XXXVII. THE OCCURRENCE AND NATURE OF THE PLANT GROWTH-PROMOTING SUBSTANCES IN VARIOUS ORGANIC MANURIAL COMPOSTS.

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THE generally accepted idea of the nutrition of an ordinary autotrophic plant is that it requires only a supply of inorganic food materials, consisting of minerals, carbon dioxide and water, from which it is able to elaborate the whole of its organic substance. This conception of plant nutrition, which forms one of the main physiological distinctions between plant and animal, has been modified during recent years by the work of Bottomley, in which he has been assisted by the writer. Experimenting on the growth of green plants of *Lemna minor* in water culture, he has found [Bottomley, 1917, 1] that the addition to the inorganic nutrients of minimal quantities of certain organic substances is absolutely essential if the plants are to grow healthily and normally for any length of time. These organic substances, which have been termed "auximones" by Bottomley, are apparently similar in function, though not necessarily so in nature, to the "vitamines," the accessory growth substances, which play such an important part in animal nutrition.

The use of water-cultures affords practically the only means of absolutely controlling the food materials supplied to a plant, and water plants are obviously the best suited to such growth, since they normally grow with their roots submerged. This fact, together with their rapid multiplication by budding, afforded the reason for the choice of *Lemna minor* for the experiment; and the result of the experiments led to the suggestion that perhaps this plant was peculiar in requiring some organic addition to its nutrient solution. A number of other water-plants have since been tested in the same way, including *Lemna major*, *Salvinia natans*, *Azolla filiculoides*, and *Limnobium stoloniferum*, and these have all been found to grow healthily and multiply rapidly in the presence of a small quantity of organic material, while in inorganic materials alone they gradually died off [Bottomley, 1920, 2], and this was the case no matter what the nutrient solution employed [Bottomley, 1920, 1]. The well-known fact that ordinary plants will grow well in water culture is explained by the comparatively large quantity of

organic food introduced in their seed, and it was shown some time ago [Bottomley, 1914] that wheat seedlings with their endosperm removed at a very early stage responded very much more quickly to the addition of these organic materials than plants which were not so treated. A number of experiments, which will be reported in due course, have more recently been carried out with cuttings; and these also demonstrate the absolute necessity of organic materials for normal healthy growth, and explain the prevalent idea of the unsuitability of cuttings for water culture. All these experiments tend to the conclusion that all green plants require the presence of organic material of a special kind for their maximum growth and development.

An entirely different type of plant, a *Saccharomyces*, has recently been the subject of similar researches on the part of Williams [1919] and Bachmann [1919]. Williams arrives at the conclusion that "a substance of unknown nature...is necessary in addition to the ordinary nutrients for the nutrition of yeast cells," and the effect of this substance is so marked that he suggests that yeast may be used as a biological test for vitamins. He also concludes that this substance is identical with the animal beri-beri vitamin. Bachmann finds that while the various races of yeast vary in their vitamin requirements, yet they all grow better and ferment more readily in a medium containing small amounts of some organic material in addition to their carbohydrate. This necessary substance appears to be a constituent of the yeast cell, and is presumably a synthetic product of the organism, which is therefore apparently stimulated by the products of its own growth.

That all of the animal vitamins are direct products of plant synthesis appears to be probable. It has been ascertained by numerous workers that animals obtain their necessary vitamins from their plant food. It is now established that plants, in their turn, require growth-promoting substances, or auximones; which, in the case of some of the lower plants, are apparently manufactured by themselves, but which in the case of green plants, must be supplied from without. Since these necessary accessory substances are essentially organic in nature, their only possible source in the case of ordinary green plants is to be found in the organic matter of the soil in which they are growing.

Bottomley [1914 and 1917, 1] has found that the auximones are produced very largely during the bacterial decomposition of peat by aerobic organisms, and that they are water-soluble, being extracted by water from peat so treated. The present writer [Mockeridge, 1917] has shown that the auximones thus extracted stimulate not only plant growth, but also the activity of the soil organisms, especially of those concerned in the bacterial treatment of the material; an observation which suggests that the bacteria concerned are stimulated by the products of their own growth, and brings them into line in this respect with the yeasts. Bottomley [1919] has found that the products of the nitrogen-fixing bacteria, *Azotobacter chroococcum* and *Bacillus radicola*, grown on pure synthetic media, can function as growth-promoting substances

for the higher plants. All of these observations tend to relegate the ultimate production of growth-promoting substances, whether plant or animal, to the soil organisms, and suggest that the moulds and bacteria of the soil, growing upon the organic matter, produce these necessary substances, which are then absorbed by the plants and utilised in their metabolism, and eventually passed on, either in the same form in which they were originally absorbed, or as some other product of plant synthesis, to the animal in its plant food.

If this suggestion be correct, then the greater the bacterial activity in the soil, the greater should be its supply of plant auximones; and the bacterial activity may be concluded to be dependent upon the amount of organic matter present. Consequently, the value of the ordinary organic manurial materials which are necessary for plant culture, and the rôle of which has given rise to so much speculation, may be due, at least partially, to the presence of growth-promoting substances which have been set free by bacterial action; for the artificial treatment of such a material as peat with various soil organisms under controlled conditions of temperature is only a hastening of the natural processes which are being carried out in these manurial composts.

CULTURE EXPERIMENTS.

A preliminary experiment was therefore carried out to ascertain whether ordinary organic composts contain water-soluble ingredients which are capable of stimulating plant growth. Samples of both fresh and well-rotted stable manures, leaf-mould, and a good well-manured garden soil were obtained. An estimation of the moisture of each sample was made, and the bulk of each material was shaken with distilled water in a shaking machine for several hours, after which the material was filtered through paper in a Buchner funnel by the aid of a pump, and the residue again shaken with distilled water. This process was repeated until all the water-soluble material had been extracted, when all the filtrates were combined, and the volume of the whole liquid measured. A little chloroform was then added to prevent bacterial action, and the whole was preserved in a stoppered bottle until ready for use.

Each of the composts was extracted in the same way, and a water extract of known strength of each material was thus obtained. The effect of these liquids on the growth of *Lemna major* in water culture was then investigated, and compared with that of bacterised peat.

Twenty flat glass crystallising dishes of 500 cc. capacity were arranged in four series of five dishes each. 250 cc. of Knop's nutrient solution (containing potassium nitrate, potassium dihydrogen phosphate, magnesium sulphate, calcium nitrate, and a trace of ferric chloride) were put into each dish, with the following additions: Series I, numbered from 1 to 5, no addition (control series); Series II, numbered from 6 to 10, the water extract of 1 g. of leaf-mould in every 500 cc.; Series III, numbered from 11 to 15, the water extract of 1 g. of soil in every 50 cc.; and Series IV, numbered from 16 to 20, the water extract of 1 g. of bacterised peat in every 1000 cc. These varying proportions of the different materials were chosen because it was anticipated that their effect would probably be in proportion to their water-soluble organic content, and so the quantities supplied were smaller in proportion as the material appeared to be richer

in organic matter. The dishes were covered to the level of the liquids with paper which was black on the inner surface and white on the outer, to prevent the entry of light through the sides and bottom of the dishes.

Ten similar plants of *Lemna major* were counted out into each dish, and 100 similar plants were counted out for an estimation of their dry weight. The dishes were placed in a well-lighted greenhouse, and a sheet of glass was supported over them in order to exclude dust as much as possible. The solutions were changed twice weekly, to prevent bacterial contamination as much as possible, and the plants were counted once each week. The figures obtained for five weeks were as follows:

Table I.

Series No.	Dish No.	Number of plants					
		At beginning	1st week	2nd week	3rd week	4th week	5th week
I. Knop's Solution	1	10	20	23	23	24	25
	2	10	23	24	26	26	28
	3	10	22	23	26	27	28
	4	10	18	23	27	27	27
	5	10	22	24	24	26	27
	Mean...10		21.0	23.4	25.2	26.0	27.0
II. Knop's Solution + leaf-mould extract	6	10	24	27	34	43	64
	7	10	27	32	42	52	65
	8	10	25	27	34	43	57
	9	10	26	30	41	50	64
	10	10	23	29	41	53	73
	Mean...10		25.0	29.0	38.4	48.2	64.6
III. Knop's Solution + soil extract	11	10	27	36	47	53	67
	12	10	23	32	48	54	71
	13	10	21	29	42	45	61
	14	10	26	39	51	53	75
	15	10	25	36	41	47	58
	Mean...10		24.4	34.4	45.8	50.4	66.4
IV. Knop's Solution + bact. peat extract	16	10	31	43	65	86	142
	17	10	29	40	63	87	132
	18	10	29	44	65	82	130
	19	10	28	38	59	75	118
	20	10	27	44	66	86	141
	Mean...10		28.8	41.8	63.6	83.2	132.6

At the end of the fifth week the plants in the two dishes of each series which were nearest the mean were thoroughly washed, and an estimation was made of their dry weight. The following figures were obtained:

Average weight of 100 plants in all series at beginning of experiment			33.4 mg.
"	"	" Series I at end of experiment	25.3 mg.
"	"	" II " "	37.4 mg.
"	"	" III " "	38.9 mg.
"	"	" IV " "	39.2 mg.

From these figures it is clear that both the extract of soil and that of leaf-mould have the power of increasing the rate of growth of plants of *Lemna major* in water-culture, and also of maintaining them in health; for

while the control plants which were successively budded off from the original ten gradually diminished in size and became sickly and yellowish, the plants in all the other series remained green, and not only maintained their original weight, but increased upon it. Both the soil and the leaf-mould thus contain substances which can be regarded as growth-promoting, since they enable the plants better to assimilate the food materials supplied to them; although, as may be expected, neither the soil nor the leaf-mould, even in the larger quantities used, contain these substances in the comparatively large proportion in which they occur in the compost in which bacterial action has been hastened.

The effect of an extract of stable manure was tested in a separate series of experiments. Samples of both fresh and well-rotted stable manure were obtained, and after an estimation of their moisture content, the materials were thoroughly extracted with water as described above for the other organic composts. A little chloroform was added to the standardised extracts, and this was driven off on the water-bath at about 80° when the liquids were required for use. In all cases the extracts were standardised on the basis of the dry weight of the compost.

Fifty dishes, each containing 250 cc. of Knop's solution, were arranged in ten series of five dishes each.

To these the following additions were made: Series I, numbered from 1 to 5, no addition (control series); Series II, numbered from 6 to 10, a water extract of 1 g. of bacterised peat per 1000 cc.; Series III, numbered from 11 to 15, the water extract of 1 g. of bacterised peat per 2000 cc.; Series IV, numbered from 16 to 20, the water extract of 1 g. of bacterised peat per 5000 cc.; Series V, VI and VII, numbered from 21 to 25, from 26 to 30, and from 31 to 35 respectively, similar proportions of extracts of fresh stable manure; and Series VIII, IX and X, numbered from 36 to 40, 41 to 45, and 46 to 50 respectively, an extract of well-rotted stable manure in similar quantity. Ten plants of *Lemna major* were counted out into each dish, and 100 similar plants were counted out for an estimation of their dry weight. The whole set was treated precisely as in the preceding experiment, the plants being counted once weekly for five weeks. The following results were obtained:

Table II.

Series No.	Dish No.	Number of plants					
		At beginning	1st week	2nd week	3rd week	4th week	5th week
I. Knop's Solution	1	10	25	31	40	52	58
	2	10	25	32	40	50	53
	3	10	22	28	36	41	43
	4	10	24	27	34	40	45
	5	10	22	30	39	47	49
	Mean...10		23·6	29·6	37·8	46·0	49·6
II. Knop's Solution + extract 1 gm. bact. peat per 1000 cc.	6	10	31	50	72	115	151
	7	10	31	45	62	102	131
	8	10	30	48	75	118	154
	9	10	29	47	70	110	150
	10	10	27	43	60	100	134
	Mean...10		29·6	46·6	67·8	109·0	144·0

Table II *continued*.

Series No.	Dish No.	Number of plants					
		At beginning	1st week	2nd week	3rd week	4th week	5th week
III. Knop's Solution + extract 1 gm. bact. peat per 2000 cc.	11	10	25	41	50	81	91
	12	10	24	40	52	80	91
	13	10	29	42	57	82	100
	14	10	31	43	61	93	105
	15	10	30	43	61	93	108
	Mean...10		27.8	41.8	56.2	85.8	99.0
IV. Knop's Solution + extract 1 gm. bact. peat per 5000 cc.	16	10	24	33	50	73	85
	17	10	28	40	56	74	91
	18	10	24	38	50	72	89
	19	10	26	40	51	73	83
	20	10	25	35	50	68	80
	Mean...10		25.4	37.6	51.4	71.8	85.6
V. Knop's Solution + extract 1 gm. fresh manure per 1000 cc.	21	10	24	33	45	54	61
	22	10	23	34	45	55	60
	23	10	20	36	48	58	64
	24	10	24	32	40	50	57
	25	10	24	34	43	51	57
	Mean...10		23.0	33.8	44.1	53.6	59.8
VI. Knop's Solution + extract 1 gm. fresh manure per 2000 cc.	26	10	26	36	44	55	61
	27	10	22	29	37	49	56
	28	10	21	31	38	49	54
	29	10	22	30	41	52	56
	30	10	24	30	40	54	57
	Mean...10		23.0	31.2	40.0	51.8	56.8
VII. Knop's Solution + extract 1 gm. fresh manure per 5000 cc.	31	10	25	35	41	54	56
	32	10	22	29	34	47	52
	33	10	23	30	36	50	55
	34	10	21	31	36	46	50
	35	10	22	33	38	45	49
	Mean...10		22.6	31.6	37.0	48.4	52.4
VIII. Knop's Solution + extract 1 gm. rotted manure per 1000 cc.	36	10	25	42	51	74	82
	37	10	26	36	45	71	81
	38	10	29	41	52	74	84
	39	10	26	40	54	71	77
	40	10	25	37	45	66	72
	Mean...10		26.2	39.2	49.4	71.2	79.2
IX. Knop's Solution + extract 1 gm. rotted manure per 2000 cc.	41	10	25	37	45	60	76
	42	10	28	38	52	70	75
	43	10	27	38	44	62	72
	44	10	28	41	50	70	78
	45	10	26	36	44	61	72
	Mean...10		26.8	38.0	47.0	64.6	74.6
X. Knop's Solution + extract 1 gm. rotted manure per 5000 cc.	46	10	25	38	49	58	64
	47	10	26	38	50	67	74
	48	10	26	35	40	58	61
	49	10	29	38	40	57	61
	50	10	28	41	46	60	63
	Mean...10		26.8	38.0	45.0	60.0	64.6

At the conclusion of the experiment an estimation was made of the dry weight of the contents of the two dishes which were nearest to the mean in each series, and the dry weight of 100 plants in each series was calculated from the figures so obtained. These, compared with the original weights, gave the following results:

Weight of 100 plants in each series at beginning of experiment		35.8 mg.
"	"	
Series	I	at end of experiment
"	II	26.4 "
"	III	52.8 "
"	IV	50.4 "
"	V	51.3 "
"	VI	36.6 "
"	VII	36.8 "
"	VIII	35.7 "
"	IX	44.9 "
"	X	45.3 "
"		43.7 "

The control plants, as in all previous experiments, had thus not only multiplied far less rapidly, but had also failed to maintain their normal size, as indicated by their weight; while in all the series containing the added extracts, in addition to showing a more rapid rate of multiplication, the plants had maintained their normal health, and even increased in size. It is noteworthy that in all the three proportions in which the extracts were used, the well-rotted stable manure was more effective than the fresh manure, thus indicating that production of growth-promoting substances is more or less proportional to the bacterial decomposition of the material.

The experiments thus far recorded point to the conclusion that all the materials examined, viz. fresh and well-rotted stable manure, leaf-mould, and well-manured soil, as well as bacterised peat, contain water-soluble substances which are capable of functioning as plant growth-promoting substances, or auximones, and the greater the bacterial decomposition of the organic matter of the compost, the greater is the quantity of these growth-promoting substances.

These organic additions appear to have a most marked effect on the nuclei of the plants so treated, and it was thought to be probable that the active agent might be some essential constituent of the nucleus. Bottomley [1917, 2] has shown that raw peat contains nucleic acid derivatives, particularly a dinucleotide, and more recently [1919] he has also shown that these nucleic acid derivatives have a marked stimulating effect on plant growth. The bacterised peat contains a very small quantity of nucleic acid, or dinucleotide, if any at all, but a proportionately larger quantity of purine and pyrimidine bases, and so it appears probable that it is the derivatives of the nucleic acid, rather than the acid itself, which has the auximonic effect.

An examination was accordingly made of the various organic composts with which the above experiments were carried out, to ascertain whether they contained either nucleic acid or its constituent groupings, which, indi-

vidually or collectively, might function as growth-promoting substances. At the same time a typical sample of the *Sphagnum* which gives rise to the peat was also examined for the occurrence of these substances; and not only was the *Sphagnum* so examined, but also a comparison was made between a sample of peat from the very surface of the deposit, and a sample from a depth of about 12 feet, in order to ascertain the effect, if any, on the nucleic acid constituents, of the changes which had resulted in the conversion of the *Sphagnum* into peat.

EXTRACTION EXPERIMENTS.

Methods of extraction.

Various methods of isolation were tried. The usual method of extracting with caustic soda and acidifying the extract with hydrochloric acid to remove the humic acids always resulted in a loss of the desired substances, which were apparently adsorbed in the humic acid precipitate, for this always contained phosphoric acid and showed an abnormally high nitrogen content. Schreiner and Lathrop's modification [1912], by which the alkaline extract was acidified with acetic instead of hydrochloric acid, to avoid the precipitation of any of the nucleic acid, gave slightly better results; but the extraction with caustic soda was very unsatisfactory, on account of the difficulty of filtration with the large quantities of material employed. Where this method was employed at all, a further modification was introduced which obviated this difficulty to some extent. The material was well shaken with a 1 % solution of caustic soda in a shaking machine for several hours, to ensure a thorough extraction, and the material was then, before filtration, acidified with the required acid (hydrochloric or acetic or both). This rendered filtration much more easy, and obviated the necessity of further precipitating and removing the humic acids. Whenever caustic soda was used, this method was followed out.

The use of sodium carbonate as the extractive was an improvement upon the caustic soda, for the mixture filtered much more readily, but in any case the extraction of the humic acid with the nucleic acid was very inconvenient, and it became desirable, if possible, to employ a method which avoided the solution of the humic acid.

The employment of sodium chloride as the extractive, according to the method of Clarke and Schryver [1917], was also tested upon all the materials examined, but the amount of material extracted in this way was much smaller than that obtained by the alkaline extraction, and this method was therefore discontinued.

After much experiment sodium bicarbonate was finally found to be the best extractive to employ for the separation of the nucleic acid constituents, for this reagent separates as large a quantity of the required substances as does the caustic soda, without dissolving the humic acids at all, so that the trouble of removing the latter, and the loss of nitrogen bases by adsorption

in the precipitate, are avoided. The method generally employed for extracting all of the composts investigated was therefore as follows. The material was air-dried and ground as finely as possible. A weighed quantity, which varied with the density of the material used, was put into a three-litre bottle, and generally sufficient was used to about half-fill the bottle, which was then almost filled with a 1 % solution of sodium bicarbonate. The bottle was tightly corked, and shaken on a shaking machine for several (four to six) hours. The contents were then filtered through paper in a Buchner funnel by the aid of a pump, and the residue again extracted with a fresh quantity of bicarbonate solution. This was repeated a third, and sometimes a fourth, time, if the third extraction showed much coloration. A fresh weighed quantity of the material was similarly treated, and this was repeated until from five to ten kilos of the compost had been extracted. The combined extracts were acidified with acetic acid, to precipitate any protein, and the slight precipitate was allowed to settle. The supernatant liquid was decanted off, just neutralised with sodium carbonate solution, and concentrated *in vacuo* to a comparatively small volume. The liquid was then again filtered by the aid of a pump, and poured into a mixture of about five times its own volume of absolute alcohol acidified with hydrochloric acid. A flocculent precipitate appeared which was more or less copious depending on the compost under examination, and this was allowed to settle for about 24 hours. The supernatant liquid was then decanted off through a filter, and more absolute alcohol added to the precipitate, which was well stirred and allowed to settle once more. The alcohol was again decanted off through a filter, and this washing was repeated once more. The precipitate was then dried in a vacuum desiccator, and the filtrate and washings were added together and preserved for examination.

This precipitate was white in appearance, or, in some cases, slightly yellowish. From the method of its preparation, and its appearance, the substance was presumed to be nucleic acid, or a derivative of it, and in each case it was examined for the typical constituent groupings of this acid. For the sake of convenience it will be referred to as the "nucleic acid precipitate."

A. *Examination of the "Nucleic Acid Precipitate."*

The material was first tested for phosphoric acid by incinerating a small portion and applying the ammonium molybdate test to the ash. The powder was then subjected in each case to two methods of hydrolysis, (a) a method of mild hydrolysis, in which the substance was heated at 100° with 10 % sulphuric acid under a reflux condenser for about two or three hours; and (b) a method of stronger hydrolysis, in which sulphuric acid was used at a temperature of 140° for about six hours. In all cases this second method was found to be more effective. Both methods always resulted in the production of a brown deposit of humic acids, indicating the presence of a carbohydrate radicle.

Separation of Purine and Pyrimidine Derivatives (I).

Two methods were then employed for the separation of purine and pyrimidine derivatives, these methods being slight modifications of those recommended by Jones [1914]. The first method involved the use of a silver salt. The hot liquid, after hydrolysis, was diluted with hot distilled water and filtered to remove the deposit of humic acids. A hot saturated solution of barium hydroxide was added until all the sulphuric and phosphoric acids had been precipitated, together with some of the colouring matter, and the liquid was alkaline to litmus. After removal of the precipitate by filtration, the excess of baryta was removed by carbon dioxide, and the pale straw-coloured liquid was concentrated *in vacuo* to a comparatively small volume. A little nitric acid was then added, followed by a saturated solution of silver nitrate, so long as a precipitate formed. This precipitate of silver-purine was filtered off, washed on the filter with very dilute nitric acid, and further examined as described below.

The filtrate and washings were thoroughly cooled, and a solution of silver nitrate further added until the addition of a drop of cold barium hydroxide solution resulted in the production of a yellow precipitate. The liquid was then made slightly alkaline to litmus with a cold saturated solution of barium hydroxide, and the silver-pyrimidine precipitate filtered off for examination.

(a) Examination of the Silver-Purine Precipitate.

The silver-purine precipitate was suspended in hot water, decomposed with dilute hydrochloric acid, and the precipitate of silver chloride removed. The liquid was then made strongly alkaline with ammonia at the boiling point, boiled briskly for a few minutes to precipitate guanine, and filtered, the precipitate being washed with dilute ammonia. The filtrate and washings were again brought to the boiling point, and a hot saturated solution of picric acid added. This precipitated adenine in the form of clusters of small needle crystals, which could be separated by filtration.

The guanine and the adenine picrate, where they occurred, were purified and used for the preparation of other salts, by which their identity was confirmed.

(b) Examination of the Silver-Pyrimidine Precipitate.

The precipitate of silver-pyrimidine was suspended in hot water and decomposed with sulphuretted hydrogen, the precipitate of silver sulphide being removed by filtration. A little barium in the filtrate was removed by sulphuric acid, and the liquid concentrated *in vacuo*. A hot saturated solution of picric acid was added in order to precipitate any cytosine present as the crystalline picrate. After standing for 24 hours, the cytosine picrate was filtered off, and purified by recrystallisation from hot water.

The filtrate was shaken with sulphuric acid and ether in order to remove

the picric acid, and the sulphuric acid was in turn removed with baryta. After concentration, the filtrate was allowed to stand, in order to allow of the separation of any uracil present.

Separation of Purine and Pyrimidine Derivatives (II).

The second method employed for the examination of the hydrolysed product concerned chiefly the purine bases. The hot liquid was diluted and filtered free of humic acids as before, and then concentrated ammonia was added at the boiling point until the guanine was completely precipitated together with the phosphate. The whole was boiled briskly for a few minutes and the precipitate then filtered off and washed with dilute ammonia. The precipitate was freed from phosphate by treating it with warm dilute caustic soda, which dissolved the guanine while the phosphate remained insoluble. The liquid was then filtered free from the latter, and the guanine precipitated from solution by means of dilute acetic acid, and used for the preparation of various salts for purposes of identification.

The filtrate and washings from the ammoniacal precipitate were added together and acidified with sulphuric acid. From this liquid the adenine was precipitated as the copper compound in the usual way by boiling the liquid, to which copper sulphate had been added, with a saturated solution of sodium bisulphite, which was added until the precipitate formed began to show a yellowish appearance. The whole was then boiled rapidly for a few minutes, and the precipitate of copper-purine filtered off, washed well with boiling water, suspended in hot water and decomposed with sulphuretted hydrogen. The filtrate from the copper sulphide was concentrated and used for the preparation of various salts of adenine.

The filtrate from the copper-purine compound, after removal of the copper with sulphuretted hydrogen and of the sulphuric acid with baryta, was either treated with silver nitrate and baryta for the precipitation of pyrimidine derivatives as above, or these bases were separated by the use of a mercuric salt. A solution of mercuric sulphate was added to the liquid, which was acidified with sulphuric acid, and the whole allowed to stand for 24 hours. The precipitate was filtered off, decomposed with sulphuretted hydrogen, and the process continued as in the examination of the pyrimidine-silver precipitate after removal of the silver.

(c) Examination for Presence of Carbohydrate Radicle.

The so-called "nucleic acid precipitate" was in every case tested by Molisch's reaction for carbohydrates. After mild acid hydrolysis, and also after hydrolysis by ammonia, the products were tested for reducing sugars by Fehling's solution, and for pentoses by the phloroglucinol and orcinol tests.

B. *Examination of the Filtrate after Removal of the*
"Nucleic Acid Precipitate."

The filtrate, after removal of the "nucleic acid precipitate," was just neutralised with ammonia, and the alcohol distilled off *in vacuo*. The aqueous fluid was tested for the presence of phosphoric acid by both magnesia mixture and ammonium molybdate. The presence of carbohydrates was also investigated by means of the tests described above.

A preliminary examination of the remainder of the filtrate showed that in order to separate the bases, acid hydrolysis was unnecessary, for they were readily separated from the fluid by the employment of the silver, copper and mercuric salts. All of these materials were used for the further examination of the filtrate for purine and pyrimidine substances, the methods employed being those already described.

RESULTS OBTAINED WITH THE DIFFERENT ORGANIC MATERIALS.

(a) *Sphagnum Moss*. A fairly copious precipitate of so-called "nucleic acid" was obtained by both the caustic soda and the sodium bicarbonate extraction. This precipitate gave a good reaction for phosphoric acid when incinerated, and gave positive results with Molisch's test for carbohydrates. After both mild acid and alkaline hydrolysis, the products reduced Fehling's solution and responded to the orcinol and phloroglucinol tests for pentoses.

Both of the purine bases which occur in yeast nucleic acid, adenine and guanine, were isolated from the hydrolysed material, the former in greater proportion than the latter. Adenine was recognised by (1) the characteristic form of its picrate, which occurred in clusters of fine needles, giving on recrystallisation elongated prisms which melted with decomposition at about 275°; (2) the hydrochloride, occurring as flat deliquescent prisms; (3) the dichromate, as six-sided plates; (4) the double salt with gold chloride, which appeared as elongated orange-coloured prisms. This base also responded to Kossel's test for purines, gave a gelatinous precipitate with ammoniacal silver nitrate, and a red colour, unchanged by heating, with ferric chloride.

Guanine was recognised by the formation of (1) the hydrochloride, as long needle crystals; (2) the dichromate, forming orange coloured prisms with truncated ends; (3) the picrate, a woolly mass of long thread-like needles when first formed, drying to a compact felt-like mass which became orange-red on heating, and decomposed without melting at about 190°; (4) the sulphate, forming needle crystals which separated out rapidly on cooling a hot solution in sulphuric acid. The solubility of this substance in hydrochloric acid and in ammonia was also characteristic; besides which, a small portion evaporated with a drop of concentrated nitric acid on porcelain left a muddy yellow spot which gave a brownish-red colour with sodium hydroxide, indicating the presence of guanine.

On examining for pyrimidine bases, both cytosine and uracil were isolated, the latter being greater in quantity than the former. Cytosine was recognised by the formation of (1) the hydrochloride, in the form of needle crystals, (2) the picrate, as almost insoluble needles, (3) the chloroplatinate, in the form of six-sided plates. The free base was also obtained in the form of plates by treating the solution of the chloride with ammonia, and this gave the colour reaction of Wheeler and Johnson, which is characteristic of pyrimidines.

The uracil was identified by its method of preparation, by its failure to form an insoluble picrate, and by the clusters of needle crystals formed from a sulphuric acid solution, which gave the colour reaction of Wheeler and Johnson.

The filtrate from this "nucleic acid precipitate" also gave the well-marked reactions for phosphoric acid and for carbohydrates (pentoses), which, of course, would not necessarily arise from nuclear material; besides which each of the purine and pyrimidine substances, adenine, guanine, cytosine and uracil, was present in fair quantity, and was recognised in each case by the preparation of the salts and the application of the tests mentioned above.

The *Sphagnum* moss, therefore, evidently contains some true nucleic acid, which is precipitated by acid alcohol from an alkaline extract; but in view of the larger proportion in which both adenine and uracil occur, as compared with guanine and cytosine, in the hydrolysed material, it is evident that the precipitate does not consist wholly of nucleic acid, but contains also an admixture of the adenine-uracil dinucleotide which occurs in peat [Bottomley, 1917, 2]. Besides these, the *Sphagnum* also contains the free purine and pyrimidine bases from nucleic acid; so that, broadly, it may be said that the nucleic acid constituents of *Sphagnum* consist of some true nucleic acid, a smaller quantity of adenine-uracil dinucleotide, and some free purine and pyrimidine derivatives resulting from the disintegration of the nucleic acid.

(b) *Surface Peat*. A peat taken from the very surface of the deposit, and still retaining something of the colour of the original *Sphagnum*, was similarly investigated. This yielded a smaller quantity of "nucleic acid" precipitate than did the *Sphagnum*, but on hydrolysis the material showed relatively much more adenine and uracil, and less guanine and cytosine than did the *Sphagnum* material. Carbohydrate (pentose) and phosphoric acid were also present in the products of hydrolysis. Evidently, therefore, it contained a smaller proportion of nucleic acid as compared with adenine-uracil dinucleotide than did the former.

The filtrate from the "nucleic acid precipitate" also contained carbohydrate, phosphoric acid, and both purine and pyrimidine bases, viz. adenine, guanine, cytosine and uracil, and these occurred in relatively larger proportion, as compared with the "nucleic acid precipitate," than in the *Sphagnum* moss. The purine and the pyrimidine substances were identified by the same products and tests as indicated above.

These results indicate that in the surface peat disintegration of the original

nucleic acid has already taken place to a certain extent, the products being a larger proportion of dinucleotide and free bases as compared with the original *Sphagnum*.

(c) *Deep Peat*. A peat taken from about 12 feet below the surface of the deposit was investigated, and a certain quantity, which was less than in either of the other cases, of "nucleic acid precipitate" obtained. On hydrolysis, this was found to contain carbohydrates and phosphoric acid, and of the nitrogen bases adenine and uracil, but no guanine or cytosine. It therefore contained no true nucleic acid at all, but consisted entirely of adenine-uracil dinucleotide. This is in agreement with the results of Bottomley.

The filtrate from this precipitate contained also carbohydrate and phosphoric acid, and a relatively large quantity of nitrogen bases. Adenine, guanine, cytosine and uracil were all isolated and identified as above, but the purine bases occurred in much larger quantity than the pyrimidines. This may be due to loss by leaching during the long period of the peat formation, or it may be due to chemical or bacterial changes during the decay of the plant substance, which changes have attacked the pyrimidine substances to a greater extent than the purines.

During the process of peat formation, it is evident that the original nucleic acid has undergone extensive disintegration, probably first of all into dinucleotides, of which a comparatively small quantity remain, and finally into the free constituent bases, which therefore occur in relatively larger quantity in the deeper peat than in either the original *Sphagnum* or the surface peat, but of which a proportion of the pyrimidine bases has disappeared.

(d) *Bacterised Peat*. An examination of bacterised peat was made by both the caustic soda and the sodium bicarbonate methods. Only a very small quantity of "nucleic acid precipitate" was obtained, but this was found to be in all probability a true nucleic acid, yielding all four nitrogen bases in approximately equal proportion. In view of the absence of nucleic acid from the deep raw peat which is used in manufacture, this small quantity is evidently a synthetic product formed during bacterisation, though at present its origin is obscure.

The filtrate from this deposit contained a fair quantity of all of the four nitrogen bases, though here again the adenine and guanine far exceeded in quantity the cytosine and uracil, as in the case of raw peat.

Thus it appears that the further bacterial action has resulted in the breaking down of the adenine-uracil dinucleotide into its constituent groupings, and these are very largely present in a water extract of the material, which does not contain any of the nucleic acid extracted by alkalis.

(e) *Leaf-mould*. A sample of good and fairly pure leaf-mould was extracted by caustic soda, sodium carbonate and sodium bicarbonate, and all three methods yielded similar results. A small quantity of material was obtained by the acid-alcohol precipitation, and this yielded on hydrolysis, adenine, guanine, cytosine and uracil, besides carbohydrates and phosphoric

acid. The guanine and cytosine, however, were present only in very small quantity as compared with the adenine and uracil, so that the material evidently contained a comparatively small proportion of true nucleic acid, the remainder being presumably adenine-uracil dinucleotide. The filtrate from this "nucleic acid precipitate" contained all four nitrogen bases in relative abundance, besides more carbohydrate and phosphoric acid. The leaf-mould, therefore, contains a fair quantity of nucleic acid derivatives, by far the greater proportion being in the form of the free bases, while only a small fraction exists as true nucleic acid, with a larger quantity of dinucleotide. In the sample investigated the disintegration of the original nucleic acid into its constituent groupings had apparently been almost complete.

(f) *Fresh Stable Manure*. This material was extracted by both caustic soda and sodium bicarbonate, and in each case a small quantity of the precipitate with acid-alcohol was obtained. This material consisted almost entirely of nucleic acid, for it gave on hydrolysis adenine, guanine, cytosine and uracil in practically equal proportions, so far as could be judged. The filtrate contained also comparatively large amounts of all these four bases, together with small quantities of xanthine and hypoxanthine.

The nucleic acid in this compost had probably been derived from the plant material constituting the animal's food, and the greater proportion had evidently been decomposed into the free bases in its passage through the animal's body. Since the animals are practically entirely herbivorous, the whole of these nucleic acid derivatives evidently owe their origin ultimately to the elements contained in their plant food. But the purine and pyrimidine bases have by no means necessarily been derived wholly, or even very largely, directly from the plant nucleic acid. On the contrary, a large proportion may be presumed to have arisen as products of metabolism in the animal's body, being elaborated from the plant materials supplied.

(g) *Well-rotted Stable Manure*. This was extracted in just the same way as for the fresh manure, and only a very slight "nucleic acid precipitate" was obtained. On hydrolysis this material yielded adenine and uracil as the only nitrogen bases, so that evidently no true nucleic acid was present, the precipitate being dinucleotide.

In the filtrate from this material were found all the free bases as indicated above for the fresh manure, but they occurred in rather larger quantity. It was noticeable here that the adenine and guanine were relatively the most abundant, occurring in greater quantity than the pyrimidine derivatives, which had evidently either been leached out, or had been transformed by the action of enzymes or micro-organisms. The filtrate also showed the presence of carbohydrate and phosphoric acid. The alkaline extract was much darker in colour, giving a much more copious precipitate of humic acids on acidification, which had, no doubt, arisen by bacterial action on the cellulose and soluble carbohydrates in the animal's food.

In the rotted stable manure, therefore, the decomposition of the nucleic

acid has proceeded to such an extent as to resolve it completely into its constituent bases.

(h) *Soil*. A sample of the same good fertile soil as that which was used in the *Lemna* experiments, and which had been well-manured for several years, was also extracted with caustic soda, sodium carbonate and sodium bicarbonate. A small quantity of the "nucleic acid precipitate" was obtained by each method, and on hydrolysis yielded in each case adenine, guanine, cytosine and uracil, the first and last being in greater proportion than the other two. Phosphoric acid and carbohydrate were also found amongst the products of hydrolysis, so that the precipitate evidently contained some nucleic acid together with adenine-uracil dinucleotide.

From the filtrate from this precipitate were isolated all of the four nitrogen bases from plant nucleic acid, viz. adenine, guanine, cytosine and uracil, together with xanthine and hypoxanthine and a little carbohydrate. All of these substances, however, occurred in relatively much smaller quantity than in any of the other materials examined, while as might have been expected, phosphoric acid occurred in much greater quantity. Among the purine bases, adenine was found to occur in greater quantity, and all of the purine bases were present in greater quantity than the pyrimidines, of which the proportions were extremely small. The occurrence of xanthine, hypoxanthine and adenine among purine bases, has already been reported in soil [Schreiner and Shorey, 1910; Shorey, 1913] and of the pyrimidine derivatives cytosine has also been identified [Schreiner and Shorey, 1910]. The soil under examination here showed the presence of all four of the purine bases mentioned, as well as both of the pyrimidine derivatives, in varying quantities, but it is not to be expected that all soils will necessarily show the presence of the same substances, nor that the composition of any one soil will remain constant for any length of time, in view of the multiplicity of the bacterial and enzymic changes which are continually taking place.

The nucleic acid derivatives which have been found in the various composts examined can be briefly summarised in the following table:

COMPOST	NUCLEIC ACID	DINUCLÉOTIDE	FREE NITROGEN BASES
<i>Sphagnum</i>	Fair amount	Small quantity	Fair quantity of adenine, guanine, cytosine and uracil
Surface peat	Less than above	More than above	More than above
Deep peat	None	Less than surface peat	More than surface peat
Bacterised peat	Trace	None	Still more than above
Leaf-mould	Very little	Small quantity	Fairly large amounts of four bases as above
Fresh stable manure	Small quantity	Practically none	Fair amount of adenine, guanine, cytosine and uracil; small amounts of xanthine and hypoxanthine
Well-rotted stable manure	Practically none	Very small quantity	Rather more adenine and guanine than above; less cytosine and uracil; small amounts of xanthine and hypoxanthine
Soil	Small quantity	Small quantity	Small amount of adenine; less guanine, xanthine and hypoxanthine; still less cytosine and uracil

SUMMARY AND CONCLUSION.

The ordinary organic manures which are generally applied to soils in agricultural and horticultural operations, viz. leaf-mould, fresh and well-rotted stable manures, have been shown to contain, in varying proportions, water-soluble substances which are effective as plant growth-promoting substances, or auximones. Such ingredients are also to be found in well-manured fertile soil, for water extracts of all these materials, when added in certain proportions to inorganic nutrient solutions, promote the healthy growth and development of plants of *Lemna major*, which without such additions rapidly become unhealthy and diminish in weight.

A comparison between fresh and well-rotted stable manure shows that in whatever proportions the materials are used, in all cases the extract of rotted manure is more effective than that of the fresh material, while an extract of peat which has been artificially subjected to bacterial decomposition under controlled conditions is more effective than either. The increase in growth following upon the addition of these materials to the culture solution is such as can not be attributed to the purely nutritive value of the materials added, and all these composts therefore must be assumed to contain growth-promoting substances, or auximones. The amount of these substances varies directly with the extent of the bacterial decomposition of the material, which decomposition is an important factor in determining the efficacy of the compost from a practical standpoint.

The exact nature of the essential substances is still very problematical, but Bottomley [1917, 2] has shown that peat contains derivatives of nucleic acid, and [1919] that these derivatives are effective in increasing the growth of plants when added to culture solutions. Microscopic examination has shown that the various auximones have a marked influence on the size and the contents of the cell, especially on the nucleus, and it seemed not improbable that they might supply some essential nuclear constituent. A detailed examination was therefore made of all of the above materials, viz. well-manured soil, leaf-mould, fresh and well-rotted stable manures, to determine whether they contain derivatives of nucleic acid; and at the same time an examination was made of *Sphagnum* in various stages of its conversion into decomposed peat, in order to ascertain what changes, if any, these materials have undergone during such conversion.

As a result it has been found that all these materials contain appreciable quantities of nucleic acid and its derivatives in various stages of decomposition. Generally speaking, nucleic acid, a dinucleotide, and the free purine and pyrimidine bases have all been found to occur in varying proportions, and the greater the bacterial decomposition of the material concerned, the greater is the resolution of the original nucleic acid into its free bases.

The greater the extent of the decomposition, the more effective is a water extract of the material when added to a culture solution, and the greater is the value of the material from a practical point of view. It would

therefore appear, assuming that the whole effect is due to nucleic acid or its derivatives, that the free bases, individually or collectively, are of more value as growth-promoting substances than the nucleic acid. Schreiner, Reed and Skinner [1907] have found that xanthine and guanine, in concentrations of 40 parts per million or less, increased the growth of wheat seedlings in water culture. Their experiments were made on ordinary seedlings with a fairly large reserve of organic matter in their endosperm, and were of only 10 to 12 days' duration, while in all the experiments carried out by Bottomley and the writer, it has been found that the beneficial effect of the added organic substance is best manifested after 21 days from the commencement of the experiments, when the original supply of auximones in the plant has presumably become either exhausted or so diffuse as to be insufficient for the needs of the plant. Similarly, Schreiner and Skinner [1912] have shown that nucleic acid, hypoxanthine, xanthine and guanine are beneficial under similar conditions, the guanine especially to root development, and a noticeable feature of the experiments recorded above was the marked improvement in the health of the roots. If the experiments of these authors had been carried out over longer periods and with plants which have not a large organic reserve to start with, it is most probable that the added organic materials would have proved even more beneficial. The effect, however, was quite marked, xanthine for instance giving an increase of 21 % in the gross weight of the plants. It is proposed to continue the present work by investigating the action of the free bases, separately and collectively, on the growth of plants which reproduce themselves in a similar manner to that of the *Lemnaceae*.

The value of organic manures is a well-recognised fact, and stress has been laid on their physical effect on the texture of the soil, and on the value of the organic matter as food for soil bacteria, but the fact that the organic materials may be of direct importance to the plant has received very little attention. Livingston [1907] acknowledged this fact when experimenting on the effect on plant growth of extracts of green and stable manures. He added these materials to a slightly modified Knop's solution, and found that "by far the most important factor in the effectiveness was the organic matter," which he found to be of direct value to the plant, although in what lay its effectiveness was not clear. In the experiments recorded here it has been shown that all of the organic manurial composts are of direct value to the plant, and that they all contain nucleic acid or its derivatives, which have been shown by Bottomley and the authors quoted above to be beneficial to plant growth, serving as growth-promoting substances, or auximones. It is evident, therefore, that part, at least, of the beneficial effect of organic manures must be due to the direct value of these substances to the plants. It is not suggested that these nucleic acid derivatives are the only auximones present in the organic composts. In such accumulations of material, the composition of which never remains constant, but is continually undergoing change on account of bacterial

and enzyme action, it is quite probable that there are many such substances capable of functioning as auximones, but the nucleic acid derivatives certainly play an important part. These auximone substances probably occur partly as degradation products of the organic debris, partly as direct products of the synthesis of moulds and bacteria, for it has been shown that auximones do occur as synthetic products of yeast [Williams, 1919, and Bachmann, 1919], and of nitrogen-fixing bacteria [Bottomley, 1919]. Whatever their origin, their liberation in the soil organic matter is evidently due to the action of micro-organisms. These organisms play a part in the auximone cycle not inferior to that of the green plant, which may be regarded as acting as a carrier, absorbing the auximones, which are liberated by the soil organisms, and utilising them in its own metabolism, in the course of which it elaborates the vitamins which in their turn are passed on to the animals that require them.

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