The action catalysed by extracts from leaves (Pirie, 1950) and sprouted barley (Shuster & Kaplan, 1953) resembles that catalysed by the enzyme from spleen and some snake venoms in being more extensive than the action catalysed by PRNase but the nature of the end products has not yet been established. Parker (1952), on the basis of the solubility of the Ba salts, recognized mononucleotides in LRNase hydrolysates but did not characterise them, and Shuster & Kaplan (1953) mention preliminary evidence that 5′-nucleotides are a product of the action of the barley enzyme, which suggests that this enzyme, unlike PRNase, splits the molecule between the phosphate and carbon-3 on the ribose. There is no published evidence that mononucleotides are the sole, or even the principal, products of the action and there may be oligonucleotides that differ from those remaining after PRNase action by being soluble in UrTCA. Finally there is no evidence that only one type of RNase is present in leaf extracts; it is only for convenience that we have referred to LRNase as if it were one enzyme. All that is clear is that LRNase is not an unspecific phosphodiesterase because it is only crude preparations that carry activity towards diesters unrelated to RNA.

This fractionation was undertaken to ascertain the range of substrate specificity of LRNase and it has only been carried far enough to satisfy us that phosphate esters and polynucleotides other than those containing ribose are not attacked. Incidentally, the activity of the enzyme per mg. of N has been increased 230-fold and the final product is as active on suitable substrates as are crystallized preparations of the pancreatic enzyme. At this stage there is a marked diminution in the stability of LRNase so that, for our purpose, there is no advantage in carrying the fractionation further. The final preparations are obviously inhomogeneous and none of the usual criteria of purity have yet been applied to them. Further work on the fractionation is in progress.

SUMMARY

1. From pea seedlings ribonuclease preparations have been made which attack P-containing substrates other than ribonucleic acid so slowly as to make it unlikely that the enzyme has an unspecific action.

2. The enzyme differs from pancreatic ribonuclease in that it hydrolyses nucleic acid so completely that no acid-precipitable 'core' is left.

3. Less thoroughly fractionated preparations have been made from tobacco leaves and some properties of the enzyme in other leaves are described.

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The Preparation of Ribonucleic Acid from Yeast, Tobacco Leaves and Tobacco Mosaic Virus

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There is no conclusive evidence that nucleic acid ever exists *in vivo* in the free state. Markham (1953) has argued that in some situations, e.g. turnip yellow mosaic virus, it is free and simply held as a clathrate complex inside a protein cage so that it is liberated when the cage is opened. But the treatments needed for the opening, though indubitably unusually gentle, denature some proteins and denaturation is often all that is needed to release a prosthetic group. In other nucleoproteins the

linkage appears to be stronger. At one time, apparently (cf. Sevag & Smolens, 1941; Greenstein, 1944), the linkage was thought to be electrovalent. If this view was indeed held it was baseless because nucleic acid had only been made from tissues after treatments that denature many proteins, and there was already evidence from work on plant virus preparations that in them, at any rate, the link was stable over a wide pH range in many different ionic environments.

If, therefore, we assume that most, even if not all, of the nucleic acid in a cell is normally linked to other materials, such as protein, by more than electrovalencies, the free acid is largely or entirely an artifact and the type of change it undergoes on liberation depends on the at present undefined nature of the links involved. The position is comparable to that of haematin and globin in haemoglobin. Nucleic acid can be liberated in many different ways, e.g. by treatment with acid, alkali, urea, guanidine, ethanol and by boiling; it may well be that these cause different changes in the nucleic acid which are incomparable. There is, therefore, no basis for trying to arrange the treatments in a sequence representing the extent of alteration. The common assumption that those preparations with the largest particle size are the least modified has no necessary foundation; one cause of apparently large size is incomplete removal of protein and some treatments may well cause aggregation rather than depolymerization.

The agents that split nucleoproteins are also, to some extent, selective and for each particular nucleoprotein some are more suitable than others. Thus, trichloroacetic acid works with the microsomes from normal tobacco leaves but not with tobacco mosaic virus (Pirie, 1950), whereas strontium nitrate works with the latter but not the former (Pirie, 1954). Similarly, detergents and urea separate nucleic acid from some viruses but not from others (Sreenivasaya & Pirie, 1938; Bawden & Pirie, 1940). It is reasonable to suppose that a starting material as complex as plant or animal tissue or a yeast cell, will contain very many different types of nucleic acid held in many different types of combination. Unless all the nucleic acid is being isolated it is therefore reasonable to suppose that each method will bring out the nucleic acids in a different ratio and this may be the cause of some of the conflict in the literature.

When nucleic acid is isolated directly from a cell without intermediate isolation of a nucleoprotein, the cell wall must be broken or damaged before even an uncombined nucleic acid can be released. This presents little difficulty with most animal and higher-plant tissues for the cells are easily destroyed by grinding. Yeast is more robust, but Chargaff *et al.* (1950) have ground it in a mill designed for bacteria. In other methods the yeast is dried and extracted with fat solvents or heated to 60–100° (Clarke & Schryver, 1917; Markham & Smith, 1952*a*). The efficacy of these treatments probably depends not only on the denaturation of the protein and consequent liberation of nucleic acid, but also on the breaking of the cell wall. Yeast cells can be broken open by digestion with the enzyme mixture from snails' crops under the conditions used with leaves by Holden, Pirie & Tracey (1950) but there is ribonuclease in the crop fluid and we have not found this treatment satisfactory as a prelude to making nucleic acid. A more refined enzyme preparation would, however, probably be admirable. The traditional method for opening yeast cells is exposure to 0·5 or 1·0N-NaOH at 0°; this treatment also denatures the protein sufficiently to make it largely insoluble in the presence of salts at pH 6–7. There are disadvantages in working with nucleic acid that has been exposed to such an alkaline environment but there seem to be comparable disadvantages in all the other treatments.

The result of any of these processes is a solution containing more or less modified nucleic acid and a variable but small amount of protein as well as other materials, polysaccharides, metaphosphates, etc., of varied molecular weight. During the last 15 years the protein has generally been removed by shaking with chloroform under various conditions derived from the method developed by Tsuchihashi (1923) and popularized by Sevag (e.g. Sevag, Lackman & Smolens, 1938). The emulsion that is formed contains a higher ratio of protein to nucleic acid than the aqueous layer, so that by repetition of the process most of the protein can be removed without great loss of nucleic acid. It should be emphasized, however, that this is potentially a method of fractionating the nucleic acid to an extent that will depend on the amount of protein being removed. It has been condemned by Jungner & Allgen (1950) as leading to depolymerization of the nucleic acid.

These comments apply equally to the ribonucleic acids and the deoxyribonucleic acids; in the remainder of this paper the unqualified term nucleic acid will be used for ribonucleic acid only.

Most published work has been done with commercial yeast nucleic acid (YNA), although the authors generally comment on its poor and variable quality. A brief search in recent patent literature shows that the acid may have been exposed to such savage treatment that these strictures are amply justified. Many methods for preparing YNA in the laboratory have been proposed and in them treatments have been advocated and condemned on grounds that do not always seem to be based on experiment. We have tried to keep conditions as mild as is compatible with getting a high yield and

we show that our product is as highly aggregated as those of other workers and that a repetition of some of the treatments does not lead to further breakdown. This suggests that the breakdown is not a progressive matter but it does not exclude the possibility that vulnerable structures were destroyed at the start of operations. As we have said, we look on nucleic acid as an artifact; if we are correct there is no possibility of separating unmodified material, and the best that can be hoped for is to minimize the modification.

It is sometimes convenient to start with commercial YNA rather than with yeast; we therefore give a method for separating from it a relatively satisfactory product. For comparison with YNA we have also made nucleic acid from normal tobacco leaf and from tobacco mosaic virus by methods similar to that used with yeast.

EXPERIMENTAL AND RESULTS

Preparation of ribonucleic acid from yeast

Pressed baker's yeast is suspended evenly in its own weight of water, cooled to 0° and an equal volume of 2N-NaOH, also at 0°, is added with vigorous stirring. After 1 hr. at 0° the mixture is neutralized by the addition of 5N-HCl with vigorous and continuous stirring. Great care is taken to prevent any regions from becoming acid and the final adjustment to pH 6·0–6·5 is made with more dilute acid. Most of the insoluble material is removed by centrifuging at 1500 *g*. The supernatant fluid is adjusted to pH 3 with HCl, 70 g. NaCl are added to each 1 l. of fluid, and the small precipitate removed by filtering through a layer of Hyflo Supercel (Johns Manville Co.). To the clear filtrate solid NaCl is added to full saturation; a turbidity develops at once and precipitation starts in a few minutes and continues for several hours. After standing for a day the precipitate is centrifuged off. More material is precipitated when the pH of the sat. NaCl soln. is adjusted to 2. Each ppt. is dissolved separately in 20–30 times its volume of water and the pH adjusted to about 4. The preparations are deproteinized by stirring with 0·25 vol. $CHCl_3$ and 0·1 vol. amyl alcohol in an 'Ato-mix' (Measuring and Scientific Equipment Co.). The emulsion is centrifuged and the lower layers discarded. At least three cycles of treatment are necessary. The solution is then dialysed for several days at 0°, against frequent changes of distilled water, in a cellophan sac protected from bursting by a sleeve of stainless-steel gauze.

The dialysed preparations of Na nucleate contain 8·0–8·5 % P and we have no evidence for any P-containing component besides ribonucleic acid. Thus, lipid solvents do not extract any P and on precipitation with HCl, although 1–2 % of the P remains in solution, the light absorption of this solution at 260 mμ. is approximately that found with solutions of partly degraded nucleic acid with the same P content. We therefore conclude that little phospholipid or metaphosphate is present. The question of deoxyribonucleic acid contamination is considered later.

The Fe content of both these preparations is less than 0·01 %. The NaCl precipitate at pH 3 contains 0·1 % of Ca and Mg but the precipitate at pH 2 contains less than 0·03 %. For these metal determinations, the nucleic acid was incinerated with H_2SO_4 until charred and then cleared with $HClO_4$. After dilution, Fe was determined colorimetrically after adding NaCNS; Ca was precipitated at pH 3 as the oxalate and this, after washing, was titrated with $KMnO_4$. Mg was determined colorimetrically on the supernatant fluid from Ca oxalate by a method based on that of Ludwig & Johnson (1942) in which the lake given with Titan Yellow in alkaline solution is stabilized by starch. Control determinations in which 10 mg. quantities of YNA were incinerated after the addition of 1–10 μg. quantities of the three metals showed that these methods were satisfactory with this material. We do not therefore agree with those who have claimed that metals, and especially Mg (cf. Jungner, 1951), are an integral part of YNA preparations made by gentle methods of isolation. The small amounts that we find, in preparations in which the primary valencies are neutralized by Na^+, may well be derived from the reagents used in the preparation.

Of the various methods used to detect small amounts of protein in materials such as nucleic acid, those based on the biuret reaction appear to be the least unsatisfactory. Of them we prefer those in which the colour is developed in a small volume and then compared qualitatively with a set of standards rather than those in which it is developed in a larger volume and measured in a colorimeter. Osborne's technique (cf. Markham, 1955) is the most sensitive, but to get satisfactory results we use less ethanol than is recommended by Markham. To 1 ml. of aqueous nucleic acid solution 0·05 ml. of 10 g./l. $CuSO_4$, $5H_2O$ and 0·3 ml. of ethanol are added. The colour is developed and the ethanol forced out of solution by the addition of approximately 0·9 g. of KOH. The biuret colour extracts into the ethanol layer whereas most of the uncombined copper and many forms of colour in the original solution, which confuse other methods of doing biuret reactions, remain in the aqueous layer. By this method 60 μg. of casein is clearly seen and 30 μg. is perceptible. With 3–5 mg. of nucleic acid the presence of 1 % of protein is readily detected. The fraction of YNA that precipitates at pH 3 contains 1 % of protein or a little less; the fraction precipitating at pH 2 contains much less than 1 % and sometimes no colour is detectable.

Purification of commercial nucleic acid

The contaminants that interfere most with enzyme measurements are degraded material and metals. The first must be removed because it is incompletely precipitated by the reagents used to precipitate YNA; this is partly achieved by re-precipitation with dilute HCl or concentrated acetic acid. Fe is not removed in this way, and Zittle (1946) found that Cu was not either.

Commercial YNA is suspended in water and neutralized with NaOH to give a 60–100 g./l. solution; this is dialysed in a sac protected by a stainless-steel gauze sleeve. During 1–2 weeks at 0° the outside water is changed frequently. The amount of material diffusing away varies from batch to batch but as much as 80 % may be lost. Protein, and also some nucleic acid, is removed by shaking with $CHCl_3$ and amyl alcohol as in the other method. NaCl is added to the fluid at pH 4 and at a concentration of 100–150 g./l. precipitation begins; it finishes in a few hours and the solid is centrifuged off. NaCl is added to saturation and another precipitate is separated off; a third comes out when the pH is adjusted to 2. The three fractions are dialysed separately; they contain approximately equal amounts of nucleic acid and together account for about half of the P present in the initially indiffusible material.

It would obviously be more convenient to reverse the sequence so that there is only one dialysis but with the commercial products that we have handled, there is little or no precipitation on saturation with NaCl at pH 3–4 unless the diffusible material has first been removed. The fraction most readily precipitated by NaCl is the most highly coloured and contains the most Fe. Thus a product which, after simple acid precipitation at the stage before the NaCl precipitation, contained 120 atoms of P for each atom of Fe, gave NaCl precipitates with P/Fe ratios rising from 40 to 350. The latter ratio amounts to 0·025 % Fe and this is more than we find in preparations made from yeast directly. Purified commercial preparations have invariably been more highly coloured than those made directly from yeast, but they are equally free from protein, Ca and Mg.

Precipitation by other salts. We have found no more convenient precipitant than NaCl. Precipitation by NH_4Cl is similar to that with NaCl; KCl and NaBr are less effective at the same saturation and $(NH_4)_2SO_4$ and Na_2SO_4 are much less so. Mg and La salts are well known precipitants for the nucleic acids but appear to be less selective.

Preparations from tobacco-leaf nucleoprotein

Nucleoprotein (NP) was made from the sap of young uninfected tobacco leaves by differential ultracentrifuging (Pirie, 1950) and used while still fresh. It can be dissociated into free nucleic acid and denatured protein in many ways. NaCl precipitation of the nucleic acid is satisfactory after fission by pouring a solution containing approximately 10 g./l. of NP at pH 8 into an equal volume of boiling 0·1 M-NaCl that is kept boiling during the addition, or by exposing the NP solution to 0·5 N-NaOH for 40 min. at 0°, or to 50 g./l. trichloroacetic acid (TCA) at room temperature for 8–12 hr. (Pirie, 1950). Most of the denatured protein is easily filtered from the boiled preparation; from the alkaline one it can be removed by adjusting the pH to 7 or by adding one-fifth volume of saturated $(NH_4)_2SO_4$ solution; after TCA fission the nucleic acid is extracted from the protein precipitate at pH 8. The three types of extract now behave similarly. A small amount of protein, accompanied by only a little nucleic acid, precipitates when the pH is adjusted to 4. This is centrifuged off and the fluid half-saturated with NaCl. There is an immediate opalescence and precipitation is complete in a few hours. This nucleic acid precipitate is centrifuged off and the supernatant is saturated with NaCl; again an opalescence is followed by precipitation. From the supernatant a further quantity of nucleic acid separates slowly on the addition of HCl.

The three products are dissolved separately at pH 4 and shaken with $CHCl_3$ and amyl alcohol; if the removal of denatured protein after the first stage of the preparation was satisfactory, little emulsion is formed. The process is repeated until there is none, and the supernatant fluids are dialysed.

For reasons that are not understood but that may well be connected with the physiological state of the original tobacco plant, the properties of NP are not constant in different preparations. The P content varies from 1·5 to 4·0 %. In general, preparations with low P contents are accompanied by chlorophyll-containing particles and contain up to 10 % of lipid. With starting material of this type the separation of denatured protein from nucleic acid is easier and more complete if the lipid is removed by precipitating the nucleoprotein with 10 vol. of a mixture of equal parts of ethanol and ether before fission by boiling or exposure to alkali. After TCA fission it is easier to remove the lipid by extracting the precipitate with ethanol–ether before the extraction at pH 8. There is no apparent advantage in removing the small amount of lipid present in preparations containing 2·7 % P or more. This variation in the starting material may in part explain the less regular distribution of the nucleic acid among the different fractions derived from NP than from other nucleic acid sources, but the point has not yet been studied in detail because these fractionations are difficult to replicate exactly. The

result of a representative experiment in which the three types of fission were compared on one preparation of NP are set out in Table 1. Three of the differences that appear in this table have been found consistently in many comparisons; TCA fission is least effective in separating nucleic acid from protein, NaOH fission leaves more of the P in a form that is not precipitable by either NaCl or HCl than the other two, and the best yield of NaCl-precipitable nucleic acid is given by boiling.

Table 1. *Distribution of phosphorus between fractions after fission of NP by three methods*

Description of the fraction	Percentage of the P in the original NP that appears finally in each fraction after fission by: TCA	Heating	Exposure to alkali
Protein coagulum at pH 7	20	11	10
Protein precipitated at pH 4	4	3	7
Protein removed from the nucleic acid preparations by $CHCl_3$ + amyl alcohol	5	7	3
Both NaCl precipitates	27	32	20
HCl precipitate	19	30	35
Final supernatant fluid	18	15	23

Preparation from tobacco mosaic virus (TMV)

Virus prepared by differential ultracentrifuging only, contains variable amounts of P that can be separated by incubation with trypsin; preparations made by precipitation with $(NH_4)_2SO_4$ and acid (Bawden & Pirie, 1943) are free from this and are, therefore, preferable as sources of virus nucleic acid. Nucleic acid is most conveniently separated from the protein by heating (Bawden & Pirie, 1937), but when separated by various other methods of fission it is also precipitable by NaCl. In Table 2 the results are summarized of an experiment in which half of a virus preparation was denatured by heating for 3 min. at 100° in 0·1 M-NaCl and half by exposure for 30 min. to 0·5 N-NaOH. Thereafter, the treatment was similar to that used with NP. The differences are clear. After heating, a larger proportion of the nucleic acid is precipitated by NaCl than after alkaline fission, and, in agreement with earlier observations (Grégoire, 1950), the proportion of the nucleic acid that remains unprecipitated even by HCl is large when NaOH as dilute as 0·5 N is used for the fission. If stronger alkali is used the proportion precipitated by NaCl is however smaller. Only 2–3 % of the nucleic acid is lost when the NaCl or HCl precipitates are shaken with $CHCl_3$ and amyl alcohol to remove residual protein, but the final products have not been so free from protein as those made from yeast.

It is clear from Tables 1 and 2 that there are losses of P in the initial protein coagulum, in the precipitates separated at pH 4, and in the emulsion that is made by shaking with $CHCl_3$ and amyl alcohol. With NP these losses are serious. A further quantity of nucleic acid can be made by suspending these protein fractions in water at pH 9–10 and adding NaCl or preferably $(NH_4)_2SO_4$ to 0·1 saturation. Most of the protein coagulates and brings out very little P, more is removed at pH 4 and the nucleic acid can then be precipitated by adding HCl; it is purified as before.

Table 2. *Distribution of phosphorus between fractions after fission of TMV by two methods*

Description of the fraction	Percentage of the P in the original TMV that appears finally in each fraction after fission by: Heating	Exposure to alkali
Initial protein coagulum and precipitate at pH 4	2–3	1·5–2
Precipitate on saturation with NaCl	70	28
Precipitate on adding HCl to lower pH to 2	14	43
Final supernatant fluid	14	27

Composition of NP and TMV nucleic acid preparations. Nucleic acid preparations made from NP and TMV are rarely so free from protein, determined by the biuret reaction, as those made from yeast. The fractions precipitating with NaCl have contained 1–3 % and those subsequently precipitated by HCl contain 1 % or a little less. The dried Na salts contain 7·5–8·0 % P. Because of shortage of material, metal determinations have not been made on many preparations. Furthermore, the nucleoproteins used for making them were not purified with any special precautions to avoid accidental contamination from traces of metal in the reagents. We have, therefore, no reason to think that the 0·10–0·16 % Ca, 0·01–0·03 % Fe, and less than 0·1 % Mg found in the preparations precipitated by NaCl are an integral part of the nucleic acid. The metal contents of preparations made by HCl precipitation were lower.

The presence of deoxyribose nucleic acid in ribose nucleic acid preparations. The diphenylamine reaction (Dische, 1930) gives a colour which can be readily seen, or measured, on a photoelectric colorimeter, with an amount of deoxyribonucleic acid (DNA) which contains 1 μg. of P. 3 mg. quantities of many YNA preparations have given less colour than this and if, as is probable, the small amount of colour that is given is the consequence of contamination, it corresponds to the presence of less than 0·4 % of DNA. Preparations

from NP have never given as little colour as those from yeast but the colour is still so faint as to be uncertain. Preparations from TMV, on the other hand, have always given a distinct colour; the intensity varies irregularly in the different fractions and corresponds to the presence of 0·5–2·0% of DNA. It is not certain that DNA is the cause of this colour, but the possibility that it is due to the presence of pectin or its breakdown products (Holden, 1953) seems to be excluded by the fact that the intensity is not increased by preliminary acid hydrolysis. Furthermore, Hoff-Jørgensen (1952), by an entirely different method of assay, found DNA in a TMV preparation. TMV preparations are commonly contaminated by a wide range of materials (cf. Pirie, 1949, 1953); we do not, therefore, claim that DNA is an essential part of the structure of TMV, and work is in progress to find out whether pretreatments that do not rob the virus of its infectivity will remove it.

Osmotic pressure measurements. The osmotic pressures exerted by these nucleic acid solutions were measured in simple osmometers with cellophan membranes at 0°. The level of the meniscus was observed regularly until equilibrium was reached; the osmometer was then disequilibrated and equilibrium attained from the other direction. Each process took 3–4 days and from the final value the height of capillary rise of the same fluid was subtracted. The concentration of nucleic acid was determined from the P content of the fluid inside the sac at equilibrium. All measurements were made at pH 6 and, to diminish the osmotic pressure set up by the uneven distribution of ions with this highly charged particle, M-NaCl was used as the outside fluid. Measurements made with the same preparation of nucleic acid against different concentrations of NaCl showed that there was no further diminution of the pressure when the NaCl concentration was increased above this value.

In Table 3 the results are set out. For convenience values for the 'molecular weight' are included; these were calculated on the assumption that all the preparations contained 8·5% P and that the van't Hoff law applies although the applicability of the law to systems which, like this one, contain highly charged, hydrated, and anisometric particles has been abundantly criticized. The 'molecular weight' figure is only included to show that these preparations fall within the range found by others with YNA that has not been subjected to extreme conditions. The values were calculated by the approximation:

$$\text{'molecular weight'} = \frac{\text{P content in g./l.}}{\text{height of fluid column in mm.}} \times 2{\cdot}6 \times 10^6.$$

YNA 'core' is the part of a YNA preparation that is resistant to attack by PRNase and was made by the method outlined in an accompanying paper (Holden & Pirie, 1955*a*). Two preparations have been used and, although it is clear that the particles are smaller than those of the parent YNA, these measurements do not support the conclusion reached by Markham & Smith (1952*b*) that the particles of core have a mean chain length of only four residues. These osmotic pressure measurements obviously only give evidence about the degree of dispersion of the preparations in M-NaCl and this is not the environment used in our enzyme experiments or in those of Markham & Smith. Furthermore, there may be an equilibrium between oligonucleotides and aggregates of them. The low value for the osmotic pressure should, however, be taken into account in any attempt to decide on the nature of all the internucleotide links in YNA.

Changes in the properties of nucleic acid brought about by the treatments used in the isolation. We have already pointed out that there is no source of 'native' nucleic acid that can be used as a standard on which to follow the effects of the various treatments used. The only apparent alternative is to repeat, on isolated products, the treatments used in the isolation. This procedure would clearly only recognize a progressive change which had not gone to completion during the isolation.

It is well known that the exposure of YNA to alkaline conditions for a few hours at room temperature or a few days at 0° diminishes its acid precipitability. This treatment also diminishes its precipitability by NaCl. The pH of the yeast suspension during the treatment with NaOH is 11·3; we do not

Table 3. *The osmotic pressure of various types of nucleic acid*

Type of preparation	P content of solution (g./l.)	Increase in height of capillary rise (mm.)	'Molecular weight'
YNA, NaCl ppt.	1·8	58	81 000
YNA, HCl ppt.	2·34	129	47 000
YNA, purified commercial	1·36	104	34 000
YNA, 'core'	0·308	44	18 000
Tobacco-leaf nucleic acid, NaCl ppt.	0·52	38	36 000
TMV nucleic acid, NaCl ppt.	0·44	17	67 000
TMV nucleic acid, HCl ppt.	0·41	27	39 500

find any change in the precipitability of YNA at pH 3·5 by NaCl after exposure for 60 min. at 0° to this pH and a change is only perceptible after 3 hr. Similarly, precipitation with HCl at pH 1·7 and exposure to that pH for the 3–5 min. necessary to centrifuge down the precipitate had no effect on the osmotic pressure of several preparations that had not, until then, been exposed to such acid conditions.

The changes that we recognize during the course of preparing YNA are in the other direction, for the process of precipitation with NaCl makes reprecipitation more easy. Thus material that has precipitated at pH 3·5 and 0·5 saturation with NaCl will partly reprecipitate at the same pH and 0·33 saturation, while material that did not precipitate till full saturation will partly reprecipitate at 0·5 saturation. Preparations from which the protein has been removed by shaking with $CHCl_3$ and amyl alcohol are largely precipitable by as little as 0·2 saturation, a concentration of NaCl with which little or nothing can be precipitated from the original extract from yeast, NP or TMV.

DISCUSSION

It is surprising that the apparently general applicability to the preparation of nucleic acids of precipitation by NaCl and some other salts in slightly acid solution has not been more widely commented on. Passing remarks in a few papers suggest that it has been noticed by others but, so far as we are aware, its use as a preparative procedure has not been advocated. The method shares with those depending on precipitation with ethanol or $MgSO_4$ the merit that exposure to a low pH is avoided and this may well be important. But the cycle of precipitation with HCl and re-solution at neutrality need not occupy more than 2 min. and can be carried out at 0°; there is no published evidence that such treatment degrades nucleic acid, and, as we have shown, it is an effective method of separating Ca and Mg from the final product. The method, like that in which the initial fission of nucleoprotein was brought about by $Sr(NO_3)_2$, may be useful because it is different from the existing methods without necessarily being better than them.

In addition to these possible advantages, precipitation by NaCl has the real advantage that it is gradual. In consequence the precipitate that separates under any particular circumstances is more likely to be in equilibrium with the environment whereas the usual acid precipitation is so nearly instantaneous that most of it probably takes place at a pH other than that of the bulk of the fluid. We have not yet shown that successive fractions, separating with increasing NaCl concentration, differ qualitatively though they differ in their precipitability and osmotic pressure, but the method holds some promise of being suitable for the fractionation of bulk preparations of nucleic acid.

We have already pointed out that all methods of separating nucleic acid from other tissue components may modify it and NaCl precipitation is no exception. Not only is there modification when the linkage with protein is broken but material that has been precipitated once with NaCl is more readily precipitated by it again. Changes in precipitability are most probably the consequence of associations between hitherto independent particles but there is no obvious method of assessing the magnitude or the detailed nature of the change. The nucleic acid in the extracts that we have studied, and these are made from yeast, leaf microsomes and virus particles, separates in a number of fractions of differing precipitability. We do not yet know whether this is a consequence of an initial diversity in the source or of partial modification during the isolation. Not only do we not know the relationship between nucleic acid in cell extracts and nucleic acid in the cell, we do not even know the relationship between nucleic acid in extracts and in purified preparations. The osmotic pressure measurements published here, and those already published by others, suggest that the mean particle weight in nucleic acid preparations can be large. This is confirmed by their other physical properties. Thus YNA that has been precipitated by NaCl gives a solution that is very viscous especially at low temperatures; some neutral solutions containing only 10 g. YNA/l. do not flow at 0° but will stay in an inverted test tube.

In an accompanying paper (Holden & Pirie, 1955*b*) we describe the effect of leaf and pancreatic ribonucleases on these nucleic acid preparations and find no striking differences. Their absorption spectra are also similar (Holden & Pirie, 1955*c*) and are similarly affected by treatments causing hydrolysis. Our results do not, therefore, throw any light on the nature of any specificity that may reside in the nucleic acids.

SUMMARY

1. Most of the nucleic acid in suitably prepared extracts from yeast, tobacco-leaf microsomes and tobacco mosaic virus (TMV) can be precipitated at pH 3–4 by NaCl. A small part of the nucleic acid in commercial products can also be precipitated.

2. Preparations of nucleic acid made from yeast in this way appear to have a high mean particle weight and to contain less than 1 % of protein.

3. Their contents of Ca, Fe and Mg are low and probably the result of contamination. Those from TMV contain significant amounts of deoxyribose nucleic acid but the other two are substantially free from it.

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A Comparison of Leaf and Pancreatic Ribonuclease

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The ribonucleases are relatively specific phosphodiesterases and their action can be followed by methods that depend either on the appearance of an acid or hydroxyl group when the ester link is broken or else by methods that detect changes in the particle size of the nucleic acid. It may be profitable to discuss the merits of the various methods that have been used, paying particular attention to their applicability to nucleic acid and nucleoprotein solutions in which the concentrations of nucleic acid or nucleoprotein fall in the physiological range, e.g. equivalent to about 10 mg. P/l. Methods that depend mainly on the first steps in the attack on the macromolecule are of more interest than those that place equal weight on all its stages.

The appearance of a new acid group has been followed by measuring the increase in buffering power in the region pH 6·8–7·9 (Allen & Eiler, 1941) or manometrically in bicarbonate buffer (Bain & Rusch, 1944). The latter method has been used in several laboratories but it calls for nucleic acid concentrations as high as 5 g. P/l.

Diminution in the molecular weight of the nucleic acid has been followed directly by Carter & Greenstein (1946), who carried out the reaction in dialysis sacs and measured spectrophotometrically the split products that diffused out. This method depends mainly on the first stages of the reaction but it is tedious if many determinations are being made and, as Markham & Smith (1952) have emphasized, variations in the salt concentration cause variation in the diffusibility of partly hydrolysed nucleic acid. For these reasons, diminution in molecular weight is generally inferred from a change in the acid precipitability of the nucleic acid. Many different conditions have been used and, in the experimental section of this paper, we compare some of them.

On hydrolysis there is a diminution in the absorption of light at 300 mμ. (Kunitz, 1946), but the effect is not large and is mainly a consequence of the later stages of the action. There is an increase in absorption at 260 mμ., which is discussed more fully in another paper (Holden & Pirie, 1955*c*); this increase depends on the extent of hydrolysis but is not proportional to it. The volume of the solution increases and then diminishes (Vandendriessche, 1951) and the ability to bind trimethyl *p*-(*p*-hydroxybenzeneazo)phenyl ammonium chloride decreases (Cavalieri, 1952). Inorganic phosphate is a product of the action of some enzyme preparations but there is reason to think that this action is due to contamination with a phosphatase so that any measurements depending on it deal with two enzymes simultaneously.

It is important to remember that the different methods of following the reaction may measure different stages of it. Thus Kunitz (1940) found that acid appeared more slowly than acid-soluble P and