SUMMARY OF LITERATURE ON NUTRIENT MEDIA USED IN CULTURING LIVERWORTS

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During the course of recent investigations into the growth of liverworts, it was found that the information pertaining to the nutrient solutions, which had been used, was widely scattered and often difficult to find. In the following an attempt has been made to give a brief survey of this material in such a way that the composition of the various media may be compared and evaluated. In several instances when the quantities were not given in grams per liter, this data has been added in parentheses in a third column, for the purpose of conformity and comparison. The arrangement is chronological.

Marchal (1906) reported that he had cultured *Cephalozia byssacea* successfully on the following nutrient solution:

$NH_4(NO_3)$	1.00 g.
K_2SO_4	0.50
$CaSO_4$	0.50
$MgSO_4$	0.50
K_2HPO_4	0.50
$Fe(SO_4)_3$	0.01
Distilled water	1000.00 ml.

Adjusted to pH 7.0 with 10 per cent KOH.

Dachnowski (1907) used Knop's solution modified in concentrations of 0.1 per cent to 0.4 per cent, with 0.3 per cent used most often, for culturing *Marchantia polymorpha* in the study of the development of rhizoids and the formation of gemmae. This was made up of the following:

$MgSO_4$	0.0075 g.
$Ca(NO_3)_2$	0.0300
K_2HPO_4	0.0075
KCl	0.0036
$FeCl_3$	trace
Distilled water	1000.00 ml.

Osterhout (1907) used two nutrient solutions for the culturing of gemmae of *Lunularia* successfully for 200 days,—the duration of the experiment.

NUTRIENT SOLUTION A

	cc. of 3/32 Molar	
NaCl	1000 cc	(5.4803 g.)
$MgCl_2$	78	(0.6964 g.)
$MgSO_4$	38	(0.4288 g.)
KCl	22	(0.1538 g.)
$CaCl_2$	10	(0.1040 g.)
Distilled water		(1000 ml.)

On solution A, which was diluted artificial sea water,[2] there was a 1204 per cent increase in the length of the thallus. Another solution (solution B) which he also used gave almost equal results, a 980 per cent increase in the length of the *Lunularia* thallus.

NUTRIENT SOLUTION B

	cc. of 3/32 Molar	
NaCl	1000 cc	(5.4803 g.)
KCl	22	(0.1538 g.)
$CaCl_2$	10	(0.1040 g.)
Distilled water		(1000 ml.)

[1]I wish to express my appreciation to Dr. Margaret Fulford for much helpful criticism in reading the manuscript.

[2]The artificial sea water was prepared from Van't Hoff's formula which has the same constituents but at 3/8 M. strength.

Killian (1911) reported favorable results using a nutrient solution devised by Marchal for the study of cultures of hepatics. This included the following:

$NH_4(NO_3)$	1.00 g.
$K_2(SO_4)$	0.50
$Mg(SO_4)$	0.50
$Ca(SO_4)$	0.50
$(NH_4)_3PO_4$	0.50
$Fe(SO_4)_3$	0.01
Distilled water	1000.00 ml.

Buch (1920) reported good results with a nutrient solution which he had used in a morphological and physiological study of *Sphenolobus Michauxi*, *Pellia epiphylla*, *Blepharozia ciliaris*, and *Cephalozia bicuspidata*. It contained the following:

K_2HPO_4	0.80 g.
$MgSO_4$	0.30
$CaCl_2$	0.30
$FeCl_3$	trace
Distilled water	1000.00 ml.

For his studies with the protonema of these species, Buch altered his nutrient solution and made a solid medium with the addition of agar as follows:

KNO_3	0.12 per cent	(1.20 g.)
K_2HPO_4	0.08	(0.80 g.)
$MgSO_4$	0.03	(0.30 g.)
$CaCl_2$	0.03	(0.30 g.)
Fe_2Cl_6	trace	(trace)
Agar	2.00	(20.0 g.)
Distilled water		(1000 ml.)

Lilienstern (1927) used both Uspenski's and Detmer's solutions plus two per cent agar for culturing *Marchantia polymorpha* in a morphological and physiological study. The composition of these two nutrient solutions is given below:

USPENSKI NUTRIENT SOLUTION

KNO_3	0.02500 g.
$MgSO_4$	0.02500
$Ca(NO_3)_2$	0.10000
KH_2PO_4	0.02500
K_2CO_3	0.03450
$Fe_2(SO_4)_3$	0.00125
Distilled water	1000.00 ml.

pH of nutrient solution 7.6.

DETMER NUTRIENT SOLUTION

$Ca(NO_3)_2$	1.00 g.
$MgCl_2$	0.25
$MgSO_4$	0.25
KH_2PO_4	0.25
$FeCl_3$	trace
Distilled water	1000.00 ml.

The pH of the solution 6.8.

Ehring (1934) used the following four solutions on *Marchantia polymorpha*, *Lunularia cruciata*, and *Riccia fluitans* with success.

"a" NUTRIENT SOLUTION

$NaNO_3$	0.0200 per cent	(0.200 g.)
$CaCl_2 \cdot 6H_2O$	0.0100	(0.100 g.)
$MgSO_4 \cdot 7H_2O$	0.0100	(0.100 g.)
KH_2PO_4	0.0100	(0.100 g.)
$FeSO_4 \cdot 7H_2O$	0.0005	(0.005 g.)
Distilled water		(1000 ml.)

(Approximate salt concentration 0.05 per cent.)

"β" NUTRIENT SOLUTION

KNO_3	0.010 per cent	(0.10 g.)
$CaSO_4 \cdot 2H_2O$	0.020	(0.20 g.)
KH_2PO_4	0.020	(0.20 g.)
$MgSO_4 \cdot 7H_2O$	0.020	(0.20 g.)
Fe_2O_3	0.003	(0.03 g.)
Distilled water		(1000 ml.)

(Approximate salt concentration 0.07 per cent.)

"γ" NUTRIENT SOLUTION

KNO_3	0.1000 per cent	(1.000 g.)
$Ca_3(PO_4)_2$	0.0500	(0.500 g.)
$MgSO_4 \cdot 7H_2O$	0.0500	(0.500 g.)
$FeSO_4 \cdot 7H_2O$	0.0005	(0.005 g.)
Distilled water		(1000 ml.)

(Approximate salt concentration 0.2 per cent.)

"δ" NUTRIENT SOLUTION

NH_4NO_3	0.020 per cent	(0.20 g.)
$Ca_3(PO_4)_2$	0.020	(0.20 g.)
KCl	0.020	(0.20 g.)
$MgSO_4 \cdot 7H_2O$	0.020	(0.20 g.)
$FeSO_4 \cdot 7H_2O$	0.002	(0.02 g.)
Distilled water		(1000 ml.)

(Approximate salt concentration 0.08 per cent.)

Müller (1939) reported a nutrient solution used by Lorbeer which is a nutrient agar modification of the one used by Benecke.

NH_4NO_3	0.200 g.
$CaCl_2$	0.100
KH_2PO_4	0.100
$MgSO_4$	0.100
$FeCl_3 \cdot 3H_2O$	0.005
Agar	15.00
Distilled water	1000.00 ml.

Also in the same year, Griggs (1939) reported a nitrogen free solution on which he had cultured *Cephaloziella byssacea* successfully for three years. The solution was a modification of one of the three salt nutrient solutions devised by Shive, but with only two-fifths the concentration.

KH_2PO_4	1.225 g.
$MgSO_4 \cdot 7H_2O$	1.848
$CaSO_4$ (anhydrous)	0.340
iron as:	
ferric phosphate, or	
ferric chloride, or	
ferric citrate	trace
Distilled water	1000.00 ml.

(The pH of the solution was between 5 and 6.)

Voth and Hamner (1940) used the following nutrient solution successfully in a physiological study of *Marchantia polymorpha*.

$MgSO_4$	0.1204 g.
$MgHPO_4 \cdot 3H_2O$	0.1744
$Mg(NO_3)_2 \cdot 6H_2O$	0.2564
$CaSO_4$	0.1722
$CaH_4(PO_4)_2$	0.1261
$Ca(NO_3)_2$	0.2362
K_2SO_4	0.1742
KH_2PO_4	0.2723
KNO_3	0.2022
Trace elements	1.00 ml.
$MnSO_4$	0.20 p.p.m.
$Na_2B_4O_7$	0.20 p.p.m.
$ZnCL_2$	0.20 p.p.m.
$FeSO_4$	0.02 p.p.m.

(Osmotic concentration approximately 0.285 atmos.)

Since then, we have successfully cultured plants of *Leucolejeunea clypeata*, for five months, on the nutrient solution described by Voth and Hamner above.

Voth (1941) later suggested the following nutrient solution as the one best for culturing *Marchantia polymorpha*.

	cc. of 0.5 Molar	
KNO_3	1.6	(0.0808 g.)
$Ca(NO_3)_2$	1.4	(0.1148 g.)
$Mg(NO_3)_2$	1.2	(0.0890 g.)
KH_2PO_4	0.8	(0.0544 g.)
$MgSO_4$	1.6	(0.0962 g.)
Distilled water		(1000 ml.)

Very recently Prát (1948) has reported cultivation of various hepaticae on the following mineral nutrient agar:

NH_4NO_3	0.200 g.
$CaCl_2$	0.100
KH_2PO_4	0.100
$MgSO_4 \cdot 7H_2O$	0.100
$FeCl_3 \cdot 6H_2O$	0.005
Agar	8.00
Distilled water	1000.00 ml.

Prát also stated that *Riella* was cultured in erlenmeyer flasks on sand to which the above nutrient solution was added, minus the agar.

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