Some Accessory Factors in Plant Growth and Nutrition.

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Recent research has demonstrated the importance of the presence of minute amounts of certain substances as accessory factors in normal dietaries of man and animals. The most striking examples of the influence of these substances are seen in their curative effect on the diseases of beri-beri and scurvy, and their stimulative effect on the growth of young animals.

Beri-beri is caused by the deficiency in a diet of polished rice of a nitrogenous substance, small amounts of which are essential for the metabolism of the nervous system. The curative substance is found in rice husks, barley, wheat, lentils, yeast, egg-yolk, milk, etc., and is precipitated from an aqueous solution of an alcoholic extract of these bodies by phosphotungstic acid. It is effective in very minute amounts, an addition to the diet of 0·02 grm. of the active fraction of the extract curing polyneuritis (beri-beri) in pigeons.

Scurvy also is caused by a diet adequate as regards proteins, carbohydrates and fats, but deficient in some constituent, small amounts of which are essential. This anti-scorbutic substance is found in lime-juice, fresh vegetables and fruits, and, like the curative substance of beri-beri, is precipitated by phosphotungstic acid.

The special importance of small amounts of substances of unknown composition in the metabolism of growing animals has been demonstrated by the recent researches of Osborne and Mendel* and Hopkins.† These investigations have shown that young rats, fed on a diet consisting of a mixture of pure proteins, carbohydrates, fats, and inorganic salts, failed to grow, but on the addition of a very small amount of certain substances obtained from milk growth was normal. Hopkins found that the fraction obtained from a phosphotungstic acid precipitation of proteid-free milk contained the active substance and gave excellent growth results. As a result of his experiments he states that "the presence of minute traces of certain

* Osborne and Mendel, Carnegie Institution Publication No. 156, Parts I and II, 1911.

† F. G. Hopkins, 'Journ. Physiol.,' vol. 44 (1912).

organic substances are, without doubt, essential for the proper nutrition of growing animals."

Very little is known as to the nature and composition of these substances. Unfortunately, the active substance appears to be largely destroyed by chemical manipulations, and it is difficult to obtain sufficient to study its chemical constitution and properties. Funk,* by a complex fractionation of the phosphotungstic precipitate of anti-beri-beri substance, succeeded in isolating a substance, melting at 233° C., which in amounts of 0·02 to 0·04 grm. cured polyneuritis in pigeons. This substance he considered to be of the nature of a pyrimidine base. Hopkins, however, states that the additions in his growth experiments were free from amino-acids, purine and pyrimidine bases. It is possible that these substances belong to a new group of nitrogenous compounds, which exist only in small amounts in food materials, but are so extremely active that minute quantities are sufficient to supply the needs of the organism.

Although these substances have been found to occur chiefly in plants, there is no record of any investigations concerning the part, if any, they play in the metabolism of the plant itself.

During the summer of last year (1913) a number of experiments were made at the Royal Gardens, Kew, on a series of plants, to test the manurial value of Sphagnum peat which had been incubated with a mixed culture of aërobic soil organisms for a fortnight at a temperature of 26° C. It had been discovered that by this bacterial treatment the humic acid in the peat is converted into soluble humates, and this bacterised peat, after sterilisation, forms an excellent medium for the growth and distribution of nitrogen-fixing organisms. As the experiments progressed it was evident that, in addition to the ordinary plant-food constituents, there was present in the bacterised peat a substance which stimulated growth in a remarkable manner. Further experiments showed that this substance was soluble in water, and was effective in very small quantities. Dr. Rosenheim, of King's College, found that seedlings of *Primula malacoides* potted up in loam, leaf-mould and sand, and treated twice with a water extract of only 0·18 grm. of bacterised peat, were, after six weeks' growth, double the size of similar untreated plants, and it was noted that flower production and root development were promoted equally with increase of foliage.

These results suggested that the growth-stimulating action of the bacterised peat might be due to the presence of a substance or substances similar in nature to the accessory food bodies concerned in animal nutrition.

* C. Funk, 'Journ. Physiol.,' vol. 45 (1912–1913).

These accessory substances essential to animal nutrition are known to be soluble in water and alcohol, and, in order to ascertain as rapidly as possible whether there are present in the bacterised peat such water- and alcohol-soluble substances which have a similar effect on plant growth, an experiment was made to test the effect of an aqueous extract of the alcohol-soluble material of the bacterised peat on the growth and fixation of nitrogen by *Azotobacter chroococcum.*

The bacterised peat was extracted with absolute alcohol in a shaking machine for three hours, and the extract evaporated to dryness *in vacuo.* The residue was taken up in warm distilled water, the liquid filtered, and the clear filtrate diluted until it contained the extract of 10 grm. of peat per litre. Portions consisting of 100 c.c. of this liquid were then transferred to each of 12 conical flasks, and the contents of six of the flasks boiled briskly over a Bunsen burner for five minutes; 100 c.c. portions of distilled water were placed in each of six similar flasks; to each of the 18 flasks of the series were added 1 grm. mannite, 0·2 grm. K_2HPO_4, 0·02 grm. $MgSO_4$, and 0·2 grm. $CaCO_3$, and each was inoculated with 1 c.c. of a uniform suspension of *Azotobacter chroococcum.* The contents of two flasks from each of the three series of six were analysed at once to serve as controls, while the remaining four of each series were incubated for eight days at 26° C., at the end of which period they were analysed by the Kjeldahl process for their nitrogen content. The results are given in the following Table :—

Table I.

Series.	Nitrogen content.			Nitrogen fixation.	Mean nitrogen fixation.
		mgrm.		mgrm.	mgrm.
I. Complete food	1. Control	0·4	Mean, 0·4 mgrm.		
	2.	0·4			
	3. Culture	4·6		4·2	
	4.	4·4		4·0	3·8
	5.	3·6		3·2	
	6.	4·4		4·0	
II. Complete food + alcoholic extract of bacterised peat	1. Control	2·6	Mean, 2·5 mgrm.		
	2.	2·3			
	3. Culture	20·7		18·2	
	4.	20·5		18·0	18·0
	5.	19·9		17·4	
	6.	20·9		18·4	
III. Complete food + boiled alcoholic extract of bacterised peat	1. Control	2·3	Mean, 2·4 mgrm.		
	2.	2·5			
	3. Culture	19·6		17·2	
	4.	19·0		16·6	17·2
	5.	20·6		18·2	
	6.	19·4		17·0	

The more rapid growth of the organism in Series II and III was rendered apparent by the fact that a scum was visible on the surface of the liquid in each flask of these series after 24 hours, while the pellicle formed in Series I only after the lapse of 72 hours.

The results obtained indicated clearly that there is present in the bacterised peat a substance which stimulates plant growth, and that this substance is of a fairly stable nature is shown by the fact that almost as good results were obtained with the extract which had been boiled for five minutes as with the unboiled extract.

In order to test whether the active substance is present as such in the original peat, or whether it is produced in the bacterised peat as a result of treatment, an extract of the raw peat was made in precisely the same manner and in the same concentration as described for the bacterised peat. Two series of cultures were prepared, the one containing complete food substances in distilled water, the other complete food in alcoholic extract of raw peat. The controls were analysed at once, while the cultures were incubated for eight days at 26° C. as before. No increased growth was apparent in the cultures containing alcoholic extract of raw peat, while the results of analysis, as given below, indicate the absence of any stimulating substance:—

Table II.

Series.	Nitrogen content.	Nitrogen fixation.	Mean nitrogen fixation.
	mgrm.	mgrm.	mgrm.
I. Complete food	1. Control 0·3 } Mean, 0·4 mgrm.		
	2. 0·4		
	3. Culture 3·8	3·4	
	4. 3·9	3·5	3·5
	5. 3·8	3·4	
	6. 4·0	3·6	
II. Complete food + alcoholic extract of raw peat	1. Control 2·4 } Mean, 2·6 mgrm.		
	2. 2·8		
	3. Culture 4·0	1·4	
	4. 4·6	2·0	1·9
	5. 4·6	2·0	
	6. 4·8	2·2	

The active substance is evidently produced in the bacterised peat as a result of treatment, and since this treatment consists essentially in the production of soluble humates by bacterial action, a test was made to ascertain whether the chemical production of soluble humates would be equally effective. Two equal portions of raw peat were saturated with solutions containing 1 per cent. of their weight of sodium carbonate, and were stirred at frequent intervals for

several hours. One portion was allowed to dry slowly at room temperature, an alcoholic extract taken and evaporated *in vacuo* as before, the residue being made up in aqueous solution to a concentration of 10 grm. of carbonated peat per litre. The other portion was leached with water until the washings were colourless, the liquid filtered, and the aqueous extract thus obtained was evaporated *in vacuo* to dryness. The residue was extracted with alcohol, and the alcoholic solution again evaporated to dryness *in vacuo*, the residue being taken up with water, filtered, and the clear filtrate diluted to the proportion of the extract of 10 grm. of the original peat per litre. The effect of both these extracts was tested on Azotobacter, three series of cultures being incubated—one containing complete food in distilled water, the second complete food in alcoholic extract of carbonated peat, and the third complete food in alcoholic extract of water-soluble substances from carbonated peat. Again no appreciable effect was observed on the growth of the cultures, while the results of the analyses as given below failed to reveal any stimulation of the organism:—

Table III.

Series.	Nitrogen content.			Nitrogen fixation.	Mean nitrogen fixation.
		mgrm.		mgrm.	mgrm.
I. Complete food..............	1. Control	0·4	Mean, 0·5 mgrm.		
	2.	0·5			
	3. Culture	4·8		4·3	
	4.	4·0		3·5	4·0
	5.	4·4		3·9	
	6.	4·8		4·3	
II. Complete food + alcoholic extract of carbonated peat	1. Control	2·2	Mean, 2·2 mgrm.		
	2.	2·2			
	3. Culture	4·6		2·4	
	4.	5·0		2·8	2·4
	5.	4·4		2·2	
	6.	4·6		2·4	
III. Complete food + alcoholic extract of water-soluble substances from carbonated peat	1. Control	1·9	Mean, 1·8 mgrm.		
	2.	1·7			
	3. Culture	4·4		2·6	
	4.	4·2		2·4	2·7
	5.	4·6		2·8	
	6.	5·0		3·2	

The results thus far obtained tend to prove that the active stimulant of plant growth which is present in bacterised peat does not exist as such in the raw peat, nor can it be liberated by a chemical production of soluble humates. It has been obtained only as a result of bacterial action.

Cooper and Funk* in 1911 showed that their curative substance was

* Cooper and Funk, 'Lancet,' p. 1267 (1911).

entirely precipitated by phosphotungstic acid from an aqueous solution of the dry residue from the alcoholic extract of rice polishings, and Hopkins* also states that he obtained the best results upon growing rats with the fraction from the crude phosphotungstic acid precipitate of protein-free milk. Consequently an experiment was made to determine how far the phosphotungstic acid fraction of the bacterised peat extract was effective in stimulating plant growth. The bacterised peat was extracted with absolute alcohol as described above, and the alcohol evaporated off *in vacuo*. The residue was taken up in water, filtered, and to the filtrate sulphuric acid was added, until the concentration of the latter reached 5 per cent. A slight precipitate of humic acid was filtered off, and to the filtrate an excess of 30-per-cent. solution of phosphotungstic acid was added. The whole was then left to stand overnight, when the liquid was decanted off through a filter, the precipitate repeatedly washed with a 5-per-cent. solution of sulphuric acid, and finally decomposed with an excess of baryta. The liquid was filtered off from the precipitate of barium phosphotungstate, and the filtrate, freed from the last traces of baryta by means of a very dilute solution of sulphuric acid, was evaporated to dryness *in vacuo*. From 7 kgrm. of bacterised peat the amount of dry substance obtained from the phosphotungstic acid fraction amounted to 12·0096 grm., and since this was made up for experimental purposes into a solution containing the fraction from 10 grm. of peat per litre, the proportion of the dry phosphotungstic acid fraction in the final solution employed consisted of 17 parts per million. This fraction was tested upon wheat-seedlings in conjunction with Detmer's complete food solution. Ten seeds were germinated in clean sand in each of nine pots, which were arranged in three series of three pots each. Series I was treated with a complete food solution, Series II with complete food plus alcoholic extract from 10 grm. of peat per litre of solution, and Series III with complete food plus phosphotungstic fraction from 10 grm. of peat per litre of solution. The food solution employed contained nitrogen, phosphorus and potash, estimated as NH_3, P_2O_5, and K_2O in the proportion of 400, 200, and 1220 parts per million respectively, so that in addition Series III had 17 parts per million of dry substance obtained from the phosphotungstic fraction. Each pot was treated with 100 c.c. of its solution one week after sowing the seed, and the treatment repeated once weekly for five weeks, at the end of which period the plants were uprooted, washed, dried, and weighed. The results were as follows:—

* Hopkins, 'Brit. Med. Journ.,' vol. 2, p. 463 (1913).

Table IV.

Series.	Weight of 30 plants.	Increase over Series I.
	grm.	per cent.
I. Complete food solution	11·94	—
II. " " " + alcoholic extract	14·46	21·1
III. " " " + phosphotungstic fraction	15·45	29·4

The results thus obtained indicate that the stimulative substance in bacterised peat is precipitated by phosphotungstic acid, and that this phosphotungstic fraction is quite as effective as the original alcoholic extract of the peat. Funk* found that, upon further fractionation of his phosphotungstic acid precipitate with silver nitrate and baryta and elimination of the reagents, he obtained a relatively pure crystalline substance, to which he gave the name "vitamine," and this he considered to be the specific curative substance. In order to determine how far the growth stimulant in bacterised peat resembled these so-called "vitamines," a further fractionation was carried out along the lines described in his paper. The phosphotungstic acid precipitate was decomposed, as before described, with baryta, and the last traces of baryta eliminated by means of sulphuric acid. To the filtrate from the barium salt silver nitrate was first added, and then baryta, until no further precipitate was produced. The brownish precipitate was filtered off, well washed, suspended in dilute sulphuric acid, and decomposed with sulphuretted hydrogen. The filtrate from the silver sulphide was then exactly neutralised with baryta, the clear liquid filtered off from the precipitate of barium sulphate, and evaporated to dryness *in vacuo*. The weight of dry substance obtained from the silver fraction from 7 kgrm. of bacterised peat amounted to 0·2452 grm., and, since this also was made up for experiment into a solution containing the silver fraction from 10 grm. of peat per litre, this solution contained the dry substance from the silver fraction in the proportion of 0·35 part per million. This fraction was also tested, concurrently with the phosphotungstic acid fraction, upon wheat seedlings; 15 seeds were germinated in clean sand in each of nine pots, which were arranged in three series of three each. Series I was treated with complete food solution, containing nitrogen, phosphorus, and potash, estimated as NH_3, P_2O_5, and K_2O, in the proportion of 400, 200, and 1220 parts per million respectively. Series II was treated with a similar solution, containing in addition 17 parts per million of the phosphotungstic fraction,

* Funk, 'Journ. Physiol.,' vol. 45 (1912–1913).

and Series III with the complete food solution +0·35 part per million of the silver fraction. The pots were first treated one week after sowing the seed, and after that each pot received once weekly 100 c.c. of its food solution for seven weeks. At the end of that period the plants were washed, dried, and weighed, and, after the gross weight had been taken, the plants were all dried in the steam oven at 100° C. until their weight was constant. The results are as follows:—

Table V.

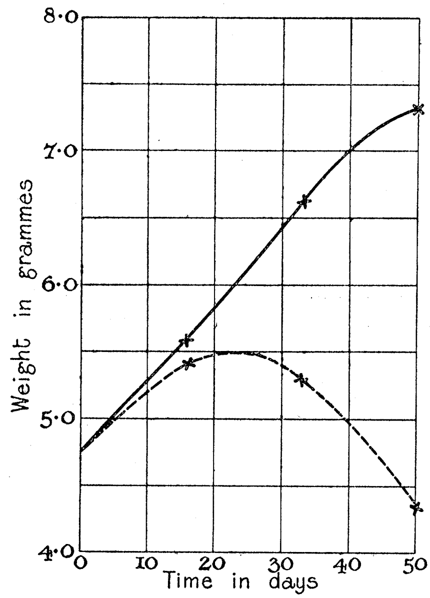
Series.	Gross weight, 45 plants.	Increase over Series I.	Dry weight.	Increase over I.
	grm.	per cent.	grm.	per cent.
I. Complete food	64·5	—	13·3480	—
II. „ „ + phosphotungstic fraction	96·8	50·0	16·3818	22·7
III. „ „ + silver fraction ...	96·5	49·6	15·7148	17·7

The silver fraction from the bacterised peat extract, corresponding with the "vitamine" fraction of Funk, having thus given results approaching those of the phosphotungstic fraction, a preliminary investigation was made to test its effect on the growth of wheat seedlings in water culture. Two sets, each consisting of 18 similar seedlings, were carefully selected, each set being originally of equal weight, viz., 4·73 grm. Each set was divided for purposes of water culture among three similar bottles of 200 c.c. capacity, six plants being inserted through notches in the corks of each bottle, so that the roots dipped into the culture solution. The three bottles of set I were filled with a nutrient solution of pure salts in physiologically pure distilled water, in which the proportions of NH_3, P_2O_5 and K_2O were 400, 200, and 1220 parts per million respectively; while those of set II contained a precisely similar solution which had received, in addition, 0·35 part per million of the silver fraction of bacterised peat extract. The bottles were aërated daily, and the solutions changed twice a week, while at the end of every 16 or 17 days the plants were taken from the jars, moisture removed from their roots by means of blotting paper, and weighed. The results obtained are shown in Table VI.

The change brought about by the addition of the silver fraction is represented by the accompanying curves, in which the dotted line represents the change in weight of the series in pure food, while the unbroken line shows the progressive increase in weight obtained upon the addition of this substance.

Table VI.

Series.	Weight of set of 18 plants.		Percentage increase on original weight.
		grm.	per cent.
I. Pure food solution	Original weight.........	4·73	—
	After 16 days............	5·42	14·7
	After further 17 days	5·29	11·8
	" " "	4·33	−8·4
II. Pure food solution + silver fraction from bacterised peat	Original weight.........	4·73	—
	After 16 days............	5·57	17·7
	After further 17 days	6·65	40·6
	" " "	7·33	54·9



Up to a certain point the two series of plants increased in weight to an almost equal extent, but beyond this point the seedlings growing in pure food solution appeared to be unable to utilise the food elements supplied to them; a condition which was apparently corrected by the addition of the silver fraction.

Experimenting with guinea-pigs in 1909, Fürst* demonstrated that seeds of barley, oats, peas and flax contained no curative substances for scurvy, but that during the germination of these seeds anti-scorbutic substances developed, which were quite as effective as extracts from green vegetables.

* Fürst, 'Zeitschr. f. Hyg. u. Infekt.,' vol. 72, p. 121.

These facts indicate the possibility of the development, during germination, of special growth substances which enable the young embryo to utilise the food material present in the seed. If this is so, the removal of the source of these growth stimulants by the cutting off of the seed as soon as possible after germination should render the effect of an addition of such substances in the food solution all the more marked. In order to test this hypothesis, two series of wheat seedlings, similar to those used above, but in a rather younger stage, were taken, and before the removal of their seeds the two sets were of equal weight, viz.: 3·97 grm. Their seeds were carefully removed, injury to the plants being avoided, and after this process the two sets weighed respectively 3·2 and 3·17 grm. These were treated in precisely the same manner as before, the first set being given complete food salts, and the second food salts with the addition of the silver fraction from bacterised peat. The weights of the two sets at various dates are shown in the following Table:—

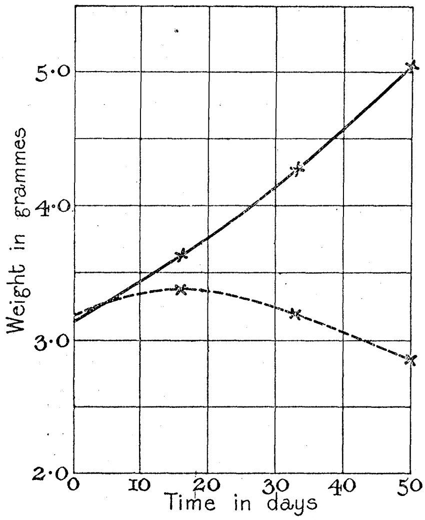
Table VII.

Series.	Weight of set of 18 plants.		Percentage of increase in weight.
		grm.	per cent.
I. Complete food solution	Original weight	3·2	—
	After 16 days	3·37	5·3
	After further 17 days	3·20	0·0
	" " "	2·85	−10·9
II. Complete food solution + silver fraction from bacterised peat	Original weight	3·17	—
	After 16 days	3·63	14·5
	After further 17 days	4·29	35·3
	" " "	5·05	59·3

The following diagram shows the variation in weight of the seedlings throughout the experiment, the dotted curve representing the series in pure food, while the unbroken curve shows the effect of the addition of the silver fraction.

These results indicate that during the germination of wheat seeds certain substances are formed which enable the young embryo to utilise the food materials present. The supply of these substances formed by the seed during germination is sufficient to establish the embryo as an independent seedling, then some other source is necessary. It has been shown that these accessory food substances are produced when peat—decayed vegetable matter—is acted upon by certain soil bacteria, and the natural inference is that during the bacterial decomposition of organic matter in the soil, that is, during humus formation, these substances are formed, hence the beneficial effect on crops of

farmyard and other organic manures. The specific action of these accessory substances is not known. They may be concerned in the metabolism of



phosphorus, they may act as catalytic agents, or may be a definite constituent of plant food—a "bau-stein."

Experiments to test these various hypotheses are in progress.

In conclusion I wish to acknowledge my indebtedness to Miss F. A. Mockeridge, B.Sc., for her valuable help in the chemical part of this investigation.