

# STUDIES ON THE COMPARATIVE PHYSIOLOGY OF CONTRACTILE TISSUES

## II. THE RELATION OF ELECTROLYTES TO ELECTRICAL CONDUCTIVITY.

BY LANCELOT HOGBEN AND CECIL GORDON.

(From the Department of Zoology, University of Cape Town.)

(Received 12th November 1929.)

(With Four Text-figures.)

### 1. INTRODUCTION.

IN a previous communication of this series an account was given of the relation of kations to contractile rhythm of certain invertebrate preparations (Hogben, 1925). The data therein recorded emphasise the conclusions derived from other researches such as those of Mines and Clark (1921) who have shown that whereas, when dealing with the same species and the same type of contractile tissue, entirely consistent phenomena are encountered, effects of a totally different character are met with in studying the relation of a particular ion to analogous muscle preparations in other species. The possibility of harmonising these discrepancies was indicated in a critical summary (Hogben, *op. cit.*) in which attention was directed to the imperative necessity of a comprehensive study of the relation of electrolytes to the physical properties of muscle. The present research was undertaken as a contribution with that end in view; and was suggested by a series of researches carried out by Osterhout on the seaweed *Laminaria* and other plant tissues.

The investigations referred to deal specifically with the study of cell permeability in relation to electrolytes by means of the conductivity method. *Inter alia* Osterhout has studied the relation of certain kations and of anaesthetics to the Kohlrausch conductivity of pieces of *Laminaria*. His data provide suggestive indications of the nature of ionic "antagonisms" in agreement with conclusions derived from the study of cell permeability by methods which are less amenable to quantitative treatment. Similar experiments have been carried out by other workers, using suspensions of cells; but as Zoond (1927) has pointed out, the suspension method has serious objections. Osterhout's most important finding from the present standpoint is the fact that pieces of *Laminaria*, which in a physiologically "balanced" solution retain a fairly constant resistance over long periods of time, exhibit in pure solutions of monovalent kations a continuous and rapid fall of resistance to the death point; but if placed in pure solutions of divalent or trivalent ions, undergo a rapid and considerable rise of resistance before falling to the lethal limit. This difference, like

the well-known antagonism of calcium and sodium ions which has been brought out in the study of cell permeability by other forms of procedure, finds a ready explanation along the general lines of Clowes' phenomenon; but like the latter gives us no clue to the striking contrasts between the action of sodium and potassium on the one hand or calcium, magnesium and barium on the other, contrasts familiar to every worker on ionic antagonisms in animal tissues. It was in the hope of applying Osterhout's method to animal tissues with a view to elucidating the specific action of ions that this investigation was undertaken.

In a short communication extensively quoted in his monograph on injury, recovery, etc., Osterhout (1919) has himself stated the substantial agreement of results obtained in experiments on frog's skin with his more comprehensive researches on plant tissues. He states that frog's skin placed in pure solutions of  $\text{CaCl}_2$ ,  $\text{MgCl}_2$ , and  $\text{HCl}$  show a rapid rise in resistance followed by a fall to the lethal limit, placed in pure solutions of  $\text{NaCl}$  and  $\text{KCl}$  they suffer a continuous diminution of resistance, while in sea water (isotonic with the tissue fluids) they remain during the same periods constant. No tables of quantitative data nor graphs are given; and the author concedes that his experimental data were less satisfactory than those obtained with plant tissues through lack of a sharp null point in the bridge measurements. This, as will be pointed out later, is a more serious criticism of the validity of his conclusions than Osterhout apparently realised. Preliminary experiments of the senior author showed in fact that to obtain a satisfactory null point with the Kohlrausch method applied to animal tissues it is essential to use variable condensers of high capacity in series with the standard resistance. As the investigation developed the necessity of a host of other precautions emerged. Some of these will now be set forth.

## 2. METHODS.

A resistance bridge of the slide-wire type, an audio hummer of 1000 cycles, a standard resistance of 1000 ohms (non-inductively wound) with head phones as the null point instrument together with a variable capacity, formed the essential parts of the physical apparatus. In addition a suitable cell for the tissue itself was designed for the purpose. In detailing the methods employed attention will be paid to the following points in order: (1) the cell for holding the tissue, (2) the solutions employed, (3) the balancing of the capacity effect, (4) the errors arising from stimulation of the tissue by the bridge arm circuit, and (5) the regulation of temperature.

### (1) *The conductivity cell.*

The present design employed for the conductivity cell was the result of numerous trials with various types with the objective of obtaining one which should combine the most suitable design for purposes of electrical measure, compatible with holding the tissue without leakage. The arrangement is illustrated in Fig. 1, which is drawn to scale. A rectangular ebonite box of internal dimensions 5 cm.  $\times$  2.3 cm.  $\times$  3.1 cm. was constructed in two halves with well-ground edges, between which two glass plates, 7 cm. high, perforated with a circular hole 0.85 cm. in diameter, the lower

edge being level with the floor of the trough, could be inserted between the two halves of the latter. The tissue was placed between the plates so that when the whole was kept in position by the spring screw *S*, current could only pass from one half of the trough into the other through the circular area of tissue between the two holes in the glass plates. Circular platinum electrodes of about 1.3 cm. diameter were applied close to the aperture on either side of the glass plates and held in position by an ebonite block grooved to rest on the top edge of the latter. The resistance of the small amount of fluid between the electrodes and the tissue was always small compared with that of the tissue itself, and the appropriate correction for this was made in all calculations of the net resistance of the tissue itself. No correction was necessary for capacity when no tissue was placed between the glass trays, since the capacity of the cell without the tissue was altogether negligible. Needless to say, all coiling of wire connections such as introduce inductances into the circuit was avoided.

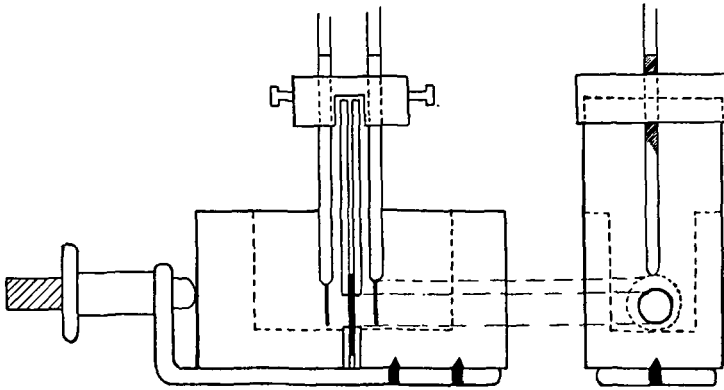


Fig. 1.

When the edges of the two halves of the trough and the surfaces of the glass plates were vaselined this device proved perfectly watertight for flat tissues such as skin, but to preclude loss of the contents of the trough by leakage the whole apparatus was inserted in a second ebonite trough just sufficiently large to contain it. So long as the level of the fluid in the outer trough was not above the upper edge of the inner trough the resistance leakage was completely negligible.

In most experiments four cells were employed, two being filled with one fluid and two with the other whose influence was to be examined.

(2) *Solutions employed.*

As the present investigation was restricted to the effects of kations, solutions of the chlorides of appropriate molarity were prepared from Merck's purest reagents (A.R. quality). As in the previous investigation of this series the formulae represent proportions by volume. The requisite hydrogen ion concentration was obtained by buffering with sodium phosphate, the latter always being added before the addition of Ca, etc., to avoid precipitation.

(3) *The capacity effect.*

In general in dealing with animal tissues in a cell of the type described, very large capacities were encountered. Osterhout in his researches on *Laminaria* employed a condenser of 0.001 mf. The insertion of a piece of toad skin or muscle in the conductivity cell in these experiments introduced a capacity in some cases more than a hundred times as great. Pieces of skin placed one over the other appear to act like condensers in series; but to eliminate the capacity effect in this way is evidently an unsuitable procedure in studying the action of electrolytes for two reasons: first, that both surfaces of the tissue should be in contact with the fluid; secondly, owing to compressibility and variation in thickness it is impracticable to remove the tissue from the same position in the course of an experiment. In his paper on "A Comparison of Permeability in Plant and Animal Cells" Osterhout gives no information as to the capacities he employed, but he does note that "the measurement of the resistance of the frog skin was less accurate than that of *Laminaria*, the point of minimum sound in the telephone was more indefinite..." At the threshold of this investigation it was realised that failure to balance the capacity of the tissue would completely invalidate results obtained, and this in all probability accounts in part for the divergence between the results obtained by Osterhout and those of the present writers. It will be seen from the theory of the bridge given below that the bridge ratio only gives a valid measure of resistance when the capacity in both arms is so adjusted that a point of complete silence is obtained, and indeed the point of minimum sound varies enormously with different capacities. But before passing on to the theoretical aspect of the question the following experimental data may be appropriately inserted to illustrate the importance of the capacity effect.

*Experiment 1.* Skin of *Xenopus* at 5° C. in Na 100 : Ca 2 : K 2. M/8.

Bridge readings for different minima with varying capacity:

Table I.

(a) Living skin.

Capacity of condenser	Bridge reading (100 cm.)	
0	27	
0.01	27	
0.02	29	
0.03	29.5	
0.04	30.5	
0.05	31	
0.06	32	
0.08	33	Complete silence
0.09	34	Complete silence
0.10	34	
0.11	35	
0.30	48	

Taking the average of the two values for which complete silence was obtained we have for the resistance 504 ohms and the capacity 0.165 mf. (*vide infra*).

Table II.

(b) Dead skin. (Same piece killed by steam.)

Capacity	Null point
0	16
0.01	16.5 Complete silence
0.02	16.5
0.06	17
0.30	20

The resistance has now become 198 ohms and the capacity 0.015 mf. The resistance of the cell without tissue in this experiment was 190 ohms and capacity 0.01 mf. In subsequent records the full experimental data will be left out and all results expressed in percentages of initial values.

In this investigation a Leeds Northrop three-dial variable condenser graduated in steps of 0.001 mf. from 1 mf. downwards proved adequate to obtain complete silence, and, since a difference of 0.002 mf. in determining the quality of the null point was detectible it will be seen from what follows below that working with capacities of 0.04 mf. the bridge method can yield measurement of changes of capacity with an error of less than 10 per cent. Since the fall in capacity at the death point is immensely greater proportionately than the fall in resistance, it is a matter of some interest to place on record capacity changes in the course of an experiment.

When introduced into the conductivity cell the tissue behaves like a leaking condenser. Campbell (1906) has applied the theory of condenser and resistance in parallel to the study of leaking condensers. The theory of our own procedure (Fig. 2) is as follows:

Let the potential between *A* and *B* be given by

$$E = E_0 e^{j\omega t}$$

$$i_2 = \frac{E_0 e^{j\omega t}}{R} ; i_1 = \frac{dQ}{dt} = C \frac{dE}{dt} = Cj\omega E_0 e^{j\omega t}.$$

$$\therefore i_{12} = \frac{E_0 e^{j\omega t}}{R} + Cj\omega E_0 e^{j\omega t}.$$

The effective resistance  $\rho = E/i_{12}$

$$= \frac{E_0 e^{j\omega t}}{E_0 e^{j\omega t} (1/R + Cj\omega)} = \frac{R}{RCj\omega + 1}.$$

If a tissue with resistance *S* and capacity *K* is placed in one arm of the bridge, the bridge ratio at null point being *A/B* against the arm with resistance and capacity *R, C*,

$$\rho' = \frac{S}{SKj\omega + 1} \quad \text{and} \quad \frac{\rho'}{\rho} = \frac{A}{B}.$$

$$\therefore \frac{S}{SKj\omega + 1} = \frac{AR}{B(RCj\omega + 1)}, \quad \text{or} \quad AR(SKj\omega + 1) = BS(RCj\omega + 1).$$

Equating real to real and imaginary to imaginary,

$$AR = BS \text{ and } AK = BC,$$

or

$$R/S = B/A; K/C = B/A.$$

From the above it will be seen that a null point is obtained when the ratio of the capacities is the inverse of the resistance ratio.

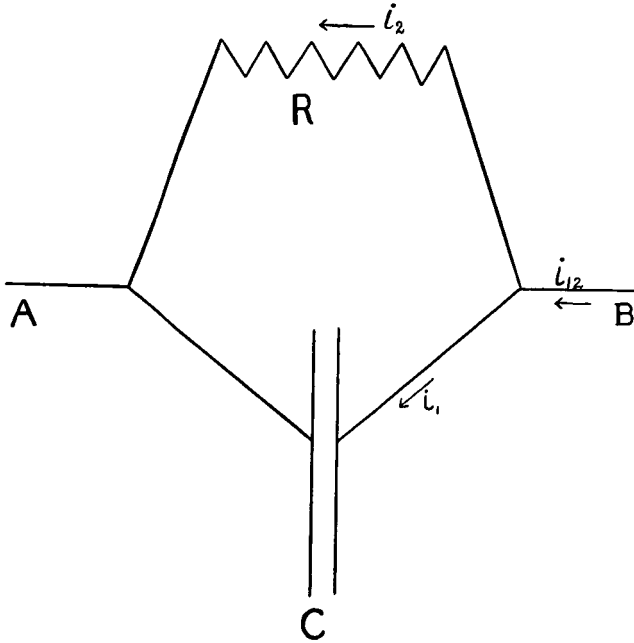


Fig. 2.

(4) *Stimulation—the Dixon effect.*

The present investigation was initiated by the senior author in 1925, and the publication of later experiments by both the collaborators after satisfactory treatment of the capacity effect was delayed by the attempt to disclose the significance of inconsistencies, the source of which eventually proved to be due to the effect on the condition of the tissue itself of the current in the arm containing the conductivity cell.

At first it was found that more uniform results were obtained by cutting down the strength of the current delivered by the audio hummer, and further, that on placing additional electrodes in the conductivity cell so that an A.C. could be passed through the tissue immediately before taking a reading, rapid changes in resistance such as occurred periodically in earlier experiments could be produced. These were at first attributed to dimensional changes in the tissue (muscle) employed, but such an explanation was invalidated partly by reasons which appear later and partly by the discovery that in general a mild alternating current when passed through a variety of tissues produces reversible changes of resistance. When this conclusion

had been reached Dixon and Clark (1927) published an independent account of the effect of electrical currents on the resistance of plant tissues. The effect they described is exactly analogous to that which the present authors had obtained, and it is perhaps of some general theoretical interest apart from the immediate issue in relation to a more precise definition of irritability.

It has become customary in biological text-books to refer to irritability as a primary property of protoplasm, and though the concept is capable of easy definition as applied to nerve, gland cell, or an amoeba, it hardly suffices as a physical definition when a connective tissue corpuscle is under consideration. Changes in resistance of filter paper soaked in oil have been found by the present writers to follow the application of direct or mild alternating currents. These probably depend, since in all cases the changes are of the nature of a fall, on facilitating polar orientation at the oil water interphase in an emulsion. But such changes were in no case found to be of a reversible type.

Table III.

*Skin of Xenopus.*

Initial resistance and capacity 100.

Time h. m.	Capacity	Resistance
55 45	120	161
56 20	120	158
56 25	120	161
	Stimulated 30 sec.	
56 26	80	126
56 28	80	137
56 29	90	149
56 30	90	151
56 33	100	151
56 57	110	154
63 20	110	220
68 45	100	243
79 35	90	244
95 25	90	232
	Stimulated 30 sec.	
95 26	70	179
95 30	80	181
95 50	80	180
113 25	80	195
127 25	90	149
135 30	90	136
140 00	70	108
	Stimulated 30 sec.	
140 01	40	80
152 00	40	69
163 40	30	61
187 15	30	42
	Stimulated 30 sec.	
187 16	30	40

It is hoped later to investigate more extensively these phenomena, particularly because in the case of some tissues the effect of stimulation was so small as to leave open the possibility that they were determined when the change was a fall in

resistance by heating effects of the current. In consequence it would be premature to put forward the universality of what may conveniently be called the Dixon effect. In these experiments an induction coil of the type employed for stimulating muscle nerve preparations could be thrown at will into circuit with the stimulating electrodes. Dixon and Clark found that by varying the strength and duration of the current a positive or negative change in the resistance of ivy leaves could be reproduced reversibly. In the present investigation a change of one or the other type was found to be characteristic of the particular tissue, and it remains to be ascertained whether either type of effect can be obtained with appropriate methods. In the case of muscular tissue from the ventral abdominal wall of the South African clawed toad, *Xenopus*, a large increase of resistance occurs after stimulation, and since it increases for an appreciable period after cessation of stimulus the possibility that it is due to dimensional changes is precluded, quite apart from the fact that the strength of the spring in the conductivity cell was such as to ensure isometric contraction.

An experiment on dorsal skin of *Xenopus* was undertaken to test how long the tissue retained its power to respond reversibly by fall of resistance after stimulation. It also shows that in the case of skin a considerable change in capacity accompanies the fall of resistance. The time in hours after the tissue was placed in Osterhout's solution for frog's skin (sea water diluted one in five) is given; and the capacities and resistances expressed as percentages of the initial values in Table III.

Table IV.

Time (min.)	Control	Stimulated tissue
35	100	—
36	—	100
		Stimulated 30 sec.
37	—	118
38	100	—
41	—	112
42	100	—
56	—	106
69	100	104
92	102	—
94	—	104
		Stimulated 30 sec.
95	—	114
97	101	—
108	101	112
129	101	109
179	101	109
		Stimulated 30 sec.
180	—	122
195	101	122
274	—	129
		Stimulated 30 sec.
275	—	134
276	102	—
283	101	134

The time is reckoned from the setting up of the experiment.



Table IV refers to an experiment on the ventral abdominal muscle of *Xenopus* in a fluid composed by volume of *M/8* chlorides of Na 100 : K 2.5 : Ca 2.5, pH 7.6, at 7° C., the resistances of a piece of stimulated and unstimulated muscle are given below. The increase of capacity on stimulation was consistent but not of sufficient magnitude to be certainly outside the limit of experimental error.

The results obtained in another experiment carried out on the ventral abdominal musculature of *Xenopus laevis*, and representative of a long series of experiments on the same tissue, are graphically presented in Fig. 3. The slow recovery, as already remarked, indicates that we are not here dealing with a dimensional change.

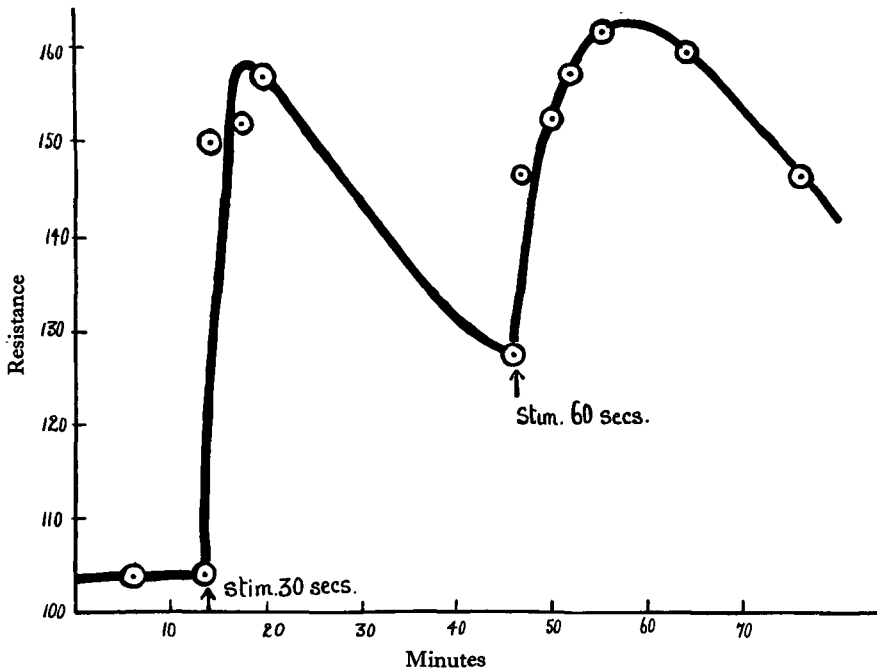


Fig. 3.

Other experiments were carried out with a variety of tissues, including liver of *Xenopus* and the thallus of *Laminaria*. A slight change in resistance occurs in the latter case of the order of less than 1 per cent. This is too small to affect the interpretation of Osterhout's researches. Of such experiments Table V, in which the immersion medium was Ringer's solution, is illustrative.

In the last experiment the capacity of the tissue, which was necessarily relatively thick, was too small to be measured with precision adequate to detect a change. In general we have found that the capacity of a thin piece of tissue is higher than a thick piece, that is to say, the different layers of cells behave like condensers arranged in series. There is no fundamental difference as regards animal and plant tissues. Though the capacities encountered by Osterhout in his experiments on *Laminaria* were low, this was simply because he used a thick piece of tissue and generally

several pieces of thallus placed one over the other. A thin leaf like Virginia Creeper may have a capacity of the same order as the animal tissues used in most of our experiments.

(5) One other point in the control of experiments of this type is the maintenance of constant temperature. As the summer temperature in Cape Town is fairly high, it was necessary to maintain a level lower than room temperature. The highest constant temperature which we could maintain satisfactorily with a Frigidaire thermostat was about 8° C. Most experiments were carried out round about this figure.

Table V.

Time (min.)	Capacity	Resistance
(i) Virginia Creeper leaf:		
0	100	100
1	100	100
	Stimulated 30 sec.	
2	115	79
4	110	82
8	115	86
12	110	93
28	120	96
	Stimulated 30 sec.	
29	130	83
31	115	86
(ii) Ovary of <i>Xenopus</i> :		
0	—	100
	Stimulated 30 sec.	
1	—	105
7	—	105
	Stimulated 30 sec.	
8	—	110
9	—	112
12	—	108
14	—	107
25	—	107
	Stimulated 30 sec.	
26	—	112
27	—	112

### 3. EXPERIMENTAL DATA.

We have dealt somewhat extensively with the methods employed to emphasise the fact that the greatest care was taken to apply successfully Osterhout's method to the study of permeability of animal tissues. The investigation was undertaken with the hope that a method which had proved so suggestive in the study of plant tissues could hardly fail to yield significant conclusions, if employed in animal physiology. After working on these lines for more than three years, during which various sources of error which do not intrude in the case of plant tissues have been eliminated, we are driven to the following conclusions, based on the study of a variety of animal tissues from a variety of species: (a) that as regards changes of

resistance of surviving animal tissues the Kohlrausch method cannot be made to yield results of adequate consistency for the purpose defined; (b) that no general antagonism between the influence of monovalent and divalent ions can be demonstrated for animal tissues by this method; (c) the results of most experiments suggest that, even when dealing with tissues whose cellular elements are homogeneous, the changes in resistance which occur either in "balanced" or "unbalanced" media are attributable to the contribution of more than one structural component—possibly within the cell itself—and do not follow any simple quantitative laws such as Osterhout has studied in the case of *Laminaria*.

Our original intention was to concentrate upon the study of resistance and capacity changes in muscular tissues, of which a variety from different species were employed. Our complete failure to obtain the results anticipated led us in addition to carry out a long series of experiments on the skin of *Xenopus laevis*. It will be remembered that Osterhout bases his belief that the Kohlrausch method is applicable to animal physiology on experiments carried out on frog's skin. It is unfortunate that he gives no graphs or tables of his experimental data in this connection. But he emphasises the conclusion that in pure solutions of sodium chloride or potassium chloride the resistance of frog's skin like that of *Laminaria* thalli continuously diminishes, whereas in solutions of calcium and magnesium chlorides the resistance of frog's skin like pieces of *Laminaria* at first increases considerably before falling to the lethal limit.

Table VI.

*Skin of Xenopus.*

A, 0.1 M NaCl. B, Sea water 1 : 5. 7°C.

Time h. m.	Capacity		Resistance	
	A	B	A	B
0 00	100	100	100	100
0 13	120	185	136	119
0 38	200	240	158	150
1 05	200	305	161	180
1 30	200	285	161	188
1 55	200	290	166	193
2 18	205	285	167	196
4 03	205	290	180	207
11 05	210	290	190	227
19 35	215	300	200	239
21 55	205	275	204	253
25 15	200	290	197	239
33 40	215	290	194	251
40 50	215	300	190	249
56 00	210	300	193	249
60 40	205	285	204	259
63 25	200	290	207	253
69 35	200	300	200	232
82 40	190	315	220	227
88 45	190	315	225	217
107 05	165	320	249	213
117 10	180	345	242	190
128 55	170	320	242	172
140 00	170	320	227	150

As a "balanced" solution for frog tissues Osterhout employed isotonic sea water. Experiments on the skin of *Xenopus*, repeatedly performed by us and safeguarded from sources of error which were uncontrolled in Osterhout's work, are in complete disagreement with all the conclusions stated above.

In the first place, it is to be noted that the one conclusion uniformly sustained by our experiments is the fact that the resistance of skin of *Xenopus* increases in pure sodium chloride. Table VI records a typical experiment in which, following Osterhout's prescription, one-tenth molar concentration was taken to be isotonic with the tissue. Two pieces of skin, *A* and *B*, were taken from the back of the same animal. The practice of taking different pieces of tissue from the same animal was generally adopted to avoid individual variability.

To emphasise the lack of uniformity obtained with tissues from the same individual, treated simultaneously in the same manner, it is worth while perhaps inserting a protocol of another representative experiment in which the immersion fluid was for both pieces of tissue Osterhout's "balanced" solution. The actual constants are appended for reference. The crude order of consistency which this experiment illustrates shows, apart from other considerations, how little may be expected from the Kohlrausch method as a means of studying permeability of animal tissues to electrolytes. In a perfectly flat plate of tissue-like skin there is little likelihood that injury owing to crushing of the cells between the plates of the conductivity cell will affect the result. A good deal of care was, as a matter of course, exercised to avoid crushing, and in the experiments with muscle referred to later additional precautions were taken.

Table VII.

*Two pieces of skin of Xenopus\**.

Sea water 1 : 5. 7° C.

Time h. m.	Capacity		Resistance	
	<i>A</i>	<i>B</i>	<i>A</i>	<i>B</i>
0 00	100	100	100	100
0 08	70	—	103	—
0 17	—	100	—	111
0 22	60	—	118	—
0 38	60	—	134	—
0 46	—	110	—	118
1 00	—	120	—	119
1 07	60	—	147	—
1 22	—	120	—	119
1 23	60	—	150	—
2 12	60	130	150	118
3 30	70	130	147	119
4 40	70	130	146	118
5 30	70	135	146	121
6 18	70	135	146	121

\* Max. capacity per sq. mm. tissue was  $1.52 \times 10^{-3}$  mf. and  $2.18 \times 10^{-3}$  mf. Fluid resistances 167 and 176 ohms. Max. resistance of tissues 785 and 341 ohms.

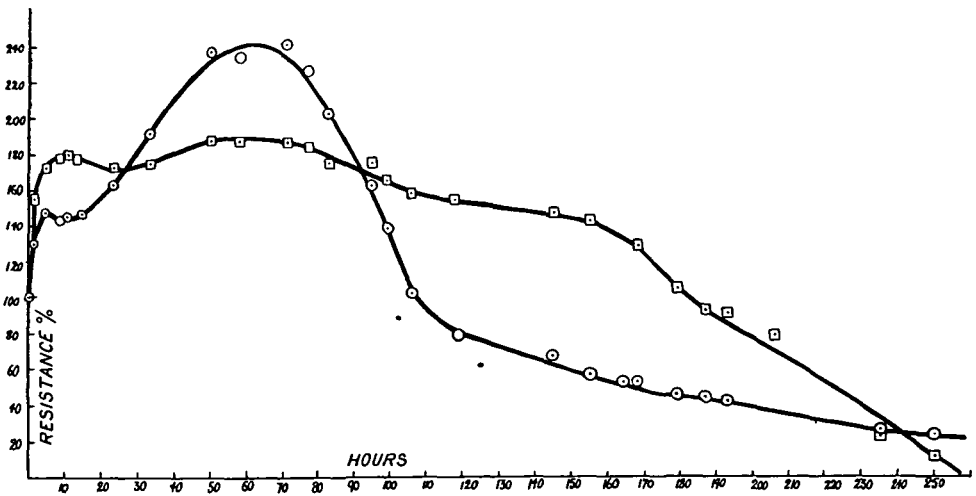
The variability obtained with different pieces of tissue immersed in tenth molar sodium chloride was of the same order, but in all cases a definite rise of resistance was recorded contrary to Osterhout's findings. An experiment carried out simultaneously on four pieces of the skin of *Xenopus* may be cited.

Table VIII.

Skin of *Xenopus*.

0.1 M NaCl. Four pieces of skin. (Resistance only.) 15° C. pH 7.2.

Time h. m.	A	B	C	D	Mean
0 00	100	100	100	100	100
0 10	102	104	100	101	102
1 00	110	113	98	108	107
3 30	111	118	98	113	110
7 00	116	127	97	117	114
11 30	111	137	98	119	116
16 45	130	137	102	123	123
23 15	141	125	111	123	125



○ = Na M/8.      □ = Sea water 1 : 4.

Fig. 4. *Xenopus* skin. March, 1929.

It is highly unlikely that the secretion of mucus by the skin enters into this discrepancy between our own observations and those of Osterhout. Indeed the experiments showing fall of resistance on stimulating the skin recorded in the previous section dispose effectively of this remote possibility. There remains the influence of osmotic pressure of the medium which would affect the dimensions of the system. It is more general to take a solution of sodium chloride about one-eighth molar concentration as isotonic with Amphibian tissues than one-tenth, as in Osterhout's experiments. This does not affect the character of the result as indicated by the experiment represented in Fig. 4 and repeatedly performed with analogous results.

To eliminate this source of disagreement completely however the following experiment was undertaken. It will be seen from Table IX that a rise of resistance accompanies immersion of skin of *Xenopus* in sodium chloride solutions of concentration within the range one-sixth to one-twelfth molar.

Table IX.

*Xenopus skin.*

pH 7.2. Readings for *M/6*, *M/10*, *M/12* based on mean of four pieces of tissue at 15° C.; *M/8* two pieces of tissue at 18° C.

Time h. m.	<i>M/6</i>	<i>M/8</i>	<i>M/10</i>	<i>M/12</i>
0 10	—	—	102	—
0 20	—	108	—	—
0 35	—	—	—	107
0 40	109	—	—	—
1 00	—	—	107	—
1 15	110	—	—	—
1 30	—	—	—	104
1 40	—	120	—	—
3 30	—	—	110	—
3 50	—	—	—	105
4 00	—	128	—	—
5 35	120	—	—	—
6 05	—	—	—	106
6 50	—	126	—	—
7 00	—	—	114	—
8 30	123	—	—	—
9 50	—	125	—	—
11 30	—	—	116	—
12 40	—	—	—	113
15 00	—	157	—	—
15 35	113	—	—	—
16 45	—	—	123	—
19 45	—	154	—	—
22 25	—	—	—	—
23 25	—	—	—	130
24 00	89	—	125	—
26 45	—	160	—	—

*Xenopus* was selected because it is easier to obtain in Cape Town than is *Rana*; it is an aquatic animal and more viable. Two experiments however were carried out on *Rana fuscigula* which did not however yield results confirmatory of Osterhout's conclusions. It is highly unlikely that the American species of *Rana* used by Osterhout is fundamentally different from *Xenopus* in this respect. If this were the case, the general applicability to animal tissues of conclusions drawn from the study of *Laminaria* would be none the less dubious.

On the other hand, it could well be argued that no less suitable tissue than skin could be selected to demonstrate their applicability. Convenient as is the skin of the frog or *Xenopus* for practical purposes on account of its toughness and flatness, it is an exceedingly heterogeneous organ from the histological standpoint. A better test is provided by the musculature of the ventral abdominal wall of the clawed toad. Very extensive experiments in which a variety of *balanced* solutions were employed

were carried out on the ventral abdominal muscle of *Xenopus*. The persistence of irritability under the conditions of the experiment was repeatedly tested by placing pieces of tissue for varying periods in the conductivity cell and subsequently removing and stimulating them with a faradic current. The results obtained were not uniform. There was never a striking difference between tissue immersed in Ringer's solution or Mines' or other *balanced* solution on the one hand and in isotonic sodium chloride on the other. Solutions containing only potassium or only calcium in addition to sodium in varying proportions were also investigated. There is no need to cite a long list of protocols. The results are best described as chaotic, as far as they bear on the end in view, namely the interpretation of the specific influence of kations. In some cases resistance increased in pure sodium chloride, as in the following experiment:

Table X.

*Ventral abdominal musculature Xenopus.*

6° C. pH 7.2.

Time h. m.	Na 100 : K 0 : Ca 0		Na 100 : Ca 2.5 : K 2.5	
0 00	100	—	100	—
0 15	100	—	105	—
0 30	—	100	—	100
0 50	100	77	103	92
1 35	100	67	104	97
2 55	107	61	113	95
5 30	110	57	113	104
7 15	107	55	123	100
10 20	110	54	129	101
22 55	107	52	132	90
28 30	107	51	123	95
37 45	91	48	100	79
46 25	107	53	91	76
49 55	109	53	86	70
54 25	119	58	78	67
61 35	137	74	72	65
71 00	123	82	69	61
79 00	95	83	64	58
83 30	74	87	59	52

A number of experiments were carried out on other suitable contractile tissues, in particular the uterus of the viviparous Cape Dogfish (*Acanthias*). Resistance increased in isotonic sodium chloride in this instance as well. But the secretion of the mucous membrane overlying the muscular coats interfered somewhat with the experiments. The mammalian diaphragm suggested itself as a suitable piece of flat and at the same time fairly homogeneous tissue. Results obtained usually, however, gave polyphasic curves when plotted graphically. In the last three tables are given changes in resistance of rabbit's diaphragm immersed in three solutions, four pieces of tissue being employed with each test fluid.

Table XI.

*Rabbit's diaphragm.*

37° C. pH 7.3.

h. m.	A	B	C	D	Mean
(i) Na 100 : M/6.					
0 00	100	100	100	100	100
0 10	93	95	102	97	97
0 30	87	95	101	100	96
0 45	84	92	101	102	95
1 00	80	90	100	100	92
1 30	84	90	109	100	96
2 15	91	120	117	100	107
3 00	96	100	114	96	101
3 45	94	94	113	96	99
5 15	98	84	105	78	91
6 30	86	68	97	54	76
11 30	19	5	33	4	15
(ii) Na 100 : Ca 3 : M/6.					
0 00	100	100	100	100	100
0 15	105	94	81	100	95
0 30	93	91	77	96	89
1 20	73	86	68	85	78
2 00	55	60	60	86	65
4 00	40	39	47	66	48
6 00	26	27	24	35	28
(iii) Na 100 : Ca 3 : K 3 : M/6.					
0 00	100	100	100	100	100
0 15	85	83	96	90	89
0 45	100	100	97	90	97
1 15	90	100	97	61	87
1 45	75	80	90	54	77
2 15	63	78	85	37	66
3 15	52	63	74	25	54
5 15	30	43	52	16	35

## 4. CONCLUSIONS.

Disappointing as the results of the present investigation have been in so far as they throw light on the nature of the action of ions on tissues, we feel that the publication of the fruits of our own enquiries may be helpful to other workers in the same field. While we do not wish to cast any doubt on the authenticity of the evidence derived from the study of *Laminaria*, we are convinced that the conductivity method employed by Osterhout in his researches is unsuited to yield evidence as to the nature of ionic antagonism in animal tissues, and that the conclusions derived from the study of *Laminaria* are not of such general physiological significance as might be hoped to be the case. In particular we are unable to confirm Osterhout's observations on frog's skin, after taking several additional precautions essential to conductivity measurements made on animal tissues. Such precautions pre-eminently include compensation of capacity changes in the course of the e-



made by use of variable condensers and elimination of all stimulation effects arising in the bridge circuit.

The expenses of this research were defrayed by a grant from the Research Grant Board of the Department of Mines and Industries (Union of South Africa).

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