The Moisture-relations of Terrestrial Algae.¹

II. The Changes during Exposure to Drought and Treatment with Hypertonic Solutions.

BY

F. E. FRITSCH

AND

F. M. HAINES.

With eight Figures in the Text.

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INTRODUCTION.

IN the first paper of this series (Fritsch, 1922) it was shown for a number of common terrestrial algae that a considerable amount of moisture is retained within the cells in the air-dry condition, so that only relatively small quantities are requisite to replace that lost by the protoplasts in drying. It was suggested that this marked retention of moisture might be due to concentration of the sap (loc. cit., p. 19). The observations

¹ From the Botanical Department, East London College. Some of the results of the present investigations have already been communicated in abstract (Journ. of Ecol., x, pp. 229-31, 1922).

communicated in the present paper are the outcome of an attempt to study the mechanism involved. The problem was first approached by an investigation of the effect of various strengths of plasmolysing solutions. For this purpose Tidman's sea-salt was selected, so as to avoid possible inaccuracies due to the toxic action of single salts on the protoplasm.

The material investigated was primarily the same as that employed in the first paper (Fritsch, 1922, p. 2), but relatively little of the *Hormidium*-stage of *Prasiola crispa* was obtainable during the course of the work, and it has consequently not been dealt with as fully as some of the other forms. Apart from these, investigations were also made on (a) a form of *Hormidium flaccidum* occurring in some quantity in different parts of the Redlands woods in Surrey; (b) *Cystococcus humicola*, Naeg., growing on wooden palings in Dorking; and (c) moss protonema (probably belonging to the moss *Hypnum cupressiforme*, L., var. *filiforme*, Brid.) from the Redlands woods. A practice was made of collecting the material for the different successive experiments from as small an area (often only a few square feet), as possible, so as to ensure relative uniformity (cf. p. 697), but this was not always feasible, and for some of the forms there were two or more collecting grounds.¹ There is, however, no reason to suppose that these habitats differed at all essentially from one another. Most of the experimental work was carried out at East London College, but some at North Holmwood, Surrey. The bulk of the observations were made during the winters of 1922 and 1923 and the early spring of 1922, since suitable material was not easily obtainable during the dry summer weather.

In the course of our investigations numerous problems have been approached, a detailed solution of which lay outside the scope of the present paper (cf. especially section E). We propose to deal with some of these in later communications.

A. THE GENERAL BEHAVIOUR TOWARDS PLASMOLYSING SOLUTIONS.

It is noticeable that, if material of these terrestrial forms is placed in plasmolysing media, some of them require solutions of very considerable

¹ The following is a list of these habitats and of the experiments in which the respective materials were used: (a) For *Zygogonium ericetorum*: (i) little-shaded pathway (Expts. I, III); (ii) unshaded pathway (Expts. IV, V, IX, X, XII, XVI, XX); (iii) somewhat shaded pathway (Expts. XXXI, XXXV, XXXVI). (b) For *Hormidium flaccidum*: (i) on sandy vertical bank at side of pathway (Expt. II); (ii) bare clayey soil, in great part shaded by a large holly (Expts. VI, XI, XXXII, XXXVI); (iii) sandy path, fully exposed to the light (Expts. XIV, XXVI, XXIX). (c) Moss protonema (all from same habitat as (ii) for *Hormidium*). (d) For *Pleurococcus*: (i) wooden palings on outskirts of Dorking (Expt. VII); (ii) wooden palings, South Holmwood (Expts. XVII, XXIV, XXVIII, XXX, XXXVI).

strength before plasmolysis is to be observed in any of the cells (cf. also Fritsch, 1922, p. 14). To this category belong *Pleurococcus Naegelii*, *Cystococcus humicola*, and under certain conditions the filamentous stage of *Prasiola crispa*,¹ all three of which are unaffected or only very slightly affected by immersion in a 10 per cent. solution of sea-salt;² as a matter of fact, to obtain decided plasmolysis in a majority of the cells, solutions of a strength of 20 per cent. and upwards have had to be employed. Not all the forms investigated, however, require solutions of such a strength to bring about pronounced plasmolysis. In many cases a 5 per cent. solution of sea-salt suffices to plasmolyse markedly many of the cells of *Zygogonium ericetorum*,³ *Hormidium flaccidum*, and of the protonema of the moss studied.

A second striking fact relating to the plasmolysis of these forms is the great inequality in the behaviour of the cells within one and the same mass of material, collected from the same spot and at the same time. In the case of the filamentous forms this is often observable within a single filament; the unplasmolysed cells are scattered singly in quite an erratic manner in filaments, most of whose cells are plasmolysed, and vice versa. In the case of the moss protonema the cells at the tips of the branches were very commonly, though not invariably, unaffected by the strength of sea-salt used (5 per cent.). By contrast with these terrestrial forms aquatic algae (*Spirogyra*, *Mougeotia*, *Oedogonium*) usually exhibit a uniform reaction of the cells throughout whole threads, although in the case of *Cladophora* the older cells of the thick main axes are usually not plasmolysed by the same strength of solution as affects the cells of the finer branches.

There is a minimum concentration of sea-salt which will produce any plasmolysis at all in the terrestrial forms studied, but with this strength the shrinkage is only very slight, and usually only to be observed in quite an insignificant percentage of the cells. Using solutions of progressive strengths above the minimum one not only observes an increase in the extent of plasmolysis in the individual cells, but a more or less steady increase in the numbers of plasmolysed cells (cf. Table I). As a general rule, however, it is necessary to employ strengths far above the minimum to bring about plasmolysis of the majority of the cells of a given mass of material. Thus, in the case of material of *Zygogonium ericetorum*,³ examined immediately after collection, the minimum concentration proved to be 2.25 per cent. sea-salt; even with 10 per cent. solutions, however, there were occasional cells that were not plasmolysed. In the case of

¹ The statement made in the first paper (Fritsch, 1922, p. 14) that the cells of the filamentous stage of *Prasiola* are markedly plasmolysed by a 10 per cent. solution of sea-salt was probably due to investigation of recently inundated threads and has not been confirmed.

² Similar high concentrations of sugars are necessary to produce plasmolysis. Thus very few cells of either *Pleurococcus* or *Prasiola* plasmolyse in 40 per cent. sucrose.

³ This is the same as the *Zygnema ericetorum* of the earlier paper.

Hormidium flaccidum the minimum was 2.5 per cent., but with a 5 per cent. solution contraction was little marked in the granular cells (cf. below).

TABLE I.

Comparison of plasmolysis of identical material of various terrestrial forms in different strengths of sea-salt.

Material.	Expt. No.	Cells counted.	Strength of sol.	Strongly plasmolysed. ¹	Slightly plasmolysed. ¹	Unaffected.
			%	%	%	%
<i>Zygogonium</i>	XVI	1,104	3.0	1.9	60.6	37.6
"	"	1,185	4.0	12.9	61.7	25.2
"	"	1,226	5.0	71.8	16.7	11.4
<i>Hormidium</i>	II	423	3.0	0.7	37.3	61.9
"	"	757	5.0	20.9	68.6	10.6
"	"	1,023	3.0	45.8	32.7	21.6
"	"	1,019	5.0	91.2	3.6	5.2
<i>Prasiola</i>	XXVII	382	10.0	7.6	54.2	38.2
"	"	1,449	20.0	74.6	11.0	14.4
<i>Protonema</i>	XV	811	3.0	17.8	60.1	22.2
"	"	851	4.0	62.8	24.7	12.6
"	"	764	5.0	70.0	17.8	12.2
				Plasmolysed.	Unaffected.	
				%	%	
<i>Pleurococcus</i>	VII (1)	687	15.0	27.1	72.9	
"	"	661	17.5	39.0	61.0	
"	"	669	20.0	39.9	60.1	
"	"	643	22.5	50.1	49.9	
"	"	645	25.0	50.2	49.8	
"	VII (2)	1,002	15.0	19.9	80.1	
"	"	1,007	17.5	22.5	77.5	
"	"	1,004	20.0	26.9	73.1	
"	"	1,003	22.5	33.6	66.4	
"	"	1,502	25.0	45.7	54.3	

Many of the cells of these terrestrial algae include larger or smaller numbers of highly refractive granules, which appear in part at least to be fatty in nature (cf. p. 721). The feature in question is often so marked that one can broadly classify the cells in a given mass of material into 'granular' and 'non-granular'; a classification often employed below. In the filamentous forms whole threads may be granular, non-granular, or mixed. The granular cells, as found in nature, differ somewhat among one another in character, some containing more or less uniform, rather evenly distributed granules, others such as are of very unequal size and often very uneven shape. But one of the most distinctive types of granular cells—apparently especially prevalent during mild periods of drought in

¹ In many cases it has been found desirable to distinguish between 'strongly plasmolysed' and 'slightly plasmolysed' cells, the latter being those in which only a slight shrinkage of the protoplasts away from the corners of the cells was recognizable. The distinction is somewhat arbitrary, but in practice not difficult to draw. It was, however, impossible to make the distinction in the unicellular forms owing to the small dimensions of the cells.

nature—is that in which the granules exhibit an essentially peripheral disposition (cf. Fritsch, 1916, p. 143); in such cases they are not uncommonly rather small and often so densely arranged as to appear like a fine moniliform thread in optical section and a coarse pitting in surface view. Granular cells have been observed in all the terrestrial algae examined, but are on the whole more striking in the filamentous than in the unicellular forms. Nothing of this kind has been seen in the moss protonema.

Particularly when working with strengths of solutions not far above the minimum, one often observes a striking correlation between the occurrence of plasmolysis and the granular or non-granular character of the cell. With these lower strengths it is in the main the non-granular cells or those with very few granules that are markedly plasmolysed (cf. Table II), and appreciable numbers of granular cells only begin to be strongly plasmolysed when we come to use the higher strengths. This is by no means without exception, but nevertheless expresses the general state of affairs. Thus, even in relatively weak solutions, one often finds a certain number of cells with plentiful granules strongly plasmolysed¹ (cf. Table II). Especially cells with prominent peripheral distribution of fine granules fail to contract until very strong solutions are used, and in *Pleurococcus* granular cells are often the ones to remain unaffected even by a 25 per cent. solution of sea-salt.

TABLE II.

Relation of plasmolysis at lower strengths of sea-salt to character of cells.

(*a* = cells with abundant granules; *b* = non-granular cells.)

Material.	Expt. No.	Cells counted.	Strength of sol. %	Strongly plasmolysed. ¹		Slightly plasmolysed. ¹		Unaffected.	
				%		%		%	
				<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
<i>Zygonium</i>	I	1,798	3.0	4.7	2.1	28.3	6.8	55.3	2.8
<i>Hormidium</i>	II	423	3.0	0.7	—	3.8	33.5	56.9	5.0
„	VI	2,100	5.0	1.1	90.6	5.0	1.4	1.6	0.3
<i>Prasiola</i>	XXVII	382	10.0	—	7.6	23.0	31.1	38.2	—
„	„	1,449	20.0	2.2	72.4	10.6	0.4	14.4	—

It has, however, become clear that the presence of granules in itself is not by any means the sole determinant of the plasmolysing qualities of the cells. This has been established by keeping material of the different forms investigated well moistened for a fortnight or more, one set being exposed to the full light of the greenhouse, the other being screened by a covering of slightly translucent brown paper (Table III). Except in the case of

¹ See foot-note on p. 686.

Pleurococcus, which in this respect proved to be as indifferent as to other treatment described later, the material grown in the dark showed a much larger percentage of plasmolysing cells. This, except in the *Hormidium*, is due to a great increase in the numbers of strongly plasmolysed¹ cells. The *Zygogonium* used was granular throughout, and remained so in both cases; that which had been kept in the dark, however, was found to present a majority of cells with very fine uniform granules, whilst the threads exposed to the light partly consisted of cells with large coarse granules. In the material that had been in the dark there were very occasional threads with relatively scanty granules, but on the whole one was struck by their apparent stability; if they constitute a food-reserve, they do not appear to be easily used during a prolonged period in which photosynthesis is impossible (cf. Piercy, 1917, p. 533). The *Hormidium* employed contained relatively few granular cells, and here too there was no sensible alteration in this respect in either lot of material at the end of the experiment. The percentage of dead cells was considerably greater in the algal mats that had been in the dark. In the case of the protonema there was likewise no obvious difference in the microscopic characters of the two sets of material.

TABLE III.

Comparison of plasmolysis of identical material kept in the light and dark respectively (Expts. VIII and XXXVI).

(l. = light; d. = dark.)

Material.	Cells counted.	Strength of sol. %	Time of exposure (days).	Strongly plasmolysed. %		Slightly plasmolysed. %		Unaffected. %	
				d.	l.	d.	l.	d.	l.
<i>Zygogonium</i>	900, 971	5.0	14.0	69.3	12.5	22.7	43.7	8.0	44.0
<i>Hormidium</i>	1,022, 1,060	3.0	29.0	39.1	42.4	43.8	28.6	17.1	29.0
Protonema	1,000, 1,000	5.0	25.0	75.3	58.7	19.4	20.3	5.4	21.0
				Plasmolysed. %		Unaffected. %			
				d.	l.	d.	l.		
<i>Pleurococcus</i>	1,000, 1,000	25.0	14.0	86.0	82.5	14.0	17.5		

The results just given may be ascribed to two possible causes. The larger number of plasmolysing cells in material kept in the dark may be a result of decreased permeability of the protoplasmic membrane. At the same time it seems probable that the differences are partly caused by the presence of abundant soluble products of photosynthesis in the cell-sap, and that the frequent absence of plasmolysis in strongly granular cells is due to such cells being rich in products of assimilation.

¹ Cf. foot-note on p. 686.

B. DESCRIPTION OF METHODS ADOPTED IN THE SUBSEQUENT INVESTIGATIONS AND DISCUSSION OF POSSIBLE SOURCES OF ERROR.

In view of the facts which have just been detailed it will be evident that little can be gleaned from comparative estimations of the minimum concentrations necessary to effect plasmolysis, since such concentrations are only to be related to a small percentage of the cells. A better comparative method of obtaining some conception of the relation to plasmolysing solutions seemed to be to employ a solution rather above the minimum and to determine by counting under the microscope the percentages of plasmolysed and unaffected cells. In order to obviate changes of concentration during the estimations a ring of marine glue dissolved in xylol was run round the edge of the cover-glass. Usually at least 1,000 cells were counted at each estimation, but occasionally this was impossible owing to lack of time; in some cases the result seemed so even that a smaller number of cells was deemed sufficient. As a general rule from fifteen to forty-five minutes were occupied in an estimation. The estimations were made with the help of a mechanical stage, and usually the material under the cover-glass was counted exactly as it appeared with the movement of the stage, thus obviating any personal bias.

The sources of possible error in this method of estimation are: (1) lack of uniformity of material, (2) personal error in the counting, and (3) effect of the xylol in the sealing medium on the permeability of the material. The error under items (1) and (2) was determined by repeated estimations of *different* mounts of supposedly identical material, and the full results are epitomized in Table IV. In general the amount of error will be found to be small in relation to the percentages upon which any stress has been laid in the subsequent matter. It will be evident that more reliance can be placed on the bigger totals than on the smaller. The only serious discrepancy among them is that shown by the first count for *Hormidium*, but we doubt very much whether an error of this magnitude has often entered into our estimations. In making the latter it was noticed again and again how the totals for the different categories maintained the same relative proportions during successive periods of the count (cf. also the analogous results often obtained with the same alga from the same habitat at different times; see Table VII). A very striking uniformity is shown by the data for *Pleurococcus*, which is no doubt due to the possibility of getting a better assortment of such a unicellular form than can be done in the case of the filamentous types.

The possible error introduced by the presence of xylol in the sealing medium was investigated by comparing the counts for material sealed with melted marine glue and with marine glue dissolved in xylol respectively.

The results are given in Table IV A, and they show quite clearly that the presence of xylol brings about no difference that is outside the limits of the ordinary experimental error above considered.

TABLE IV.

Calculation of possible error.

Material.	Cells counted.	Sol. %	Strongly plasmol. ¹	Deviat. from mean.	Error.	Slightly plasmol. ¹	Deviat. from mean.	Error.	Unaffected.	Deviat. from mean.	Error.
			%	%	%	%	%	%	%	%	%
Zygonium	1,035	5.0	76.9	-2.6	3.4	14.4	+4.9	34.0	8.7	-2.3	26.4
"	1,000	"	83.6	+4.1	4.9	6.4	-3.1	48.5	10.0	-1.0	10.0
"	1,046	"	86.3	+6.8	7.9	4.0	-5.5	137.5	9.7	-1.3	13.4
"	1,051	"	71.2	-8.3	11.6	13.2	+3.7	28.0	15.7	+4.7	30.0
Average			79.5			9.5			11.0		
Zygonium	1,093	3.0	68.6	+2.8	4.1	19.4	-0.5	2.6	12.0	-2.3	19.2
"	1,262	"	64.1	-1.7	2.7	19.6	-0.3	1.5	16.4	+2.1	12.8
"	1,023	"	64.7	-1.1	1.7	20.7	+0.8	3.9	14.6	+0.3	2.1
Average			65.8			19.9			14.3		
Hormidium	1,023	3.0	45.8	-13.0	28.4	32.7	+3.2	9.8	21.6	+9.9	45.9
"	1,008	"	65.8	+7.0	10.6	26.6	-2.9	10.9	7.6	-4.1	54.0
"	1,012	"	67.8	+9.0	13.3	23.7	-5.8	24.5	8.5	-3.2	37.6
"	1,004	"	55.7	-3.1	5.6	35.2	+5.7	16.2	9.1	-2.6	28.6
Average			58.8			29.5			11.7		
Protonema	1,010	5.0	76.1	-3.1	4.1	14.3	+1.0	7.0	9.7	+2.1	21.6
"	1,000	"	83.0	+3.8	4.6	10.7	-2.6	24.3	6.3	-1.3	20.6
"	1,000	"	75.2	-4.0	5.3	17.2	+3.9	22.7	7.6	—	—
"	1,000	"	82.4	+3.2	3.9	11.0	-2.3	20.9	6.6	-1.0	15.2
Average			79.2			13.3			7.6		

			Plasmol.	Deviat.	Error.	Unaffected.	Deviat.	Error.
			%	%	%	%	%	%
Pleurococcus	1,000	25.0	87.5	+2.1	2.4	12.5	-2.1	16.8
"	"	"	83.9	-1.5	1.8	16.1	+1.5	9.3
"	"	"	86.2	+0.8	1.0	13.8	-0.8	5.8
"	"	"	84.1	-1.3	1.5	15.9	+1.3	8.2
Average			85.4			14.6		

TABLE IV A.

Investigation of error due to xylol in the sealing medium.

(5 % sea-salt solution.)

Material.	Method of sealing.	Cells counted.	Strongly plasmolysed.	Slightly plasmolysed.	Unaffected.
			%	%	%
Zygonium	(in xylol)	1,056	94.7	2.0	3.3
"	(melted)	1,208	92.7	3.3	4.0
Protonema	(in xylol)	949	83.2	12.4	4.4
"	(melted)	1,000	84.7	7.6	7.7

¹ Cf. foot-note on p. 686.

C. THE BEHAVIOUR WHEN EXPOSED TO DROUGHT.

An appreciable alteration in the behaviour towards plasmolysing solutions, in most of the forms investigated, is to be observed during a spell of drought. Using the same strength of sea-salt solution, the number of plasmolysed cells and the extent of plasmolysis in the affected cells gradually diminish day by day till ultimately very little plasmolysis is to be observed. In the experiments in question, a large number of which were performed, the initial material was usually obtained during damp weather and a strength of salt solution selected sufficiently concentrated to bring about marked plasmolysis in a large majority of the cells. The material was then allowed to dry exposed to the air of the laboratory in the earlier experiments, but in the later ones was dried in a desiccator over suitable strengths of sulphuric acid. Except in a few cases, in which a constant temperature was maintained, the experiments were exposed to ordinary room temperature. Relatively small quantities of material were employed, all collected under the same conditions, on the same day, and from the same habitat—usually not more than could be placed in an ordinary watch-glass. The material was left on the patch of soil as collected. The experiments were almost always started on the same day or on the day after collection. As far as possible daily estimations were made of the drying material until it appeared that no further appreciable change was taking place.

The results of a number of these experiments are epitomized in Table V. They are essentially uniform as regards the three filamentous algae, and fall far outside the limits of our experimental error. Only in the case of Experiment VI with *Hormidium flaccidum* was there any appreciable percentage of strongly plasmolysed cells at the end of the period of drought, and even in this case the very considerable reduction as compared with the original material is quite obvious.¹ It will be noticed that, whilst in all these cases there is a great increase in the percentage of unaffected cells after drought, there is no such uniformity as regards the slightly plasmolysed ones, which sometimes show a decided decrease and sometimes an increase. We are inclined to think that this depends partly on the condition of the original material, and on the duration and intensity of the drought. Attention must also be drawn to the fact that, after prolonged drought, a certain number of the cells of all the filamentous forms are found to show a permanent slight contraction of the protoplasts, visible even when material is mounted in ordinary tap-water (cf. below, p. 716). This fact was not realized until a considerable number of the estimations had been carried out,

¹ The presence of strongly plasmolysing cells in this case is probably due to the high humidity of the air during this experiment (cf. p. 696).

FIG. 1. Influence of drought on plasmolysis of *Zygonium ericetorum* (Expt. XII). *a* = granular, *b* = non-granular cells.

FIG. 2. Effect of drought on *Zygonium* (Expt. XVI).

TABLE V.

Percentages of plasmolysed and unaffected cells in material of various terrestrial forms before and after a period of drought.

(Under each experiment the first line gives the original condition, the second the final condition.)									
Material.	Expt. No.	Cells counted.	Strength of sol. %	Strongly plasmolysed. %	Slightly plasmolysed. %	Unaffected. %	Duration of drought.	Nature of drought.	
<i>Zygonium</i>	I-III	1,798	3	6.8	35.1	58.1	28 days	air	
"	IV-V	990	3	—	2.6	97.4	14 "	air	
"	"	2,008	5	12.5	38.0	49.3	4 "	pure H ₂ SO ₄	
"	IX	1,839	5	0.1	7.2	92.7	3 "	"	
"	"	1,987	5	69.9	12.2	17.9	5 "	34.5 % H ₂ SO ₄	
"	XII	636	5	0.3	50.5	49.2	7 "	air	
"	"	1,886	5	71.8	21.7	6.5	2 "	45 % H ₂ SO ₄	
"	XVI	926	5	—	5.1	94.9	7 "	pure H ₂ SO ₄	
"	"	1,226	5	71.8	16.7	11.5	4 "	"	
"	VI	841	5	0.5	15.1	84.4	11 "	34.5 % H ₂ SO ₄	
<i>Hornidium</i>	"	2,100	5	91.7	6.4	1.9	7 "	air	
"	"	1,663	5	6.6	53.1	40.3	2 "	45 % H ₂ SO ₄	
"	XIV	1,870	5	41.0	48.4	10.6	7 "	34.5 % H ₂ SO ₄	
"	"	1,400	5	—	12.9	87.1	2 "	pure H ₂ SO ₄	
"	XXVI	1,503	5	56.0	39.0	3.0	5 ½ "	34.5 % H ₂ SO ₄	
<i>Prasiola</i>	XXVII	626	5	—	40.2	53.8	4 "	"	
"	"	1,449	20	74.6	11.0	14.4	11 "	pure H ₂ SO ₄	
<i>Protonema</i>	XV	905	20	—	28.3	71.7	45 "	"	
"	"	1,215	5	58.0	27.9	14.1	9 weeks	"	
"	XXIX	655	5	2.9	83.8	13.3	4 days	34.5 % H ₂ SO ₄	
"	"	2,006	5	82.8	12.3	4.5	"	"	
"	"	519	5	5.2	63.9	30.9	"	"	
<i>Plourococcus</i>	XVII	1,852	25	77.9	22.1	22.1	"	pure H ₂ SO ₄	
"	"	904	25	68.2	31.8	31.8	"	"	
"	XXVIII	1,000	20	67.0	33.0	33.0	"	"	
"	"	641	20	61.8	38.2	38.2	"	"	
"	XXX	2,034	25	88.5	11.5	11.5	"	"	
"	"	994	25	80.5 ¹	19.5	19.5	"	"	
<i>Cystococcus</i>	XXII	818	25	87.2	12.8	12.8	"	"	
"	"	613	25	81.6	18.4	18.4	"	34.5 % H ₂ SO ₄	

¹ Cf. foot-note on p. 699.

and under the heading of 'slightly plasmolysed' cells in the final drought condition there are therefore comprised a more or less considerable percentage of cells which are in this permanently contracted state. Probably, therefore, a considerably larger percentage of cells than is shown in the alternate lines of Table V is to be regarded as unaffected by the plasmolysing solution. In the case of the protonema, which otherwise falls into line with the filamentous algae, the great apparent increase in slightly plasmolysed cells seems due to a relatively larger number of the cells being in this permanently contracted state than in the algae investigated.

TABLE VI.

Successive estimations of material of Zygonium (habitat II) exposed to drought over 34.5 per cent. sulphuric acid.

(Experiment XVI, 5 % sea-salt.)

<i>Date.</i>	<i>Cells counted.</i>	<i>Strongly plasmolysed.</i>	<i>Slightly plasmolysed.</i>	<i>Unaffected.</i>	<i>Dead¹ cells.</i>
		%	%	%	%
April 1 ²	1,226	71.8	16.7	11.5	16.3
" 2	913	49.5	41.0	9.5	15.0
" 3	860	30.8	45.6	23.6	18.3
" 4	900	13.8	32.3	53.9	24.7
" 5	823	6.6	25.6	67.8	34.7
" 6	841	0.5	15.1	84.4	20.7
Material at this stage of drought transferred to damp air (cf. p. 708).					
" 7	571	0.2	14.4	85.4	28.0
" 8	398	0.3	16.1	83.6	25.6
" 19	1,042	1.1	51.2	47.7	35.3
" 28	518	0.2	48.2	51.5	31.9
May 3	495	0.8	59.6	39.6	27.9
" 10	520	1.7	70.9	27.3	29.9
" 10 (10 % sol.)	264	37.5	53.8	8.7	—

In most of the drought experiments repeated estimations were made during the period of desiccation—daily in the case of the short-period experiments. The progressive effects of drought could thus be studied, but the results are too numerous to be reproduced in full here. In illustration we give one table (VI) and a number of figures (1-5). When the estimations are made at daily intervals, and provided a large percentage of the cells is strongly plasmolysed in the original material, one observes, in the case of the filamentous algae, for one or two days a more or less considerable increase in the number of slightly plasmolysed cells (cf.

¹ Where given, the dead cells are stated as a percentage calculated on the total number of living cells, instead of on the total number of cells, so that the percentages of plasmolysed cells recorded should represent a percentage of the living cells, i. e. of those still having the capacity to plasmolyse.

² The first line gives the condition of the material on the day of collection.

FIG. 3. Effect of drought on *Hormidium flaccidum* (Expt. XXVI).

FIG. 4. Effect of drought on *Hormidium*-stage of *Prasiola* (Expt. XXVII).
a = granular, b = non-granular cells.

especially Figs. 2 and 3), whilst the percentage of unaffected cells for the first day or two increases but little. Subsequently the number of slightly plasmolysed cells again decreases, and that of the unaffected cells rises rapidly (cf. Figs. 1–3). In the case of moss protonema the same relation holds good (Fig. 5), but here the appearance of permanent contraction in many of the cells (cf. p. 694) ultimately leads again to an apparent increase in the numbers of slightly plasmolysed and a decrease in the number of unaffected cells. In these forms, therefore, there is evidently a gradual loss of the tendency to plasmolyse, many of the formerly

FIG. 5. Influence of drought on plasmolysis of moss protonema (expt. XV).

strongly plasmolysed cells, during the first days of a period of drought, still exhibiting slight plasmolysis. In the final stages, however, the majority show no plasmolysis.

In the case of the filamentous forms the behaviour during drought nearly always follows the sequence just described and irregularities were rarely noticed. In some of the earlier experiments, where the material was merely dried by exposure to the air of the laboratory, certain variations were, however, noticed which appear to be related to changes in the humidity of the air. Thus, after a week's drying in the laboratory, the *Hormidium* in Experiment VI gave on Feb. 20: 6.6 per cent. strongly plasmolysed, 53.1 per cent. slightly plasmolysed, and 40.3 per cent. unaffected cells; on Feb. 27, however, the figures were: 19.4 per cent. strongly plasmolysed,

75.4 per cent. slightly plasmolysed, and 5.2 per cent. unaffected cells. The air showed a very high relative humidity between the 20th and 27th, and this probably accounts for the result. Somewhat similar figures were obtained in an experiment with *Zygogonium* under the same conditions at the same time, and it was after these experiences that desiccation over sulphuric acid was substituted for mere drying in air.

TABLE VII.

Comparison of plasmolysis in material of terrestrial algae obtained from the same habitat on different occasions.

Material.	Habitat.	Date.	Cells counted.	Strength of sol. %	Strongly plasmolysed. %	Slightly plasmolysed. %	Unaffected. %
<i>Zygogonium</i>	II	Feb. 1	2,008	5	12.5	38.0	49.2
"	"	" 26	1,987	5	69.9	12.2	17.9
"	"	Mar. 20	1,886	5	71.8	21.7	6.5
"	"	Apr. 1	1,226	5	71.8	16.8	11.4
"	III	Feb. 4	1,544	5	66.8	16.7	16.5
"	"	" 11	4,132	5	79.5	9.5	11.0
<i>Hormidium</i>	II	Feb. 13	2,100	5	91.7	6.4	1.9
"	"	Mar. 1	2,053	5	62.1	33.1	4.8
"	III	Mar. 27	1,870	5	40.9	48.5	10.6
"	"	May 1	1,503	5	56.0	39.0	5.0

It is evident that an analogous alteration in behaviour towards plasmolysing solutions is to be observed also in a state of nature. In Table VII are given some data that bear on this point. The most striking feature about the four estimations of *Zygogonium* from habitat II is the astonishing uniformity of the last three. The first one, on Feb. 1, was made just after a dry spell, whilst the remaining three all fell within a period of intermittent wet weather. The two estimations of this alga from habitat III were likewise made during a spell of wet weather. In the case of the *Hormidium* from habitat II the first estimation was made after wet weather, the second after a few days' lull. It is hoped in a later communication to produce more data with reference to the changes in terrestrial algae under varying conditions of humidity in nature, the data just offered not having been definitely collected with this object in view.

In all of the experiments described above sea-salt was used as the plasmolysing medium, but in one experiment with *Hormidium* (XXIX) 40 per cent. sucrose was employed. In the fresh material (1,000 cells counted) there were 88.7 per cent. strongly, and 11.3 per cent. slightly, plasmolysed cells, none being unaffected. After two days in a desiccator over 34.5 per cent. sulphuric acid the result of the count (378 cells) showed 4.6 per cent. slightly plasmolysed and 95.4 per cent. unaffected cells; none were strongly plasmolysed and about three-quarters of the cells were dead. This may be compared with the result of Experiment XIV.

The question still arises as to how far changes might take place in material kept damp and merely exposed to the conditions extant in the laboratory. There is no doubt that under these circumstances the filamentous forms in some cases tend to exhibit a reduction of the number of strongly plasmolysed cells, perhaps as a result of favourable conditions for photosynthesis. Thus, in Experiment XII, an estimation of some of the original material which had been kept damp afforded on the day of the final drought determination 18.2 per cent. strongly plasmolysed, 70.0 per cent. slightly plasmolysed, and 11.8 per cent. unaffected cells; but this is quite a different result from that obtained with the drought material (cf. Table V). In other experiments, however, no such alteration of the original material was found; in Experiment I, for example, an estimation, after 28 days, of part of the original material that had been kept damp gave 11.3 per cent. strongly plasmolysed, 46.7 per cent. slightly plasmolysed, and 42.0 per cent. unaffected cells, a result which does not differ appreciably from that given by the material at the outset of the experiment (cf. Table V).

TABLE VIII.

Successive estimations of material of Pleurococcus (habitat II) exposed to drought over pure sulphuric acid.

(Experiment XXVIII, 20 % sea-salt.)				
<i>Date.</i>	<i>Cells counted.</i>	<i>Plasmolysed.</i>	<i>Unaffected.</i>	<i>Dead cells.¹</i>
		%	%	%
May 15	1,000	67.0	33.0	25.4
„ 16	1,000	83.7	16.3	11.6
„ 17	1,151	69.5	30.5	9.6
„ 18	922	76.0	24.0	18.0
„ 19	1,015	88.2	11.8	8.0
„ 22	1,030	86.2	13.7	9.1
„ 23	1,095	91.3	8.7	8.2
„ 30	582	85.9	14.1	9.5
June 12	840	45.5	54.5	33.6
„ 29	641	61.8	38.2	71.8

The results so far obtained with *Pleurococcus* and *Cystococcus* appear quite anomalous and difficult to understand (cf. Tables V and VIII and Fig. 6). It must remain doubtful whether in these forms there is any alteration in the behaviour towards plasmolysing solutions comparable with that above described for the filamentous forms, since no really decisive results have been obtained. If analogous changes do occur, they certainly only ensue after very prolonged and intensive drought over pure sulphuric acid. It would seem that in some cases death of the cells intervenes before any decisive response to the conditions of drought has taken place (cf.

¹ Cf. foot-note on p. 694.

Table VIII). A marked feature, moreover, of our consecutive estimations of *Pleurococcus* subjected to drought has been the peculiar variability of the figures at successive periods, of which both Table VIII and Fig. 6 afford testimony. Yet Table IV demonstrates that the error in the case of *Pleurococcus* is remarkably small, and one can only conclude that other factors come into play which have at present not been recognized. In Experiment

FIG. 6. Effect of drought on *Pleurococcus Naegeli* (Expt. XXX).
 Material soaked with water after nearly four weeks.

XXX, the results of which are reproduced in Fig. 6, the material was kept throughout in the dark and at a constant temperature of 18°C., but nevertheless the figures are as erratic as in the other cases.¹ It remains to mention that the cells of *Pleurococcus* show none of that permanent contraction of the protoplast during the later stages of drought, such as has been recorded above for the filamentous forms; whenever the material was examined in tap-water the living cells looked quite normal. Although few experiments have been performed with *Cystococcus* there is every reason to believe that its behaviour is similar to that of *Pleurococcus*.

¹ An average of 2,000 cells was counted at each estimation (except for the last two) in this experiment. A further estimation made after sixteen weeks' exposure to pure sulphuric acid produced no different result, 75.8 per cent. of the cells still plasmolysing.

D. THE EFFECT OF PROLONGED ACTION OF THE PLASMOLYSING SOLUTION.

In a considerable number of cases material, with the edge of the cover-glass sealed as described above (p. 689), was estimated at daily intervals; between the successive determinations the slide was usually exposed to room temperature and to a moderate illumination. Some of the results are epitomized in Table IX (cf. also Fig. 7). It will be seen that in the filamentous forms there is invariably a recovery from plasmolysis, analogous to but not always as marked as the final response on the part of drought material to plasmolysing solutions. In both cases there is a very appreciable reduction in the number of strongly plasmolysed cells, and the usual increase in the number of slightly plasmolysed cells in the final stage is here, too, no doubt due to the fact that a larger or smaller number of the cells have their protoplasts in a permanently contracted condition (cf. pp. 691, 716); that this was the case could naturally only be established at the end of each experiment. The moss protonema again stands out more strikingly in this respect than do the filamentous algae. Attention may be drawn to the fact that a larger percentage of dead cells was invariably found at the end of the experiment in the filamentous forms. Whilst we do not attach much importance to the estimations of dead cells, since the liability to error is much greater here, the results are so uniform that they can scarcely be due to chance.

With the unicellular forms the results are almost as difficult to understand as in the case of the drought experiments considered in the preceding section. Thus, whilst in Experiments VII and XXVIII with *Pleurococcus* an obvious recovery was noted, Experiment XVII and others not included in Table IX showed no alteration in the material that could be regarded as beyond the limits of experimental error, a similar conclusion being reached in the case of *Cystococcus*. In Experiment XXVIII there was no appreciable recovery during the first four days, but after that a rapid change took place, so that on the eighth day practically no plasmolysed cells were to be found. It thus appears that *Pleurococcus* is usually capable of remaining unaffected by the unfavourable conditions in a sealed slide for a much longer time than the filamentous forms, in which the recovery is very rapid (cf. below), and the duration of its resistance may well depend on the condition of the original material. This would explain the discrepancy between the different experiments. In this connexion attention may be drawn to the fact that in Experiment XVII, in which no recovery was noted, there is no difference between the percentages of dead cells at the beginning and end of the experiment, implying considerable power of resistance on the part of the material. In Experiment XXVIII, on the other hand, there were far more dead cells at the end of the experiment than at the outset.

TABLE IX.

Recovery from plasmolysis, during prolonged exposure to the plasmolysing solution, on the part of various terrestrial forms.

(In the case of each experiment the first line gives the original, the second the final condition.)

Material.	Expt. No. ¹	Cells counted.	Strength of sol.	Strongly plasmolysed.	Slightly plasmolysed.	Unaffected.	Dead. ²	Duration of experiment (days).
			%	%	%	%	%	
<i>Zygogonium</i>	IX, X	1,987	5	69.9	12.2	17.9	2.1	1
"	"	864	5	23.6	39.6	36.8	2.4	
"	XVI	1,226	5	71.8	16.7	11.5	16.3	5
"	"	756	5	2.8	25.6	71.6	32.7	
"	XX	884	6 (cold)	55.3	19.7	25.0	14.1	2
"	"	887	6 (cold)	7.9	33.1	59.0	18.1	
"	XX	882	6 (warm)	45.6	28.4	26.0	17.0	2
"	"	759	6 (warm)	0.1	12.0	87.9	25.5	
<i>Hormidium</i>	XI	2,053	5	62.1	33.0	4.9	2.5	2
"	"	503	5	—	86.3	13.7	4.2	
"	XXVI	1,503	5	56.0	40.0	4.0	9.9	4
"	"	524	5	—	43.7	56.3	14.7	
<i>Prasiola</i>	XXVII	1,449	20	74.6	11.0	14.4	1.3	4
"	"	455	20	—	1.3	98.7	2.4	
<i>Protonema</i>	XV	1,215	5	58.0	27.9	14.1	—	6
"	"	406	5	22.2	74.4	3.4	—	
"	XXIII	887	5	53.7	30.9	15.4	—	4
"	"	618	5	25.1	64.7	10.2	—	
<i>Pleurococcus</i>	VII	1,301	25	57.1		42.9	—	3
"	"	632	25	32.1		67.9	—	
"	XVII	1,852	25	77.9		22.1	8.8	7
"	"	1,028	25	77.7		22.3	8.0	
"	XXVIII	1,000	20	67.0		33.0	25.4	8
"	"	1,008	20	0.8		99.2	43.9	
<i>Cystococcus</i>	XXII	818	25	87.2		12.8	—	4
"	"	613	25	81.6		18.4	—	

The recovery in the filamentous forms is as a general rule very rapid. Thus, in Experiment XVI on *Zygogonium* the estimation of the original material (cf. Table IX) was made at 4.30 p.m.; at 9.30 p.m. on the same day the figures obtained (866 cells counted) were: 19.2 per cent. strongly plasmolysed, 61.6 per cent. slightly plasmolysed, and 19.2 per cent. unaffected. During the subsequent days (cf. Fig. 7) there was quite a gradual diminution of the strongly plasmolysed cells, whilst the number of slightly plasmolysed ones remained practically constant till three days later, when a sudden rapid decrease was noted. Perfectly similar results were obtained in all the estimations of the filamentous algae, but in the case of the protonema the change is always more gradual and a relatively large number of the strongly plasmolysed cells fail to recover (cf. Table IX and Fig. 7).

¹ In many cases a drought series and a recovery from plasmolysis estimation were carried out in the same experiment, the determination of the original material serving both as the starting-point of the drought estimations and as the first estimation of the material whose recovery from plasmolysis was to be studied.

² Cf. foot-note on p. 694.

It may be that the sudden drop in the number of slightly plasmolysed cells, which is to be observed in the filamentous forms after the experiment has been going on for some days, marks the death-point of a large number of the cells.

The rapidity of recovery would seem to depend to some extent on the temperature. In Experiment XX with *Zygogonium* the second estimation carried out on a sealed slide kept in a warm room shows a much higher total of unaffected cells in the final condition than in any of the other similar experiments made with this alga. The experiment with *Prasiola*

FIG. 7. Recovery from plasmolysis in a sealed slide. The heavy lines show the results for *Zygogonium* (Expt. XVI), the thin lines those for moss protonema (Expt. XV).

was also conducted in May, whilst the others were performed in March or April.

It has already been indicated that in *Pleurococcus*, if recovery occurs at all, it usually sets in only after some days. In Experiment VII, however, some results were obtained which seem to indicate that, when the material is in a suitable condition, some at least of the cells may recover very rapidly.

These results are set out in Table X and were all obtained on the same day with the same mass of material. They are so uniform, without any exception, that they can scarcely be due to experimental error, yet on other occasions (e. g. in Experiment XVII) nothing of the kind was to be observed. It is, however, to be noted that in the latter case there was no recovery even after seven days (cf. Table IX), whereas in Experiment VII recovery was evidently continued.

TABLE X.

Estimation at half-hour intervals of percentages of plasmolysed cells of Pleurococcus (Experiment VII).

Strength of sol. %	Cells counted.	Percentage plasmolysed.	
		1st half-hour.	2nd half-hour.
15.0	500, 502	20.8	19.1
17.5	502, 505	24.7	20.4
20.0	502, 502	30.5	23.3
22.5	502, 501	37.3	29.9
25.0	501, 401	48.7	44.4

In another case (Experiment XXIV) estimations of 1,000 cells were made at two-hourly intervals on a sealed slide with *Pleurococcus* kept in a warm room. There were some indications here of a daily cycle, the percentage of plasmolysed cells sinking towards midday and rising again in the evening and, during the two days of the experiment, showing a slight downward tendency. The results are not at all conclusive, but owing to the large amount of time consumed in such experiments it has not been possible to repeat them up to the present.

Recovery from plasmolysis was also established in a very large number of cases by direct microscopic observation of small groups of initially plasmolysed cells over a period of several days. In the case of *Zygogonium* such recovery was repeatedly noted in 3, 5, and 10 per cent. solutions, being complete in about three days; the recovery of granular cells was almost invariable, that of non-granular cells not so frequent, especially in the stronger solutions. *Hormidium* showed frequent complete recovery both in 5 and 10 per cent. solutions. Moss protonema usually fails to recover, but in one set of cells complete recovery was observed in a 5 per cent. solution, never in a 10 per cent. solution; in most cases the extent of plasmolysis was found to increase at first and then to decrease again, although, as just mentioned, this only led to complete recovery in one thread. Analogous observations were also carried out on the cells of the leaves of a moss (*Hypnum cupressiforme*, L., var. *filiforme*, Brid.) and gave results quite identical with those just recorded for the protomena, recovery being only noted in one case in a 5 per cent. solution. In a few cases cells of a narrow species of *Spirogyra* showed recovery both in 5 and 10 per cent. solutions; more usually, however, they gave a negative result (cf. also Table XI); cells of *Cladophora* and *Chaetophora* in a 3 per cent. solution showed no decrease in shrinkage of the protoplasts during a period of ten days. The epidermal cells of *Saxifraga sarmentosa* lastly, though occasionally recovering in 3 per cent. solutions, failed to show any recovery either in 5 or 10 per cent.

solutions; here again, as in the protonema, a slight increase in plasmolysis was often observed at first, to be followed by a subsequent slight decrease. These results bring out very clearly the great capacity for recovery on the part of the two terrestrial algae studied, by contrast with the moss (protonema and leaf), aquatic algae, and the flowering plant (see also Table XI).

TABLE XI.

Behaviour of the protoplasts of various forms when exposed to prolonged action of the plasmolysing solution in a sealed slide.

(The figures give the areas of the protoplasts in percentages of the total areas of the cells.)

Material.	Strength of sol.	Time in days.					
		1	2	3	4	5	7
<i>Zygogonium</i> (granular)	%						
	5	82.2	88.3	100.0	—	—	—
	5	90.1	100.0	—	—	—	—
	10	82.2	82.4	87.5	95.8	—	—
<i>Zygogonium</i> (non-granular)	10	72.0	93.6	100.0	100.0	—	—
	5 % NaCl	85.1	85.3	98.6	99.6	—	—
	5	57.0	78.5	94.5	—	—	97.3
	10	52.2	70.1	50.8	60.8	—	—
<i>Hormidium</i>	10	45.9	60.2	96.8	100.0	—	—
	5	85.2	87.9	99.2	—	—	100.0
	5	81.7	—	81.9	90.9	94.1	—
	10	82.2	100.0	100.0	100.0	—	—
Protonema	10	76.0	85.5	80.1	81.8	—	—
	5 % NaCl	81.4	92.8	94.3	87.7	—	—
	5	75.8	—	100.0	80.2	85.3	—
	5	71.7	—	65.2	66.3	—	—
<i>Spirogyra</i> (narrow sp.)	5 % NaCl	83.0	80.4	82.9	85.2	—	—
	5	88.7	—	90.5	92.1	93.7	—
	5	47.9	—	100.0	100.0	100.0	—
	10	66.1	100.0	100.0	100.0	—	—
<i>Spirogyra</i> (broad sp.)	10	91.3	72.3	43.5	43.5	—	—
	10	96.6	92.7	92.3	92.8	—	—
	5 % NaCl	67.5	56.6	55.5	64.7	—	—

The data given in the preceding paragraph were in part obtained merely by daily observation, but in a considerable number of cases more accurate means were employed in order to follow the stages in recovery. The method most usually employed, and that by which the results given in Table XI were obtained, consisted in making camera lucida drawings of the outlines of the protoplasts of selected cells on successive days and estimating the degree of contraction by finding the areas of the protoplasts as represented in the drawings with the help of a planimeter. In Table XI these results are expressed as percentages of the total area of the interior of the cell as seen on the same drawings. Whereas the results epitomized in Table IX and Fig. 7 indicate the aggregate behaviour of the cells of a given mass of material, those in Table XI indicate the behaviour of individual cells. The most striking feature in the latter case is again the usually very rapid

recovery, if recovery is to occur at all. Another fact of some interest is that the extent of the initial plasmolysis bears no relation to the occurrence or non-occurrence of a subsequent recovery, nor to the rapidity with which the latter sets in. There are indications that recovery does not so easily take place in a 5 per cent. sodium chloride as in the balanced solution. Attention should finally be drawn to the fact that the conclusions detailed in the preceding paragraph were drawn from a much larger number of observations than are shown in Table XI, which would have become too unwieldy if all had been included.

Some rather peculiar results were obtained in analogous experiments in which sugars were used. If threads of *Zygonium* are placed in a 20 per cent. sucrose solution, no plasmolysis at all is to be observed at first, although such a solution has an osmotic pressure exceeding that of a 3 per cent. sea-salt solution which plasmolyses many of the cells of the alga. A day later, however, slight plasmolysis is to be seen in many of the cells and remains apparent for some days, though ultimately mostly disappearing. The same results were obtained with 10 per cent. glucose, whilst 30 per cent. glucose causes immediate violent plasmolysis, from which the cells in a sealed slide recover in about two days. This matter has so far not been further followed up.

In most of the cases described above, in which recovery was established by direct microscopic observation, a more or less marked central aggregation of the chloroplast or chloroplasts, as well as of the granules when present, was noted, the whole periphery of the newly expanded protoplast appearing clear, opaque, and whitish. Frequently, too, a change in the character of the granules was established, those in the final condition being large and irregular (cf. p. 716 on condition of the cells during drought). On the whole this condition was more evidently seen when recovery was rapid than when it was slow.

It is probable that recovery from plasmolysis in a sealed slide is partly to be regarded as a pathological phenomenon, implying a state of decreased vitality on the part of the material, chiefly perhaps as a result of interference with respiration. There are two possible methods by which such a pathological condition could lead to the observed recovery. Firstly, it might be expected to bring about increased permeability of the cells, thus allowing penetration of the surrounding solution. Secondly, the protoplast might swell by simple imbibition, this process being augmented by degradation-products such as acids produced as a result of the unhealthy condition of the cell. Emphasis must, however, be laid upon the fact that, if such factors come into play, their action is different in the case of terrestrial and aquatic algae and also different in the moss and the flowering plant (cf. p. 703). Moreover, the very marked similarity in the response to drought and in the reaction to a continued exposure to the plasmolysing solution,

upon which stress has already been laid, appears very indicative of a capacity on the part of these terrestrial forms to accommodate their cells to extreme conditions of their environment.

The possibility of the xylol present in the sealing medium being responsible for the results recorded in this section was disproved by placing two mats of *Zygogonium* respectively in 5 per cent. sea-salt solution and a similar solution to which a small amount of 5 per cent. sea-salt, previously shaken up with xylol, had been added. The dishes containing the solutions were covered over with glass plates and estimations of the two sets of material were made two days later in the usual way. The results were :

<i>Solution.</i>	<i>Cells counted.</i>	<i>Strongly plasmolysed.</i>	<i>Slightly plasmolysed.</i>	<i>Unaffected.</i>
		%	%	%
5 % sea-salt	1,021	22.9	23.4	53.7
Ditto + xylol	966	31.5	37.4	31.0

It is seen that the effect of the xylol is in the opposite direction to that noted in the above experiments, and its presence cannot therefore be responsible for the observed results.

The frequent recovery of the cells of the terrestrial forms studied from plasmolysis in hypertonic solutions led us to attempt their cultivation in such solutions. With this end in view, small patches of soil bearing mats of *Zygogonium*, *Hormidium*, and protonema were placed in wide crystalizing dishes in solutions of sea-salt of various strengths (usually 5 per cent.), partly out of doors and partly in a warm greenhouse; the dishes were covered with glass plates and exposed to varied illumination. Except in one experiment with *Zygogonium* in a 5 per cent. solution, however, no further growth of any of the forms was noticed. In the one case small tufts of threads arose from the edge of the mat and trailed out into the surrounding fluid. Such growth was, however, limited to a fortnight, and some weeks later a large number of the constituent cells proved to be dead, whilst others (nearly always granular cells) were perfectly healthy and green, though mostly plasmolysed.

The general result of the remaining experiments of this kind may be briefly summarized as follows. A large number of the cells of the three forms examined remain in a healthy condition for periods of six weeks to three months and upwards, the duration of resistance probably depending *inter alia* on the condition of the original material. Those of the cells that suffer from the conditions of the experiment die in a relatively short space of time, probably usually within the first week, and show brown, often uncontracted contents. The surviving cells are very commonly the granular ones, and in the case of protonema, where the mortality is much greater, especially those situated at the tips of the branches. A variable number of the surviving cells are found to be plasmolysed, and this is often the condi-

tion of the majority; as a general rule the plasmolysis is not very extreme and its extent appears to undergo no appreciable alteration after the experiment has been in progress for some weeks. That these plasmolysed cells are otherwise perfectly healthy is shown by their capacity for immediate and complete recovery, even after weeks in the salt solution, when placed in tap-water. It should be especially emphasized that cells with uncontracted though quite healthy protoplasts were always to be found in the threads, even after many weeks' sojourn in the salt solution, and this again implies on the part of a larger or smaller number of the cells a marked capacity for resistance to the high concentration of the surrounding fluid. Such cells may obviously be the starting-points for growth, and that this has only been observed in one instance may well be due to other conditions of the experiment being unsuitable. We hope to return to this problem at a later stage.

The observations just detailed indicate that material permanently immersed in hypertonic solutions shows a failure on the part of many of the initially plasmolysed cells to recover. This conclusion is obviously at variance with the results obtained in the case of algal threads kept in a sealed slide which were considered in the earlier part of this section. It is for this reason that we regard these results as probably in part due to pathological causes. There is no doubt, however, that even in open solutions a gradual recovery takes place during the first days (cf. Table XII), but the recovery is probably again in great part pathological and followed by the death of the cells in question. Thus, whilst in the first days there is

TABLE XII.

Successive estimations of material of Zygonium immersed in 5 per cent. sea-salt solution (Experiment XXXV).

Date.	Cells counted.	Strongly plasmolysed.	Slightly plasmolysed.	Unaffected.
		%	%	%
Feb. 5	1,544	66.8	16.7	16.5
„ 6	1,021	22.9	23.4	53.7
„ 7	1,000	27.2	10.7	62.1
„ 9	1,030	47.3	21.8	30.9

a very obvious decrease in the numbers of plasmolysed cells, the percentages of such cells tend subsequently to increase again in the direction of a maximum. This increase is, however, a deception and due to the fact that there is a great mortality among the unaffected cells, the plasmolysed ones which constitute the main mass of the healthy cells thus, although actually remaining constant in number, appearing to undergo a great relative increase. Whilst the majority of the healthy surviving cells are often plasmolysed, there are, as already mentioned above, always a number of healthy unaffected cells. It is probable that these are cells which were

unaffected from the very first. In Experiment XXXV all the surviving cells had small granules, whilst the moribund unaffected cells were distinguished by the possession of coarse irregular granules.

In concluding this section attention may be drawn to the most important conclusion reached, viz. that the cells surviving prolonged treatment with hypertonic solutions are either those which are impermeable to the solution as implied by the persistence of plasmolysis or more rarely such as are possessed of a mechanism inhibiting the occurrence of plasmolysis as implied by their remaining unaffected. The close correspondence between the reaction to drought and the reaction to hypertonic solutions would seem to make it probable that the surviving cells are of the same kind in both cases, a matter which is further considered in the next section (cf. p. 717).

E. RECOVERY FROM THE DROUGHT CONDITION AND THE STATE OF THE MATERIAL DURING DROUGHT.

In several cases, after the material had been subjected to more or less intense drought, it was placed either in saturated air or was actually soaked with water.

TABLE XIII.

Comparison of plasmolysis in drought material of various terrestrial forms and in the same material some time after exposure to moisture.

(The first line in each case shows the drought condition,¹ the second that after exposure to moisture.)

Material.	Expt. No.	Cells counted.	Strength of sol.	Strongly plasmolysed.	Slightly plasmolysed.	Unaffected.	Duration of exposure to moisture (days).
			%	%	%	%	
<i>Zygogonium</i>	V	1,839	5	0.1	7.2	92.7	
"	"	715	5	—	81.3	18.7	3
"	XII	926	5	—	5.1	94.9	
"	"	827	5	1.2	22.5	76.3	3
"	XVI	841	5	0.5	15.1	84.4	
"	"	1,042	5	1.1	51.2	47.7	13
"	III	990	3	—	2.6	97.4	
"	"	960	3	0.4	68.8	30.8	12
<i>Hormidium</i>	XIV	1,400	5	—	12.9	87.1	
"	"	503	5	—	13.7	86.3	2
<i>Prasiola</i>	XXVII	905	20	—	28.3	71.7	
"	"	921	20	45.8	27.3	26.9	21
<i>Protonema</i>	XV	655	5	2.9	83.8	13.3	
"	"	931	5	32.3	37.6	30.1	14
"	XXXIV ²	887	5	—	62.3	47.7	
"	"	1,105	5	80.7	14.3	5.0	1
"	"	640	5	—	34.2	65.8	
"	"	565	5	68.7	28.8	2.5	1

¹ Regarding the nature and duration of the previous drought, cf. Table V.

² In Experiment XXXIV patches of soil with protonema were removed from the desiccator on successive days and soaked with water.

Its relation to the same strength of plasmolysing solution as had been used for the previous drought determinations was investigated a few days later. Some of the results are epitomized in Table XIII (cf. also Table VI and Figs. 6 and 8). Although in most cases some alteration in the behaviour of the material was to be recorded, a pronounced recovery was only observed when the duration of the previous drought had been short, as in the case of the *Prasiola* and protonema. In such cases, moreover, the recovery is rapid, and one can feel sure that it is actually due to an alteration of the cells that had previously been affected by the drought. On the

FIG. 8. Recovery from drought in the case of *Zygogonium* (Expt. XVI).

other hand, if material during exposure to drought has once reached the state in which a large number of the cells exhibit the slight permanent contraction of the protoplast mentioned on p. 691, recovery is very slow, and one is in some doubt as to whether increasing plasmolysis is due to an actual recovery of the tendency to plasmolyse or to the gradual formation of new threads by some of the surviving normal cells.¹ Those recovering from drought, however, are generally distinguished by microscopic characteristics (cf. p. 715) from those due to new growth. That there is an actual recovery, even in such cases, is indicated by observations made in Experiment XVI (cf. Table VI and Fig. 8), where the gradual increase in the number of slightly plasmolysed cells proved to be due to an actual increase in the

¹ Moreover, when recovery is slow, the deleterious effect of laboratory conditions (cf. p. 716) may be just as responsible for the state of the protoplast as the original drought.

capacity of the cells to plasmolyse. This was shown by the fact that on May 10 a 10 per cent. sea-salt solution produced far more plasmolysis than a 5 per cent. one. The frequent failure to recover, or the slow recovery noted in many of our experiments, is no doubt to be ascribed to the fact that the intensity of the drought to which the material was subjected was in general rather great, probably far greater than ordinarily occurs in nature. That the difference between drought and wet material is not confined to the filamentous forms is illustrated by Fig. 6, which gives the results of experiments with *Pleurococcus*.

The question may now be approached as to the actual nature of the change that takes place in the cells of the forms investigated during a period of drought. There are obviously various explanations that could be advanced to account for the gradual disappearance of the tendency to plasmolyse. Thus, there may be an increase in the permeability of the protoplasts, production of additional osmotic material may take place, or the viscosity of the protoplasts may increase to such an extent that the latter become sufficiently rigid to resist the tendency to undergo contraction. In the case of recovery of material kept in hypertonic solutions there is the further possibility that the protoplasts are slightly permeable from the beginning, but this is unlikely to apply to more than a minority of the cells, in view of the persistence of plasmolysis in the majority in open hypertonic solutions.

(a) *Observations on Permeability to Stains.*

1. *Zygogonium*. In the case of *Zygogonium* (material from habitat III) experiments have been conducted with a number of different stains, dissolved in small quantity in a 5 per cent. solution of sea-salt, in order to investigate a possible relation between the penetration of the stain and the occurrence of plasmolysis. After treatment for twenty minutes with such a solution to which a little methyl violet had been added, the dead and unplasmolysed cells of the alga showed deep staining of the walls and the protoplasts, whilst in the plasmolysed cells the latter were practically unaffected. After five minutes in a 5 per cent. solution with four drops of aqueous 1 per cent. erythrosin the unaffected cells were deeply stained and the majority of the plasmolysed cells unstained; treatment for half an hour afforded the same result, though the staining was much more pronounced. When a little eosin was dissolved in the plasmolysing solution, only the unaffected cells were stained as a general rule; the result was apparent already after five minutes, but much more marked after half an hour.

On the other hand, methyl green, nigrosin, and Bismarck brown afforded no such results, even after an hour's treatment; in the first two cases there was no evident penetration of the stain into any of the cells, whilst with Bismarck brown there was faint staining of the chloroplast in all cases.

A solution made up of one drop of 1 per cent. Congo red in 1 c.c. 5 per cent. sea-salt produced a very deep coloration of the wall after three-quarters of an hour in all the dead cells, but only a very slight one, if any, in the living cells; there was no penetration into the protoplast of the latter whether plasmolysed or not. It may be added that neutral red does not differentiate between different cells of this alga, as it does in *Hormidium* (cf. below); the stain is taken up very rapidly, even from a very dilute solution, and appears in the chloroplasts, which become deeply tinted.

The cells of *Zygogonium* are thus permeable to different stains and, in the case of several of these, the degree of permeability varies, the stain penetrating readily into some of the cells and not into others. Eosin, as giving the most pronounced results and apparently not staining the wall, was selected for further investigations. The number of cells becoming stained after half an hour's treatment differs very much (cf. the first column of Table XV) and evidently depends on the condition of the material.

TABLE XIV.

*Relation between penetration of eosin and response to 5 per cent. sea-salt solution on the part of fresh material of Zygogonium.*¹

(Habitat II, Experiment XXV.)

	<i>Strongly plasmolysed.</i>		<i>Slightly plasmolysed.</i>		<i>Unaffected.</i>	
	<i>Stained.</i> %	<i>Unstained.</i> %	<i>Stained.</i> %	<i>Unstained.</i> %	<i>Stained.</i> %	<i>Unstained.</i> %
Immediately	3.2	35.0	9.2	33.6	19.0	—
After one hour	15.4	2.1	42.1	2.5	37.9	—

The rate of penetration of the stain varies. In Experiment XXV (cf. Table XIV) the unaffected cells stained immediately, and so did some of the plasmolysed ones. The majority of these, however, required a varying time to take up the stain, but it had penetrated into most of them at the end of an hour. This was, however, an extreme result, partly due perhaps to the experiment being performed in a very warm room, and in other cases many of the cells resisted the penetration of the stain for a much longer time. There is often, as mentioned above, a considerable degree of parallelism between the permeability to the stain and to a 5 per cent. sea-salt solution, as indicated by the occurrence or non-occurrence of plasmolysis. It may be pointed out, however, that the degree of correspondence between the two sets of phenomena will of course depend on the strength of plasmolysing solution used and the condition of the material. Threads which had been for a few days immersed in 5 per cent. salt solution (cf. p. 706) gave very definite results,

¹ Slightly over 200 cells counted at each estimation. Since the experiment was performed in a very warm room the material shows recovery at the second estimation.

the contents of the plasmolysed cells all being unstained after half an hour, whilst the majority of the unaffected cells were stained. In later stages of such experiments, however, the few unaffected cells are not stained, whilst a certain number of the strongly plasmolysed ones are.

Some of the drought material of Experiment XVI (cf. Table V) was subjected to the same treatment. The contents of all the dead cells and of the non-granular ones were immediately deeply stained, whilst the numerous cells with large irregular granules (cf. below) required but little longer to stain. The protoplasts of the few perfectly healthy green cells with fine granules, on the other hand, remained unstained, although it was very noticeable that in these last the wall became distinctly coloured, much more so than in the other cells or in the original fresh material. Similar results were obtained with the material that had been in a sealed slide for five days (cf. Table IX, Experiment XVI), the protoplasts of the majority of the cells being immediately stained, whilst the few healthy green cells with fine peripheral granules remained unstained.

TABLE XV.

Percentages of cells¹ stained with eosin (treatment as described in text) in Zygonium during drought and subsequent recovery.

(Experiment XXXI, habitat III.)

	<i>Original material.</i>	<i>Days of drought.</i>					<i>Days after soaking.</i>		
		1	2	3	4	5	2	3	5
Series I	11.0	83.7	99.1	—	—	—	72.5	—	46.6
„ 2	2.5	5.4	5.4	87.4	99.2	100.0	—	96.4	97.1
„ 3	0.0	15.6	94.2	99.7	—	—	83.2	—	—

There can hardly be any doubt that there is an appreciable alteration in the degree of permeability to eosin on the part of the cells of *Zygonium* during a period of drought.² This has been established by keeping material over 34.5 per cent. sulphuric acid in a desiccator, in the dark and at a constant temperature of 18° C., and making daily estimations of the numbers of stained and unstained cells. At each estimation some of the filaments, well teased apart, were placed for half an hour in a watch-glass in 0.2 per cent. eosin in tap-water, and the material was then mounted in ordinary water. The results are reproduced for three separate series in Table XV. A marked feature of all three series is the suddenness with which the change in per-

¹ 2,000 or more cells were counted at each estimation in this experiment, no dead ones being taken into account.

² The only other explanation that could be advanced for the phenomena about to be described is that the increased tendency to take up the stain is due to a change in the reaction, and therefore in the electrical condition of the protoplasmic substance, rather than to an increased permeability. Such an explanation, whilst possibly applying in the case of *Hormidium* (cf. p. 713), is not established for *Zygonium* by the available data.

meability to the stain sets in. It will be seen that, if the drought is sufficiently prolonged, all the cells may ultimately take up the stain (series 2). A further noteworthy point is that soaking of the material with water, after a brief period of drought, leads to a more or less rapid decrease in the percentage of stained cells (cf. series 1 and 3), whilst there is no such decrease if the drought has been prolonged (series 2). This falls into line with the observations detailed above on the recovery of drought material when placed in damp air (cf. p. 709), where in the vast majority of cases the previous drought was as intense or even more intense than in series 2. Dilute erythrosin gives quite similar results to those described for eosin, but no such alteration in the behaviour of the protoplasts with respect to neutral red, such as is recorded below for *Hormidium*, was found in the case of *Zygogonium*; ten minutes' treatment with 0.2 per cent. neutral red stained all the cells, and even after three days' drought there was no change in this respect.

TABLE XVI.

Percentages of cells¹ stained and unstained by neutral red (treatment as described in text) in Hormidium during drought and subsequent recovery.

(Experiment XXXII, habitat II.)

	Series 1.			Series 2.		
	Whole stained.	Protopl. stained.	Whole unstained.	Whole stained.	Protopl. stained.	Whole unstained.
Original material	25.5	8.3	66.2	25.6	36.0	38.4
1 day's drought	11.3	5.2	83.5	—	—	—
2 days' "	0.0	1.5	98.5	4.9	3.1	92.0
3 " "	—	—	—	1.2	9.4	89.4
5 " "	—	—	—	0.2	10.0	99.8
3 days after soaking	—	—	—	7.5	19.2	73.0

2. *Hormidium*. This alga gives results with 0.2 per cent. eosin and dilute erythrosin which are identical with those obtained for *Zygogonium*, the healthy cells of fresh material altogether excluding the stain, whilst nearly all the cells of drought material take it up rapidly. Neutral red affords different results. With this stain the cells fall into three groups, viz. those which are unstained, those with the protoplast but not the chloroplast stained, and those in which the whole contents are stained.

The effect of drought is rather contrary to expectation (cf. Table XVI). The material in this case was stained for 10 minutes in 0.4 per cent. (series 1) or 0.2 per cent. (series 2) neutral red dissolved in tap-water, the estimation again being made in tap-water. The conditions of drought were the same as in the experiments above described for *Zygogonium*. The progressive change in the material is again quite obvious, but in this case it is in the direction of a decrease to a minimum of the number of stained cells.

¹ 2,000 cells counted at almost every estimation; dead cells ignored.

The result could be explained as due to a decrease in the permeability of the protoplasts, but this is not very likely in the face of other conclusions reached in the present paper. It seems more probable that it is to be ascribed to a change in the reaction of the protoplast, so that the stain is not accumulated. Evidence for this view is afforded by the fact that stains of opposite sign (eosin and erythrosin on the one hand, neutral red on the other) are respectively only capable of accumulation when the material is in a condition in which it is unaffected by the opposite stain. *Zygogonium*, as already mentioned, does not show this change in behaviour towards neutral red during drought, which is indicative of the diversity and complexity of the phenomena involved. Soaking of the drought material with water, as in *Zygogonium*, leads after some days to a recovery in the original direction (cf. series 2).

3. *Moss protonema*. Treatment of the original material of Experiment XV (cf. Table V) with 5 per cent. sea-salt, to which a very small quantity of eosin had been added, gave results analogous to those above described for *Zygogonium*. The vast majority of the unaffected cells were deeply stained, the slightly plasmolysed cells stained after a little time, and most of the strongly plasmolysed ones were unstained, although a few here and there were deeply coloured. The drought material of this experiment treated in the same way showed the majority of the unaffected cells unstained, the slightly plasmolysed ones mostly stained, whilst a few of the strongly plasmolysed cells were stained, though most of them were not. Since the statistical observations in Experiment XV (cf. p. 693) indicate that some at least of the originally strongly plasmolysed cells can recover, and since direct microscopic observation of protonema sealed in a 5 per cent. sea-salt solution (cf. pp. 703, 704) shows that some of these cells are incapable of recovery, it would appear that there are in the protonema used two types of strongly plasmolysing cells differing in their degree of permeability. Some are altogether impermeable and remain plasmolysed, others are slightly permeable, so that, though plasmolysed at first, they ultimately recover. It should be emphasized that there does not appear to be any great difference in the behaviour towards eosin on the part of the strongly plasmolysed cells in the fresh and the drought material, the principal difference lying in the unaffected cells.

Material that had been for four days in 5 per cent. sea-salt in a sealed slide (Experiment XXIII, Table IX) showed, after twenty minutes' treatment as above, practically all the slightly plasmolysed cells stained, whilst the unaffected and strongly plasmolysed ones were each about half stained, half unstained (cf. with the results for drought material). In another experiment (XV, Table IX), with six days' exposure to the solution, all the cells stained deeply in a short time, although some of the strongly plasmolysed ones more slowly than the others.

Two separate investigations (Experiment XXXIII) on the progressive changes in staining of the cells of protonema with eosin during drought (34.5 per cent. sulphuric acid in the dark at 18° C.) have been undertaken.¹ In the first series the percentages of stained cells on successive days were: 17.5 (original material), 20.3, 87.5, 18.5, 17.2; in the second series they were: 29.4 (original material), 38.7, 99.8, and, after four days' drought, approximately half the cells stained (actual count not made). In both series the result, for the first two days of drought, was as in *Zygogonium*, but after that there was in each case a great decrease in the percentage of stained cells, a fact for which there is at present no satisfactory explanation. In the second series parts of the protonema mat were removed at various stages of drought and soaked with water, though otherwise left under the same conditions. Material thus removed after two days' drought (then showing 99.8 per cent. cells stained) gave one day after soaking 81.7 per cent. and two days after soaking 79.3 per cent. stained cells, after three days' drought and one day's soaking with water 86.4 per cent. stained cells. It is evident, therefore, that with reference to the capacity to exclude the stain there is very little recovery on the part of drought material in this case. Only one investigation of the effect of dilute erythrosin has been undertaken, and this afforded results which were practically identical with those obtained with *Hormidium* and *Zygogonium*; after five days' drought the majority of the cells stained deeply in a short space of time.

4. *Pleurococcus* and *Cystococcus*. No clear results have been obtained with either of these algae. If cells of *Pleurococcus* are mounted in 0.2 per cent. eosin, only very few take up the stain, and simultaneous treatment with a 25 per cent. sea-salt solution shows that the strongly plasmolysed cells never stain, the dead cells invariably, whilst of the unaffected cells roughly half are stained. Nor is there any sensible difference in drought material; thus, in Experiment XXX (cf. Table V) an estimation, made after ten weeks' drought, showed that only 6.4 per cent. of the cells stained, which corresponds to about one-third of the unaffected cells present. In the same experiment, material that had been subjected to four weeks' drought and had then been soaked with water for six weeks gave 6.0 per cent. cells stained, corresponding to about one-half the unaffected cells. Examination of material which had been in a 25 per cent. solution in a sealed slide for seven days (Experiment XVII, Table IX) afforded identical results, the ratio of stained to unstained unaffected cells being 41:59. In the case of *Cystococcus* the unaffected cells on the whole stain more strongly with eosin than the plasmolysed ones, but this is not an invariable rule. No difference could be detected in this respect between fresh and drought material.

The general conclusions to be drawn from the above observations are considered in the next section.

¹ Method as above described for *Zygogonium*; 1,000 cells or more counted at each estimation.

(b) Microscopic Characters of Drought Material.

The cells to be found in drought material can be broadly divided into three groups: (a) the dead cells which have discoloured, though not necessarily shrunken, contents; (b) living cells with a slightly contracted and apparently rigid protoplast, since the contraction does not disappear in ordinary water and plasmolysis does not ensue even after treatment with very strong solutions (25 per cent. sea-salt); and (c) healthy pure green cells with a quite uncontracted protoplast and not affected by similar strong plasmolysing solutions. In *Pleurococcus* and *Cystococcus*, as far as our observations go, the second type of cell is not found and many of the cells remain capable of plasmolysis even after very prolonged drought. In the filamentous forms the relative numbers of the different types of cells present depend on the intensity and duration of the drought; at first there are relatively many of the third type, later the second type predominates, and under extreme drought the majority of the cells may die. What extent of drought may be necessary to lead to the death of all the cells in a given mass of terrestrial alga or moss protonema we are unable to say (cf., however, Schroeder, 1886), but we are inclined to think it would have to be very intense or very prolonged.

The cells of the second type are not dead (cf. section (c)), although a large number of them may be moribund, if the drought has been intense or prolonged. These cells, when granular, invariably contain large coarse granules of unequal size and showing an irregular distribution, some parts of the periphery of the protoplast being crowded with them, others practically free. Non-granular cells, especially in *Zygogonium*, often have a peculiar whitish opaque look about the edge of the protoplast. Material that has been in a sealed slide presents similar features, but cells of either type are rarely found in material out of doors and then only in small numbers. In *Hormidium* during drought a large spherical granule is often to be seen in these cells in the clear area outside the chloroplast.

It is evident, however, that this rigid state of the protoplast can arise also as a result of conditions other than drought. In the first place it would seem to develop after some weeks' sojourn in the laboratory, even in material that has been kept continuously moist. Further, it has been observed to appear in a large number of the cells of *Zygogonium* as a result of exposure to bright light. Thus, in one of our experiments on the influence of darkness and illumination on the plasmolysing capacities of the cells of this alga, in which the experiment had been inadvertently allowed to continue for four weeks, the cells of the 'light' material were found to be mainly in this condition, whilst those of the 'dark' material showed it to a much lesser extent. It may be added that in the former the cells were nearly all purple

as a result of the development of phycoporphyrin, whereas in the latter they were pure green.

The only cells which after drought can be regarded as really healthy are those whose protoplast is quite uncontracted, which possess a healthy green colour, and in the filamentous algae usually have a very regular layer of fine, even granules at the periphery of the protoplast. These cells, which after prolonged drought are not very numerous or may be completely absent, are the ones which prove to be impermeable to eosin in the case of *Zygogonium* (cf. p. 712) and the protonema (p. 714), whilst the slightly contracted cells are all readily permeable, although not generally as rapidly as the dead cells.

In the case of material kept in hypertonic solutions (cf. p. 706 et seq.) there are likewise usually a certain number of cells with uncontracted protoplasts, and, from the similarity in microscopic characteristics, it is probable that they are the same cells as appear quite unaffected at the end of a drought experiment. Side by side with the unaffected cells in material kept in hypertonic solutions there are, however, far more numerous strongly plasmolysed cells. Some at least, if not the majority, of these must correspond to the numerous cells with permanent slight contraction of the protoplast in drought material. These cells are therefore more resistant to a prolonged sojourn in a hypertonic solution than to drought, since the great permeability of these cells in the latter case is probably an indication of their being in a more or less unhealthy condition, leading sooner or later to death, and from which a recovery is perhaps only possible in early stages (cf. p. 706). Moreover, although plasmolysed, the protoplasts of the cells in open hypertonic solutions are not rigid, since they recover in tap-water (cf. p. 707).

The healthy uncontracted cells remaining after drought and prolonged treatment with hypertonic solutions, in view of their practical impermeability to stains, are probably also impermeable to the salt solution. Since they remain unplasmolysed, the concentration of their sap in osmotic material would seem to be at least as great as that of the 5 per cent. sea-salt solution used. In fresh material, however (cf. Table XIV), it is the plasmolysed and not the unaffected cells that fail to take up stains. To account for the presence of unplasmolysed and non-staining cells in the final condition, therefore, there are two possible explanations: These cells may be the unaffected ones of the original material (which might be supposed to have an osmotic strength greater than 5 per cent. sea-salt solution) which, at first readily permeable, have become impermeable. Alternatively these cells may be the original strongly plasmolysed ones which have remained relatively impermeable but have met the conditions to which they have been subjected by the formation of additional osmotic material. The failure of both suggestions to cover the facts completely, since the cells in question are often also unaffected by solutions far stronger than 5 per cent., hints at the

possibility of imbibition phenomena being also concerned. In the case of *Prasiola*, *Pleurococcus*, and *Cystococcus* such an explanation would appear to be the only feasible one to account for the absence of plasmolysis in a considerable number of the cells in solutions of a strength of 20 per cent. and upwards.

(c) *Observations with Dark-ground Illumination.*

For these investigations the illuminant used was an electric arc taking from fifteen to twenty-five amperes, in conjunction with a Watson paraboloid condenser. Fresh material of *Spirogyra* was always examined first with the object of obtaining an idea of the condition of the normal algal protoplast. This shows complete milkiness and vigorous Brownian movement throughout the cytoplasm.

When fresh material of *Zygogonium* is examined, vibratory Brownian movement of minute particles in the protoplasm is distinct in all except the dead cells. Non-granular cells are seen to have but a thin cytoplasmic lining beneath the cell-wall, with a wide vacuole between it and the central chloroplast, whilst in granular cells there is a much wider cytoplasmic lining and a smaller vacuole.

Examination of the drought material of *Zygogonium* from Experiment XII showed a general appearance of milkiness in the granular cells, but no Brownian movement was recognizable. Diffraction lines were observed around the granules, but were definitely limited to them, and the milky appearance of the protoplast throughout demonstrated that no coagulation had taken place. In none of the granular cells of drought material could a vacuole be recognized, an observation which was confirmed on other occasions using other material. Similarly in the case of *Zygogonium* that had been for two months in a solution of 5 per cent. sea-salt, the protoplasts of the healthy cells, though mostly contracted (cf. p. 706), were milky throughout, showed no Brownian movement, nor could vacuoles ever be distinguished. After shorter periods of exposure to drought or hypertonic solutions some of the cells still show Brownian movement.

In the healthy cells of fresh *Hormidium*-material observation with dark-ground illumination again shows general milkiness of the protoplast, vigorous Brownian movement of the minute particles present, and a distinct vacuole. In the drought material from Experiment XIV and others, the milkiness was less marked, no Brownian movement could be recognized, and no vacuole was discerned. The cells showed progressive degrees of decreasing milkiness, but in no case were they as milky as in the fresh material; the slight diffraction discernible again proved to be due to the granules rather than to coagulation of the protoplast. The healthy cells of filaments that had been immersed in 5 per cent. sea-salt for two months presented just the same

features as the cells of drought material. The obviously dead cells in all cases looked quite different from the others.

Only fresh material of *Prasiola* has been examined with this type of illumination. The cells showed pronounced milkiness of the protoplast, no Brownian movement, and usually no indication of vacuoles. In some of the longer cells, however, there appeared to be very small vacuoles around the periphery of the protoplast, which is contrary to what is usually described for this alga (cf. Fritsch, 1922, p. 14), and may be due to the fact that the material examined had been completely submerged during the previous days as the result of heavy rains.

A comparison of fresh and drought material of protonema showed the same contrast as above described for the two filamentous algae: Brownian movement and marked vacuoles in the fresh cells, no movement and no vacuoles in the drought cells, which, however, appeared quite healthy. Material that had been submerged for two months in 5 per cent. sea-salt solution showed in the healthy cells near the tips of the branches (cf. p. 706) a very well-defined cytoplasmic lining which was typically milky throughout; there was a clear vacuole in this case.

In *Pleurococcus*, lastly, one recognizes no vacuoles, even in fresh material (cf. Fritsch, 1922, p. 14); the protoplasts have a general milky appearance, but the large granules in the cells make it difficult to say whether there is any Brownian movement or not. It is probable, however, that there is none.

The general conclusions to be drawn from these observations are that the granular cells of drought material still possess a healthy protoplast, which has, however, undergone a marked increase in viscosity, so that Brownian movement can no longer be recognized in it. It would appear, too, that an increase in the area occupied by the protoplast takes place at the expense of the vacuole. In all of these respects *Pleurococcus* and *Prasiola*, in the normal condition as found in nature, resemble the drought condition of the other forms, and to this many of their peculiarities and their great power of resistance may be due. Extreme viscosity of the protoplast may well account for the failure of the majority of the cells of drought material to undergo plasmolysis. On the other hand, the recovery from plasmolysis of strongly plasmolysed cells that have been immersed for long periods in hypertonic solutions, when mounted in tap-water (cf. p. 707), shows that the protoplasts of these cells, though otherwise usually showing similar characteristics to the protoplasts of cells in the drought condition, are not as viscous. The same remarks also apply to the fresh protoplasts of *Pleurococcus* and *Prasiola*, since plasmolysis of these takes place when high concentrations are used.

(d) Investigation of Centrifugalized Material.

As a further method of investigating the supposed change in viscosity of the protoplasts (cf. above), the effect of centrifugalizing at a speed of 2,500 revolutions per minute on the position of bodies within the cells was examined. If fresh material of *Spirogyra*, *Zygogonium*, *Hormidium*, or moss protonema, placed together with a little water in the bottom of the centrifuge tube, be subjected to the centrifugal force mentioned for 15 minutes, a pronounced displacement of nucleus and chloroplasts to the side of the cell takes place. In the case of the two terrestrial algae the granules 'cream' in the opposite direction to the chloroplast, thus affording additional evidence of their fatty nature. On the other hand, in *Pleurococcus* and *Prasiola* no displacement of the cell-contents was to be observed, even after one hour's centrifugalizing at the above speed.

A similar test was applied to material of *Zygogonium*, *Hormidium*, and moss protonema, which had been exposed to four days' drought over 34.5 per cent. sulphuric acid in the dark at 18° C. Before centrifugalizing, the material was thoroughly soaked and was then placed in water in the centrifuge tube. In this case neither one-quarter hour nor one hour's centrifugalizing served to bring about any alteration in the cell-contents, which appeared in the same position as in uncentrifugalized material. The mats were then thoroughly soaked and some of the material subjected to the same treatment three days later, but no difference as compared with the drought material was to be noticed. It should be added that, at the time when the drought material was centrifugalized, parts of the same mats which had been kept moist in the thermostat under otherwise similar conditions to those to which the drought material was exposed were also centrifugalized, and after a quarter of an hour showed the same kind of displacement as the original material. This proved that the features observed in the drought material are not a result of exposure to the conditions extant in the laboratory.

The observations made on centrifugalized material thus afford a striking confirmation of the view, derived from the examination with dark-ground illumination, that as a result of drought there is a great increase in the viscosity of the protoplasm in *Zygogonium*, *Hormidium*, and the moss protonema. In *Pleurococcus* and *Prasiola*, on the other hand, such high viscosity of the protoplasm is evidently the normal condition. This feature accounts for the inert character of drought material and, in the case of the two forms last mentioned, even of the fresh condition.

The effects of centrifugalizing on material which had been immersed for some time in sea-salt solutions was only tried on *Zygogonium* two days after Experiment XXXV had been in progress. After a quarter of an hour's centrifugalizing most of the cells did not show any displacement, but in a few cases it was quite marked.

F. THE NATURE OF THE GRANULES FOUND IN THE CELLS OF TERRESTRIAL ALGAE.

We have been able to obtain evidence which shows that in the cells of several of the terrestrial algae examined the granules are of at least two kinds. Those of the one kind are minute, relatively uniform in their size and distribution, which is frequently peripheral, and are characterized by the fact that, whereas they fail to give any of the usual fat reactions, they stain a dark colour after a longer or shorter treatment with silver nitrate without exposure to light. In the case of *Zygogonium* half an hour's treatment with the reagent suffices, whilst in *Hormidium* and *Pleurococcus* a much longer period is requisite and it has been found necessary to add glycerine to the reagent in order to facilitate its penetration. In some cases there are relatively few of these granules (especially *Pleurococcus*). They have not been observed in *Prasiola*.

The granules of the other kind are larger and irregular in distribution and appear to differ somewhat in their reactions in the different forms. In *Zygogonium* they give the normal reactions for fats, both with Sudan III and osmic acid; in *Hormidium* they stain after some time in the former, but appear unaffected by the latter; in *Pleurococcus* a certain number of these granules stain with osmic acid, but Sudan III has no effect. Possibly, however, these differences are only due to differences in the ease of penetration. In practically all cases the majority of the larger granules are soluble in ether, chloroform, and acetone, although in *Prasiola* solution is only attained after a long time (cf. Piercy, 1917, p. 526). Alcohol dissolves them in *Zygogonium* and to some extent in *Prasiola*, but apparently not in the other cases. The large granules are evidently of the nature of fats and are possibly the only kind present in *Prasiola*.

Ether and acetone appear quite often to remove all the granules in the cells, whilst with chloroform, at least in the case of *Zygogonium*, the small ones remain behind, and, after such treatment, can be brought out clearly by subsequent staining with silver nitrate. In the case of *Hormidium* and *Pleurococcus* we have been unable to arrive at any definite conclusion on this point.

G. GENERAL CONCLUSIONS.

The methods adopted in the present investigations are described on pp. 683, 689-91, 700, 704, 706, 712, 718, and 720, where also the extent of error involved is considered. The most important facts that have been established are:

1. With a given strength of sea-salt solution there is great inequality in the behaviour of the cells of the terrestrial algae investigated, as well as of

moss protonema. Some of the cells become strongly plasmolysed, others only slightly, whilst a varying percentage are totally unaffected. In order to bring about plasmolysis of a majority of the cells, very high concentrations are often necessary, the outstanding forms in this respect being *Prasiola*, *Pleurococcus*, and *Cystococcus*. With the latter two even 25 per cent. solutions of sea-salt leave about 10–20 per cent. of the cells unplasmolysed. In both of these respects aquatic algae afford a marked contrast, the cells plasmolysing with low strengths of solution and usually showing an almost uniform reaction.

2. With increasing concentrations of sea-salt solution there is a progressive increase, both in the numbers of cells plasmolysed and in the extent of plasmolysis in the individual cells, a fact which implies that in the terrestrial forms investigated there is a more or less complete grading of the cells as regards their response to plasmolysing solutions.

3. When subjected to concentrations not far above the minimum, the majority of the plasmolysed cells are those which lack the granules which are so characteristic of terrestrial algae, whilst with high concentrations the unaffected cells are almost invariably such as are rich in these granules. The granular or non-granular character of the cell is, however, not the only determinant of its plasmolysing qualities, as shown by the fact that far more plasmolysed cells are to be found in material grown for some days in the dark than in similar material that has remained exposed to the light.

4. During a period of drought there is a progressive daily decrease in the degree of plasmolysis in the filamentous forms, as well as in the numbers of plasmolysed cells, culminating after some little time, as a general rule, in a complete absence of strongly plasmolysed cells and a majority of unaffected cells. There is thus evidently a gradual loss of the tendency of the cells to plasmolyse.

5. In *Pleurococcus* (probably also *Cystococcus*), on the other hand, there is no such definite response to drought. If the plasmolysing tendencies of the cells change at all, they do so only after very prolonged and intensive drought, and apparently death of the cells may often intervene before any decisive response has occurred. In our numerous experiments with *Pleurococcus* very erratic, fluctuating results have been obtained which are at present inexplicable.

6. If threads of the filamentous forms are permanently mounted in sea-salt solutions of varying strength in a sealed slide (cf. p. 689), the cells show a recovery from plasmolysis which is rapid at first, but subsequently slower, until after some days no decided plasmolysis is to be found. This fact has been established both by means of comparative estimations and direct microscopic observation. The rate of recovery depends on the temperature.

7. If material of *Pleurococcus* or *Cystococcus* is sealed in this way in a 25 per cent. sea-salt solution, there is often no change for several days.

The data given in this paper indicate that at certain times these two algae are capable of remaining unaffected for a long time by the unfavourable conditions prevailing in such a sealed slide.

8. The recovery referred to in 6 and 7 is probably in part pathological, but emphasis must be laid on the similarity of the response to that observed during drought, and on the fact that aquatic algae, as well as the epidermal cells of *Saxifraga sarmentosa*, show a very much smaller capacity for recovery under such conditions. The cells of the protonema and leaves of mosses seem in this respect to occupy a somewhat intermediate position.

9. If small patches of soil bearing mats of the filamentous forms are placed in open vessels containing various concentrations of sea-salt (usually 5 per cent.), a large number of the cells remain alive for weeks or even months. In the first days after such treatment a considerable number of the cells recover from plasmolysis, but the majority of these at least die within a week or so. The remainder are of a healthy green colour, but usually most of them are plasmolysed, though not very strongly. Even after weeks of the treatment these cells recover instantaneously if the threads are mounted in tap-water. Side by side with the plasmolysed cells there are always to be found a smaller or larger number of cells with quite uncontracted, healthy green protoplasts. Such might serve as the starting-points for growth, which has, however, so far been observed in one case only (*Zygogonium*).

10. Recovery from the effects of drought on subsequent access of moisture takes place only very slowly, unless the previous drought has been of short duration or relatively mild. In *Pleurococcus* it appears to be more rapid than in the others (cf. Fig. 6).

11. The cells of the forms studied show marked differences among one another in their degree of permeability to certain stains. In the case of *Hormidium* treated with neutral red some cells are altogether unstained, others exhibit staining of the protoplasts except for the chloroplasts, whilst in a third group the entire contents are stained.

12. In *Zygogonium* and the protonema the permeability to eosin (and other stains) runs more or less parallel with the behaviour of the cells towards a 5 per cent. sea-salt solution, the majority of the unaffected cells staining almost immediately, whilst the plasmolysed ones remain unstained for a much longer time. In *Pleurococcus* only the unaffected cells (using 25 per cent. sea-salt solution) take up the stain, and only about half of these.

13. During the progressive stages of drought a great increase in the permeability of the cells of *Zygogonium*, *Hormidium*, and the protonema to various stains is observable, the number of stained cells ultimately reaching nearly 100 per cent., although subsequently falling off again in an inexplicable manner in the protonema (cf. p. 715). In the final drought condition of the algae the only unstained cells are the few perfectly healthy ones. If the

period of drought has not been too prolonged, there is, in the case of the algae, an increasing percentage of unstained cells during the days succeeding access of moisture.

14. In *Hormidium* there is a progressive decrease in the number of cells stained with neutral red during a period of drought, the decrease being evident in both the categories above distinguished (cf. 11). If the material is wetted before the drought has lasted too long, there is a reversal. There is no obvious difference in the staining reactions with eosin of fresh and of drought material of *Pleurococcus*.

15. Material of *Zygogonium* and protonema that has been in a sealed slide shows staining properties similar to those of drought material.

16. Three kinds of cells are distinguishable in drought material of the filamentous forms, viz.: (a) dead cells; (b) living, though perhaps in part moribund, cells which have a rigid, slightly contracted protoplast and, in the algae, contain coarse granules; (c) healthy green cells, in the algae commonly provided with numerous fine peripheral granules, with an uncontracted protoplast which, like that of the cells under (b), is not affected even by strong (25 per cent. sea-salt) plasmolysing solutions. The same types of cells are recognizable in material that has been for some days in a sealed slide. The second kind of cell also appears at times in material that has been for a prolonged period in the laboratory or exposed to strong illumination. Such cells have not been observed in *Pleurococcus*.

17. Examination with dark-ground illumination shows that the (b) and (c) cells possess a living protoplast which is not coagulated, but no Brownian movement is to be recognized within it, and, by contrast with fresh material of *Zygogonium*, *Hormidium*, and the protonema, the cells fail to show any vacuoles. Even fresh material of *Prasiola* and *Pleurococcus* exhibits these characteristics, which in the other forms are only to be seen in the drought condition.

18. If fresh material of these different forms is centrifugalized for a quarter of an hour at 2,500 revolutions to the minute the chloroplasts and nuclei are found to be displaced towards one side of the cell in all the filamentous forms except *Prasiola*, the granules, when present, creaming in the opposite direction. Drought material is, however, unaffected even by an hour's centrifugalizing. Fresh material of *Pleurococcus* and *Prasiola* behaves just like drought material of the other forms—that is to say, one hour's centrifugalizing produces no effect.

19. The granules found in the cells of these terrestrial forms probably in large part consist of fats, which, however, appear to vary somewhat in their solubilities in the usual fat solvents. At the same time in all the algae investigated, except *Prasiola*, we have obtained evidence for the presence of a second type of granule, characterized by small size, usually peripheral distribution, and the assumption of a dark, almost black colour after treat-

ment with silver nitrate in the absence of light. These granules are apparently the ones that are particularly prevalent in the healthy cells surviving drought.

The general conclusion to be arrived at is that, in fresh material of the terrestrial forms investigated, the cells are in diverse states as regards drought resistance, resistance to hypertonic solutions, and permeability to stains. There would, however, probably be no means of grouping these cells in sharply delimited categories, since in all these respects there appears to be a practically complete grading. The numbers of cells consequently that are killed or become unhealthy during a period of drought or exposure to hypertonic solutions will depend on the condition of the original material, and in this respect *Pleurococcus* and *Cystococcus* differ from all the others in possessing a far larger number of resistant cells.

The experiments with material placed in open vessels in hypertonic solutions show that a certain, and no doubt varying, percentage of the cells, which are characterized by rapid recovery from plasmolysis, are little resistant to these conditions and die in a short space of time, whilst a relatively large percentage, though in great part remaining plasmolysed, continue in a healthy condition for many weeks or even months. Under conditions of drought or in a sealed slide (cf. p. 689), on the other hand, practically all the cells in the final stage exhibit no plasmolysis, though a very large number of them show the permanent slight contraction of the protoplast which does not disappear even in tap-water. These cells, which are readily permeable to such stains as eosin and erythrosin, are apparently in an unhealthy state though not dead, and their abundant occurrence both in drought material and in a sealed slide indicates that, in both these cases, the conditions are more deleterious than in open hypertonic solutions. In all probability these cells correspond to those which remain plasmolysed under the latter treatment, since the numbers of perfectly healthy unaffected cells, always rather small, are approximately the same in all three cases. The cells with slightly contracted protoplasts are probably in a moribund condition, from which recovery is either very slow or altogether impossible; recovery may depend on the intensity and duration of the previous treatment. There are some data that indicate that a relatively slight drought produces a condition that is reversible, a more pronounced one an irreversible condition. In any case it may be well to point out that the drought to which these forms have been subjected in our experiments was either more prolonged or more intense than anything that is likely to occur in nature.

With all kinds of treatment, however, there usually remain a certain number of healthy green cells, not plasmolysed by even high concentrations (25 per cent.), generally impermeable to the various stains used, and in the filamentous algae usually characterized by the possession of numerous fine peripheral granules. Even supposing that all the cells above described as

moribund really die, the few healthy surviving cells would probably serve as a sufficient basis for new growth when conditions again became favourable. During the prolonged drought of 1921 many of the filamentous forms disappeared altogether (as far as macroscopic observation went) from their normal habitats. Yet, in the autumn or later, they appeared again in quantity in the old situations.

Whatever the treatment may have been, the surviving cells, whether healthy or unhealthy, are characterized by the possession of a more or less rigid, highly viscous protoplast, absence of vacuoles, and in the algae usually abundant granular contents. Most of these features, distinctive only of drought material in other cases, are exhibited already by the fresh cells of *Pleurococcus* and, to a less extent, of *Prasiola*. The very marked powers of resistance of the unicellular alga, a familiar fact of observation in nature and strikingly exemplified in our experiments, is no doubt to be related to the peculiar characteristics of its protoplasts. This form is, so to say, permanently in the drought condition. It is probable that *Cystococcus* altogether resembles it in these respects.

The problem as to the mechanism of water retention, which we set out to investigate, can be regarded as only partially solved. It is plain that a high osmotic concentration of the sap, if it plays any rôle at all, is not a fundamental part of the mechanism.¹ The gel-condition of the protoplast, with absence of all large vacuoles, indicates the possibility of imbibition phenomena being principally concerned. That such a gel would retain a certain quantity of moisture on drying under any ordinary atmospheric conditions is undoubted, but it is open to question whether the amount would be as large as has been found to be the case. Since the protoplasts of *Pleurococcus* are apparently always in this state, the behaviour of this form is of particular interest, for we know that its cells will resist drought for months without apparently losing any of their normal vital properties. This alga, however, exhibits a very small moisture-content (cf. Fritsch, 1922, p. 16) and a low capacity for absorbing moisture when dry (loc. cit., p. 4). Moreover, it shows a higher permanent loss of moisture after heating to 100° C. than either *Zygogonium* or *Prasiola*. All these facts tend to show that the moisture is imbibed within the gel-like protoplast, that it is held tenaciously against dry air, and that it is only after heating that its imbibitional efficiency is destroyed. On the other hand, *Prasiola*, whose protoplast seems to be in much the same condition, does not appear to possess as efficient a mechanism.

In all the other forms, where the gel-condition is only reached during drought, a majority of the cells at these times are probably in a far less healthy state than in *Pleurococcus* and their imbibitional efficiency may

¹ The observations made since the writing of the article on terrestrial algae (in *Journal of Ecology*, vol. x, 1922) have led us to modify the view expressed on p. 230 of that paper.

be more or less impaired. At the same time the fact that the really healthy surviving cells are apparently always those that are provided with fine peripheral granules of unknown nature hints at the probability of still other factors coming into play. It is not impossible that moisture-retention may be partly due to a change in the cell-walls, since alterations in staining reactions, sometimes shown by the walls during drought (cf. p. 712), indicate that they also are capable of some modification under these conditions.

H. SUMMARY.

The present paper deals with the changes occurring in terrestrial algae and moss protonema during drought and when exposed for long periods to hypertonic solutions. During drought there is a gradual diminution in the tendency of the cells to plasmolyse with a given hypertonic solution until finally there is no appreciable plasmolysis at all. At the same time there is a marked alteration in the permeability of the cells to stains. Subsequent access of moisture brings about changes in the reverse direction, unless the drought is severe or very prolonged. If material is sealed in a hypertonic solution and investigated day by day, a similar gradual disappearance of plasmolysis is noted, an effect which is probably partly pathological. In all these respects, however, *Pleurococcus* shows far less tendency to change than the filamentous forms examined.

In material exposed to hypertonic solutions, but unsealed, only a certain number of the cells recover from plasmolysis and the majority of these subsequently die; the rest, often a large percentage, remain plasmolysed, though retaining their vitality for weeks or months. At the same time there is usually a small number of healthy cells with uncontracted protoplasts.

The cells surviving after a period of drought or of prolonged treatment with hypertonic solutions have a more or less rigid, highly viscous protoplast without obvious vacuoles. This has been demonstrated especially by the use of dark-ground illumination and the examination of 'centrifugalized' material; it is the normal state of the cells of *Pleurococcus* and *Prasiola*. It is suggested that this gel-condition of the protoplast may in part explain the marked retention of moisture by such terrestrial forms in the air-dry condition, although there are some indications that other factors also are concerned.

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