FURTHER NOTES ON THE PHYSIOLOGY OF POLYMORPHISM IN GREEN ALGAE.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XXXII.

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SINCE the publication of the previous article on this subject[1] several additional lines of experimentation have been completed. The results of these will be given in the present paper. They corroborate the conclusions already expressed, and also throw some further light upon the interesting response with which we are dealing.

I. PHYSIOLOGICAL EXPERIMENTS.

The experiments here recorded were all performed upon the organism used in the previous work, that is, *Stigeoclonium tenue* (?), and the same methods were employed as far as the culture media would allow. The cultures may be classified according to the media used into the following groups: (1) sugar solutions, (2) solutions containing both sugar and mineral salts, (3) porous plate cultures, (4) gelatine cultures, (5) cultures in darkness, (6) evaporation cultures. These categories will be considered in order.

1. SUGAR SOLUTIONS.

The work already published shows clearly that for changes in concentration of Knop's solution the factor controlling the response of the alga is the osmotic pressure. Whether this acts upon the organism merely through a change in the relation of water to the cell, or in some more complex way, could not be decided as long as the pressure was always produced by mineral salts. Thus the next step to be taken was to determine the effect upon the plant of a solution of non-electrolytes. To this end some

[1] LIVINGSTON, B. E.: On the nature of the stimulus which causes the change of form in polymorphic green algae. BOT. GAZ. **30**: 289. 1900.

substance had to be chosen in which the plant would live and grow for some length of time. Also it was necessary that the culture media be of such nature as not to be readily attacked by bacteria and fungi. After several failures, the two saccharoses, lactose and cane sugar, were hit upon for this purpose. The former is far the better, but the latter serves very well. Cultures in these may be continued for two, three, or even four weeks without undergoing fermentation sufficient to affect the alga appreciably.

Tables I and II give the results of some representative cultures in these solutions. At the left are those in lactose, at the right those in cane sugar. In the first table the original material was of the palmella form; in the second, of the filamentous. The concentrations are given in terms of a normal solution ($n = 1$ gram molecule per liter), and are placed in the columns headed "concentration." In the columns headed "first response," Sp. F. denotes that zoospores were produced in great numbers, and germinated as usual to form filaments. A single asterisk (Sp.*) shows that the spores did not germinate. Filaments produced from the original masses are denoted by F in this column. Two asterisks (Sp.**) show that few zoospores were produced, and that these failed to germinate. P denotes the palmelloid form; $P >$ the rounding up of cells as if going over into palmella. In the columns following those indicating the response is given the time of response in days; 2–12 denotes that the response was observed two days after the making of the culture, and continued for ten days. The second response is the result of evaporation, and comes after a somewhat longer time than the first. The time for it is given in days after the making of the culture.

In general, solutions of low osmotic pressure produce zoospores and filaments, while those of high pressure produce only the round-celled form. This is in accord with the results obtained with solutions of mineral salts. However there is one difference in the behavior of the palmella form in solutions of electrolytes and non-electrolytes. This is brought out by Table

TABLE I.

PALMELLA IN SUGAR SOLUTIONS.

Lactose					Cane Sugar				
Concentration	1st Response	Time	2d Response	Time	Concentration	1st Response	Time	2d Response	Time
$\frac{n}{100}$	Sp. F.	2–12	P	30	$\frac{n}{100}$	Sp. F.	5–10	..	..
$\frac{n}{100}$	Sp. F.	2–7	..	..	$\frac{n}{100}$	Sp. F.	2–9	..	..
$\frac{n}{10}$	Sp. F.	4–9	P	30	$\frac{n}{10}$	Sp. F.	3	..	..
$\frac{n}{10}$	Sp. F.	9	..	..	$\frac{n}{10}$	Sp. F.	2–18	..	..
$\frac{2n}{10}$	Sp. F.	2–12	..	..	$\frac{2n}{10}$	Sp. F.	2–18	..	..
$\frac{3n}{10}$	Sp.* F.	2–7	P	9	$\frac{3n}{10}$	Sp. F.	2–18	P	30
$\frac{4n}{10}$	Sp.** F.	2	P	14	$\frac{4n}{10}$	Sp. F.	8–14	..	..
$\frac{5n}{10}$	No spores P	14	..	..	$\frac{5n}{10}$	Sp.* F.	8	P	14
$\frac{7n}{10}$	P	10	..	..	$\frac{7n}{10}$	P	10	..	..

TABLE II.

FILAMENTS IN SUGAR SOLUTIONS.

Lactose			Cane Sugar		
Concentration	Response	Time	Concentration	Response	Time
..	..	..	$\frac{n}{100}$	Sp. F.	6–13
..	..	..	$\frac{n}{10}$	No spores F	6
..	..	..	$\frac{n}{10}$	Sp.* F P>	4–11
$\frac{3n}{10}$	No spores P>	10	$\frac{3n}{10}$	No spores P>	14
$\frac{5n}{10}$	P	14	$\frac{5n}{10}$	P	8–14
$\frac{5n}{10}$	P	10	$\frac{5n}{10}$	P	10–12
$\frac{7n}{10}$	P	10	$\frac{7n}{10}$	P	10
..	..	..	$\frac{75n}{100}$	P	9

III, which shows the maximum limit for zoospore production in each case. It is to be compared with Table V of the former article,[2] from which the figures for mineral solutions have been taken. The figures have been altered to round numbers to avoid confusion.

TABLE III.

MAXIMUM LIMITS FOR ZOOSPORE PRODUCTION.

Original Form	Mineral Solutions	Solutions of Non-electrolytes	
		Lactose	Cane Sugar
Palmella	$\frac{2n}{10}$	$\frac{5n}{10}$	$\frac{6n}{10} - \frac{7n}{10}$
Filamentous	$\frac{3n}{10}$	$\frac{3n}{10}$	$\frac{3n}{10}$

The filamentous form responds in exactly the same manner whether the solution is of electrolyte or non-electrolyte. But to bring about an inhibition of zoospore production in the palmella form requires a much higher osmotic pressure when this is produced by non-electrolytes than in the other case. Also the two sugars used differ in that the limit for cane sugar is much higher than for lactose. Why is this? The volume of the cells in the culture is so small, as compared with that of the surrounding fluid, that the difference just spoken of cannot be explained by the supposition that the carbohydrate is absorbed and thus the concentration of the medium lowered. The amount of absorption possible would be entirely inadequate to alter the concentration to any appreciable degree. It seems more probable that by absorption of sugar the concentration of the cell sap is increased, thus decreasing the difference between the osmotic conditions within and without the plant, and so weakening the stimulus. This would occur if the carbohydrate molecules were to penetrate the cells (*i. e.*, be absorbed) more rapidly than the ions of an electrolyte. It is possible that cane sugar is absorbed

[2] *Loc cit.*, p. 313.

much more readily than lactose; hence might arise the difference between the effect of these two substances upon the organism.

Considering these results, together with those heretofore published, there seems little room for doubt that the response of this plant is brought about by a change in its relation to water. An increase in the osmotic pressure of the surrounding fluid must invariably extract water from the cell (since plant membranes are readily permeable to water), and a decrease of such pressure must cause the cell to take up more. However, an increase in the amount of sugar in the medium might influence the plant otherwise than in the way just mentioned. There might be, for instance, a chemical effect produced by the carbohydrate molecules. Also, it is readily conceivable that an increase in the number of electrically charged ions in the medium might exert some specific influence upon the protoplasm aside from mere change of water relation. But it is hardly conceivable that any chemical influence exerted by sugar molecules could be identical in its effect with an influence exerted by electrolyte ions. Thus we are almost driven to the following conclusions: (1) since solutions of electrolytes and non-electrolytes affect the organism in the same way, they must exert a common influence upon the cells; and (2) since it is inconceivable that there is any chemical influence common to the two forms of solution, the response must be due to the *one factor which is common to both*, namely, change in the water relation. Whether this change in the water content of the cells acts merely through the mechanical effects of a change in the turgor pressure of the cell sap, or whether the response is brought about by a more subtle adjustment within the protoplasm itself, we have at present no means of telling. It seems probable that both these factors are operative.

It is of interest to note here that while the organism often dies in a mineral solution whose pressure is $\frac{4\pi}{10}$, and invariably dies in stronger mineral solutions, yet it lives indefinitely and apparently without injury in a normal sugar solution. This is

probably due to the poisonous action of some of the electrolyte ions used. To determine, if possible, what this action may be, will be the object of further research.

The same culture may be made to change its form several times in sugar solutions just as in mineral solutions, by adding water or allowing it to evaporate. Nearly all the cultures made in sugar solutions have been controlled by others made at the same time in mineral solutions, and the control cultures have not deviated at all from those discussed in my previous paper. Further, healthy material taken from sugar solutions behaves in water and in mineral solutions precisely as though it had been grown in a mineral solution.

2. MIXED SOLUTIONS.

These solutions contained both sugar and mineral salts. The response of the alga to the weaker ones is the same as though they were composed either of sugar alone or of salts alone. For the stronger solutions the same is true of the filamentous form. But the palmella form gives something of its characteristic response to sugar or electrolytes, according as one or the other of these substances predominates. However, this was not very well marked in the experiments. In general, the plant behaves in the same manner in a mixed solution as in a simple one.

2. POROUS PLATE CULTURES.

These were made on unglazed porcelain, such as is used in chemical work. A piece of plate four or five centimeters square was laid on the bottom of the culture dish, and sufficient solution was poured over to stand within a millimeter or two of the upper surface of the plate. Thus the plate was saturated, but no free liquid was upon its surface. The alga cells were placed upon the plate and the whole was covered as usual. The results of these cultures are perfectly uniform, and are exactly what would have been expected. The osmotic pressure of the solution which saturates the plate determines the response of the plant. Typical results from the series of plate cultures are presented in table IV. The abbreviations are those used in tables

I and II. Ev denotes an uncovered culture with rapid evaporation.

TABLE IV.

POROUS PLATE CULTURES.

Solution.	Original form.	Response.	Time.	Solution.	Original form.	Response.	Time.
$\frac{n}{100}$ L	P	Sp. F.	9	$\frac{3n}{10}$ K	P	P.	10
$\frac{n}{100}$ S	P	Sp. F.	9	$\frac{4n}{10}$ S	P	Sp. F.	9
$\frac{n}{100}$ K	F	Sp. F.	10	$\frac{4n}{10}$ K	P	P. F.	16
$\frac{n}{10}$ K	F	P. F.	10	$\frac{5n}{10}$ L	P	P.	16
$\frac{2n}{10}$ L	P	Sp. F.	9	$\frac{75n}{100}$ L	P	P.	46
$\frac{3n}{10}$ K	F	P. F.	10	$\frac{n}{100}$ L, Ev	F	P.	6

4. GELATINE CULTURES.

These were made like the plate cultures just described, excepting that in the place of the porcelain support the solution to be used was thickened with gelatin until it would give a firm surface on which to place the alga. The result was uniform with the foregoing. The gelatin itself has no influence. The osmotic pressure of the solution determines the response of the organism. Gelatin cultures cannot be continued very long because of bacterial action, and are therefore not very satisfactory.

5. CULTURES IN DARKNESS.

In darkness the alga remains green and healthy for three to five weeks. At last the cells become plasmolyzed and go to pieces. Putting the culture in darkness has no effect whatever upon the response of the plant. This probably indicates that the mechanism of polymorphism is not connected with that of photosynthesis.

6. EVAPORATION CULTURES.

Weak solutions, with their contained filaments, may be left open to the air, and within a few days become very much concentrated through evaporation. The response of the plant is

rapid and marked in these cases. If one has filaments and wishes the palmelloid form, he has but to leave the dish uncovered for a few days to attain this end. Evaporation affects the plant in the same way whether it be in the case of sugar solution, mineral solution, or porous plate culture. It was impracticable to get results by evaporation from gelatin on account of bacteria, by the growth of which the culture was destroyed before a response could be expected.

SUMMARY.

1. Non-electrolyte solutions have the same effect as electrolyte solutions; the osmotic pressure is the controlling factor in determining the form of the plant. This is effective through changes in the water content of the cells.

2. The threshold of stimulation for inhibition of zoospore production is at a higher concentration in a solution of non-electrolytes than in one of electrolytes. Also, the plant withstands the killing effect of the solution at a higher concentration for non-electrolytes than for electrolytes.

3. This threshold of stimulation for the palmella form is at a higher concentration for cane sugar than for lactose.

4. Cells supported upon gelatin or porcelain plates moistened by a solution respond in the same way as though they were immersed in the solution.

5. Prolonged darkness has no effect upon the form of the plant; the response of polymorphism does not depend upon the photosynthetic process.

6. Increase in concentration caused by evaporation from any solution or from porcelain plates brings about the normal response for a concentrated solution.

II. PHYSICO-CHEMICAL TESTS.

In order to determine the extent of the error introduced into these experiments by the author's assumption[3] of complete ionization in the mineral solutions, a series of tests of the actual

[3] *Loc. cit.*, p. 297.

osmotic pressures developed therein has been carried out during the past summer.

Owing to the as yet insurmountable difficulties in determining directly the osmotic pressure of a solution, the indirect method by freezing points was resorted to. It has been well established[4] that for dilute solutions a uniform relation obtains between the osmotic pressure, the depression of the freezing point, and the elevation of the boiling point. Thus if Δ_f denotes the depression of the freezing point, the osmotic pressure is given by the formula $P_f = 12.07\,\Delta_f$, wherein P_f is the osmotic pressure at the freezing point of the solution. Similarly, $P_b = 57\,\Delta_b$, wherein P_b is the osmotic pressure at the boiling point and Δ_b the elevation of the boiling point. The depression of the freezing point was determined by means of Beckmann's apparatus,[5] for the mineral solutions described in the previous paper, as far as these were at hand when the tests were made. From these data the osmotic pressure at the freezing point (practically 0° C.) was derived by the formula given above, and from this the pressure at 25° C. was obtained by the formula $P_t = P_f\,(1 + 0.00367\,t)$,[6] wherein P_t is the pressure at any given temperature (t).

Since ionization is usually more complete at high temperatures than at low ones, it was thought advisable to make some sample tests of osmotic pressure by determining the elevation of the boiling point. This was done for a limited number of solutions only. For this purpose the improved apparatus of Beckmann[7] was used. After the determination of P_f and P_b (*supra*), P_{25} was obtained by interpolation between them thus: $P_{25} = P_f + 25 \times 0.00367\,(P_b - P_f)$. The results show that the difference

[4] NERNST, W.: Theoretical chemistry, translated by C. S. Palmer, p. 123 *et seq.* 1895.

[5] BECKMANN: Zeitschr. Physik. Chem. **2**: 638. 1888.

[6] Simply the law of Gay-Lussac, which holds for osmotic pressures of weak solutions. NERNST: *op. cit.*, p. 134 *et seq.* Also OSTWALD, W., Outlines of general chemistry, translated by J. Walker, p. 128 *et seq.* 1895.

[7] BECKMANN: Zeitschr. physik. Chem. **8**: 223. 1891.

between the degree of dissociation at 0° C. and at 25° C. is so small as to be neglible for our present purpose.

The data obtained by these tests of the actual molecular conditions within these solutions are given in Table V. This is a supplement to the table given in the author's previous paper on this subject. In the first column are given the so-called percentage strengths of the solutions employed. In the second the solutions are designated by the letters previously used for that purpose (*loc. cit.*, p. 599). The next two columns contain the depressions of the freezing point and the elevations of the boiling point as directly observed. Then follow the two columns containing the osmotic pressures at the freezing and boiling points given in atmospheres. These numbers were obtained from Δ_f and Δ_b by the formulae given above. In the next two columns are given the pressures at 25° C., measured in centimeters of mercury, 1 being found from P_f by direct application of the law of Gay-Lussac, 2 by interpolation between P_f and P_b. The last column contains the pressures originally calculated by the assumption of complete ionization. They are introduced here for comparison.

It will be noted that there is a considerable range in the actual osmotic pressures of the different solutions whose calculated pressures are the same. This would be expected and is partially due to variations in the degree of ionization of the different salts according to their influence upon one another. It is also in part brought about by the fact that K_2HPO_4 dissociates completely only in very weak solutions. But this range of pressures lies entirely within the range of the pressure limits found for the several responses of the plant (*loc. cit.*, pp. 301, 306, 313). That is, the errors of calculation, while very large from a physical point of view, are not large enough to affect the physiological conclusions already expressed. This is on account of the comparatively low degree of sensitiveness of the organism with which we are dealing.

Discussion of the reasons for the curious variations from the calculated pressure manifested by these tests will be reserved

TABLE V.

PRESSURE DATA FOR NUTRIENT FLUIDS.

Per cent.	Solution	Δf	Δb	P_f (Atm.)	P_b (Atm.)	P_{25} (cm. Hg) (1)	P_{25} (cm. Hg) (2)	P (Calc.) (cm. Hg.)[8]
0.005	A	.023	....	.2776	....	23.0288	.....	16.19
	C	.012	.005	.1448	.285	12.0148	13.6701	
	D	.032	....	.3862	....	32.0475	.....	
0.1	A	.032	....	.3862	....	32.0475	.....	32.37
	C	.058	....	.7001	....	57.9861	.....	
	D	.041	....	.4949	....	41.0605	.....	
0.5	K	.154	.061	1.9088	3.477	158.3460	172.9608	161.86
	A	.124	....	1.5967	....	132.4809	.....	
	B	.155	....	1.8708	....	155.2300	.....	
	C	.165	....	1.9915	....	165.2392	.....	
	D	.185	....	2.2329	....	185.2728	.....	
1.0	K	.3	....	3.621	....	299.896	.....	323.71
	A	.235	.06	2.8364	3.42	235.3416	228.5578	
	B	.26	....	3.1382	....	260.3836	.....	
	C	.295	....	3.5606	....	295.4348	.....	
	D	.322	....	3.8865	....	322.4756	.....	
1.5	K	.4	.09	4.828	5.13	400.368	369.0180	487.59
	A	.32	....	3.8634	....	320.5604	.....	
	B	.381	....	4.5987	....	374.1936	.....	
	C	.433	....	5.2263	....	433.6408	.....	
	D	.465	.12	5.6125	6.84	465.7100	449.8644	
2.0	A	.415	.1	5.0007	5.7	414.9296	393.3380	647.42
	B	.46	....	5.543	....	459.9216	.....	
	C	.56	....	6.8444	....	567.9024	.....	
	D	.682	.15	8.2317	8.55	683.009	631.6588	

for a future paper. These data are published here merely as corroboration of the general conclusions already published. I wish to express my thanks here to Dr. Felix Lengfeld, of the Kent Chemical Laboratory of this University, for much kind advice and for the unrestricted use of the necessary facilities for such determinations as the above.

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[8] See the author's previous paper, *loc. cit.*, pp. 301, 306.