Table 1

	Control	Amount of 7·2 per cent gibberellic acid used			
		1 μgm./ml.	100 μgm./ml.	500 μgm./ml.	1,000 μgm./ml.
Length of protonemata (mm.)	1·152	1·069	3·161	5·680	6·981
Cell length (mm.)	0·0477	0·0376	0·0739	0·0977	0·1116
Cell width (mm.)	0·0192	0·0186	0·0172	0·0170	0·0165

The difference between these two ratios indicates that the growth-promoting effect of gibberellic acid on the protonemata is brought about both by an increase in the number of cells and the size of the cells. While the length of the cells increased greatly with an increase in the concentration of gibberellic acid, cell width decreased slightly.

It was observed earlier that the presence of fungal contaminants in cultures containing protonemata greatly increased the growth of these protonemata. To determine the specificity of this 'fungal effect', cultures of isolated leaves of the moss, growing on Beijerinck's inorganic medium, were inoculated with various fungi, obtained from airborne fungal spores. The different fungi had a rather similar growth-promoting effect on the protonemata. The 'fungal effect' was therefore an unspecific one obtainable with a variety of different fungi.

An attempt was made to duplicate this effect by means of the addition of different individual amino-acids and vitamins and various combinations of them, including also various concentrations of yeast extract and casein hydrolysate, to the inorganic medium in the absence of the fungus. In no case was there any marked increase in the growth of the protonemata. A complete series of concentrations of indole-acetic acid showed similarly that the latter does not have any growth-promoting effect on the protonemata. With the exception of the great similarity between the effect of gibberellic acid and the 'fungal effect' on the growth of protonemata, it was not possible, therefore, to replace the 'fungal effect' by means of any of the other compounds tested. Although it seems established that gibberellic acid has to be considered as a growth-promoting factor for the protonemata of *Splachnum ampullaceum*, the unspecificity of the 'fungal effect' makes the similarity between this effect and that of gibberellic acid difficult to explain.

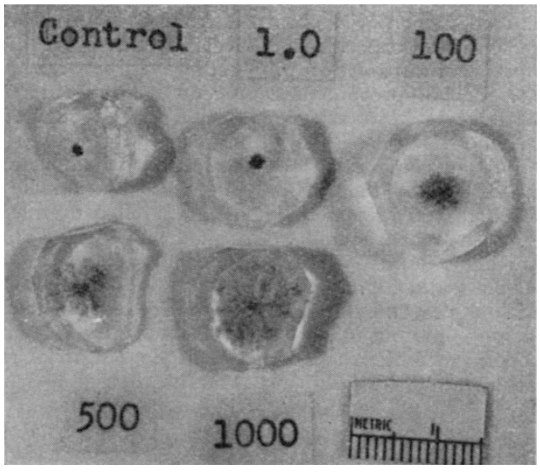

Fig. 1. Effect of gibberellic acid on the length of protonemata (1 month after planting)

It should be mentioned that Sironval[2] reported that gametophyte development in *Funaria hygrometrica* passes through three distinct stages: chloronema → caulonema → gametophore. He states that with low light intensities, in which the chloronema → caulonema transition does not take place, this transition can be brought about in the presence of *Penicillium*. Protonemata developed by way of regeneration have, according to Sironval[3], always the characteristics of caulonema. Hence both 'fungal effect' and the growth-promoting action of gibberellic acid should be an effect on caulonematal growth if Sironval's developmental scheme applies also to *Splachnum*. Although we have no material for comparing chloronema with caulonema in *Splachnum*, our regeneration protonemata do not show the characteristics which Sironval describes as being typical for caulonema (see also ref. 4). We can therefore only speak in terms of the effect of gibberellic acid on the growth of protonemata rather than of its effect on caulonemata in *Splachnum ampullaceum*.

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Effect of Anaerobic Conditions on Imbibed Lettuce Seeds

In investigations into the influence of soil conditions on the establishment of vegetable crops from seed, experiments on the effect of the atmosphere on seed germination have shown that seeds of lettuce (*Lactuca sativa* L., var. Cobham Green) are capable of germinating, though not of growing rapidly, when exposed to an atmosphere of 80 per cent nitrogen, 5 per cent oxygen and 15 per cent carbon dioxide. Published evidence[1,2] suggests that concentrations of carbon dioxide in the surface layers of cultivated soils are rarely, if ever, higher than 4 per cent, and it might be assumed that any effect of soil anaerobiosis on crop plants is due to lack of oxygen rather than excess of carbon dioxide. However, Hack[2] has pointed out that the smaller the sample of soil air taken, generally the higher is its carbon dioxide content.

To test the respective effects of a deficiency of oxygen and an excess of carbon dioxide, seeds were allowed to imbibe in air for various periods, then exposed to atmospheres of nitrogen or carbon dioxide for 1 or 2 weeks, during which time no seeds germinated, and then returned to air and maintained until no further germination occurred. Moisture was non-limiting throughout. Some of the effects on lettuce seeds at 18 ± 2° C. are shown in Table 1.

Anaerobic treatment for one week in nitrogen after 0–12 hr. or in carbon dioxide after 0–6 hr. imbibition in air did not affect germination capacity, but there was an abrupt reduction in the proportion of seeds surviving the carbon dioxide treatment following 12 hr. as compared with 6 hr. imbibition, suggesting a

Table 1. PERCENTAGE GERMINATION ON RETURNING SEEDS TO AIR

Prior imbibition in air (hr.)	Nitrogen		Carbon dioxide	
	7 days	14 days	7 days	14 days
0	86	32	80	5
6	70	40	80	10
12	87	2	20	2

The interaction of prior imbibition period × gas × anaerobic period was highly significant ($P < 0.01$).

specific effect of carbon dioxide from a certain stage of seed activation onwards. In this connexion, Bendall *et al.*[3], working with germinating seeds of *Ricinus*, have recently shown how concentrations of carbon dioxide of more than 20 per cent can affect respiration by interfering with the mitochondrial redox system of cytochrome *c*. In the experiment described here, non-germination after carbon dioxide treatment was accompanied by excessive swelling of the seed and necrosis of the unemerged radicle. In the few affected seeds which escaped microbial invasion, all the organs, except the root, emerged after some delay and grew normally. All seeds not surviving the nitrogen treatment softened and disintegrated completely.

Lettuce seeds with blackened radicles are frequently found in seed boxes in which the soil has been too wet. In view of the present results, this is circumstantial evidence for the existence of high concentrations of carbon dioxide even in shallow layers of soil; although these may be confined to conditions where the carbon dioxide produced, for example, by the respiration of seeds in the initial stages of germination, cannot escape rapidly from its source of origin.

Full details of these investigations will be published elsewhere.

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Chloride Effect on the Growth of *Chlorella pyrenoidosa*

A CHLORIDE (anion) effect has been reported for washed chloroplasts[1] and for lyophilized *Chlorella* cells[2]. Recently there has been evidence reported to show that chlorine is a micronutrient element[3]. No definite growth requirement by freshwater algae for chloride has yet been shown. The purpose of this communication is to report that, with certain media, the growth of *Chlorella pyrenoidosa* is appreciably increased by the addition of chloride. Furthermore, stimulation by sodium has also been observed.

A special medium for *Chlorella* was formulated on the basis of the critical concentrations of nutrients for autotrophic growth[4]. The critical concentration of a nutrient element is defined as the minimum amount of the element which will produce maximum growth. The special medium had, in addition to the usual amount of iron (as sulphate) and trace elements (manganese, copper and zinc as sulphates): $1 \cdot 8 \times 10^{-4}$ *M* KH_2PO_4, 25 mgm./l.; $1 \cdot 95 \times 10^{-4}$ *M* K_2SO_4, 34 mgm./l.; $1 \cdot 25 \times 10^{-2}$ *M* $Mg(NO_3)_2.6H_2O$, 3,200 mgm./l. All the elements in the special medium were approximately at the critical concentration except that magnesium was ten times more concentrated.

Table 1. COMPARATIVE GROWTH OF *Chlorella pyrenoidosa* IN THE SPECIAL MEDIUM AND IN THE SPECIAL MEDIUM WITH VARIOUS ANION SUPPLEMENTS

Medium	Relative yield* (per cent)
Warburg and Burk (with sodium chloride)	100
Warburg and Burk (without sodium chloride)	102
Special	25
Special + 500 mgm. $MgSO_4.7H_2O$/100 ml.	64
Special + 250 mgm. KH_2PO_4/100 ml.	84
Special + 200 mgm. sodium chloride/100 ml.	122
Special + 352 mgm. sodium bromide/100 ml.	100
Special + 144 mgm. sodium fluoride/100 ml.	21
Special + 513 mgm. sodium iodide/100 ml.	4

* Based on packed cell volume

The special medium was found to be seriously inadequate (Table 1). The growth of *Chlorella pyrenoidosa* in the special medium was only 25 per cent of its growth in the regular Warburg and Burk medium[5]. The cultures were bubbled with 5 per cent carbon dioxide in air, were kept at a temperature of 25° C., and were illuminated with about 1,000 ft. c. of fluorescent light. In an attempt to improve the growth of *Chlorella*, in the special medium, separate additions of magnesium sulphate, potassium dihydrogen phosphate and sodium chloride were made to successive aliquots of medium in such a way as to make the nutrient the same strength as in the Warburg and Burk medium. Each of the nutrients improved the growth of *Chlorella*, but the greatest improvement occurred with the addition of sodium chloride. Afterwards, it was shown that sodium bromide was almost as effective as sodium chloride, and that sodium fluoride and sodium iodide were quite ineffective. The Warburg and Burk medium has consistently shown no difference in the yield of *Chlorella pyrenoidosa* with or without the addition of sodium chloride to the medium. The improvement of the special medium was really an anion effect rather than a specific chloride effect, inasmuch as growth was stimulated by sulphate and phosphate as well as by chloride.

A logarithmic gradient of sodium chloride concentrations superimposed upon the special medium showed: (1) that the amount of growth of *Chlorella* could be doubled with the addition of merely 0·02 mgm./100 ml. ($3 \cdot 4 \times 10^{-6}$ *M*); (2) that the critical concentration for sodium chloride was about 200 mgm./100 ml. ($3 \cdot 4 \times 10^{-2}$ *M*); (3) that the optimum additions of sodium chloride produced a medium which was fully 25 per cent better than the Warburg and Burk medium.

The role of sodium in the growth of *Chlorella pyrenoidosa* seemed to have been ruled out when it was found that a supplement of 250 mgm. sodium dihydrogen phosphate/100 ml. special medium produced a growth increase of only 40 per cent in contrast to the 400 per cent increase produced by 200 mgm. sodium chloride/100 ml. special medium. However, it became quite puzzling when equimolar potassium chloride (255 mgm./100 ml.) brought about increases of no more than 150 per cent.

A medium was formulated whereby it was possible to investigate the effect of sodium on the autotrophic growth of *Chlorella pyrenoidosa*. 2,550 mgm. potassium chloride/l. were added to the special medium in