The Isolation from Peat of Certain Nucleic Acid Derivatives.

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In a previous communication* describing the stimulating effect of certain organic substances, extracted from "bacterised" peat, on the growth of *Lemna minor* in water-culture solution, it was suggested that some of the substances may act directly as organic nutrients, being absorbed as such and utilised directly for building up the protein and other complex nitrogenous constituents of the plant.

The marked effect of these substances on the development of the nucleus in the cells of the *Lemna minor* plants also suggested the possible presence of some nuclear constituent, such as nucleic acid, in the extracts. An examination of the aqueous extract of "bacterised" peat showed that, although it contained no nucleic acid, certain purine and pyrimidine bases, together with phosphoric acid, were present. As the presence of these free radicles indicated the possibility that nucleic acid exists as such in raw peat, and is decomposed by further bacterial treatment, an attempt was made to isolate nucleic acid from raw peat.

The usual method of obtaining nucleic acid from soil is by precipitating an alkaline extract of soil with hydrochloric acid, filtering off the precipitate, and pouring the filtrate, after concentration *in vacuo*, into an excess of alcohol containing sodium acetate. By this method, however, there is danger of precipitating some of the nucleic acid with the humic acid.

Schreiner and Lathrop in their soil investigations used acetic acid instead of hydrochloric acid to acidify the alkaline extract. This precipitates humic acid, but not the nucleic acid.

A combination of these methods was first employed in the extraction of nucleic acid from raw peat. An alkaline extract was obtained in the usual way by treatment with a 1-per-cent. solution of caustic soda. This was just neutralised with hydrochloric acid, then acidified with acetic acid, and the humic acid filtered off. The filtrate was treated by the ordinary method for the separation of nucleic acid, and a flocculent precipitate obtained. This substance was much darker in colour than yeast nucleic acid, and was

evidently impure, for on solution and reprecipitation much of the colouring matter was removed.

This method was found to be laborious when working with such a substance as peat, owing to the large amount of humic acid brought into solution by the caustic alkali employed, and the difficulty of rapidly removing this after precipitation.

After numerous experiments it was found that when peat is extracted with a solution of sodium bicarbonate the humic acid remains undissolved, and there is separated from the filtrate a product similar to that obtained by extraction with caustic soda.

Accordingly, a quantity of finely-ground air-dried raw peat was saturated with a 1-per-cent. solution of sodium bicarbonate, and allowed to stand for 24 hours. The liquid was then expressed, and the peat again extracted with a similar solution. The combined extracts were filtered, just neutralised with hydrochloric acid, concentrated in vacuo to a small volume, and then poured into about four volumes of absolute alcohol containing sodium acetate and hydrochloric acid. The flocculent precipitate was allowed to settle for 24 hours, and after decanting the supernatant liquid through a filter, the precipitate was washed and dried in a vacuum desiccator. The combined filtrate and washings were preserved for examination.

Examination of the Precipitate.

From the method of preparation, the substance obtained was thought to be nucleic acid, and on incineration of a portion a considerable amount of phosphoric acid was obtained from the ash. Mild hydrolysis with mineral acids, however, failed to yield more than a trace of a purine base, but the hydrolysed product reduced Fehling's solution and gave Molisch's reaction for carbohydrates. The failure to obtain definite purine substances indicated that the product was not similar in nature to yeast nucleic acid, which readily yields purine bases on mild hydrolysis with mineral acids. An examination of the alcoholic filtrate showed that it also contained phosphoric acid, sugar, and a purine base.

As this mild hydrolysis failed to yield an appreciable quantity of purine bases, although the substance was highly nitrogenous, the method of stronger hydrolysis described by Jones* for obtaining pyrimidine derivatives was employed. This method also separates the purine bases, if present, as a silver compound.

(a) Separation of Purine and Pyrimidine Bases.—Twenty grammes of the substance were heated with 150 c.c. of 25-per-cent. sulphuric acid in an

* Jones, W. 'Nucleic Acids,' p. 90 (1914).
autoclave for five hours at 140° C. The product was diluted with distilled water to 400 c.c., and hot saturated barium hydroxide added in excess to the boiling solution. This precipitated the sulphuric and phosphoric acids, together with much of the colouring matter. Excess of barium hydroxide was removed by carbon dioxide, and the yellow filtrate concentrated to 150 c.c. This was acidified with nitric acid, and silver nitrate added as long as a precipitate formed. This silver-purine precipitate was removed by filtration, and to the cooled filtrate silver nitrate was further added until a drop of cold saturated barium hydroxide produced a yellow precipitate. Barium hydroxide was then added until the solution was permanently alkaline and precipitation ceased.

(b) Examination of the Silver-Pyrimidine Precipitate.—The precipitate was filtered by means of a pump, suspended in hot water and decomposed with hydrogen sulphide. A trace of barium was quantitatively removed with sulphuric acid, and the filtrate from the silver sulphide concentrated to a small volume. A hot saturated solution of picric acid was then added, but after standing for 48 hours no precipitate of cytosine picrate appeared. The picric acid was therefore extracted with sulphuric acid and ether, and, after removal of most of the sulphuric acid by barium hydroxide, the liquid was again concentrated, when fine needle clusters formed. The liquid also gave the pyrimidine colour reaction of Wheeler and Johnson.

The method of preparation of this substance, its failure to form an insoluble picrate, the needle clusters formed in sulphuric acid solution, together with the Wheeler and Johnson colour reaction, identify it as uracil.

(c) Examination of the Silver-Purine Precipitate.—The silver-purine compound was suspended in hot water, decomposed with hydrochloric acid, and the precipitate of silver chloride filtered off. A portion of this filtrate was tested for guanine by adding excess of ammonia at the boiling point. As no trace of a precipitate of guanine was obtained, to another portion of the filtrate picric acid was added and a drop or two of ammonia, when a copious precipitate of fine needles at once appeared. This was filtered off and recrystallised from hot water, when the crystals appeared in the form of prisms. These were dried and were found to melt with effervescence and decomposition at 277° C., the melting point of adenine picrate. This substance was further identified as adenine by the characteristic formation of the following salts: the bichromate, as six-sided plates; the double salt with gold chloride, as long orange coloured prisms; the hydrochloride, as flat deliquescent prisms. The substance also gave a gelatinous precipitate with ammoniacal silver nitrate, a fine red colour with ferric chloride, unchanged by heating, and responded to Kossel’s test for purine bases.
During the process of hydrolysis a copious precipitate of humic acid was formed, presumably from the carbohydrate radicle. The filtrate, however, still gave a strong reducing reaction with Fehling's solution and responded to Molisch's test for carbohydrates. It also gave positive results with the phloroglucin and orcin tests for pentoses.

The hydrolysed product also gave a strong reaction for phosphoric acid with both magnesia mixture and ammonium molybdate.

The material therefore contains phosphoric acid, a pentose sugar, one purine and one pyrimidine base. This indicates that it is a dinucleotide—an adenine-uracil dinucleotide—and not the typical tetranucleotide, nucleic acid.

Examination of the Filtrate.

(a) Separation of the Purine Base.—A preliminary test having shown that the filtrate from the dinucleotide contained phosphoric acid, sugar and a purine base, a further examination of the liquid was made. The alcohol was distilled off and a small portion of the aqueous residue was made alkaline with ammonia. A flocculent precipitate formed which settled as a crystalline sediment. After standing for 24 hours this was filtered off, dissolved in a small amount of boiling hydrochloric acid, its colour discharged with animal charcoal, and the base, together with the phosphoric acid, reprecipitated with ammonia at the boiling point. A small proportion of this precipitate, evaporated on porcelain with a drop of nitric acid, left a muddy yellow spot which gave a brownish-red colour with sodium hydroxide. This indicated the presence of guanine.

The whole of the filtrate was then treated in a similar way and the precipitate dissolved in hydrochloric acid. From this solution of the chloride a mixture of elongated tetrahedral and needle crystals was obtained on long standing in a desiccator. This chloride was used for the preparation of the following salts: the picrate, as a woolly mass of long, fine, thread-like needles, which dried to a felt-like mass and on heating became orange-red, decomposing without melting at 190° C.; the bichromate, as bright orange coloured prisms with truncated ends, which became dark violet when heated at 100° C. These reactions, together with its solubility in ammonia and hydrochloric acid and the formation of a gelatinous precipitate with ammoniacal silver nitrate, identified the substance as guanine.

Guanine was also isolated from the filtrate by precipitation with copper sulphate and sodium bisulphite in the ordinary way. No other purine base could be isolated from the filtrate.

(b) Separation of the Pyrimidine Base.—A small portion of the filtrate after
the precipitation of the guanine by ammonia was examined by the method of Jones, already described, for pyrimidines, and cytosine was obtained.

A simpler method for the isolation of this base was found in the use of mercuric sulphate, which dispenses with the need of a silver salt. The bulk of the filtrate was therefore acidified with sulphuric acid and a solution of mercuric sulphate added. After standing for 24 hours the precipitate was removed by filtration, decomposed with hydrogen sulphide, and the filtrate from the mercuric sulphide concentrated. From this the following salts of cytosine were obtained: an almost insoluble picrate in the form of needles; a chloroplatinate, as six-sided plates; a chloride, as needle crystals. The free base was obtained in the form of pearly plates by precipitating with ammonia, and this gave the colour reaction of Wheeler and Johnson. By these reactions the substance was identified as cytosine.

Thus the filtrate contains the four radicles of another dinucleotide—phosphoric acid, sugar, guanine and cytosine—a guanine-cytosine dinucleotide.

Various kinds of peat from different localities and varying depths have been extracted, and all have given similar results.

Conclusions.

It is evident from the above results that all the constituents of a true nucleic acid are present in raw peat, but nucleic acid as such has not been isolated. Nucleic acid must have been present in the plants from which peat has been formed, and since it is improbable that hydrolysis could have been brought about by the methods of extraction employed, the original nucleic acid has evidently been decomposed by bacterial or other agencies during the process of peat formation, into the products which have been isolated.

It is generally assumed that the first step in the decomposition of nucleic acid results in the formation of four mononucleotides. Levene and Medigreceanu* state that the first phase in the enzymatic decomposition of yeast nucleic acid is the formation of four mononucleotides by the action of a ferment called "nucleinase." It is evident, however, that the decomposition of peat nucleic acid in situ has not followed these lines, but has yielded a stable adenine-uracil dinucleotide and the component parts of a guanine-cytosine dinucleotide.

This is interesting in view of the recent work of Jones and Germann,† who have shown that when yeast nucleic acid is submitted to alkaline hydrolysis it is split up into two very definite dinucleotides: a guanine-cytosine dinucleotide and an adenine-uracil dinucleotide. They state that the former

The Substance, the easily hydrolyses into its component mononucleotides, but the latter is comparatively stable. Evidently a similar decomposition of nucleic acid has taken place during peat formation, and the relatively unstable guanine-cytosine dinucleotide has been further decomposed into its constituent parts. In view of the stable nature of this adenine-uracil dinucleotide and the ease with which it can be extracted from peat, it is surprising that its occurrence as such in nature has hitherto escaped observation.

So far the work has been purely qualitative, but a more exhaustive examination of the substances obtained is now in progress, to determine not only the quantitative proportions of the various radicles, but also their elementary composition.

The Distribution in Wheat, Rice, and Maize Grains of the Substance, the Deficiency of which in a Diet causes Polyneuritis in Birds and Beri-beri in Man.

By Harriette Chick and E. Margaret Hume.

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Our attention was turned to this subject by the reported occurrence of beri-beri among our forces in the Dardanelles and Mesopotamia during the autumn and winter of 1915 (see Wilcox, 1916, I and II). Owing to the exigencies of the military situation, many individuals in these regions subsisted for considerable periods mainly on tinned meat, jam and white bread (or biscuit baked principally from white flour). Beri-beri has in recent years been included among the deficiency diseases, and there was therefore good presumptive evidence that the diet had been at fault. We examined two samples of tinned meat-and-vegetable ration by the methods described below, and found, as was to be expected, that the substances preventing beri-beri in the fresh materials had not survived the heat-sterilisation undergone in the process of canning. The case of bread and biscuit demanded a more extended investigation, and the present work is concerned with the nutritive properties of cereals generally, especially wheat.

One member of this group, viz., rice, has been exhaustively studied in recent years, and the work, among others, of Eijkman, Grijns, and Braddon has established the etiology of tropical beri-beri in a deficiency in the diet of