exception of C-5, the same configuration as D-glucopyranose. The formation of this disaccharide thus parallels that of laminaribiose. This behaviour is similar to that reported by Barker *et al.* (1957) for *A. niger* (152) and differs from that of another strain of this mould (E19) which was unable to effect appreciable trans- β -glucosylation to D-xylose (Crook & Stone, 1957). It is presumed that the hydroxyl groups at C-2 and C-4 of D-xylose, and at C-2, C-3 and C-4 in the other pentoses are less favourable acceptor sites for the algal cellobiase.

D-Xylose is not an acceptor substrate for trans- α -glucosylation by extracts of *C. rupestris* (Duncan & Manners, 1958).

SUMMARY

1. Extracts of *Cladophora rupestris* and *Ulva lactuca* catalyse the synthesis of oligosaccharides from concentrated solutions of cellobiose. Laminaribiose, gentiobiose and cellobiose have been identified by paper chromatography.

2. On incubation of *C. rupestris* extract with cellobiose-pentose mixtures, glucosylpentose disaccharides are produced. 3-O- β -D-Glucopyranosyl-D-xylopyranose has been isolated from a cellobiose-xylose mixture and characterized.

3. The acceptor specificity of cellobiases from various biological sources is discussed.

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Metal Ions as Precipitants for Nucleic Acids and their Use in the Isolation of Polynucleotides from Leaves

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Many genetical, virological and metabolic studies will require better methods for isolating nucleic acids than are yet available. It is necessary to aim at complete extraction of the polynucleotides from the tissues, since it cannot be assumed that even a large fraction is representative of the whole; and the product should be obtained as nearly as possible in its native state, uncontaminated by other cell polymers. In the procedure to be described an attempt is made to fulfil these requirements. It is assumed that the principal variables affecting the solubility of polynucleotides are polynucleotide concentration, temperature, hydrogen-ion concentration, ionic composition, ionic concentration and dielectric constant of the medium.

It has been found that a high proportion of the nucleic acid extracted from leaves in 1.7 M-sodium chloride at 100° is precipitated when the solution is

kept at 0°. Williams (1945) found that comparable extracts from plants made with 2.1 M-sodium chloride precipitated the extracted nucleic acid at pH values from 0.4 to 3.4 when kept at 2–5°, maximum precipitation occurring at pH 0.4–1.6. Holden & Pirie (1955a) obtained fractions of nucleic acids from leaves and yeast by precipitation from extracts by sodium chloride at pH 3.0. Crestfield, Smith & Allen (1955) used this salt at pH 7.0 to precipitate nucleic acid from extracts of yeast in an attempt to prepare undegraded yeast ribonucleic acid. No detailed study of this type of method has been published, nor has there been a systematic study of the interaction of multivalent cations with nucleic acids, although La³⁺, Cu²⁺ and some other multivalent metal ions are known to be good polynucleotide precipitants. A study of certain details of these reactions has been made, primarily as a guide to the

perfection of isolation procedures. The close analogy with proteins which it has revealed shows the possibility of a much wider application of metal precipitations to the chemical manipulation of polynucleotide solutions.

METHODS

Dry weights. Nucleic acid solutions were freeze-dried and then heated in an oven at 105° overnight.

Total phosphorus. The method of Allen (1940), as modified by C. S. Hanes (unpublished work), was used for the estimation of phosphorus in nucleic acids and nucleic acid hydrolysates.

Nitrogen. This was estimated by the micro-Kjeldahl method.

Protein. An approximate value for the upper limit of protein in nucleic acid preparations was obtained by Osborne's method (Coghill, 1931), with the quantities recommended by Holden & Pirie (1955a). A sample of serum albumin (Armour's Fraction 5) was used as a standard.

Nucleotides and purine bases. The purine bases and pyrimidine nucleotides from ribonucleic acid were determined after hydrolysis for 1 hr. at 100° in *N*-HCl and chromatography in solvent A, made by taking 700 ml. of 2-methylpropan-2-ol at 26°, adding 132 ml. of constant-boiling HCl and making to 1 l. at 20° (Smith & Markham, 1950). Nucleotides were estimated after alkaline hydrolysis and chromatography in solvent B [propan-2-ol-water (7:3, v/v; Markham & Smith, 1952), with which NH_3 is present in the vapour phase] and also in solvent C [sat. $(\text{NH}_4)_2\text{SO}_4$ -water-propan-2-ol (79:19:2, by vol.; Markham & Smith, 1951)]. The nucleotides were also detected and estimated by electrophoresis on Whatman no. 3 MM paper in the apparatus of Markham & Smith (1952).

Nucleotides and purine bases were detected on the papers by the photographic method of Markham & Smith (1949, 1951) and by the direct observation of fluorescence quenching (Holiday & Johnson, 1949). This required the addition of a disk 2 mm. thick of Chance glass filter OX 7 to the light-filter system of Markham & Smith (1949, 1951). For guanine and guanylic acid the fluorescence printing method of Smith & Markham (1950), see also Matthews (1954), was also used.

Deoxyribonucleic acid. This was detected and estimated by a modification (Burton, 1956) of the Dische diphenylamine reaction.

Buffers. Sørensen buffers were prepared according to Britton (1955). Other buffers used were: 0.05M-potassium phthalate-HCl (pH 3-4); 0.05M-potassium phthalate-NaOH (pH 4-6); 0.05M-2-amino-2-hydroxymethylpropane-1,3-diol (tris) maleate (pH 6-8); 0.05M-boric acid (in 0.05M-KCl)-NaOH (pH 8-10).

Nucleic acid preparations

The proportion of three different preparations precipitated by metal ions was measured under standard conditions.

Preparation 1. This was obtained by dissolving commercial yeast nucleic acid in a small volume of water and dialysing at pH 7.0 for 48 hr. with repeated changes of water. The dialysed solution was stored at -10°. The phosphorus-containing component of this material was mainly polyribonucleotide containing the usual bases, since 97% of the total P of samples hydrolysed for 1 hr. at 100° with

N-HCl was accounted for in terms of the purine bases and pyrimidine nucleotides separated by chromatography with solvent A.

Preparation 2. Highly polymerized thymus nucleic acid was obtained by a slight modification of the method of Hammarsten (1924) and was kindly presented by Dr K. Bailey, F.R.S.

Preparation 3. Nucleic acid from the foliage of lucerne, *Medicago sativa*, was chosen as a representative of the nucleic acid preparations from leaves with which this work is concerned. The methods of preparation differed essentially only in the method of precipitation of the polynucleotide from the crude plant extract. The best general method for destroying hydrolytic enzymes in the higher plants is treatment of the coarsely chopped tissues with boiling aqueous ethanol. Holden & Pirie (1955b) found that a ribonuclease obtained from leaves differs from the better-known pancreatic ribonuclease in being comparatively heat-labile, but as no detailed study of the stability of leaf nucleases has been made the method of inactivation employed here is arbitrary.

Freshly picked tips (279 g.) of young growing shoots of *Medicago sativa* were washed in tap water and 30 g. batches chopped and dropped into 300 ml. of boiling aqueous 80% ethanol. The boiling was continued for 2 min. and the leaves were disintegrated in fresh, cold, aqueous 80% ethanol for 5 min. in a high-speed macerator. The fine suspension was filtered through a Büchner funnel prepared with a thin layer of kieselguhr. The pad was washed with aqueous 80% ethanol until the filtrate ran colourless, then with acetone until it again ran colourless. The pad was sucked dry and the dried leaf powder was extracted successively with 50, 30 and 30 ml. of Sørensen 0.04M-phosphate buffer, pH 7.0 at 100°. Each extract was squeezed out through muslin and the three extracts were combined and filtered through kieselguhr. The solution was adjusted to pH 3.0 with dilute HCl and NaCl (10 g. for each 100 ml.) was dissolved in it. The nucleic acid precipitated slowly and after being kept overnight at 0° it was removed by centrifuging and dissolved in the minimum volume of Sørensen 0.06M-phosphate buffer, pH 7.0. The nucleic acid was precipitated with 3 vol. of ethanol and a few drops of saturated MgSO_4 solution and kept overnight at -10° to ensure complete precipitation.

The precipitate was removed by centrifuging, dissolved in water and reprecipitated three times with ethanol. The aqueous solution of the third precipitate was centrifuged to remove insoluble matter and dialysed against distilled water with stirring at room temp. for 3 hr. and then for 24 hr. at 0° without stirring. The dialysed solution was made up to 200 ml., centrifuged at 0° for 30 min. at 20 000 *g* to remove the remaining traces of suspended matter and stored at -10°. The solution contained 0.9 g. of solid material [N, 14.1%; P, 7.1%; $\epsilon(\text{P})$ 8950; $\epsilon(\text{P})$ after alkaline hydrolysis, 11 300] of which 4.5% was deoxyribonucleic acid (DNA) and about 5% protein. A sample hydrolysed for 48 hr. at room temp. with *N*-KOH was brought to pH 1.0 with HClO_4 and the DNA was removed by centrifuging at 20 000 *g* for 30 min.; the pH was brought to 4.0 with KOH, insoluble KClO_4 was removed and samples of the nucleotide solution were evaporated to dryness; the purine nucleotides were hydrolysed to the free bases by heating at 100° with *N*-HCl for 1 hr. Chromatography in solvent A, measurement of the absorption spectra of the components at

pH 1.0, 7.0 and 12.0 and phosphorus determinations showed that 95% of the total P of the ribonucleic acid (RNA) fraction could be accounted for in terms of components very similar to, if not identical with, the known products of RNA hydrolysis.

RESULTS

Precipitation of nucleic acids with the chlorides of alkali metals and ammonium chloride

Most of the samples of nucleic acid were obtained from leaf powders, prepared by methods similar to those used in preparation 3, by extraction with unbuffered 1.7M-NaCl at 100°. When diluted ten times with CO₂-free deionized water these extracts had a pH of about 6.3. The undiluted extracts contained about 0.4 mg. of polynucleotide/ml., which precipitated slowly at 0° until about 80% of it had come out of solution. However, contrary to expectation, no nucleic acid was precipitated when equal volumes of a diluted solution of preparation 3, containing 1.16 mg. of dry matter/ml., of 0.05M-tris maleate buffer, pH 7.0, and 6.9M-NaCl, or 3.9M-KCl or 5.7M-NH₄Cl were mixed at 0°, but up to 70% of the polynucleotide was precipitated by LiCl under similar conditions (Fig. 1).

The effect of pH on the precipitation of nucleic acid (preparation 3) by LiCl is shown in Fig. 2. The same effect of pH was obtained with a higher final concentration of LiCl when the P in the precipitate was measured. When the 1.64M-LiCl was replaced by 2.3M-NaCl, 1.3M-KCl and 1.9M-NH₄Cl there was no precipitation of nucleic acid from preparation 3 buffered between pH 3.0 and 10.0.

Fig. 1. Relation between the precipitation of leaf nucleic acid (diluted preparation 3) and the molarity of LiCl. O, Nucleic acid solution (1 ml. containing 1.16 mg. of dry matter, i.e. 0.0825 mg. of P), 0.05M-tris buffer, pH 7.0 (1 ml.), and LiCl solution (1 ml.) were mixed and the solution was kept at 0° overnight. ●, The same mixtures kept at 0° for 2 hr., then mixed with dioxan (1 ml.) and the solution kept at 0° overnight.

The addition of dioxan, acetone, ethanol or methanol greatly increased the precipitation of nucleic acid from preparation 3 by the above-mentioned salts. To 1 ml. of a diluted solution of preparation 3 containing 1.16 mg. of dry matter/ml. were added 1 ml. of buffer and 1 ml. of salt solution (9.0M-LiCl, 6.9M-NaCl, 3.9M-KCl or 5.7M-NH₄Cl). These solutions were kept at 0° for 2 hr. before the addition of 1 ml. of dioxan and then were kept overnight at -5°. About 90% of the nucleic acid was precipitated by LiCl, NaCl and KCl at pH 3.0 and 8.0 and 75-80% at intermediate pH values. NH₄Cl caused precipitation of about 70% of the nucleic acid from pH 3.0 to 5.0 and much less at

Fig. 2. Relation between the precipitation of leaf nucleic acid (diluted preparation 3) by 1.64M-LiCl and the pH of the solution. A portion (2.5 ml.) of aqueous solution of diluted preparation 3, containing 1.16 mg. of dry matter/ml., was mixed with 2 ml. of buffer of chosen pH and 1 ml. of 9M-LiCl was added. The mixture was kept at -5° overnight and the nucleic acid precipitate dissolved in Sørensen 0.033M-phosphate buffer, pH 6.8. Buffers whose components absorb ultraviolet light were removed by precipitating the nucleic acid twice from water by the addition of 3 vol. of ethanol and a few drops of saturated MgSO₄; the nucleic acid was determined spectrophotometrically by measuring the extinction at 230 and 257 mμ.

higher pH values. With LiCl at pH 7.0 complete precipitation could not be achieved by increasing the salt concentration (see Fig. 1), but this was obtained with 3M-LiCl by increasing the volume of added dioxan to 1.33 vol. Precipitations of preparations 1, 2 and 3 at pH 3.0 and 7.0 are recorded in Table 1.

Precipitation of nucleic acids with multivalent cations

Precipitation occurred with all multivalent metal ions at salt concentrations much lower than those

Table 1. *Effect of the addition of dioxan on the precipitation of nucleic acids by 3M-lithium chloride*

Nucleic acid solution (1 ml. containing 0.0825 mg. of nucleotide P), buffer [0.05M-phthalate, pH 3.0, or 0.05M-tris, pH 7.0 (1 ml.)] and 9M-LiCl (1 ml.) were mixed and the solution was kept at 0° overnight before collection of the precipitate. Dioxan (1 ml.) was added 2 hr. after the solution had been made.

Nucleic acid preparation no.	Nucleic acid precipitated (%)			
	pH 3.0		pH 7.0	
	Without dioxan	With dioxan	Without dioxan	With dioxan
1	28	38	0	36
2	0	79	0	81
3	93	95	67	86

Table 2. *Precipitation of nucleic acid of preparation 1*

Nucleate solution (1 ml. containing 0.0825 mg. of P), 1 ml. of buffer (glycine-HCl for pH 3.0 and 0.05M-tris maleate for pH 7.0) and 1 ml. of 0.01M-cation solution were mixed and kept at room temp. for 1 hr. The precipitate was separated by centrifuging and the total P of the pellet was determined. In some experiments ethanol (1 ml.) was added 1 hr. after the addition of cation solution. The precipitate was collected at least 1 hr. later. The results with Pb²⁺ at pH 3.0 were obtained with unbuffered solutions adjusted with acetic acid.

Cation	Nucleic acid precipitated (%)			
	Without ethanol		With ethanol	
	pH 3.0	pH 7.0	pH 3.0	pH 7.0
La ³⁺	91	85	—	—
Al ³⁺	82	—	—	—
UO ₂ ²⁺	95	0	—	0
Fe ³⁺	83	0	—	0
Pb ²⁺	82	0	—	0
Fe ²⁺	58	—	75	—
Co ²⁺	27	0	67	0
Ni ²⁺	36	0	67	0
Mn ²⁺	38	0	72	0
Cu ²⁺	53	0	73	0
Ca ²⁺	0	0	65	0
Ba ²⁺	0	0	68	0
Zn ²⁺	0	0	69	0
Hg ²⁺	0	0	61	0
Mg ²⁺	0	0	49	0

required with alkali metal ions and it was possible to work with dilute ionic solutions and degraded nucleic acid preparation 1. The results are recorded in Table 2. The relative effectiveness of Al³⁺, Co²⁺ and Ba²⁺ ions was the same with preparations 2 and 3 as with preparation 1 (Table 2), but the proportion of nucleic acid precipitated was increased in all cases.

Isolation of nucleic acid from leaf extracts by salt precipitation

Leaf powders were prepared as described for preparation 3. A minor modification, introduced in later preparations, was to precede the acetone extraction by extraction with warm ethanol-ether (3:1, v/v), which increases the extraction of lipid substances. Method 1 (below) gives erratic results unless the following modification is made. After maceration in aqueous 80% ethanol the solid leaf material is removed by centrifuging for 20 min. and washed twice by resuspending in 80% ethanol and centrifuging for 20 min. It is then transferred to a Büchner filter and washed with acetone. In the methods described below the extractions of the powder with Sørensen 0.04M-phosphate buffer, pH 7.0, at 100° were replaced by four extractions with unbuffered 1.7M-NaCl at 100°.

Method 1: precipitation by 1.7M-NaCl. The combined, filtered 1.7M-NaCl extracts were kept at 0° for at least 24 hr. The nucleic acid precipitate was removed by centrifuging and extracted four times with borate buffer, pH 9.7, and the combined extract was adjusted to pH 4.0. Ethanol (3 vol.) and a few drops of saturated MgCl₂ solution were then added. The precipitation of nucleic acid was repeated twice and the third precipitate was dissolved in a small volume of water, brought to pH 7.0 and then dialysed against distilled water with stirring at room temp. for 3 hr., then at 0° for 4 days with daily changes of water. The dialysed solution was made to a known volume (usually 50–100 ml./300 g. fresh wt. of leaves) and stored for analysis. During storage some non-nucleotide material was precipitated and was removed before analysis by centrifuging at 0° for 1 hr. at 20 000 g.

Method 2: precipitation with Al³⁺ ions in 0.57 M-NaCl. The combined 1.7M-NaCl extract from the leaf powder was filtered through kieselguhr and mixed with 1 vol. of glycine buffer, pH 3.0 (glycine-NaCl-HCl; all 0.1M) and 1 vol. of 0.1M-Al₂(SO₄)₃. The mixture was kept overnight at 0° and the precipitated polynucleotide component was removed by centrifuging at 0°. The precipitate was dissolved in 0.05M-tris maleate buffer, pH 7.0, and the nucleate reprecipitated by the addition of ethanol (3 vol.). This precipitation was repeated twice with the addition of a few drops of saturated NaCl solution. The third precipitate was dissolved in water and insoluble matter was removed by centrifuging for 30 min. at 0° and 20 000 g.

Method 1 suffers from the disadvantage of giving incomplete precipitation and the product appears to be inferior to that obtained by method 2, which gives the better product by the analytical criteria with virtually complete precipitation of the polynucleotide from leaf extracts.

The preparations were stored at -10° as nucleates in water. These solutions contained 1–4 mg. of nucleate/ml. and appear to undergo no major degradation during storage, since their ε(P) value remained unchanged for at least

Table 3. Analyses of nucleic acid preparations from leaves

Source	Method	Fresh wt. of leaves (g.)	Dry wt. of leaves (g.)	Nucleic acid isolated (mg.)	Percentage of total nucleic acid*	Light absorption (m μ)	Analyses of isolated nucleic acid							
							N (%)	P (%)	N/P	ϵ (P)	ϵ (P) after alkali	Increase of ϵ (P) (%)	DNA (%)	Protein (%)
<i>Beta maritima</i> . Young leaves	1	264.2	24.2	164.5	82	min. 231 257.5 max. 231 257.5	15.3	8.6	3.92	8 000	11 100	39	4.0	<2
<i>B. vulgaris</i> , strain KDNB. Young leaves	1	307.5	27.1	159.4	78	231 258	14.7	8.6	3.79	8 000	11 700	46	4.0	<2
<i>B. vulgaris</i> , strain T49/147. Polyploid. Young leaves	1	307.3	28.0	168.0	77	230.5 258	15.4	7.65	4.45	7 950	11 000	39	4.2	<2
<i>B. vulgaris</i> , strain Battie's. Young leaves	1	326.0	25.7	141.5	76	230 257.5	15.0	8.4	3.93	7 600	11 100	46	4.3	<2
<i>B. vulgaris</i> , strain Battie's infected with beet yellows virus. Young leaves	1	317.4	31.0	201.5	85	230 257.5	16.7	7.2	5.15	7 550	10 900	44	5.1	2
<i>B. vulgaris</i> 1. Very young leaves	2	30	—	20.1	>98	231 259	12.4	7.3	3.76	7 750	10 800	39	5.2	<3
<i>B. vulgaris</i> 2.	2	30	—	35.4	>98	231 259	13.3	7.6	3.88	—	—	—	3.7	<2
Kale 1. Very young leaves	2	30	—	52.8	>98	230 257.5	13.8	8.0	3.82	7 800	9 400	21	1.9	<1
Kale 2. Very young leaves	2	30	—	45.9	>98	230.5 257.5	13.2	7.5	3.91	7 920	10 900	38	2.4	1
<i>B. vulgaris</i> . Very young leaves	2†	60	—	78.4	>98	231 257.5	12.2	8.05	3.35	8 750	9 100	4	2.5	<1

* Values obtained from the absorption curves at 257 m μ of suitably diluted solutions of the precipitated nucleic acid and unprecipitated nucleic acid. Interference by non-polynucleotide material in the solution of unprecipitated nucleic acid was minimized by precipitation with ethanol and centrifuging as described in the text. Where necessary an allowance was made for unspecific general absorption.

Fig. 3. (a) Absorption curves of leaf extracts: 103 ml. of 1.7M-NaCl extract from 30 g. fresh wt. of beet leaves. ○, solution diluted 1:3 [100 ml. was precipitated with $Al_2(SO_4)_3$ by method 2 and nucleic acid removed (see Fig. 3b)]. □, Supernatant solution (300 ml., pH 3.0) before and after centrifuging at 20 000 g for 30 min. ●, Supernatant solution at pH 12.0; 50 ml. of supernatant was redissolved in 50 ml. of water; ▲, the same at pH 12.0. (b) Absorption curve of nucleate of Fig. 3(a) in 300 ml. of water.

1.5 years. The degree of purity, the proportion of RNA and DNA, the degree of polymerization and the yield of the products of these two methods may be judged from Table 3. A third method was similar to method 1 except that after the addition of NaCl 0.4 vol. of dioxan was added. Since the yield was not appreciably increased and the products had a comparatively low contents of N and P (N, 6-8%; P, about 4%) the method was abandoned.

Until much more is known about nucleic acids and their function the relative merits of different methods of preparation cannot be evaluated. However, as shown by the curves in Figs. 3a and 3b, method 2 has the advantage of completeness and this was confirmed by using Al^{3+} ions to precipitate a further fraction of polynucleotide from the five preparations by method 1 shown in Table 3. In this method, after the separation of nucleic acid from the NaCl extract of leaves a portion of both the RNA and the DNA remained in solution. After centrifuging, the supernatant fluids from the five preparations were reprecipitated four times with 3 vols. of ethanol, dissolved in 50 ml. of water and treated with Al^{3+} ions according to method 2. The nucleic acid precipitate was removed by centrifuging at 20 000 g for 1 hr.

Many preparations have been made by methods 1 and 2, and the supernatant liquids, after the removal of the nucleic acid precipitate, were tested spectrophotometrically for the presence of polynucleotide. Method 1 never precipitated the whole of the polynucleotide and method 2 never left a detectable quantity of it in solution. The material employed in all cases was immature foliage, usually young leaves of sugar beet, and it is improbable that differences in leaf material were responsible for the observed differences in precipitation by the different methods. A direct comparison of the two methods on three separate samples of leaf material showed that method 1 precipitated 74, 71 and 76% of the amount of nucleic acid precipitated by method 2.

Completeness of separation of nucleic acids from leaves

Leaf residues were extracted from 1 to 2 days at room temperature with 2N-KOH. The extracts were neutralized with 60% $HClO_4$ and, after concentration, chromatographed in solvents B and C and submitted to electrophoresis on paper in ammonium formate buffer, pH 3.5, to separate the ribonucleotides. The DNA, which remained unhydrolysed, was precipitated with ethanol and measured colorimetrically by Burton's (1956) method, which permitted the easy detection of DNA in concentrations down to 20 $\mu g./ml.$ The limits of detection by the other methods are shown in Table 4. The results for DNA and electrophoresis of ribonucleotides in extracts of the residue from 30 g. fresh weight of leaves showed that the amounts of both types of components, if present, were beyond the limits of the tests, i.e. the residue from the 30 g. of fresh leaves contained less than 0.16 mg. of RNA and less than 0.1 mg. of DNA. These quantities are respectively less than 1.6 and 1.0% of the total nucleic acid usually isolated from a 30 g. sample of young leaves. Since some components of the leaf residues ran in positions overlapping those normally occupied by the ribonucleotides the chromatographic methods were unsuitable for this material.

DISCUSSION

Under conditions which avoid the known dangers of degradation, 97% of the nucleic acid has been extracted from sugar-beet leaves. It was separated from the extracts by salts in proportions ranging from approximately 75% to at least 98% of the total polynucleotide, depending on the salt used. It is still impossible to determine the extent to which such preparations are artifacts and the relative values of the methods depend very largely upon the use for which the preparations are intended and the nature of the plant material from

Table 4. *Smallest quantities of purines and ribonucleotides detected after chromatography and electrophoresis*

Material was applied in bands approx. 6×1.5 cm. to Whatman no. 3 paper for chromatography and to Whatman no. 3 MM paper for electrophoresis. Results are expressed in $\mu g.$ detected per band.

Substance	Chromatography			Electrophoresis at pH 3.5 in ammonium formate buffer
	Solvent A	Solvent B	Solvent C	
Adenine	4-6	—	—	—
Guanine	4-6	—	—	—
	(0.5-1)*			
Adenylic acid	—	4-10	36	4
Guanylic acid	—	4-10	40	4-8
		(4)*	(4)*	(4)*
Cytidylic acid	—	3-10	25	3-6
Uridylic acid	—	3-10	30	3-6

* Limit when the bands were detected by fluorescence printing.

which the extracts are made. The methods yield products containing a high proportion of polynucleotide which compare favourably with good preparations of ribonucleic acid from sources other than higher plants. The ϵ (P) values are low and increase by about 40 % on alkaline hydrolysis, which, by currently accepted standards, is an indication that the preparations are not highly degraded.

The interactions of metal ions with proteins have been studied in the light of modern concepts, but there have been no comparable studies of their reactions with nucleic acids. However, the work of Smith & Alberty (1956) and others has shown the existence of ionic complexes between metals and such substances as adenosine di- and tri-phosphate. The work of Hammarsten (1924), Hammarsten, Hammarsten & Teorell (1928), Chargaff, Vischer, Doniger, Green & Misani (1949) and Caspersson, Hammarsten & Hammarsten (1935) suggests that DNA forms complexes with Fe^{3+} , La^{3+} and Cu^{2+} ions; Stern & Steinberg (1953) studied compounds of this type formed by DNA and several of the ions of rare-earth metals. Davidson & Waymouth (1944) showed that a similar reaction occurs between RNA and La^{3+} ions. Metal-sequestering properties of nucleic acids were demonstrated by Neuberg & Roberts (1949) and the present work demonstrates the high affinity of nucleic acids for some metal ions.

Among biological polymers the polynucleotide molecule has the largest number and variety of potential electron-pair donors available for complex formation, and the best explanation of the results in this paper would be that complexes of the predominantly ionic type are formed between the polynucleotides and most, if not all, of the true metals. The metal ions may be divided into three main groups according to their ability to precipitate polynucleotides from aqueous solutions. Alkali-metal ions and NH_4^+ ions are the weakest precipitants and require about 2M-concentrations of salt to cause appreciable precipitation. This type of precipitation seems to be a salting-out phenomenon. The alkaline-earth metal ions and Zn^{2+} , Hg^{2+} and Mg^{2+} (all elements in group II of the Periodic Table) occupy an intermediate position and the ions of the transitional elements, lead and tervalent metals such as Al^{3+} and La^{3+} are the most powerful precipitants. In this respect the metal ions fall into the same groups as when they are classified according to their ability to form chelate compounds with inorganic polyphosphates in which the combining groups of the ligand are the phosphoryl oxygen and the free acidic groups of the adjacent phosphate groups in the chain. Van Wazer & Campanella (1950) found that alkali metals and similar ions form weak complexes with inorganic polyphosphates; the other metals form stronger complexes of which the most stable are those formed by UO_2^{2+}

ions and the transitional elements. The parallel suggests that nucleic acids may form similar chelate complexes. Whatever the explanation of the observations which have been described, a thorough investigation of the interaction of polynucleotides with metal ions and with organic solvents appears to be necessary.

SUMMARY

1. The precipitation of nucleic acids by metal ions has been studied.
2. Two methods have been devised for the isolation of nucleic acids from leaves, based on precipitation by metal ions.
3. Analytical results on ten preparations from leaves by these methods are given. These suggest that relatively pure, undegraded polynucleotides are produced.
4. Unextracted nucleic acid in leaf residues could not be detected.
5. The mechanism of the interaction between metal ions and nucleic acids is discussed.

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