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ON THE IMPORTANCE OF PHYSIOLOGICALLY BALANCED SOLUTIONS FOR PLANTS.[1]

I. MARINE PLANTS.

W. J. V. OSTERHOUT.

RINGER demonstrated that animal tissues live longer in a solution of NaCl to which a small amount of KCl and $CaCl_2$ is added than in a solution of NaCl alone. Various explanations of this fact were given by different investigators, all of whom, however, agreed upon the essential point that KCl and $CaCl_2$ are essential for the maintenance of life.

HOWELL assumed that $CaCl_2$ is the stimulus for the heart beat, while NaCl is an indifferent substance, necessary only for the maintenance of osmotic pressure. Similarly RINGER concluded that Ca is the stimulus for the systole, while K is necessary for the diastole of the heart beat.

HERBST made experiments on the influence of the composition of the sea water on sea urchin eggs, eliminating in each successive experiment a different constituent of the sea water. He found that the eggs would not develop in any solution which did not contain all the salts of the sea water. From this he concluded that each of the salts found in sea water is necessary for the development of the egg. LOEB called this view in question as the result of his experiments on Fundulus. He found that this marine fish cannot live in a pure NaCl solution of the same osmotic pressure as the sea water, but that it can live indefinitely in a mixture of NaCl, KCl, and $CaCl_2$, in the same proportions in which these salts are contained in sea water. The fish can also live indefinitely in distilled water. This proves that it does not need any of the three salts mentioned for the maintenance of its life, and that the Ca and K are only required to overcome the poisonous effects which would be produced by the NaCl if it alone were present in the solution (at the above mentioned concentration).

[1] I wish here to express my sincere thanks to Professor LOEB, who kindly placed the facilities of his laboratory at my disposal and assisted me in every way during these investigations.

It is noteworthy that the Ca and K, which are added to inhibit the toxic effect of NaCl, are themselves poisonous at the concentration at which they are here employed.

These antagonistic effects of Ca and K toward a pure NaCl solution were illustrated still more strikingly in experiments on the egg of Fundulus. The newly fertilized eggs of this fish develop equally well in sea water and in distilled water, but die in a pure *m*/2 NaCl solution without forming an embryo. If, however, a small but definite amount of a salt with a bivalent kation, even of such poisonous salts as $BaCl_2$, $ZnSO_4$, and $Pb(CH_3\text{-}COO)_2$, is added, the eggs will produce embryos. From these and similar observations LOEB was led to formulate his conception of the necessity of physiologically balanced salt solutions, in which are inhibited or counteracted the toxic effects which each constituent would have if it alone were present in the solution.

The blood, the sea water, and to a large extent RINGER'S solution, are such physiologically balanced salt solutions. The observations of HERBST, as well as those of RINGER, are easily explained on this basis. The fact that the elimination of any one constituent from the sea water makes the solution unfit to sustain life does not prove that the eliminated substance is needed by the animal for any purpose other than to counteract the poisonous action of some other constituent of the solution.

Botanists have not thus far made use of these conclusions, for the obvious reason that facts similar to those mentioned above have not been observed in plants. I have recently made a number of experiments which show that there exist in plants phenomena similar to those observed by LOEB on Fundulus and other marine animals.

The species of marine plants chosen for investigation may be divided into two groups:

Group 1 comprises plants which can live a long time in distilled water. It includes the following: BLUE-GREEN ALGAE, Lyngbya aestuarii; GREEN ALGAE, Enteromorpha Hopkirkii; FLOWERING PLANTS, Ruppia maritima.

Group 2 is composed of plants which quickly die in distilled water. It includes the following: GREEN ALGAE, Enteromorpha intestinalis; BROWN ALGAE, Ectocarpus confervoides; RED ALGAE, Ptilota filicina, Pterosiphonia bipinnata, Iridaea laminarioides, Sarcophyllis pygmæa, Nitophyllum multilobum, Porphyra naiadum, Porphyra perforata, Gelidium sp., Gymnogongrus linearis, Gigartina mammillosa.[2]

If plants of either group be placed in a solution of pure sodium chlorid (isotonic with sea water), they die in a short time. This might be attributed to the lack of certain salts which are necessary for their metabolism, rather than to the toxicity of the sodium chlorid. In the case of the plants of Group 1 there can be no doubt on this point, for these plants live a long time in distilled water. If we add pure sodium chlorid to the distilled water it kills them in a very short time. An inspection of the tables will show that these plants in their behavior toward sodium chlorid and other salts, closely agree with those of Group 2, which can live but a short time in distilled water. Sodium chlorid is certainly toxic to the first group, and there can be little doubt that it is so to the second group as well.

The plants of the first group were found in a ditch in a salt marsh through which the tide ebbs and flows; there is always a foot or so of water even at low tide. The salt content of the water fluctuates around a mean of approximately 2.3 per cent.

The plants of the second group were collected at the entrance to San Francisco Bay, where the salt content of the water fluctuates about a mean which is probably not far from 2.7 per cent. The only exceptions are *Enteromorpha intestinalis* and *Ectocarpus confervoides*, which came from wharves in the bay, where the mean salt content is about 2.3 per cent.

All the plants used in the experiments were transferred from the sea water directly to distilled water. After rinsing in this they were placed in glass dishes, each containing 200^{cc} of the solution to be tested. The dishes were then covered with glass plates to exclude dust and check evaporation. Only a small amount of material was placed in each dish. The temperature during the experiments did not vary far from 18° C.

Artificial sea water was prepared[3] according to VAN 'T HOFF'S

[2] The determinations were kindly made by Professor SETCHELL.

[3] The water used was distilled in glass only and the first part of the distillate rejected. The purity of each salt was carefully tested before using.

formula[4] as follows: 1000^{cc} NaCl, $3m/8$; 78^{cc} $MgCl_2$, $3m/8$; 38^{cc} $MgSO_4$, $3m/8$; 22^{cc} KCl, $3m/8$; 10^{cc} $CaCl_2$, $3m/8$.[5]

This closely approximates the bay water. The plants thrive almost as well in it as in sea water, especially when a very little $NaHCO_3$ or $KHCO_3$ is added to produce a neutral or faintly alkaline reaction.

A series of solutions was tried, beginning with pure NaCl $3m/8$ and adding to it in turn $MgCl_2$, KCl, and $CaCl_2$, either singly or in combination, in the proportions given above. These salts were also used in pure solutions of the same concentration at which they exist in the artificial sea water described above.

It should be said that little difficulty was experienced in determining the death point with sufficient precision. The color reactions and the microscopic appearance of the cells allowed this to be done with sufficient accuracy, so that the results were not in doubt on this account.

The results of the experiments are set forth in the tables. The figures represent the average of four parallel series carried on simultaneously. A control series was also carried on in which each solution was made faintly alkaline by the addition of $NaHCO_3$, $KHCO_3$, or $Ca(OH)_2$. This had a beneficial effect during the first two or three days of the experiment, but the final results were practically the same as in the other series.

From a consideration of the results for Group 1 we may draw the following conclusions.

1. The plants die much sooner in a pure sodium chlorid solution (isotonic with sea water) than in distilled water. The poisonous effect of the NaCl largely disappears if we add a little $CaCl_2$ (10^{cc} $CaCl_2$ $3m/8$ to 1000^{cc} NaCl $3m/8$); in this mixture the plants live nearly as long is in distilled water. Addition of KCl to this mixture enables them to live longer than in distilled water. Further addition of $MgCl_2$ and $MgSO_4$ enables them to live practically as long as in sea water.

[4] VAN'T HOFF, J. H., Physical chemistry in the service of the sciences 101. Univ. of Chicago Press, 1903.

[5] This corresponds approximately to the proportion of Ca in the sea water of the bay.

TABLE I.

DURATION OF LIFE IN DAYS.

Culture solution.	Group 1			Group 2		
	Lyngbya aestuarii	Entero-morpha Hopkirkii	Ruppia maritima	Ptilota filicina	Pterosi-phonia bipinnata	Iridæa laminar-ioides
Sea water (total salts 2.7 %)	95	150+	150+	11	24½	24
Artificial sea water: 1000 cc $NaCl$ $3m/8$ 78 " $MgCl_2$ " 38 " $MgSO_4$ " 22 " KCl " 10 " $CaCl_2$ "	90	150+	150+	10½	24½	23
Distilled water..........	30	30	80	1	3½	2½
Tap water.............	32+	36	85	2½	9½	10
$NaCl$ $3m/8$	22	15	23	1¼	3½	4
1000 cc $NaCl$ " 10 " $CaCl_2$ "	29	23	65	2½	6	5
1000 " $NaCl$ " 22 " KCl " 10 " $CaCl_2$ "	35	32	88	3½	10	9
1000 " $NaCl$ " 78 " $MgCl_2$ " 10 " $CaCl_2$ "	29	23	45	3	6	6
1000 " $NaCl$ " 78 " $MgCl_2$ " 22 " KCl "	25	13½	30	2	4	4
1000 " $NaCl$ " 22 " KCl "	23	13½	23	1	2	5
1000 " $NaCl$ " 78 " $MgCl_2$ "	22⅝	13½	25	1½	2	2
1000 " Dist. H_2O 78 " $MgCl_2$ "	15½	16½	19	1	2	2¼
1000 " Dist. H_2O 38 " $MgSO_4$ "	17½	13	23	1	2	2
1000 " Dist. H_2O 22 " KCl "	21	13½	56	1	1⅝	5⅓
1000 " Dist. H_2O 10 " $CaCl_2$ "	26+	12½	58	2½	5	2

TABLE II.

DURATION OF LIFE IN DAYS. GROUP 2.

CULTURE SOLUTION.	Enteromorpha intestinalis	Ectocarpus confervoides	Sarcophyllis pygmæa	Nitophyllum multilobum	Porphyra naiadum	Porphyra perforata	Gelidium sp.	Gymnogongrus linearis	Gigartina mammillosa
Sea water (total salt 2.7 %.)	240	25	11	4½	6	21	33+	11	11
Artificial sea water: 1000 cc NaCl 3*m*/8; 78 " $MgCl_2$ "; 38 " $MgSO_4$ "; 22 " KCl "; 10 " $CaCl_2$ "	220	20	7½	4½	6	20	33+	10	9¼
Distilled water	3	1¼	1⅚	2½	2½	3⅓	1⅚	2½	3½
Tap water	10	2¼	3¾	3¾	2½	4½	5½	4½	5¼
NaCl 3*m*/8	4¾	⅔	1⅔	⅚	2⅙	3	3	5⅚	2½
1000 cc NaCl "; 22 " KCl "; 10 " $CaCl_2$ "	68	8	5½	3½	5	14⅙	33+	9	6
1000 " Dist. H_2O; 22 " KCl "	4¾	4	1⅚	..	4¾	3	3	4	3

2. The pure solution of each of the salts added to inhibit the poisonous effects of NaCl is itself poisonous at the concentration at which it exists after its addition, since the plants die in such a solution much sooner than in distilled water.[6] A mixture of solutions which are individually poisonous produces a medium in which the plants live indefinitely.

That the plants die so quickly in solutions containing a single salt might be attributed to the fact that the osmotic pressure of some of these solutions is much lower than that of sea water. This supposition is disproved by the fact that in general the plants live longer in tap water than in any solution containing but a single salt, although the tap water has a lower osmotic pressure than that of any solution used in the experiments. (The plants of Group 1 live longer in distilled water also. The tap water is to be regarded as a physi-

[6] This statement does not apply in all cases to $CaCl_2$, which is the least toxic of the salts employed and for some forms quite harmless in dilute solutions.

ologically balanced solution; this will be more fully discussed in the second portion of the paper.)

3. The poisonous effect of NaCl is inhibited little or not at all by KCl or $MgCl_2$ added singly.

4. The combination $NaCl+KCl+CaCl_2$ is superior to $NaCl+MgCl_2+CaCl_2$, but the latter is better than $NaCl+MgCl_2+KCl$.

5. These effects must be due to the metal ions, since the anion is in nearly all cases the same.

The plants of Group 2 agree with those of Group 1 except in their behavior toward distilled water.

Essentially similar results were obtained from the study of fresh water algae and other plants, the details of which will be given in the second part of this paper.

These results agree in striking fashion with those obtained from the study of marine[7] and freshwater animals[8].

The combination $NaCl+KCl+CaCl_2$ (in the same proportions as in sea water) seems to be quite generally beneficial for animals and plants.

We may in conclusion briefly consider the effects of concentrated solutions. A series of experiments were made on *Enteromorpha Hopkirkii* in which the plants were placed in dishes with a very little sea water. This quickly evaporated, so that the plants became covered with salt crystals in 24 to 48 hours. In this condition some of them remained alive for about 150 days. This means that Enteromorpha plants which remain alive only 15 days in $3m/8$ NaCl solution can live 150 days in an NaCl solution of 10 to 12 times higher concentration, provided the other salts of the sea water are present in the solution (at corresponding concentration) to inhibit the toxic effect of NaCl. Experiments on Lyngbya, Ptilota, and Pterosiphonia gave essentially the same results.

In view of these results, and others of a similar character shortly to be published, it appears certain that physiologically balanced salt solutions have the same fundamental importance for plants as for animals.

[7] LOEB, Pflüger's Archiv 107:252. 1905, and the literature there cited.

[8] OSTWALD, Pflüger's Archiv 106:568. 1905. Univ. of California Publications, Physiology 2:163. 1905.

RESULTS.

1. Each of the salts of the sea water is poisonous where it alone is present in solution.

2. In a mixture of these salts (in the proper proportions) the toxic effects are mutually counteracted. The mixture so formed is a physiologically balanced solution.

3. Such physiologically balanced solutions have the same fundamental importance for plants as for animals.

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ON THE IMPORTANCE OF PHYSIOLOGICALLY BALANCED SOLUTIONS FOR PLANTS

II. FRESH-WATER AND TERRESTRIAL PLANTS

(WITH SEVEN FIGURES)

W. J. V. OSTERHOUT

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ON THE IMPORTANCE OF PHYSIOLOGICALLY BALANCED SOLUTIONS FOR PLANTS

II. FRESH-WATER AND TERRESTRIAL PLANTS

W. J. V. Osterhout

(WITH SEVEN FIGURES)

If the facts set forth in the first part[1] of this paper prove to be valid, not for marine plants only, but also for all other kinds, we cannot suppose them to be merely the result of adaptation to a particular environment, but must consider them to be the direct expression of certain fundamental characteristics of living matter. In order that the evidence on this important point might be as complete as possible, a wide range of material was studied. It includes both lower and higher algae, liverworts, Equisetaceae, and several species of flowering plants, embracing among the latter both fresh-water aquatics and land plants. The solutions were made up with all the precautions regarding distilled water and purity of salts described in the first part of this paper. The solutions had the same compositions as there described, except that lower concentrations were employed. A control series was always made, in which all solutions were made faintly alkaline. The material was always rinsed in distilled water before being placed in the solutions. The plants were in all cases exposed to fairly bright light, but not to direct sunlight. The temperature averaged between 18° and 20°C., and was not subject to much fluctuation.

ALGAE

The most extensive series of experiments on algae was made with Vaucheria and Spirogyra. A form of *Vaucheria sessilis*, abundant in running water, was chosen because it readily gives off zoospores when brought into the laboratory. Tufts of this material, washed free from all adhering dirt, were placed in glass dishes and covered with tap water. Glass slides were placed upright in the dishes. On the following morning numerous zoospores were found

[1] Botanical Gazette 42:127–134. 1906.

attached to each slide at the water level. As many as fifty to a hundred zoospores were commonly found arranged in a row across the slide, so that subsequent observation was an easy matter. The slides were thoroughly rinsed in distilled water and transferred to the solutions. The solutions were contained in glass tumblers, in which the slides were placed in an upright position, care being taken to have the zoospores always at the same depth below the surface. The volume of the solution, 100^{cc}, was very large compared with that of the zoospores. The tumblers were covered with glass plates to exclude dust and hinder evaporation. Under these circumstances the plants thrive excellently and in favorable solutions produce normal mature fruit.[2]

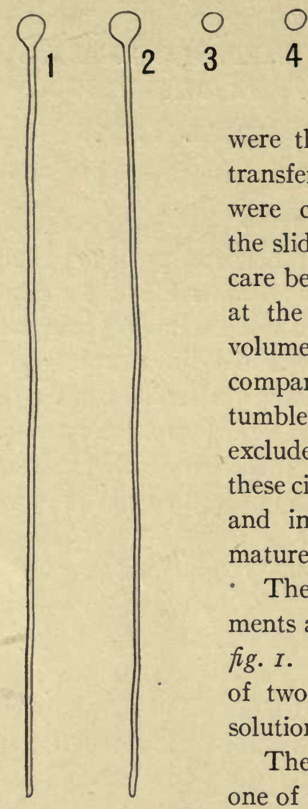

FIG. 1.—Growth of zoospores of Vaucheria during 25 days in various *m*/100 solutions. The quantities are stated in cubic centimeters, the length in millimeters, and the gain in length in per cent. 1, distilled water, length 9.4, gain 5000. 2, NaCl 1000 + $CaCl_2$ 10, length 9.4, gain 5000. 3, NaCl, length 0.18, gain 0. 4, $CaCl_2$, length 0.18, gain 0.

The average results of six series of experiments are given in Table III and illustrated in *fig. 1*. The figure shows clearly how a mixture of two poisonous substances may produce a solution as harmless as distilled water.

The species of Spirogyra employed is a large one of the *majuscula* type. The material was transferred from the pond directly to distilled water; after being rinsed in this it was placed in covered glass dishes containing each 200^{cc} of solution.

It will be seen that these results agree in the most striking way with those already described for marine plants.

Further experiments were made with a variety of other algae, including a species of blue-green alga (Oscillatoria), Chlamydomonas, Closterium and two other species of desmids,

[2] *Cf.* OSTERHOUT, Extreme toxicity of sodium chloride and its prevention by other salts. Jour. Biol. Chemistry 1:363. 1906.

a diatom (Navicula), and a species of Oedogonium. The results agree closely with those given below. There can be little doubt, therefore, that the algae in general, both fresh-water and marine, obey the same law.

TABLE III. ALGAE

A plus sign indicates that the plants were alive when the experiment was stopped. All quantities given are cubic centimeters of $3m/32$ solutions.

Culture Solution	Duration of Life in Days	
	Vaucheria	Spirogyra
Dilute sea water (total salts 0.6 per cent.)	40+	95+
Dilute artificial sea water: 1000 $NaCl$, 78 $MgCl_2$, 38 $MgSO_4$, 22 KCl, 10 $CaCl_2$	40+	95+
Distilled water	40+	95+
Tap water	40+	95+
$NaCl$	$\frac{1}{100}$	$\frac{3}{4}$
1000 $NaCl$, 10 $CaCl_2$	21	60
1000 $NaCl$, 22 KCl, 10 $CaCl_2$	40+	65
1000 $NaCl$, 78 $MgCl_2$, 10 $CaCl_2$	40+	65
1000 $NaCl$, 78 $MgCl_2$, 22 KCl	$\frac{2}{3}$	2
1000 $NaCl$, 22 KCl	$\frac{1}{8}$	2
1000 $NaCl$, 78 $MgCl_2$	$\frac{2}{3}$	$1\frac{3}{4}$
1000 Dist. H_2O, 78 $MgCl_2$ $= m/147$ $MgCl_2$	$1\frac{1}{2}$	2
1000 Dist. H_2O, 22 KCl $= m/495$ KCl	$2\frac{1}{2}$	3
1000 Dist. H_2O, 10 $CaCl_2$ $= m/1077$ $CaCl_2$	9	95+

LIVERWORTS

The gemmae of Lunularia furnish ideal material for studies on the effects of solutions. They can be obtained at any time of year, they grow readily when floating on the surfaces of solutions, and are fairly uniform in behavior. The material used in these experiments was obtained in part from a greenhouse and in part from moist banks of earth along streams. Material from different sources was never mixed together in any series of experiments.

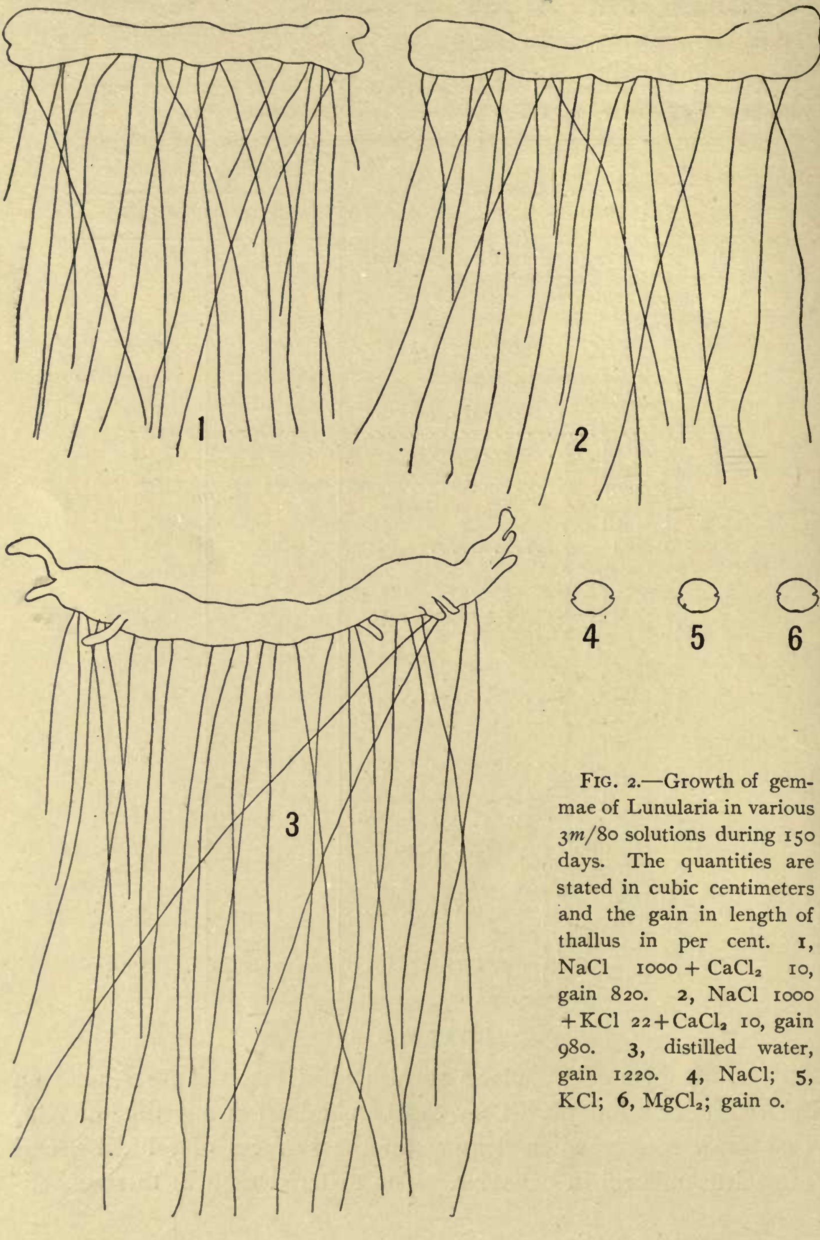

FIG. 2.—Growth of gemmae of Lunularia in various $3m/80$ solutions during 150 days. The quantities are stated in cubic centimeters and the gain in length of thallus in per cent. 1, $NaCl$ 1000 + $CaCl_2$ 10, gain 820. 2, $NaCl$ 1000 + KCl 22 + $CaCl_2$ 10, gain 980. 3, distilled water, gain 1220. 4, $NaCl$; 5, KCl; 6, $MgCl_2$; gain 0.

The gemmae were removed from the cups with the point of a knife and sprinkled on the surface of the solution, care being taken to exclude particles of dust and dirt. Each tumbler contained 200[cc] of solution and was covered with a glass plate. The gemmae may be easily removed from the surface of the solution for purposes of observation by dipping into it a clean slide and slowly raising it at an angle so as to take up the material upon one side only. The material should not be replaced in the solution unless extreme care be taken to prevent contamination.

The average results of six series of experiments are given in Tables IV and V, and illustrated in *figs. 2* and *3*.

TABLE IV. LUNULARIA

A plus sign indicates that the plants were alive when the experiment was stopped. All quantities given are cubic centimeters of $3m/32$ solutions.

Culture Solution	Duration of Life in Days
Dilute sea water; total salts 0.6 per cent	200+
Dilute artificial sea water: 1000 $NaCl$, 78 $MgCl_2$, 38 $MgSO_4$, 22 KCl, 10 $CaCl_2$	200+
Distilled water	200+
$NaCl$	4
1000 $NaCl$, 10 $CaCl_2$	100
1000 $NaCl$, 22 KCl, 10 $CaCl_2$	200+
1000 $NaCl$, 78 $MgCl_2$, 10 $CaCl_2$	200+
1000 $NaCl$, 78 $MgCl_2$, 22 KCl	9
1000 $NaCl$, 22 KCl	4
1000 $NaCl$, 78 $MgCl_2$	7
$MgCl_2$	2
KCl	12
$CaCl_2$	100

TABLE V. LUNULARIA

All quantities given are cubic centimeters of $3m/80$ solutions

Culture Solution	Growth in 150 Days		
	Length of thallus in mm.	Per cent. increase in length of thallus	Aggregate length of rhizoids per thallus in mm.
Dilute artificial sea water: 1000 Nacl, 78 $MgCl_2$, 38 $MgSO_4$, 22 KCl, 10 $CaCl_2$	6.52	1204	168
Distilled water	6.60	1220	180
NaCl	0.50	0	0
1000 NaCl, 10 $CaCl_2$	4.60	820	114
1000 NaCl, 22 KCl, 10 $CaCl_2$	5.40	980	123.5
1000 NaCl, 78 $MgCl_2$, 10 $CaCl_2$	5.50	1000	125
1000 NaCl, 78 $MgCl_2$, 22 KCl	0.54	8	0.3
1000 NaCl, 22 KCl	0.50	0	0
1000 NaCl, 78 $MgCl_2$	0.52	4	0.1
$MgCl_2$	0.50	0	0
KCl	0.50	0	0
$CaCl_2$	4.55	810	90

EQUISETUM

The spores of Equisetum retain their vitality for only a few days. The fruiting cones were brought into the laboratory and allowed to stand for a day or two. The freshly shed spores were then placed on the surfaces of solutions in covered glass dishes. They germinate rapidly and in a few days produce prothallia of fair size. The average results of four series of experiments are shown in Table VI and *fig. 4*.

FLOWERING PLANTS

The most extensive series of experiments was made with wheat. The variety selected is known as Early Genesee. The percentage of germination is very high and the growth is vigorous from the start. The plan first tried was that of carefully placing the seeds on the surface of the solutions so that they float. This worked well with

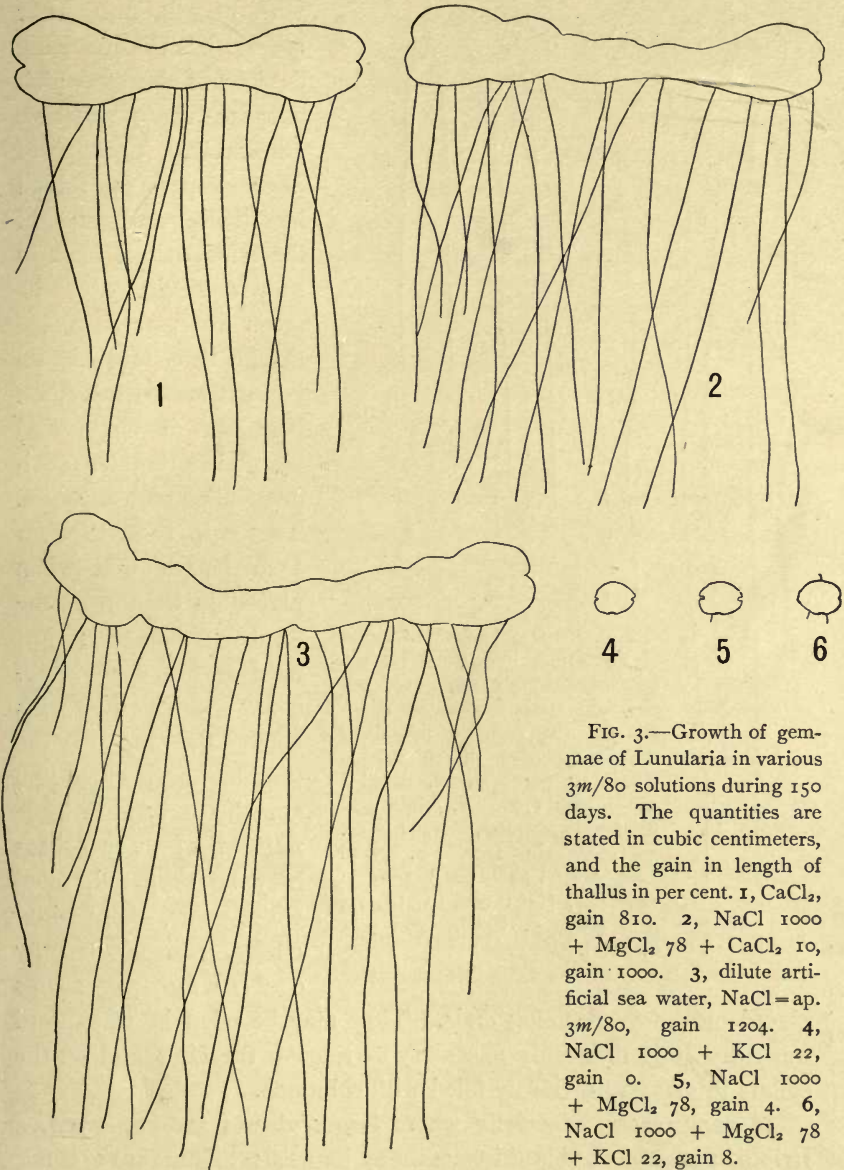

FIG. 3.—Growth of gemmae of Lunularia in various $3m/80$ solutions during 150 days. The quantities are stated in cubic centimeters, and the gain in length of thallus in per cent. 1, $CaCl_2$, gain 810. 2, NaCl 1000 + $MgCl_2$ 78 + $CaCl_2$ 10, gain 1000. 3, dilute artificial sea water, NaCl = ap. $3m/80$, gain 1204. 4, NaCl 1000 + KCl 22, gain 0. 5, NaCl 1000 + $MgCl_2$ 78, gain 4. 6, NaCl 1000 + $MgCl_2$ 78 + KCl 22, gain 8.

wheat and other small seeds during the first stages of germination; but if the experiments are to be carried beyond this stage, the seedlings must be supported so that the leaves do not come into contact with the solution. After some trials the following device was hit upon which answers the purpose admirably. A strip of filter paper is folded lengthwise and one of the folds turned back as shown in *fig. 5*. The seeds are placed in the trough thus formed and the whole strip is then bent into a circle and placed in a tumbler previously filled with solution. The strip should be of such length that when placed in the top of the tumbler the ends just meet and so form a stiff collar which just fits inside the top of the tumblers and which will not slip down. A large number of these collars may be prepared, filled with seeds, bent into circles, and secured by ordinary paper-clips placed on the overlapping ends. They may be piled in trays until wanted. It is then only necessary to remove the clips and set the collars in glasses previously filled with solutions.

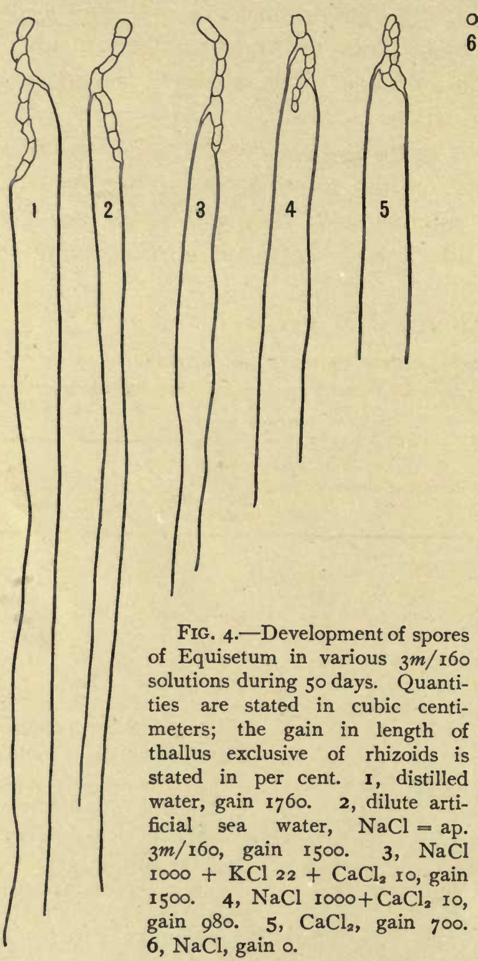

FIG. 4.—Development of spores of Equisetum in various $3m/160$ solutions during 50 days. Quantities are stated in cubic centimeters; the gain in length of thallus exclusive of rhizoids is stated in per cent. 1, distilled water, gain 1760. 2, dilute artificial sea water, NaCl = ap. $3m/160$, gain 1500. 3, NaCl 1000 + KCl 22 + $CaCl_2$ 10, gain 1500. 4, NaCl 1000 + $CaCl_2$ 10, gain 980. 5, $CaCl_2$, gain 700. 6, NaCl, gain 0.

In some cases, especially where larger glasses are employed, a strip of paper of double thickness may be used; this makes a stiffer collar. It is then advisable to perforate the bottom of the seed trough by means of a tracing wheel such as is used for patterns. This allows the roots to penetrate the paper freely and without delay.

Care should be taken that the solution does not cover the seeds. The paper must be spread open at the top so as to allow the air to come into direct contact with the seeds. The micropyle should be in contact with the moist filter paper.

Careful experiments were made to determine whether the filter paper exerted any influence on the solution (by absorption, etc., or by concentration of the solution about the seed as the result of evaporation) which might affect the results, but no such influence

TABLE VI. EQUISETUM

All quantities given are cubic centimeters of $3m/160$ solutions.

Culture Solution	Growth in 50 Days		
	Length of thallus in mm.	Per cent. increase in length of thallus, exclusive of rhizoids	Aggregate length of rhizoids per thallus in mm.
Dilute artificial sea water: 1000 NaCl, 78 $MgCl_2$, 38 $MgSO_4$, 22 KCl, 10 $CaCl_2$	0.80	1500	8.1
Distilled water	0.93	1760	9.0
NaCl	0.05	0	0
1000 NaCl, 10 $CaCl_2$	0.54	980	4.7
1000 NaCl, 22 KCl, 10 $CaCl_2$	0.80	1500	5.2
1000 NaCl, 78 $MgCl_2$, 10 $CaCl_2$	0.93	1760	9.0
1000 NaCl, 78 $MgCl_2$, 22 KCl	0.07	40	0
1000 NaCl, 22 KCl	0.05	0	0
1000 NaCl, 78 $MgCl_2$	0.07	40	0
$MgCl_2$	0.05	0	0
KCl	0.05	0	0
$CaCl_2$	0.40	700	3.2

could be detected. The solutions were renewed from time to time and the concentration ascertained by occasional titration.

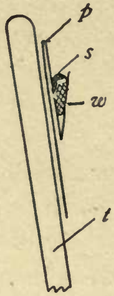

FIG. 5.—Sectional view of wall of tumbler and seed supported by folded filter paper; *p*, paper; *s*, seed; *t*, tumbler; *w*, water line.

It should be said that in general the growth of roots (or any parts in direct contact with the solution) furnishes a much better criterion of the effect of solutions than the aerial portions of the plant. In certain solutions which are so poisonous that the roots cannot develop, the leaves may grow fairly well for a time. In these cases the poisonous solutes are apparently filtered out by the tissues of the seed as the solution passes through them on its way to the leaf. For this reason the figures for the growth of roots only are here given. The results are shown in Table VII and *figs. 6* and *7*, which give the average of five series of experiments. Each number represents average measurements of at least four or five hundred seeds. This is necessary in order to do away with the individual variation so common in seeds.

TABLE VII. WHEAT

All quantities given are cubic centimeters of $3m/25$ solutions.

CULTURE SOLUTION	GROWTH IN 40 DAYS Aggregate length of roots per plant in mm.
Dilute artificial sea water: 1000 NaCl 78 $MgCl_2$ 38 $MgSO_4$ 22 KCl 10 $CaCl_2$	360
Distilled water	740
NaCl	59
1000 NaCl 10 $CaCl_2$	254
1000 NaCl 22 KCl 10 $CaCl_2$	324
1000 NaCl 78 $MgCl_2$ 10 $CaCl_2$	327
$MgCl_2$	7
KCl	68
$CaCl_2$	70

A similar though less extensive series of experiments was carried out with flax, alfalfa, red-beet, and radish seeds. Another series was made by placing pieces of the fresh-water aquatics, Zannichellia and Potamogeton, in solutions, or in the case of Lemna, by allowing the plants to float on the surface. The results in all these cases were similar to those given above.

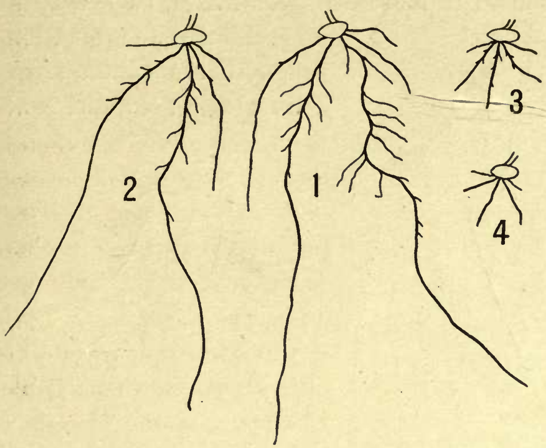

FIG. 6.—Growth of roots of wheat in various $3m/25$ solutions. Quantities are stated in cubic centimeters, and the aggregate length of roots in millimeters. 1, NaCl 1000 + KCl 22 + $CaCl_2$ 10, length 324. 2, NaCl 1000 + $CaCl_2$ 10, length 254. 3, $CaCl_2$, length 70. 4, NaCl, length 59.

It is thought desirable to see how cuttings would behave under similar treatment. Cuttings (about nine inches in length) of Tradescantia and Tropaeolum were placed upright in bottles, the lower three inches of the plant being submerged

TABLE VIII. CUTTINGS

All quantities given are cubic centimeters of $3m/32$ solutions.

Culture Solution	Growth in 10 Days	
	Tradescantia	Tropaeolum
Dilute artificial sea water: 1000 NaCl, 78 $MgCl_2$, 38 $MgSO_4$, 22 KCl, 10 $CaCl_2$	Long roots	Long roots
Distilled water	Very long roots	Very long roots
NaCl	No roots	No roots
1000 NaCl, 10 $CaCl_2$	Short roots	Short roots
1000 NaCl, 22 KCl, 10 $CaCl_2$	Medium length roots	Medium length roots

in the solution. Absorbent cotton was packed in the neck of the bottle to exclude dirt and hinder evaporation. The results were similar to those described above, as will be seen from Table VIII.

Finally the question was raised whether the tissues of the stem and leaf, if brought into direct contact with the solution, would behave like the root. To answer this, sections of considerable (but uniform) thickness were cut with a microtome and placed in the solutions. The results appear in Table IX.

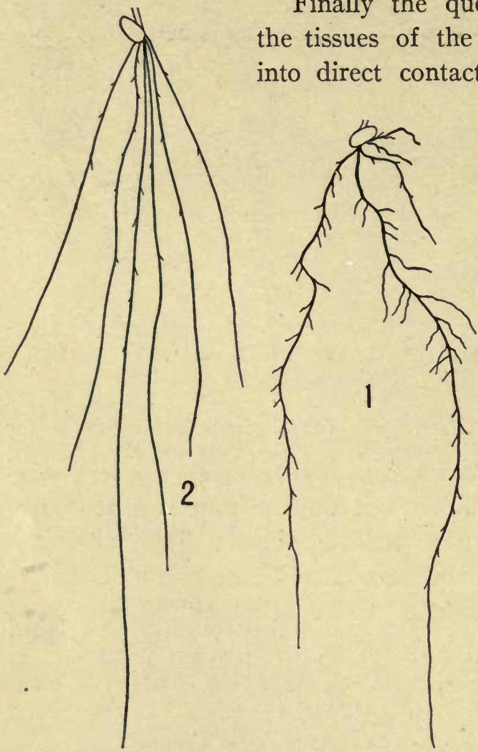

FIG. 7.—Growth of roots of wheat for 40 days. 1, in dilute artificial sea water (NaCl = ap. $3m/25$), aggregate length of roots 360^{mm}. 2, in distilled water, aggregate length of roots 740^{mm}.

The results described in this paper are in all essentials in striking agreement with those obtained from the study of marine plants, as well as from the study of marine and fresh-water animals as referred to in the first part of this paper. This agreement shows that the principle of balanced solutions is of general validity.[3] The application of this principle to soil and river water[4] and to nutrient solutions, I hope to take up in a subsequent paper.

[3] LOEW and his pupils have shown that calcium antagonizes magnesium (cf. Bull. No. 18, Div. Veg. Phys. and Path. U. S. Dept. Agric. 1899). See also the antagonistic effects noted by KEARNEY and CAMERON (Report No. 71, U. S. Dept. Agric. 1902) in their studies on the salts of alkali soils. The method employed by them (observation of the root-tip only) is so different from mine that I have not attempted to compare the results.

[4] In the first part of this paper I have referred to the composition of tap water, but it seems advisable to defer the discussion of this point.

TABLE IX. SECTIONS

All quantities given are cubic centimeters of $3m/32$ solutions.

Culture Solution	Duration of Life in Days			
	Red beet: Cross-sections of root	Mesembryanthemum: Cross-sections of leaf	Tradescantia: Cross-sections of stem	Tropaeolum: Cross-sections of leaf
Dilute artificial sea water: 1000 NaCl, 78 $MgCl_2$, 38 $MgSO_4$, 22 KCl, 10 $CaCl_2$	35	16	19	25
Distilled water	35	18	20	32
NaCl	18	9	12	14
1000 NaCl, 10 $CaCl_2$	25	12	18	20
1000 NaCl, 22 KCl, 10 $CaCl_2$	30	15	19	22

For the sake of clearness it seems desirable to call attention to the distinction between balanced solutions and ordinary nutrient solutions. A nutrient solution may be used in such dilute form that none of its components could exert any toxic action even if the other constituents were removed. In this case there are no poisonous effects to be inhibited and consequently no balancing of the solution is required. Our only concern is to supply all the substances needed for nutrition, irrespective of any balancing action, and so form a *complete* nutrient solution.

If we increase the concentration of this solution, however, we soon reach the point where some or all of the components begin to exert their individual toxic effects, whereupon it may become necessary to inhibit these effects by proper adjustment of the relative proportions of the substances present or by the addition of other substances. The substances added to produce a balance do not necessarily have a nutritive value. For example, LOEB[5] was able to balance certain solutions by adding zinc, cobalt, aluminum, etc.[6]

[5] Am. Jour. Physiology 6:411–433. 1902.

[6] To make clear this distinction between balanced and nutrient solutions is more necessary, since LOEW and ASO (Bull. Coll. Agr. Tokyo Imp. University 7:395. 1907) confuse the two kinds of solutions. Their criticisms are wholly based on this misconception and do not affect the matter as I have presented it. The distinction between nutrient and balanced solutions is due to LOEB, who has explained it clearly in his *Dynamik der Lebenserscheinungen* 115–120.

In general we may know when the solution is properly balanced by comparing its effects with those of *pure*[7] distilled water. In a properly balanced solution we expect the organism to live approximately as long as in distilled water, and while it will not grow so fast (on account of the osmotic pressure), the ultimate development reached should be comparable with that attained in distilled water.[8]

Why all these effects are so, we are not at present prepared to say in detail. LOEB has gone farther than any other in the explanation of these phenomena, referring them to the effects of salts and ions on proteids[9]. According to his conception any metal must be poisonous when it alone is present in the solution, for it will enter the proteids and drive out other metals in accordance with the law of mass action. This will of course alter the properties of the proteids and so cause disturbances in function. The only way to prevent this is to maintain a proper balance between the various metals in the solution. It may be pointed out that an analogy exists between the effects described here and various reactions in which proteids are involved. Antagonism between Na and Ca, for example, is seen in the clotting of blood, which is hindered by Na and favored by Ca.

The thing of chief importance is the agreement in behavior of such a great diversity of plants with the fresh-water and marine animals already studied. Thereby is brought to light a new point of similarity between animals and plants which is fundamental in character and which must be taken into consideration in attempting to formulate a theory of living matter.

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7 Water twice distilled from glass, the first third of the distillate being rejected is usually regarded as pure. But such water may be quite poisonous if any part of the apparatus, including stoppers, be new. The longer the apparatus is used the less poisonous the water becomes, until it finally ceases to be toxic.

8 Higher concentrations excepted.

9 See references in the first part of this paper, BOT. GAZ. 42:134. 1906