

but French bean seedlings grown in water culture secreted material with gibberellin-like activity into the culture solution for at least two weeks after germination. At least 0.01  $\mu\text{gm.}$  gibberellic acid equivalent per plant was detected. Runner beans, peas, wheat and radish secreted smaller quantities into the culture medium.

These results suggest that the nodule-inhibiting factor produced by nodules and root tips is probably a gibberellin, and this may diffuse out into the surrounding medium in some circumstances.

The plants inoculated with *Rhizobium* in water culture were provided by Mr. W. K. Smith.

<sup>1</sup> Nutman, P. S., *Ann. Bot.*, N.S., **16**, 79 (1951).

<sup>2</sup> Nutman, P. S., *Ann. Bot.*, N.S., **17**, 95 (1953).

<sup>3</sup> Nutman, P. S., *Ann. Bot.*, N.S., **21**, 321 (1957).

<sup>4</sup> Turner, E. R., *Ann. Bot.*, N.S., **19**, 149 (1955).

<sup>5</sup> Galston, A. W., *Nature*, **183**, 545 (1959).

<sup>6</sup> Thurber, G. A., Douglas, J. R., and Galston, A. W., *Nature*, **181**, 1082 (1958).

<sup>7</sup> Fletcher, W. W., Alcorn, J. W. S., and Raymond, J. C., *Nature*, **184**, 1576 (1959).

<sup>8</sup> Radley, M., *Chem. and Indust.*, 877 (1959).

### Seaweed (*Fucus vesiculosus*)

ALTHOUGH gibberellin-like substances have been found in many species of flowering plants, none has yet been reported from lower plants, apart from the fungus *Gibberella fujikuroi* and the yeast *Candida pulcherrima*<sup>1</sup>. Nevertheless, there are reports of a response to applied gibberellin in ferns<sup>2,3</sup>, a moss<sup>4</sup>, a liverwort<sup>5</sup> and green algae<sup>6,7</sup>, suggesting that gibberellins may play some part in regulation of growth. A substance with gibberellin-like activity has now been shown to occur in a seaweed.

4,000 gm. of *Fucus vesiculosus* was collected and stored at  $-18^{\circ}\text{C}$ . For the first experiment 500 gm. was extracted with ethyl alcohol for 18 hr. at room temperature. The alcohol was removed by filtration and pressing, and the tissues were re-extracted with fresh 70 per cent aqueous alcohol for 4 hr. The alcoholic solutions were combined and were then treated as previously described<sup>8</sup>. The purified extracts were applied in alcohol to the leaves of dwarf pea seedlings of the variety Meteor. Three doses of the extract were compared with known amounts of gibberellic acid. This test was carried out in duplicate, one batch of plants being grown in a greenhouse in the natural lighting conditions of July, and the other batch grown under continuous artificial light only at a temperature of  $15^{\circ}\text{C}$ . The results are given in Table 1.

Table 1. GIBBERELIC ACID EQUIVALENT ( $\mu\text{GM./KGM. FRESH WEIGHT}$ )

Fresh weight equiv. of extract (gm.) per plant	Bioassay conditions	
	Greenhouse	Artificial light
50	7.2	0.76
10	12.0	2.1
2.5	6.8	5.2

Thus all extracts promoted growth considerably; this is evidence for the presence of gibberellin-like substances. The relative decrease in activity in the higher doses tested, when the plants were receiving artificial illumination, suggested that inhibitors might be interfering with the response.

The remaining 3,500 gm. of material was then extracted twice with alcohol, filtering but not pressing the tissue each time, and the alcoholic solution was concentrated in a climbing film evapora-

tor. The acidic fraction of this was adsorbed on to a column of charcoal/Celite' (5 : 1), and was eluted with water containing increasing amounts of acetone. Six fractions were recovered from the aqueous acetone and were dissolved in ethyl alcohol.

In a preliminary series of bioassays aliquots of all eluates were concentrated and applied to dwarf pea plants. Gibberellin-like activity was found only in the 50 per cent acetone eluate. In a similar experiment carried out under artificial light in which 1.0  $\mu\text{gm.}$  gibberellic acid was added to each eluate it was found that the 90 per cent acetone eluate (an aliquot equivalent to 100 gm. of tissue) reduced the response to gibberellic acid to about half that expected; there was no evidence for the presence of an inhibitor in the 50 per cent acetone eluate.

The 50 per cent acetone eluate was then bioassayed at three dosage-levels (Table 2). The lower level of activity found, compared with that recorded in Table 1, may have been due to loss in storage or to less-efficient extraction. Part of this eluate was chromatographed on paper in *n*-butanol/1.5 *N* ammonia (3 : 1). The paper was divided into 10 parts, eluted with alcohol and bioassayed on dwarf peas. All the activity was found between  $R_F$  0.3 and 0.4. Under these conditions the  $R_F$  of gibberellic acid was 0.38 and that of gibberellin  $A_5$  was 0.59. The other known gibberellins with a similar  $R_F$  to gibberellic acid are gibberellins  $A_1$  and  $A_6$  (ref. 9); thus the active material present in *Fucus vesiculosus* may be one of these.

Table 2. GIBBERELIC ACID EQUIVALENT ( $\mu\text{GM./KGM. FRESH WEIGHT}$ )

Fresh weight equiv. of extract (gm.) per plant	Bioassay conditions	
	Greenhouse	Artificial light
125	0.45	0.36
25	0.52	0.40
4	trace	trace

Chromatograms of the 90 per cent acetone eluate were run in the same solvent system. An inhibitory substance present at  $R_F$  0.6-0.7 was demonstrated by a lettuce germination test and by the lettuce seedling assay<sup>10</sup> in which the response to gibberellic acid was reduced. On the other hand, there was no significant reduction of the response of dwarf peas to gibberellic acid, and it is not clear whether this is in fact the substance causing the reduction in gibberellin response in the earlier experiments. Inhibitor  $\beta$ , which is found in many higher-plant tissues, has a similar  $R_F$  in this solvent system<sup>11,12</sup>. An inhibitor of gibberellin-induced growth found in seed extracts has been described by Corcoran and Phinney<sup>13</sup>. Its mobility in this solvent system is not known.

<sup>1</sup> Krassilnikov, N. A., et al., *Doklady Akad. Nauk SSSR*, **123**, 1124 (1959).

<sup>2</sup> Knobloch, I. W., *Amer. Fern J.*, **47**, 134 (1957).

<sup>3</sup> Wardlaw, C. W., and Mitra, G. C., *Nature*, **181**, 400 (1958).

<sup>4</sup> Mitra, G. C., and Allsopp, A., *Nature*, **183**, 974 (1959).

<sup>5</sup> Asprey, G. F., Benson-Evans, K., and Lyon, A. G., *Nature*, **181**, 1351 (1958).

<sup>6</sup> Conrad, H., Saltman, P., and Eppley, R., *Nature*, **184**, 556 (1959).

<sup>7</sup> Provasoli, L., *Biol. Bull.*, **113**, 321 (1957).

<sup>8</sup> Radley, M., *Chem. and Indust.*, 877 (1959).

<sup>9</sup> MacMillan, J., Seaton, J. C., and Suter, P. J., 138th Meeting of the Amer. Chem. Soc. (1960), *Advances in Chemistry*, **28**, 18 (1961).

<sup>10</sup> Frankland, B., and Wareing, P. F., *Nature*, **185**, 255 (1960).

<sup>11</sup> Luckwill, L. C., *Nature*, **169**, 375 (1952).

<sup>12</sup> Kefford, N. P., *J. Exp. Bot.*, **6**, 129 (1955).

<sup>13</sup> Corcoran, M. R., and Phinney, B. O., *Plant Physiol.*, **33**, Supp. xl (1958).