EXTREME ALTERATIONS OF PERMEABILITY WITHOUT INJURY

(WITH FOUR FIGURES)

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It has been pointed out in a previous paper[1] that in the opinion of some writers permeability is a relatively fixed property of the cell, and that it is altered only as the result of injury; the alteration is then irreversible. Others assume[2] that there are reversible changes in permeability which may form a normal part of the activities of the cell. In view of the fact that such changes may control metabolism, it seemed important to establish the truth or falsity of this assumption by rigorous proof.

This was successfully accomplished by the use of quantitative methods. The previous paper contained a brief statement of some of the results obtained; the present paper adds important data and describes subsequent experiments in which an extreme range of permeability was attained and very rapid changes were investigated.

The permeability was measured by determining the electrical resistance of living tissues of *Laminaria saccharina* by a method which has been previously described.[3]

It has been shown that the electrical resistance of the living tissue falls rapidly where it is transferred from sea water to a solution of NaCl of the same conductivity, and that within certain limits this effect is reversible. Tissue which in sea water had a resistance of 1020 ohms[4] was placed in a solution of NaCl 0.52M which had the same conductivity as the sea water. In the course of five minutes the resistance fell to 830 ohms. The tissue was replaced in sea water; the resistance soon rose to normal and so continued during the remainder of the day.

[1] Science N.S. **36**:350. 1912.

[2] Cf. HÖBER, Physikalische Chemie der Zelle und Gewebe. Kap. 7 und 10. 1911.

[3] Science N.S. **35**:112. 1912.

[4] All readings were taken at 18° C. unless otherwise stated.

As the electrical conductivity of the tissue is a measure of the permeability of the protoplasm to ions, we may calculate the percentage of increase of permeability by finding the change in conductivity. In the present instance it is more convenient to use the change in conductance without reducing this to specific conductivity. The resistance at the start was 1020 ohms, but this includes the resistance of the apparatus with its contained sea water. Evidently this should be subtracted from the total resistance; the remainder will be called the *net resistance*.[5]

In this case the resistance of the apparatus was 250 ohms. The net resistance of the tissue at the start, therefore, was $1020-250=770$ ohms; the net conductance was $1\div770=0.00130$ mho. At the end of five minutes in NaCl the net resistance was $830-250=580$ ohms; the net conductance was $1\div580=0.00172$ mho. The increase in permeability, therefore, amounts to $0.00172-0.00130=0.00042$ mho, or 32.3 per cent.[6]

It might be objected that this increase in conductance was not due to an increase in permeability but to an increase in the ions of sodium chloride, to which the tissue might be assumed to be normally more permeable than to some of the other ions of the sea water. This, however, cannot be the case, as is shown by the following experiment. Tissue having a resistance of 1020 ohms was placed in a mixture of 1000 cc. NaCl 0.52M+20 cc. $CaCl_2$ 0.278M. The resistance remained at 1020 ohms in this mixture. The tissue was then transferred to NaCl 0.52M for five minutes. At the end of this time the resistance had fallen to 860 ohms; on being placed in sea water the resistance rose to the normal and so remained for some time.

In this case the resistance of the apparatus was 230 ohms. The net resistance at the start was $1020-230=790$ ohms; and the net

[5] In the previous paper it was suggested that the tissue should be killed, that the resistance of the apparatus should be measured while the dead tissue remained in it, and that this should be subtracted from the total; the remainder was called the net resistance. It seems better, however, to take the resistance of the apparatus after the tissue has been removed, to subtract this from the total, and call the remainder the net resistance.

[6] Complete recovery after such a large increase of permeability is not always obtainable unless the material is in good condition and is freshly collected. Even in such material a lot will occasionally be found which shows poor recovery.

conductance 1÷790=0.00127 mho. After treatment with NaCl the net resistance was 860−230=630 ohms, and the net conductance was 1÷630=0.00159 mho. The increase in net conductance, therefore, was 0.00159−0.00127=0.00032 mho, or 25.2 per cent. The increase in the percentage of sodium ions was only 2 per cent, while the content of chlorine ions remained unchanged. It is evident, therefore, that there was a great increase in permeability.

In order to see whether this increase of permeability is accompanied by injury, an experiment was made in which the same piece of tissue was exposed to the action of NaCl several times during the same day. The resistance of the tissue in sea water was 1010 ohms; after five minutes in NaCl the resistance fell to 880 ohms; the tissue was then placed in sea water and a reading ten minutes later showed that the resistance had risen to 1010 ohms. During the next 95 minutes it showed no change. It was then placed in NaCl for five minutes and the resistance fell to 870 ohms. It was replaced in sea water; a reading taken ten minutes later showed that it had returned to normal, where it remained for 90 minutes without change. It was then placed in NaCl for five minutes. The resistance fell to 900 ohms and returned to normal during the ensuing ten minutes in sea water. After 105 minutes in sea water, during which no change occurred, it was again exposed to NaCl for five minutes. The resistance fell to 870 ohms and returned again to normal during the following ten minutes in sea water. On the following day its resistance was only 30 ohms below the resistance of the control, which at the beginning of the experiment was 1040 ohms. The results are presented graphically in fig. 1.

The successful outcome of this experiment lead to an attempt to carry on such an experiment for several days in succession, giving the tissue one treatment daily with NaCl. The material was selected with especial care. The fronds were fairly thick, without reproductive organs. The experiment was made at Woods Hole, Mass., in July, at which time such fronds may be easily obtained. The disks cut from these fronds were slightly curved, so that when placed in the apparatus they separated spontaneously, thus allowing the running sea water in which they were kept to circulate freely between them. Care was taken to keep them only about two-thirds submerged, so that they had free access to air without any risk of drying.

The tissue had in sea water a resistance of 1020 ohms at 20° C. As the temperature of the sea water varied but slightly from this during the experiment, all readings were taken at 20° C. On being placed in NaCl 0.52M at this temperature, the resistance fell in five minutes to 890 ohms; it was then placed in sea water and a reading taken ten minutes later showed that it had risen again to the normal. The resistance of the apparatus was 240 ohms; hence

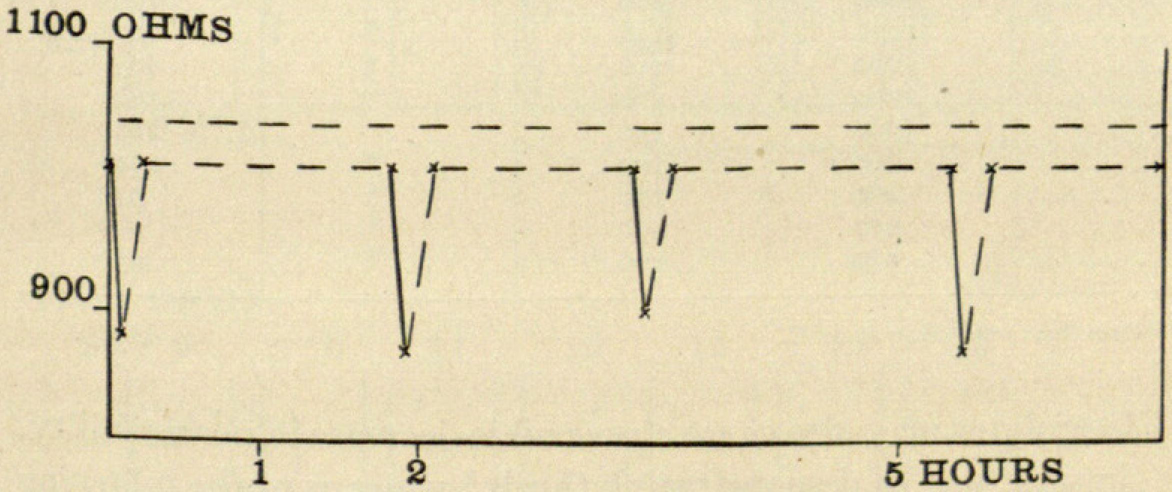

FIG. 1.—Alterations of permeability shown by curves of the electrical resistance of *Laminaria saccharina* in NaCl 0.52M (unbroken line) and in sea water (dotted portion of the curve); the horizontal dotted line (above) shows the resistance of the control.

the net resistance at the start was 1020−240=780 ohms, and the net conductance 1÷780=0.00128 mho. The net resistance after treatment with NaCl was 890−240=650 ohms, and the net conductance 1÷650=0.00154 mho. The increase in permeability, therefore, was 0.00154−0.00128=0.00026 mho, or 20.3 per cent.

The tissue was then placed in running sea water for 22 hours, with the precautions mentioned above. At the end of 22 hours the resistance was 1020 ohms at 20° C. An exposure of five minutes to NaCl resulted in a drop to 920 ohms, with complete recovery within ten minutes. The same treatment was given once each day for 15 days. On the tenth day the resistance began to fall off, but as this falling off was also shown by the control, which remained in sea water through the experiment, it was not due to the sodium chloride but to other causes. The results are shown in table I and fig. 2.

TABLE I*

Day	Resistance before exposure	Fall of resistance after 5 minutes in NaCl	Recovery	Control in sea water
1	1020	130	Complete	1030
2	1020	100	"	1030
3	1020	110	"	1030
4	1020	140	"	1030
5	1020	120	"	1030
6	1020	120	"	1030
7	1020	100	"	1030
8	1020	130	"	1030
9	1020	120	"	1030
10	1000	120	"	1020
11	1000	110	"	1010
12	980	100	"	1010
13	960	110	"	970
14	950	120	"	960
15	930	100	"	950

* All readings were taken at 20° C.

Electrolytes may also cause a reversible decrease in permeability. The simplest way of demonstrating this is by means of the following very striking experiment. The resistance of a cylinder of living tissue in sea water was found to be 750 ohms. It was tested an hour later and found to be the same. Sufficient lanthanum nitrate (8.7 gm. to 1000 cc. sea water) was then added in solid form to make its concentration[7] in the sea water 0.01M. After five minutes the resistance rose to 900 ohms. As the resistance of the apparatus was 250 ohms, the net resistance before the addition of lanthanum was $750-250=500$ ohms, and the net conductance $1\div 500=0.002$ mho. After treatment with lanthanum nitrate, the net resistance was $900-250=650$ ohms, and net conductance $1\div 650=0.00154$ mho, a loss of 23 per cent.

[7] The concentration was reduced by the precipitation of a small amount of lanthanum sulphate; this had practically no influence on the subsequent result, since the outcome is the same if we use in place of sea water a mixture of 1000 cc. NaCl 0.52M + 20 cc. $CaCl_2$ 0.278M, in which case no precipitate is formed. It should be noted that the addition of lanthanum chloride has the same effect as the addition of lanthanum nitrate.

In order to ascertain whether this change in permeability is reversible, the tissue was replaced in sea water. In the course of an hour its resistance returned again to the original condition.[8] The experiment was then repeated three times on the same lot of material with practically the same result; it was then allowed to stand over night in sea water. On the following day there was no

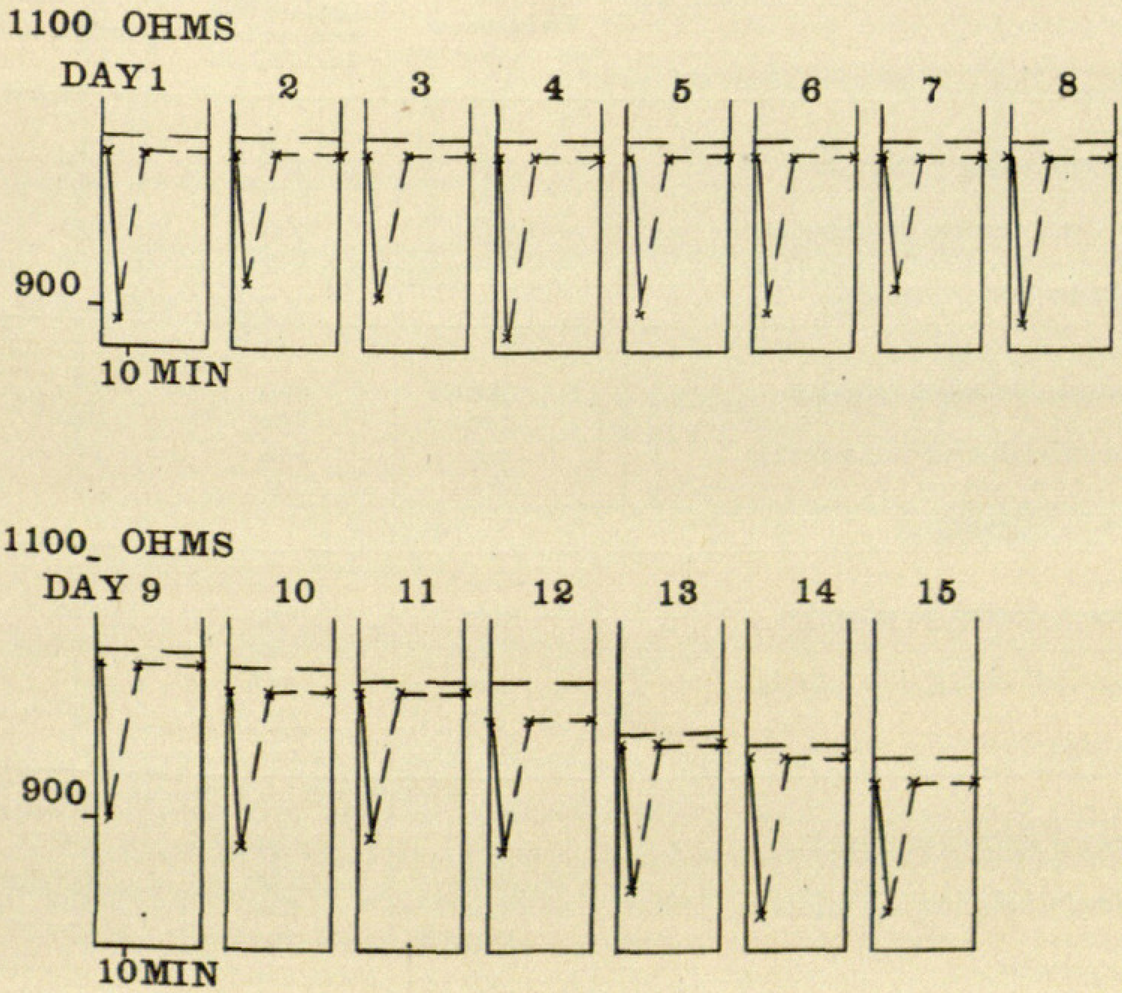

FIG. 2.—Alterations of permeability shown by curves of the electrical resistance of *Laminaria saccharina* in NaCl 0.52M (unbroken line) and in sea water (dotted portion of curve); upper dotted line, control in sea water.

appearance of injury, and its resistance was the same as that of the control, which had remained in sea water throughout the experiment. The tissue was then placed in the sea water plus lanthanum and left until its resistance had increased 100 ohms; it was then put back into sea water and left until the resistance fell to nearly normal. This was repeated three times, and the tissue

[8] If the material is left in sea water plus lanthanum nitrate the increased resistance is maintained for a long time unaltered.

was then allowed to stand over night in sea water. On the third, fourth, and fifth days the same experiment was repeated four times. On the fifth day the tissue appeared to be in as good condition as the control, and had a resistance which was slightly higher. There

TABLE II*

	Resistance at start of exposure	Resistance rose in sea water $+La_2(NO_3)_6$ to	Recovered to
Day 1—			
Exposed during 5 minutes	750	900	750
	750	870	750
Recovered during 55 minutes	750	900	750
	750	850	750
Control=730			
Day 2—			
Exposed during 20 minutes	700	860	710
	710	830	710
Recovered during 100 minutes	710	850	710
	710	840	710
Control=690			
Day 3—			
Exposed during 30 minutes	690	790	710
	710	800	720
Recovered during 100 minutes	720	790	710
	710	790	700
Control=660			
Day 4—			
Exposed during 30 minutes	670	760	680
	680	750	670
Recovered during 100 minutes	670	780	680
	680	770	680
Control=650			
Day 5—			
Exposed during 40 minutes	660	760	660
	660	780	660
Recovered during 120 minutes	660	770	660
	660	760	660
Control=650			

* All readings were taken at 20° C.

was no reason, therefore, to suspect that the changes in permeability had been attended by any injurious effect. The results are shown in detail in table II and fig. 3.

Similar experiments were performed in which calcium chloride was used in place of lanthanum nitrate. In this case 3.3 gm.

$CaCl_2$ were added to each 1000 cc. of sea water. Owing to the fact that the rise in resistance took place more slowly[9] than when lanthanum was used, the experiment was performed twice on each of the five successive days. On the sixth day the material was in as good condition as the control and had the same resistance.

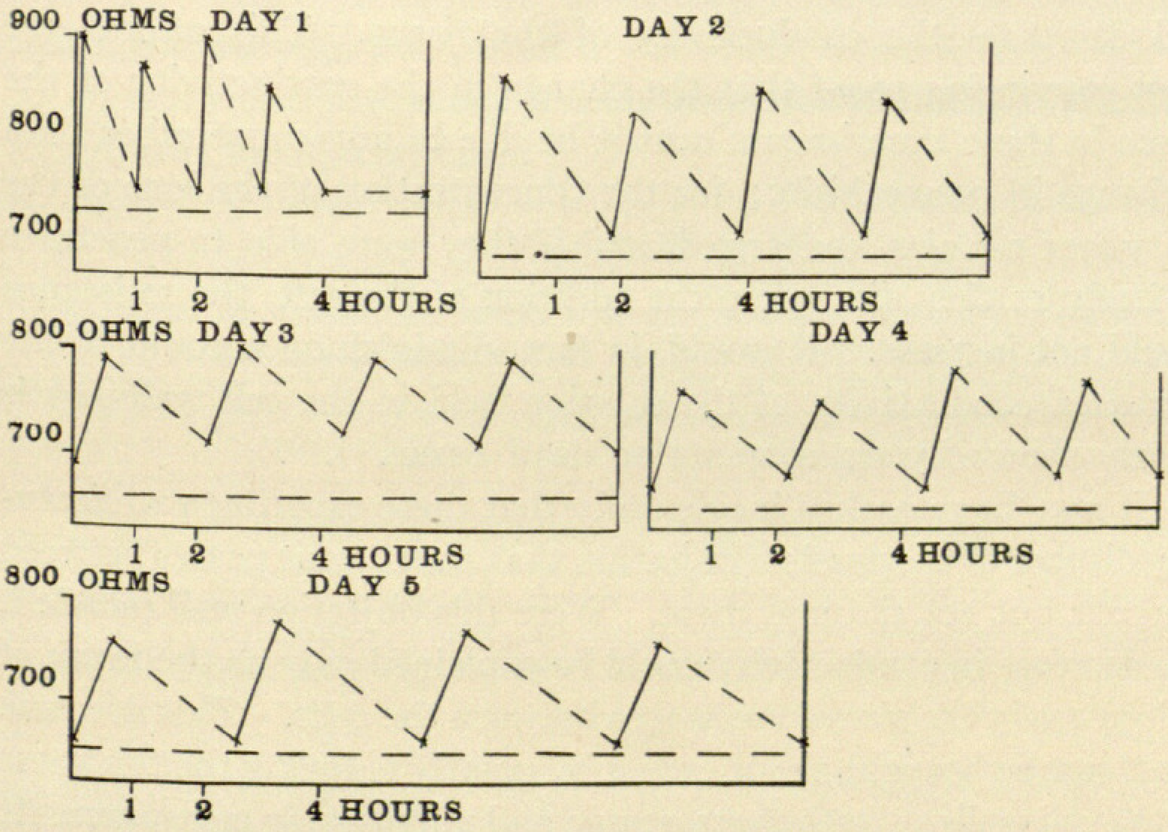

FIG. 3.—Alterations of permeability shown by curves of the electrical resistance of *Laminaria saccharina* in sea water, and in 1000 cc. sea water+2.6 gms. $La_3(NO_3)_6$ (=0.01M); the same lot of material was exposed four times daily on five successive days to the action of 1000 cc. sea water+$La_2(NO_3)_6$; unbroken part of curve, resistance in sea water+$La_2(NO_3)_6$; dotted part of curve, resistance in sea water; lower horizontal dotted line, control in sea water.

It is evident, therefore, that the permeability may be greatly decreased and then restored to the normal several times on five successive days without any trace of injury. Further experiments showed that the permeability may be alternately increased and decreased twice daily for five days without injury. The amount of increase and of decrease was about the same as in the experiments just described.

[9] If in place of solid $CaCl_2$ a strong solution is added, the rise is more rapid and reaches a higher figure.

Experiments on dead tissue (killed by heat or by formalin or allowed to die a natural death) showed that the results described above are due entirely to the living cells.

A very marked decrease of permeability may be produced by a considerable variety of other salts. The addition of these salts in solid form simultaneously increases the conductivity of the solution and decreases the conductivity of the tissue. This affords the most convincing proof that the change in the conductivity of the tissue in these experiments cannot be due to any cause other than a change in permeability; for the concentration of the ions of the sea water remains unchanged, and if they were able to penetrate as freely as they did before the addition of the salt, the resistance would not increase. It would, in fact, diminish on account of the increased conductivity of the solution held in the cell walls, as is clearly shown by experiments on dead tissue.

It may be remarked incidentally that these experiments effectually dispose of the possible objection that the current passes between the cells but not through them. Were this objection well founded, the decrease in conductivity could be explained only as the result of a decrease in the size of the spaces between the cells. This decrease could not be brought about except by greatly reducing the thickness of the cell walls. Both macroscopic and microscopic measurements show most conclusively that this does not occur. The contrary effect would be produced by the addition of salts in solid form, for they would tend to produce plasmolysis and thereby to increase the space between the cells.

As these remarkable changes in permeability seemed to produce no bad effects, it occurred to the writer to see whether the protoplasm could endure still more violent alterations without permanent injury. In order to test this the following experiment was performed. A lot of tissue was found to have in sea water a resistance of 1010 ohms. It was placed in $CaCl_2$ 0.278M, which had the same conductivity as the sea water. At the end of ten minutes a reading was taken which showed that the resistance had risen to 1500 ohms. The material was then placed in NaCl 0.52M, which had the same conductivity as the sea water; at the end of ten minutes the resistance was 880 ohms. The experiment was continued by placing the material for ten minutes alternately in $CaCl_2$ and NaCl, with the results shown in table III and fig. 4. After 80 minutes the material was placed in sea water, where it soon regained its normal resistance of 1010 ohms. Twenty hours later the resistance was found to be unaltered and the experiment was repeated. After 80 minutes of alternate exposure to $CaCl_2$ and NaCl, the material was placed in sea water, where it soon regained its normal resistance, which it maintained for three days, when the experiment was discontinued.

TABLE III*

ALTERATIONS IN ELECTRICAL RESISTANCE OF *Laminaria saccharina* EXPOSED FOR 10 MINUTES ALTERNATELY TO $CaCl_2$ 0.278 AND NaCl 0.52M

Day 1			Day 2		
Time in minutes	Solution	Resistance	Time in minutes	Solution	Resistance
0	....	1010	....	....	1010
10	$CaCl_2$	1500	10	$CaCl_2$	1490
20	NaCl	880	20	NaCl	880
30	$CaCl_2$	1470	30	$CaCl_2$	1500
40	NaCl	900	40	NaCl	900
50	$CaCl_2$	1500	50	$CaCl_2$	1460
60	NaCl	860	60	NaCl	900
70	$CaCl_2$	1470	70	$CaCl_2$	1480
80	NaCl	890	80	NaCl	880
95	Sea water	1010	95	Sea water	1010
115	" "	1010	....	....	....
Control in sea water 990			Control in sea water 990		

* All readings were taken at 18° C.

The resistance of the apparatus was 240 ohms. The net resistance of the tissue at the start, therefore, was $1010-240=770$ ohms, and the net conductance $1\div770=0.00130$ mho. After the first exposure to $CaCl_2$, the net resistance was $1500-240=1260$ ohms, and the net conductance was $1\div1260=0.00079$ mho. The loss in conductance was $0.0013-0.00079=0.00051$ mho, or 39.2 per cent.

After the first exposure to NaCl the net resistance was $880-240=640$ ohms, and the net conductance $1\div640=0.00156$ mho.

This was greater than the normal by 0.000156−0.0013=0.00026 mho, or a gain of 20 per cent.

The fact that protoplasm is able to endure such violent alterations of permeability throws a new light on the normal life processes of the cell. In the course of metabolism a great variety of substances are produced which affect the permeability of the protoplasm. Since it is clear that the permeability may be increased or

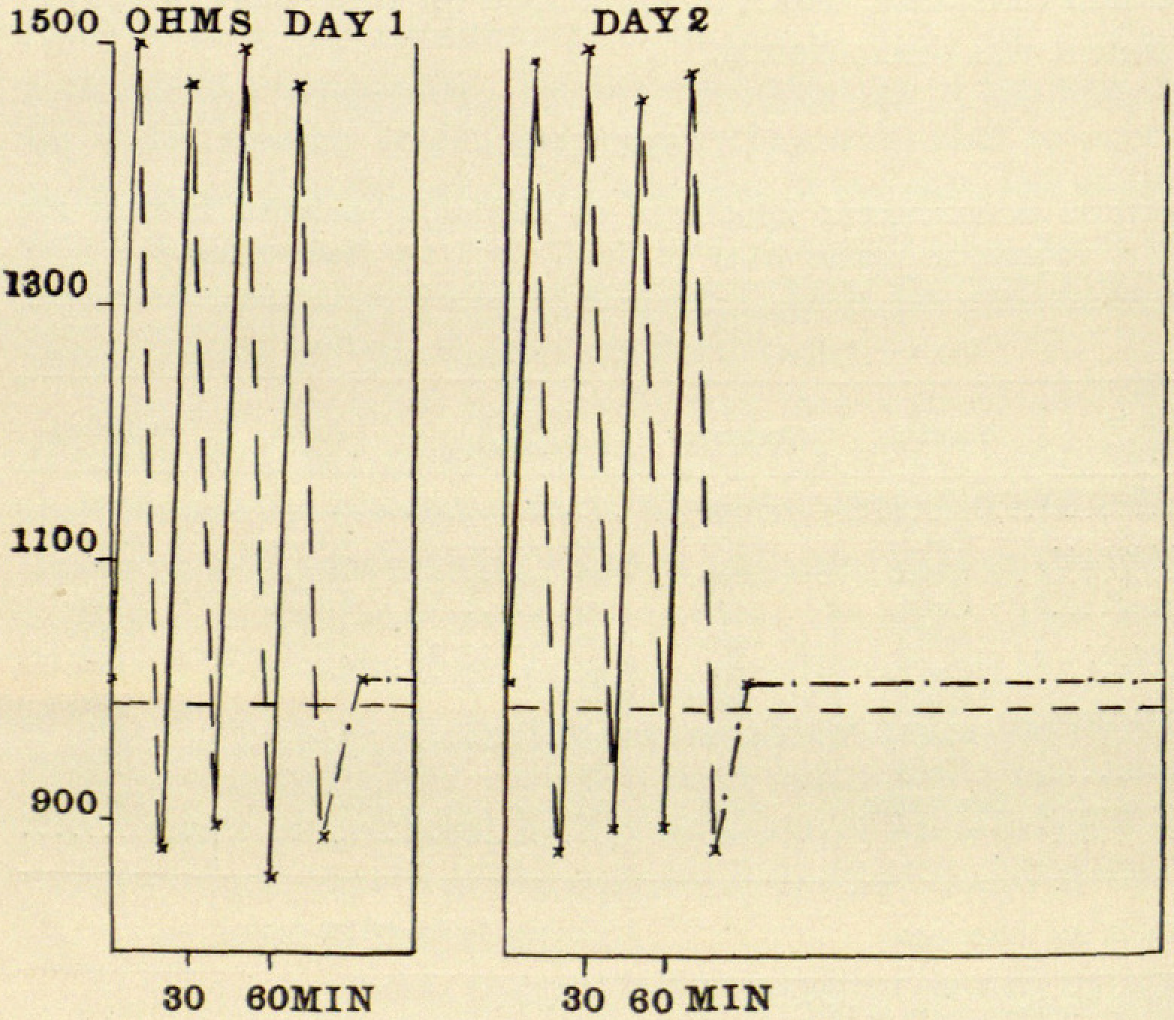

FIG. 4.—Extreme alterations of permeability shown by curves of electrical resistance of *Laminaria saccharina* in $CaCl_2$ 0.278 M (unbroken line), in NaCl 0.52 M (dotted portion of curve), and in sea water (dotted line with points); horizontal dotted line, control in sea water.

decreased 30 per cent or more without rendering a return to normal permeability impossible, it is evident that considerable fluctuations in permeability may form a normal part of the life processes of the protoplasm. In this way the whole course of metabolism may be controlled, since this evidently depends on the exchange of substances between the cell and its environment.

Summary

Results obtained by the use of quantitative methods prove that the permeability of protoplasm may be greatly increased or diminished without injury. A rapid alternation of increase (amounting to 20 per cent above normal) and decrease (amounting to 39 per cent below normal) did not produce injury.

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