Table 1. THE NUMBER OF RATS SHOWING RETARDED HEALING OF BURNS IN AN AREA OF SKIN IRRADIATED WITH 1,600 AND 2,400 r.

Time thermal burn given after X-rays (weeks)	No. of rats	Days after branding 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total observed differences
		1,600 r.																				
0	10	0	0	0	0	2	2	4	7	10	6	4	2	2	3	4	3	3	1	0	0	53
1	10	0	0	0	0	2	5	7	9	8	7	4	2	1	1	1	1	0	0	0	0	48
2	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		2,400 r.																				
0	10	0	0	0	0	0	3	5	8	10	10	9	8	6	6	5	4	2	1	1	1*	79
1	10	0	3	4	6	8	7	3	3	5	7	6	6	5	5	5	5	3	1	0	0	82
2	10	0	0	0	0	0	0	0	0	1	2	2	2	2	1	0	0	0	0	0	0	10
3	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* 0 at 21 days

burns in the two areas were made over a 20-day period for each animal and for each brand separately.

The degree of healing in both the X-irradiated and non-irradiated burned areas was determined using as criteria the degree of inflammation, width of the brands, and presence of scar tissue. In no case did the burns heal more quickly in the irradiated area.

Healing was retarded when branding was done up to one week after irradiation with 1,600 r.; but not when done two and three weeks later (Table 1). With 2,400 r., retardation was observed with animals branded up to two weeks after irradiation, although this represented only a small proportion of the animals branded. These lingering effects seemed to coincide with the length of time that erythema caused by the X-irradiation itself was visible.

Although there was only a slight difference between 1,600 and 2,400 r. with respect to the lingering effect of radiation, there was a pronounced difference in intensity of the injury. This is indicated by the number of cumulative healing differences recorded during a 20-day period following branding which was greater with 2,400 r. than with 1,600 r. (Table 1). In addition the proportion of brands (there being three brands per animal) on individual rats showing retarded healing was twice as large with 2,400 r. as with 1,600 r.

Where retardation of the healing of burns was observed, the maximum difference in healing under most conditions was observed 8–10 days after the burns were inflicted. This coincided with complete healing of the burns on the non-irradiated area of skin. In Table 1, therefore, the period of time in which differences were recorded represents the healing time of burns on the irradiated side. This latter is 16–20 days or about twice the time taken for healing on the control side. This is similar to that recorded by Hawkins and Clark[1] using suberythemal doses of X-rays and heat. Merwin and Hill[4] using a different criterion of healing found that the revascularization of small burns, which were inflicted immediately after irradiation, was retarded about four times the normal period (7 days) with doses of 1,500 r.

In one case where the thermal burns were inflicted one week after 2,400 r., two maxima of healing difference were observed, at 5 days and 10 days after branding. The second maximum was due to recurrence of differences already recorded in the 5-day maximum. It is assumed that this effect resulted from varied rates of healing among the three brands.

We conclude that X-rays do not retard the healing of burns inflicted beyond one to two weeks after irradiation with the doses used and that radiation effects, when present, delay healing to twice its normal time.

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Effect of Gibberellic Acid on the Growth of Protonemata in *Splachnum ampullaceum* (L.) Hedw.

Stowe and Yamaki[1] wrote, in a recent review, of the work on the physiological action of the gibberillins: "Reminiscent of the auxins, no convincing action of the gibberillins has yet been shown on any organisms except higher plants". In the course of studies on protonematal regeneration and growth in the moss *Splachnum ampullaceum* (L.) Hedw., the effect of gibberellic acid on the growth of the protonemata was also tested.

Leaves of comparable age and position in the gametophore were isolated and planted under sterile conditions on Beijerinck's inorganic medium with 1 per cent agar. They were kept under artificial illumination with fluorescent (day-light) lamps. The chemical, which was obtained from the Nutritional Biochemical Corporation, Cleveland, Ohio, contains the potassium salt of gibberellic acid at 7·2 per cent. The protonemata regenerating from the isolated leaf tend to grow radially from the leaf, so that the tips of the protonemata form a circle around the leaf (Fig. 1). The radius of this circle formed by the longest protonemata was taken as a measure of protonematal length. Measurements of protonematal length were taken one month after planting.

Table 1 and Fig. 1 show the influence of gibberellic acid on protonematal growth. To establish the relative contribution of cell division compared with cell enlargement in this increase in the length of the protonemata, the following ratios were used:

$$\frac{\text{Radius of system at 1,000 } \mu\text{gm./ml.}}{\text{Radius of system at 0·0 } \mu\text{gm./ml.}} = \frac{6·981}{1·152} = 6·07$$

and

$$\frac{\text{Cell length at 1,000 } \mu\text{gm./ml.}}{\text{Cell length at 0·0 } \mu\text{gm./ml.}} = \frac{0·1116}{0·0477} = 2·34$$

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.

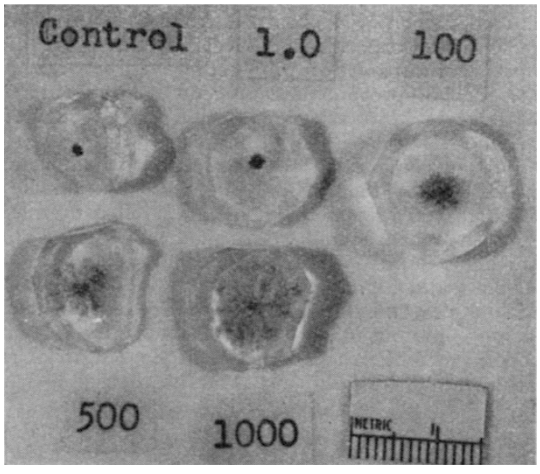

Fig. 1. Effect of gibberellic acid on the length of protonemata (1 month after planting)

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