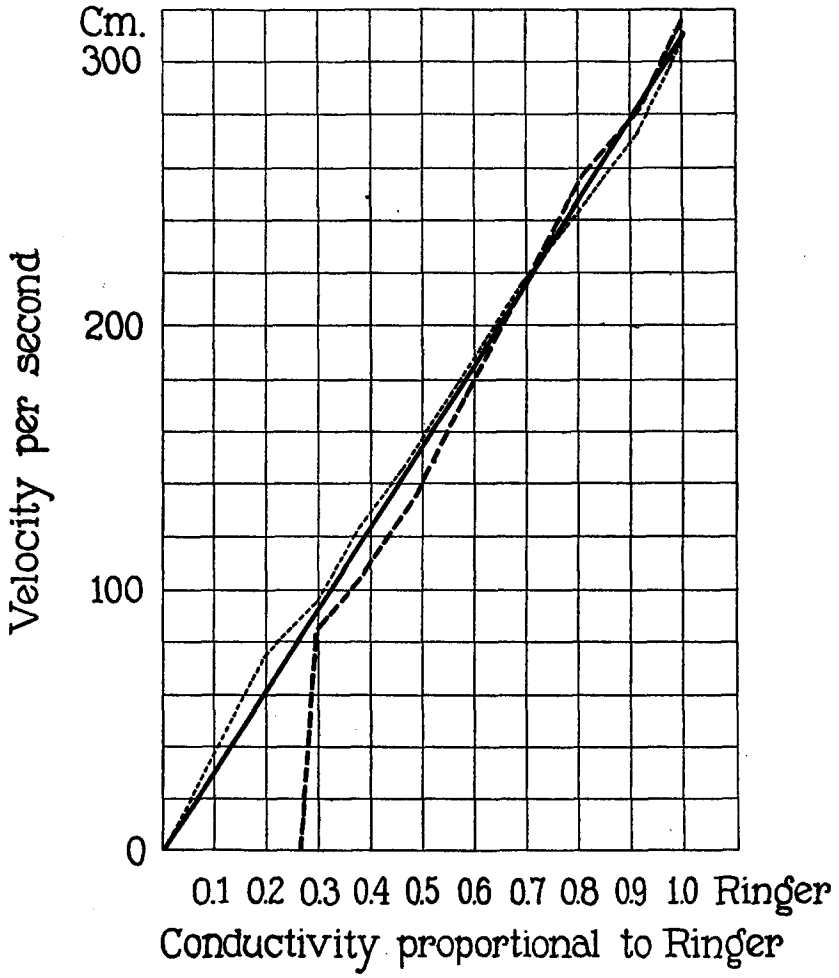

FIG. 1. Sartorius of frog in Ringer-sugar solution. Long dashes indicate aerated mixtures (Table IV); short dashes, oxygenated mixtures (Table VI).

In Fig. 1 the velocities of the contraction-wave in the sartorius of the frog are plotted against the conductivities of the solutions.

It will be seen that the dotted line runs closely parallel with the straight line which represents an ideal dependence of speed of conduction upon electrical conductivity. The dotted line connects the values secured when the muscle is immersed in a solution saturated with oxygen; while the dash line connects those obtained when the muscle is exposed to media containing oxygen at air tension.

The fact that in the presence of a stream of oxygen the transmission of the contraction-wave is possible even in solutions of very low conductivity is an indication that processes of oxidation are directly concerned in stimulation and in the propagation of the excitation state. If the primary change in stimulation is an alteration of the protoplasmic surface film, it would appear that in a medium of a given electrical conductivity a certain minimal concentration of oxygen is necessary for the completion of this surface reaction. Lillie has pointed out that such a relation of oxygen to a chemical reaction at the cell surface is suggestive of conditions similar to those of an electrolysis at an electrode. In this case it is "possible to reduce the current-strength through a wider range, and still have a high rate of decomposition at the electrode, if the concentration of the reacting substance is high, than if it is low."[14] In the present state of knowledge of the chemical conditions determining the formation of an active or "stimulated" region in living tissue, it is difficult to define clearly the rôle of oxygen in the stimulation process. In general it seems most probable that free oxygen is required in the return of the stimulated region to the resting state and in the propagation of the contraction-wave after the local stimulation has been aroused. If the analogy between activation in living tissues and in oxidisable metals bathed by electrolyte solutions, *e.g.* iron in nitric acid, is at all close, then the destruction of the surface film at any region during the rise of the activation-wave should depend upon a local reduction rather than upon an oxidation; apparently when the reduction has reached its maximum an oxidation process occurs, reforming the film and the passive or resting state is regained. In other words, the local excitation state is to be regarded as an effect resulting from the expenditure of energy in the stimulated region—

[14] Personal communication.

accompanying a reduction process; after the excited region has reached a maximum negativity (electrically negative with respect to adjacent regions) the reverse or oxidative process automatically follows, with the effect of reestablishing the passive or resting state. According to this hypothesis the free oxygen enters directly into only one part of the local stimulating process; but this oxidation must be repeated at each successive region during the propagation of the excitation state along the tissue.

If transmission in the living tissue is in fact dependent upon secondary electric stimulation of the resting region by the local bioelectric current between that region and the active region adjoining, there should be a direct proportionality between the electrical conductivity of the medium and the rate at which the state of activity spreads from region to region. The electrical conductivity of the first local stimulating circuit, other conditions being equal, determines the intensity of the current at any point in the circuit; and if the conductivity is uniform throughout the tissue and medium the rate of propagation will be uniform throughout the tissue. Differences in the electrical conductivity of the medium will be associated with differences in the rate of propagation because of the effect upon the intensity of the local bioelectric current traversing the tissue at any point adjacent to the active (electrically negative) area. The greater the conductivity of the medium, the greater will be the distance (from the already active region) at which the current traversing the resting tissue is sufficiently intense to effect the stimulating reaction. It is on the basis of such general considerations that Lillie believes we may provisionally disregard the special chemical nature of the local reaction (at the cell-medium boundary), on the ground that at present we know very little about it. Whatever the nature of the local stimulation process may happen to be, if it is initiated electrically by the current of the local bioelectric circuit it should spread from region to region at a rate proportional to the electrical conductivity of the circuit. This conductivity depends chiefly on the conductivity of the external medium.

In the preliminary work before any oxygen had been employed experimentally and when conditions were constant (as with air bubbled through the solutions or with fresh solutions) it seemed

possible that sugar might have some direct toxic action upon the tissues. Later considerations, however, make this doubtful. In the first place the parallelism between the electrical conductivity and propagation rate is closer with higher than with lower tensions of oxygen, although the sugar solutions are of the same concentration. In the second place if sugar acts toxically the toxic effect should increase proportionately to the increase in the length of exposure to the more concentrated sugar solutions. That this is not the case is indicated by the fact that the frog and *Limulus* tissues after immersion in pure sugar solution for periods up to 3 and 4 hours recover their irritability, upon return to Ringer's solution, just as rapidly and completely as when exposed to the pure sugar solution for periods just sufficient to abolish irritability. A further peculiarity in the action of sugar solutions, namely, the marked difference between the time required for loss of irritability in sugar solutions and for recovery of irritability on return to the normal conducting solution—the latter interval being many times shorter than the former—is not readily explained. It is beyond the aim of the present paper to discuss this problem, and it may be that the conditions must be more carefully investigated.

The author desires to thank Dr. Ralph S. Lillie for constant help and advice in the study of the present problem, and the Trustees of Clark University for the arrangements under which the research has been prosecuted.