XXIX. THE PREPARATION OF PLANT NUCLEIC ACIDS.

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The methods described in the literature for the preparation of plant nucleic acids involve the extraction and separation of nucleoproteins containing varying amounts of phosphorus, and subsequent digestion with pepsin hydrochloric acid mixture until a product giving no biuret reaction and containing 8-9 % phosphorus is obtained. Several fermentations and treatment with picric acid are required to obtain a biuret-free substance. The methods are somewhat cumbersome and necessitate the manipulation of large volumes of liquid.

Nucleic acid of plant origin was required in some quantity for biological experiments and the following work was undertaken with the object of finding out if its preparation could be simplified and a biuret-free product obtained without peptic digestion.

Preliminary experiments were made with the raw material used—wheat embryos and dried yeast—by quantitative extraction with water and with solutions of sodium acetate or sodium chloride and afterwards with very dilute caustic potash. The proportion of the total phosphorus extracted by the various solutions was thus ascertained and a rough indication obtained of the form in which this element existed.

The results are given below.

Wheat embryo (1.13 % total phosphorus).

Percentage of the total phosphorus extracted by 10 % sodium acetate =				63.7
"	"	"	"	N/10 caustic potash = 32.5
Total				= 96.2
Percentage of the total phosphorus extracted by 10 % sodium chloride =				85.2
"	"	"	"	N/20 caustic potash = 12.4
Total				= 97.6
"	"	"	"	Water = 75.7

Wheat embryo (another sample) (0.78 % total phosphorus).

Percentage of the total phosphorus extracted by 10 % sodium chloride = 80.4

Dried, alcohol-extracted yeast (2.33 % total phosphorus).

Percentage of the total phosphorus extracted by 10 % sodium chloride = 57.2

In the above experiments the extraction was carried out at a temperature 60–80°, and in cases where a second extraction of the same material was made the residue was thoroughly washed with water on the centrifuge.

A very large proportion of the total phosphorus of wheat embryos is soluble in a 10 % solution of sodium chloride and if this extraction is followed by extraction with very dilute alkali, practically the whole of the phosphorus is removed. Some of the phosphorus thus extracted is combined in the form of nucleic acid and can be separated by the addition of hydrochloric acid to the NaCl solution, the nucleic acid being combined as a salt with varying amounts of protein.

The remainder of the phosphorus is present as phytin ($C_{12}H_{22}O_{44}P_{10}Ca_7Mg$) and can be isolated in this form after the separation of nucleic acid by the methods already described by one of the authors [Clarke, 1914].

From dried yeast less of the phosphorus is extracted with sodium chloride solution, although a somewhat larger yield of nucleic acid is obtained from this material than from wheat embryos. A considerable proportion of the phosphoric acid appears to exist in dried yeast in an uncombined form.

As pointed out by Osborne and Harris, nucleic acid exists in plant tissues combined with varying amounts of proteins in the form of salts, the number of which known to exist in plants is exceedingly large. These salts, which contain in many cases only a small amount of phosphorus, retain their protein characters and the phosphorus was formerly regarded as part of the protein molecule.

During the course of the present investigation in which untreated raw material was extracted with 10 % sodium chloride solution and the clear extract treated with hydrochloric acid, substances were obtained in which the phosphorus varied from an inconsiderable trace to 3 %, and which all contained large amounts of protein and gave a well defined biuret reaction.

The problem of obtaining protein-free nucleic acid by direct extraction therefore, resolved itself into devising some means of so altering the protein complex that it was no longer extracted with sodium chloride solution. It was found that boiling the material used with a large excess of 95 % ethyl

alcohol for two hours and subsequently extracting the dried material with warm 10 % sodium chloride solution gave a clear extract from which by the addition of hydrochloric acid a characteristic precipitate of nucleic acid could be obtained that quickly settled and could be ground to a fine powder. This substance gave no biuret reaction and contained in the case of yeast 7.79 % phosphorus, and in the case of wheat embryo 4.6 % phosphorus. The wheat embryo employed was mixed with a considerable amount of endosperm and the crude nucleic acid obtained from this source was mixed with starch. This could be removed by suitable treatment during the subsequent purification but the presence of gelatinised starch in the extract was very inconvenient and caused a loss of a certain amount of nucleic acid.

In later preparations from wheat embryo the starch was gelatinised by placing the alcohol-extracted material in boiling water and heating. The starch was then fermented with taka-diasatase in the presence of toluene until the solution no longer gave the starch reaction with iodine. Salt was added in sufficient quantity to give a 10 % solution and the extraction and separation of the nucleic acid continued in the usual way. This method gives extracts that can be easily filtered and a starch-free product.

Preparation of nucleic acid from yeast.

30 lbs. of freshly pressed yeast were treated with a large excess of 95 % alcohol, allowed to stand 24 hours, filtered on a Buchner funnel and dried in air. The air-dried material, in successive batches of 1000 g. was boiled for two hours with 95 % alcohol, filtered, pressed and dried in a current of air at 37°. It was then ground to a fine powder in a laboratory mill. 1000 g. of finely ground material, prepared in the above manner was extracted with 10 litres of 10 % sodium chloride at a temperature 60–80°. The extraction was continued with frequent stirring and intermittent heating to 60–80° for 4–5 days. The residue was separated by pouring through muslin and pressed, the turbid extract being filtered clear through paper. 90 cc. hydrochloric acid (1 pt. conc. HCl: 1 pt. water) were then added to the clear extract and the solution vigorously stirred. A characteristic precipitate of nucleic acid separated, quickly settling to a hard cake at the bottom of the vessel. After standing for two hours the liquid was syphoned off and the crude nucleic acid was washed with 50 % alcohol till free from chlorine, left standing over night in 95 % alcohol and finally washed with absolute alcohol and ether. The yield was 15 g. In other experiments the yields varied from 1.4–1.6 % of the dry alcohol-extracted yeast. The crude nucleic acid thus obtained

was a light brown powder and contained 7.8 % phosphorus. It gave no biuret reaction and had the characteristic properties of nucleic acid.

The crude substance was best purified by a method described by Osborne and Harris [1902] by solution in warm 10 % sodium acetate solution and precipitation with excess of hydrochloric acid. 20 g. of the crude acid were dissolved in 500 cc. 10 % sodium acetate by warming for a short time on a water-bath. A small amount of insoluble matter was separated by centrifuging and a clear solution obtained, to which 100 cc. alcohol and excess of hydrochloric acid were added. The nucleic acid separated out as a white mass which settled quickly. It was well washed with 50 % alcohol, 95 % alcohol and ether, and finally dried in a vacuum. It was a perfectly white powder. There is no loss in this method of purification which gives a much whiter product than purification from dilute potash.

The pure nucleic acid thus prepared gives no biuret reaction. Dried at 110° in air it gave the following results on analysis:

0.5626 g.; 0.189 g. $\text{Mg}_2\text{P}_2\text{O}_7$ P = 9.3 %

0.3905 g.; 45.9 cc. $N/10 \text{ H}_2\text{SO}_4$ N = 16.4

Calculated for $\text{C}_{38}\text{H}_{50}\text{O}_{29}\text{N}_{15}\text{P}_4$ P = 9.5

N = 16.1

5 g. of the above preparation were suspended in 200 cc. water and 100 cc. of a 0.1 % solution of caustic potash added. A clear solution was obtained from which the nucleic acid was precipitated by hydrochloric acid (20 cc. $N/2 \text{ HCl}$). After the nucleic acid had separated, an equal volume of 95 % alcohol was added to the solution and the pure nucleic acid separated and washed with alcohol and ether in the usual way. Dried at 110° in air it gave on analysis the following results:

0.359 g.; 0.124 g. $\text{Mg}_2\text{P}_2\text{O}_7$ P = 9.6 %

0.2903 g.; 34.7 cc. $N/10 \text{ H}_2\text{SO}_4$ N = 16.5

These results are in good agreement with the formula proposed by Levene [1909] for yeast-nucleic acid.

5 g. of pure nucleic acid were heated with 20 cc. of 10 % sulphuric acid solution on a boiling water-bath for two hours and strong ammonia in slight excess was added to the dark coloured solution. Guanine separated out on cooling as a dark coloured powder. It was purified by dissolving in dilute sulphuric acid, clearing with a little animal charcoal, and reprecipitating with strong ammonia. It gave a characteristic salt with hydrochloric acid and the usual reaction for guanine.

Preparation of nucleic acid from wheat embryos.

1000 g. of fresh wheat embryos (containing 0.78 % P) were boiled for two hours with 3 litres of 95 % alcohol, pressed free from the solvent and dried in air. The dry material was extracted with 10 litres of 10 % sodium chloride at 60–80° for five days¹. The extract, which was somewhat viscid owing to the presence of gelatinised starch, was separated from the residue of wheat embryos by filtering through muslin and washing the latter with water, and was then centrifuged as clear as possible. 100 cc. hydrochloric acid (1 pt. conc. HCl : 1 pt. water) were added. Crude nucleic acid separated out and was washed with alcohol and ether in the usual way. Yield = 14 g. of a white powder containing a large amount of starchy impurity. It gave no biuret reaction and contained 4.6 % phosphorus which corresponded to approximately 50 % nucleic acid. In subsequent experiments yields varying from 1.1–1.4 % dry material were obtained.

50 g. of the crude nucleic acid (P = 4.6 %) was dissolved in 1000 cc. of a 10 % sodium acetate solution by gently warming on a water-bath. A large amount of starchy impurity remained undissolved and was easily separated by centrifuging. Nucleic acid was precipitated from the sodium acetate solution by the addition of excess of hydrochloric acid. The precipitated acid was washed with 50 % alcohol, 95 % alcohol and finally with ether. The yield of purified nucleic acid was 22.5 g.

5 g. of the nucleic acid thus purified was dissolved in 400 cc. 0.1 % caustic potash solution. A clear solution was obtained from which the nucleic acid was precipitated by adding 5 cc. of strong hydrochloric acid and an equal volume of 95 % alcohol. The precipitated acid was washed with alcohol and ether in the usual way and dried in a vacuum.

Dried at 110° it gave on analysis the following results :

0.3916 g.; 0.116 g. $\text{Mg}_2\text{P}_2\text{O}_7$ P = 8.3 %

0.329 g.; 45.2 cc. $N/10 \text{ H}_2\text{SO}_4$ N = 16.1

It has been mentioned that loss of nucleic acid and considerable inconvenience was caused in this method of preparation by the presence of gelatinised starch in the extract. In subsequent preparations this difficulty was overcome by digesting the starch with taka-diastrase before extracting the nucleic acid with sodium chloride solution.

1500 g. of wheat embryo (P = 1.13 %) were treated in the usual manner with boiling alcohol and dried in air. The air-dried material was then added

¹ The mixture was not kept constantly at 60–80°, but was heated up every day to this temperature and allowed to cool slowly.

in small portions at a time to 10 litres of boiling water and heating continued for two hours in a boiling water-bath. A thick paste was formed. This was transferred to two large flasks, cooled to 40° and a solution of taka-diastase containing 2.5 g. was added to each flask. The fermenting liquid was covered with toluene and the fermentation continued until no starch reaction with iodine was given. The liquid was then heated to 100° cooled to 60° and sufficient sodium chloride added to make a 10 % solution. The extraction with salt solution was continued for four days at 60–80° (intermittent heating). A perfectly clear extract was obtained by filtering through paper pulp and on pressing the residue practically the whole of the extract could be recovered. Nucleic acid separates from the clear extract in the usual characteristic way. Yield = 7 g.

The crude product was purified by dissolving in 400 cc. 0.1 % caustic potash solution, precipitating with hydrochloric acid, washing with alcohol and ether and drying in air. Dried at 110° it gave on analysis the following results:

$$\begin{array}{ll} 0.3717 \text{ g.}; 0.119 \text{ g. Mg}_2\text{P}_2\text{O}_7 & \text{P} = 8.9 \% \\ 0.3997 \text{ g.}; 46.6 \text{ cc. N/10 H}_2\text{SO}_4 & \text{N} = 16.3 \end{array}$$

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SUMMARY.

General methods have been worked out for preparing protein-free nucleic acid from plant tissues without peptic digestion by first boiling the material used with alcohol and then extracting the alcohol-treated material with warm 10 % sodium chloride solution. Protein-free nucleic acid is precipitated from the sodium chloride extract on the addition of hydrochloric acid.

In the case of material containing starch, such as wheat embryos obtained in the modern processes of milling, the starch was removed by fermentation with diastase before extracting with sodium chloride solution. In this way extracts were obtained which could easily be filtered.

The analytical results obtained were in good agreement with the formula $\text{C}_{38}\text{H}_{50}\text{O}_{29}\text{N}_{15}\text{P}_4$ suggested by Levene for plant nucleic acid.

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