II. ACCESSORY FACTORS FOR PLANT GROWTH.

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In his endeavours to find a suitable medium for the growth of the nitrogen-fixing bacteria of the "Azotobacter" group, Prof. Bottomley observed that although these organisms failed to grow in natural peat, they flourished when grown in sphagnum peat which had previously been "rotted" by the agency of ordinary aërobic soil organisms. Sterilised rotted peat, inoculated with a mixed culture of nitrogen-fixing bacteria was subsequently found to produce striking results in the development of pot grown plants, results which could not be ascribed to the manurial contents of the treated peat nor to the beneficial effect of the development of the nitrogen-fixing bacteria. It was found later, indeed, that the presence of these bacteria was not an essential factor. Prof. Bottomley was at a loss to explain these results, which had been confirmed by impartial experienced horticulturists, and in discussion with me suggested an examination of the chemical composition of the treated peat.

Before entering into this necessarily lengthy investigation I desired to convince myself of the activity of the material. In order to eliminate a possible beneficial effect on the soil porosity, etc., due to the increased water-retaining power of the peat-soil mixture, I decided to use an aqueous extract of the material. Although the actual manurial properties of the treated peat are small and its contents of nitrogen, phosphorus and potassium are low as compared with any ordinary fertiliser, I purposely chose an amount of the extract which should exclude its possible manurial effect.

As it was hardly to be expected that the small quantity used in the first experiment could produce any effect, it was my original intention gradually to raise the amount until the minimal effective dose was reached. The surprising result of the experiment to be described, however, made this unnecessary

and led me to the assumption that a substance is present in the treated peat which plays a rôle in plant growth similar to that played by the now generally accepted food accessories in animal growth. Owing to various causes I had no opportunity of continuing this work, and as my experiments, which formed the starting point of Prof. Bottomley's subsequent researches, were necessarily only briefly alluded to by him, a somewhat fuller account seems desirable at this stage.

My experiments were begun in the late autumn of 1913, a season unfavourable for plant growth under ordinary conditions and for that reason more likely to show the effect of any stimulating agent. The only plants I had available at that time for experiments were a number of seedlings of *Primula malacoides*. It was recognised that this primula is by no means an ideal plant for this work, as it is well known to practical gardeners that a considerable variation in its character and rate of growth may occur. An experimental error due to this cause was guarded against by selecting from a large batch of seedlings a considerable number of plants of apparently equal development and by beginning the treatment only at such a relatively advanced stage of their growth that any inequality in their character would have been noticeable. The seedlings were raised during the summer, 1913, and a large number of them pricked out into boxes. Some of the plants were potted up on Oct. 5th in ordinary potting compost (loam, leaf-mould and sand). Shallow porous pots, so-called dwarf alpine pots, of uniform size (12.5 cm. diam. and 500 cc. capacity) were used. A layer of 1.5 cm. washed granite chips served as drainage. On Oct. 24th a series of twelve plants were selected which were as far as possible in a uniform stage of development. Six of these were used as controls, whilst the other six were watered with 60 cc. each of the diluted peat extract (see below). The plants were kept under the ordinary conditions in an unheated greenhouse. During the course of the experiment the outside temperature fell only on a few occasions below freezing point, without affecting the development of the plants.

No difference could be detected between the treated and untreated plants during the first three weeks, and therefore on Nov. 15th three of the treated plants received another dose of 60 cc. of the extract¹. During the fifth week, however, the difference became very marked indeed. The treated plants not only grew considerably taller, but showed a remarkable luxuriance

¹ It may be mentioned that this second treatment did not produce any additional beneficial effect, an observation which speaks in favour of the view of "accessories."

of growth. They were of a much deeper green and had larger leaves. They produced flower stems in greater abundance and also the size of the individual flowers and the number of whorls was increased. One each of the plants of each series was photographed on Dec. 7th, Pl. 1, Fig. 1, 14th, Fig. 2, and 26th, Fig. 3, and the last photograph shows clearly the increased development of the root system which corresponds to the increase in the foliage. The effect was uniform on all the plants of the series and the control plants also were very uniform in their behaviour.

For the preparation of the extract 15 g. of treated peat, which was kindly put at my disposal by Prof. Bottomley, was left standing for 18 hours with 2 litres of tapwater. 500 cc. of the brown clear solution were decanted and diluted to 1250 cc. The extract contained:

0.045 % Total Solids,	0.011 % Inorganic Substances,
0.034 % Organic Substances,	0.003(2) % Nitrogen.

The plants received therefore in one application the extract of 0.18 g. of treated peat containing 20 mg. of organic matter and 1.9 mg. of nitrogen, i.e. roughly 4 per million nitrogen to soil.

The effect produced on plant growth by this small quantity of soluble substances suggested to me an analogy to that due to "vitamines" or accessory food substances in animal growth and led me to investigate the behaviour of the peat towards reagents which are known to dissolve or precipitate "vitamines." Some of these substances are soluble in alcohol and are precipitated, or carried down in an adsorbed condition, by phosphotungstic acid.

Extracts were made for comparison from garden soil, natural peat and treated peat by allowing 15 g. of each of the materials to stand for one hour with 100 cc. of water. To 20 cc. of each filtrate 2.5 cc. conc. H_2SO_4 in 10 cc. water was added and the volume made up to 50 cc. A brown floccular precipitate of humic acid formed in the treated peat extract and was filtered off. Equal volumes of a 30 % phosphotungstic acid solution were added to each filtrate. The extract from treated peat gave an immediate copious precipitate, whilst that of natural peat only gave a faint opalescence and the extract of garden soil remained clear.

Extracts were made from the materials in a similar way with boiling alcohol or acetone. The solvent was evaporated and the residue taken up with water. Again a heavy precipitate formed on adding phosphotungstic acid to the extract of the treated peat. It was further noted that a considerable part of the phosphotungstic acid precipitate was insoluble in acetone,

a fact which may be of use for the isolation of the active substance [see Funk, 1916].

It had been suggested by Funk and Macallum [1913] that the blue colour reaction which is given by Folin and Macallum's uric acid and phenol reagents, may serve as an index for the presence of "vitamines" [see also Williams and Seidell, 1916]. I was unable to test the original extract used for these experiments in this direction. A fresh supply of treated peat, whose activity on plants had not, however, been tested and was considered doubtful, did not give the uric acid reaction, but developed a blue colour with the phenol reagent. Prof. Bottomley has since stated that active preparations show the uric acid reaction distinctly.

The chemical examination of the treated peat extract lends therefore strong support to my view which was arrived at from its effect on plant growth and demonstrated the presence of substances similar to the "vitamines" in their general behaviour. At my suggestion the action on plant growth of alcoholic extracts and the fractions obtained from them by phosphotungstic acid were tested in Prof. Bottomley's laboratory. It was found that the active substances were contained in the alcoholic extract and its sub-fractions, and that in minute quantities they exerted a remarkable stimulating effect on plant growth (Bottomley, 1915, 1, 2).

As far as the activity of the treated peat is concerned these observations therefore supply a working hypothesis which is being further tested experimentally. They suggest besides many interesting problems regarding plant nutrition in general, and I am at present engaged in some experiments on the effect on plant growth of the substances contained in yeast and milk, which are known to possess growth promoting properties in animals.

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Fig. 3

Fig. 2

Fig. 1