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FACTORS AFFECTING NUCLEIC ACID EXTRACTABILITY IN PLANTS*

JOE H. CHERRY and HANNA CHROBOCZEK

Horticulture Department, Purdue University, Lafayette, Indiana

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Abstract—Factors affecting the amount and type of isotopically labelled nucleic acid extracted from peanut cotyledons by a two-phase partition system employing phenol were investigated. Nucleic acids isolated at 37° contained some degraded ribosomal RNA, while at 60° ribosomal RNA was severely degraded and DNA was completely eliminated from the aqueous extract. Changing the pH of the extraction medium over a range between 6·0–8·5 had little measurable effect on the type of nucleic acid extracted. However, addition of dupanol (sodium lauryl sulfate) to the extraction medium increased the amount of nucleic acids by 40 per cent, and the specific activity by 50 per cent; little DNA was isolated without dupanol. Differential extraction techniques involving variations in temperature, pH or dupanol gave two different samples of nucleic acid in each case. One contained most of the extractable nucleic acid while the second contained only 4–5 per cent of the total nucleic acid which was composed mostly (as judged by its relatively high specific activity) of newly synthesized ribosomal and messenger RNAs and DNA.

INTRODUCTION

WE ARE investigating the changes in different RNAs during growth of higher plants. In our work, as well as that reported from other laboratories, the yield of isotopically labelled RNA extracted with phenol may vary depending on temperature, pH and presence of organic anions. From published work, it is clear that the conditions used in extracting RNA with phenol are quite important. Georgiev *et al.*[1] have fractionated RNA into three classes depending on the temperature of extraction. When cell suspensions or tissue homogenates were mixed with an equal volume of phenol, pH 6, cytoplasmic RNA was obtained at 10° while R-RNA (ribosomal) and D-RNA (often referred to as messenger RNA) were extracted at between 10°–40° and 55°–60°, respectively. Even though the RNA extracted at high temperatures shows signs of degradation, it has a different base composition than that extracted at lower temperatures.

Brown and Littna[2] also used this type of procedure to extract different RNAs. They claim that there are no obvious differences between RNA extracted in phenol at low and high temperatures as judged by sucrose gradient profiles. However, their data indicate that heating causes degradation of the ribosomal RNA.

Brawerman[3] obtained different types of RNAs from *Euglena gracilis* by extracting the cells with phenol at two different pH values. RNA very similar to ribosomal RNA was obtained at pH 7·6; when the phenol was adjusted to pH 8 or 8·5 a small fraction of RNA

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[1] G. P. GEORGIEV, O. P. SAMARINA, M. I. TERMAN, M. N. SAMIRNOV and A. N. SEVERTZOV, *Nature* **200**, 1291 (1963).

[2] D. D. BROWN and E. LITTNA, *J. Mol. Biol.* **8**, 669 (1964).

[3] G. BRAWERMAN, *Biochim. Biophys. Acta* **76**, 322 (1963).

(about 4 per cent of the total RNA) was obtained with a much different base composition than the ribosomal RNA. Even though the author referred to this fraction as D-RNA, it has a much different base composition than DNA. Nevertheless, this fraction of RNA is suggested to be involved with cell differentiation. Brown and Littna[2] also preferentially extracted an RNA fraction at pH 7·3, that in radioactive experiments contained much more radioactivity than the fraction extracted at pH 5·1.

Kirby[4] found that the addition of certain salts to the phenol enhanced the extraction of both DNA and RNA. Of the chemicals tested, *p*-amino salicylate, sodium dodecyl sulfate and sodium benzoate increased the amount of DNA extracted. Kay *et al.*[5] and Kay and Dounce[6] had previously shown that dupanol (sodium lauryl sulfate) improved the extraction of both DNA and RNA in aqueous system. Georgiev[7] has showed that the addition of certain organic anions or the elevation of the pH increased the yield of RNA and greatly enhanced the extraction of DNA.

It is therefore apparent that Kirby's method[4] of extracting RNA with phenol has been modified by many investigators. If D-RNA (RNA with base composition similar to DNA) can be preferentially separated from soluble and ribosomal RNA by varying some element of the extraction procedure such as temperature, pH and dupanol, it would be a very useful technique. Therefore, we initiated this study to determine the effect of varying extraction conditions on the types of isotopically labelled RNA and DNA extracted from peanut (*Arachis hypoqaea* L.) cotyledons by the phenol method.

RESULTS

Effects of Temperature

It has been reported[1-3] that different classes of nucleic acids may be extracted from animal tissue, *Euglena*, etc., depending on the temperature. In order to establish whether this technique is satisfactory for a tissue from higher plants, we extracted the nucleic acids from peanut cotyledons at temperatures ranging from 2° to 60° and then fractionated them on MAK (methylated albumin on kieselguhr) columns. An elution profile typical of nucleic acids from plant tissue[8,9] and bacteria[10,11] using this technique is shown in Fig. 1A (extracted at 2°). As indicated, there are at least five major fractions. The soluble RNA is separated into two u.v. absorbing peaks; but since the radioactivity does not coincide with either of the peaks, it appears that the whole soluble RNA area is quite heterogenous. The second fraction contains at least three components, namely RNA, rapidly metabolized DNA (^{32}P-labelled), and non-metabolic DNA. The characterization of this DNA-RNA fraction has been reported earlier.[8]

Most of the RNA of peanut cotyledons is ribosomal. Since sedimentation constants have not been determined, we have designated this RNA as light ribosomal (lr) and heavy ribosomal (hr) (fractions III and IV), respectively. These two fractions should be equivalent to 16*S* and 23*S* RNAs of bacteria.[12] The so-called messenger (m) RNA is the last fraction eluted from

[4] K. S. KIRBY, *Biochem. J.* **64**, 405 (1956).
[5] E. R. KAY, N. S. SIMMONS and A. L. DOUNCE, *J. Am. Chem. Soc.* **74**, 1724 (1952).
[6] E. R. KAY and A. L. DOUNCE, *J. Am. Chem. Soc.* **75**, 4041 (1953).
[7] G. P. GEORGIEV, *Biokhimiya* **24**, 472 (1952).
[8] J. H. CHERRY, *Science* **146**, 1066 (1964).
[9] J. L. KEY and J. INGLE, *Proc. Natl Acad. Sci. U.S.* **52**, 1382 (1964).
[10] J. E. M. MIDGLEY and B. M. MCCARTHY, *Biochem. Biophys. Acta* **61**, 696 (1962).
[11] S. SPIEGLEMAN and M. HAYASHI, *Symp. Quant. Biol.* **28**, 161 (1963).
[12] S. A. YANKOFSKY and S. SPIEGLEMAN, *Proc. Natl Acad. Sci. U.S.* **49**, 538 (1963).

the MAK column. This fraction of RNA has a half-life of about 2 hr and its formation is inhibited by actinomycin D.[9, 13] Based on the work of Spiegleman's group,[11] therefore, this RNA is referred to as messenger RNA. Thus, fractionation of nucleic acids on MAK columns was used to determine the effect of the extraction procedure on the amounts of various nucleic acids isolated and the relative degree of degradation.

The elution profiles on MAK columns of the nucleic acids extracted in phenol–0·5% dupanol at 2° and 60° are presented in Fig. 1. Elution profiles not shown in this paper

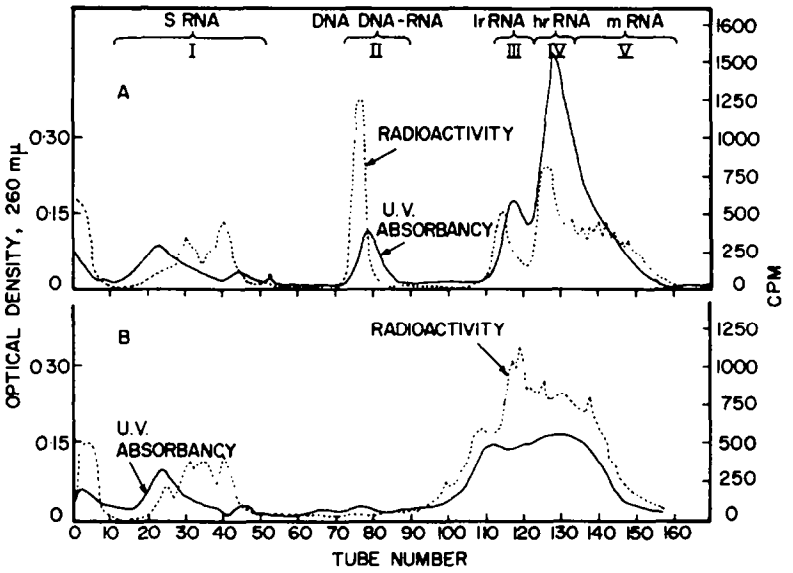

FIG. 1. SEPARATION ON MAK COLUMNS OF NUCLEIC ACIDS EXTRACTED AT 2° AND 60° FROM PEANUT COTYLEDONS.

After 2 hr of incubation with ^{32}P, 10 g of peanut cotyledon slices were homogenized in a medium containing 20 ml 0·01 M tris–HCl, pH 7·6, 0·06 M KCl, 0·01 M $MgCl_2$; 1 ml bentonite (40 mg); 3·1 ml 5·5% dupanol (sodium lauryl sulfate) and 34 ml of phenol (washed with tris–buffer). All samples were homogenized in an ice-bath. Subsequently, a series of the homogenates were incubated for 10 min at 2° and 60° C, Fig. A and B, respectively. Then the samples were centrifuged and the aqueous solutions were collected and extracted twice with cold phenol. Finally, the nucleic acids were precipitated from the aqueous solutions in ethanol. The precipitates were dissolved in 0·05 M sodium phosphate, pH 6·7 and dialyzed for two days against the same buffer. Two mg samples of each of the nucleic acid extracts were placed on a MAK column and eluted with a linear gradient of NaCl from 0·35 M to 1·00 M in 0·05 M sodium phosphate, pH 6·7. Fractions containing 5 ml each were collected.

indicate that extraction of nucleic acids at 27° and 37° causes degradation of the ribosomal and messenger RNAs. Also, treatment at 60° causes much degradation (Fig. 1B and is in agreement with the work of Georgiev.[1] Even though there is degradation of RNA while the extraction temperature is increased, the yield of nucleic acids increases with temperature up to 37° (Fig. 2A); however, the yield at 60° is reduced about 5 per cent.

Examination of the five major nucleic acid fractions separated by chromatography on MAK columns show that raising the extraction temperature reduces the amount of DNA extracted, with complete elimination of the ^{32}P-labelled DNA and its associated RNA

[13] H. CHROBOCZEK and J. H. CHERRY, *J. Mol. Biol.* In press.

fraction at 60° (Fig. 1B and Fig. 2B). As previously reported[8], the ^{32}P-labelled material is confined to the first half of the DNA peak and is composed of about 75 per cent rapidly metabolized DNA and 25 per cent RNA. Since both these labelled DNA and RNA fractions are degraded to the same extent and more readily than the other RNA fractions, this may indicate along with the previous report[8] that the rapidly metabolized DNA and RNA have a chemical association and perhaps form a native DNA–RNA hybrid. The extraction temperature also affects the relative amount of the various RNA fractions (Fig. 2B). The

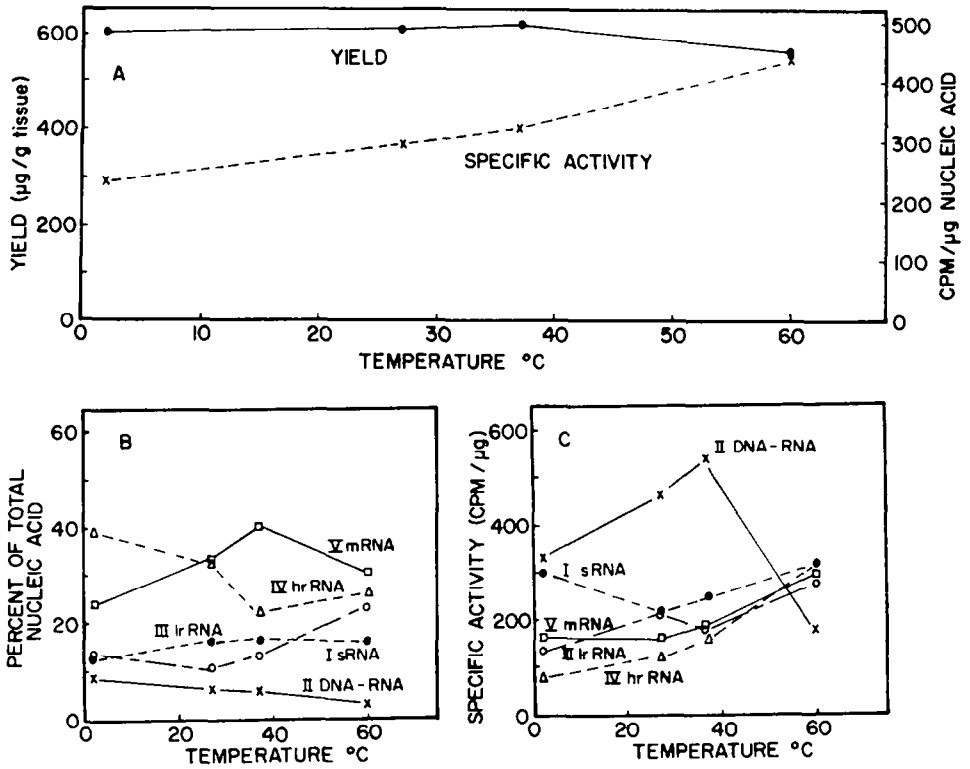

FIG. 2. EFFECT OF THE EXTRACTION TEMPERATURE ON YIELD AND SPECIFIC ACTIVITY OF NUCLEIC ACIDS.

A Yield and specific activity of the dialyzed nucleic acids (total) extracted at the four temperatures; B Relative amounts (per cent of total O.D.) of each of the five fractions of nucleic acids obtained from chromatography on MAK columns; C Specific activities of each of the fractions of nucleic acids obtained from chromatography on MAK columns.

amount of mRNA and sRNA increases with extraction temperature up to 37°, while raising the temperature to 60° reduces mRNA but has no affect on sRNA. The amount of hrRNA decreases with extraction temperature, while lrRNA increases between 2° and 37°. While it is shown that the relative amounts of RNAs change with extraction temperature, the major effect appears to result from wide-spread degradation of the RNAs at high extraction temperature, and thus causes a change in the amounts of RNA in each fraction. This is in agreement with Kurland[14] who has shown that the 16*S* and 23*S* ribosomal sub-units are broken-up by temperature into many heterogenous pieces.

[14] C. G. KURLAND, *J. Mol. Biol.* **2**, 83 (1960).

Although extraction of nucleic acids with hot (over 37°) phenol–dupanol causes degradation of RNA, the specific activity (cpm/μg) of the extracted nucleic acid increases with temperature (Fig. 2A). Perhaps the most recently synthesized RNA is more tightly bound to proteins and requires a rigorous treatment to free it. Apparently, this highly labelled fraction of RNA is not confined to any particular fraction (Fig. 2C). With the exception of sRNA, all the fractions increase in specific activity when the extraction temperature is raised from 2° to 37°. The ribosomal RNAs and mRNA increase in specific activity to 60°; the specific activity of the DNA peak drastically declines between 37° and 60° because the high temperature destroys the ^{32}P-labelled DNA and associated RNA. Therefore, from these data with peanut cotyledons, it is concluded that extraction at high temperatures is not a useful technique because it causes widespread degradation.

Effects of pH

In order to determine the effect of pH on the amount and type of nucleic acid extracted with the phenol method, ^{32}P-labelled samples of peanut cotyledon slices were homogenized

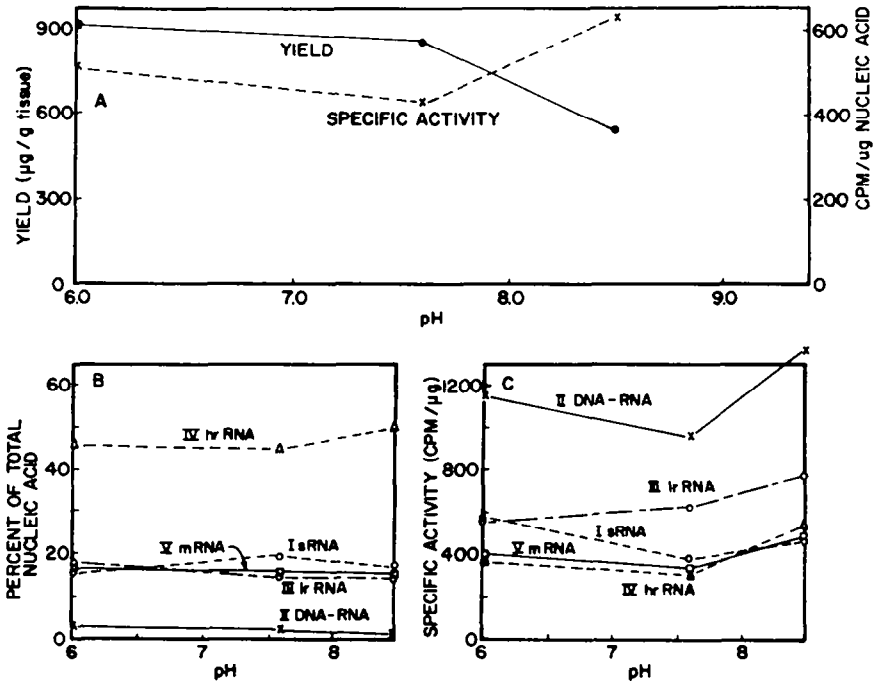

FIG. 3. EFFECT OF THE EXTRACTION pH ON YIELD AND SPECIFIC ACTIVITY OF NUCLEIC ACIDS.

A Yield and specific activity of the dialyzed nucleic acids (total) extracted at the three pHs; B Relative amounts (per cent of total O.D.) of each of the five fractions of nucleic acids obtained from chromatography on MAK columns; C Specific activities of each of the fractions of nucleic acids obtained from chromatography on MAK columns.

in media containing buffered phenol at pH 6·0, 7·6 and 8·5 in the absence of dupanol. The extraction was carried out at about 2° and the pH for each of the samples was maintained. As shown in Fig. 3A, the total yield decreases as the pH of the extraction medium is increased, whereas contrary to this, the specific activity increases. Results obtained by fractionation on

MAK colums of the nucleic acids extracted at the three pHs show little differences in the relative amounts of the five fractions (Fig. 3B). However, it is to be noted that twice as much DNA was extracted at pH 6·0 as at pH 8·5. Contrary to these results, Georgiev[7] and Kirby[15] found that more DNA was extracted at pH 6·6–7·1 than at 4·0–6·0. This difference may be because a difference in hydrogen bonds between DNA and protein of peanut cotyledons (20% protein) as compared to rat liver and also possibly to the difference in the solubility of the proteins in phenol.

In agreement with the analysis of the total nucleic acid (Fig. 3A), the specific activity of all the fractions separated on MAK columns were higher as a result of raising the pH of the extraction medium (Fig. 3C).

Effects of Dupanol

Since Kirby[15] and Georgiev[7] have shown that the addition of dupanol to phenol increases the extraction of both RNA and DNA, many investigators have chosen an arbitrary concen-

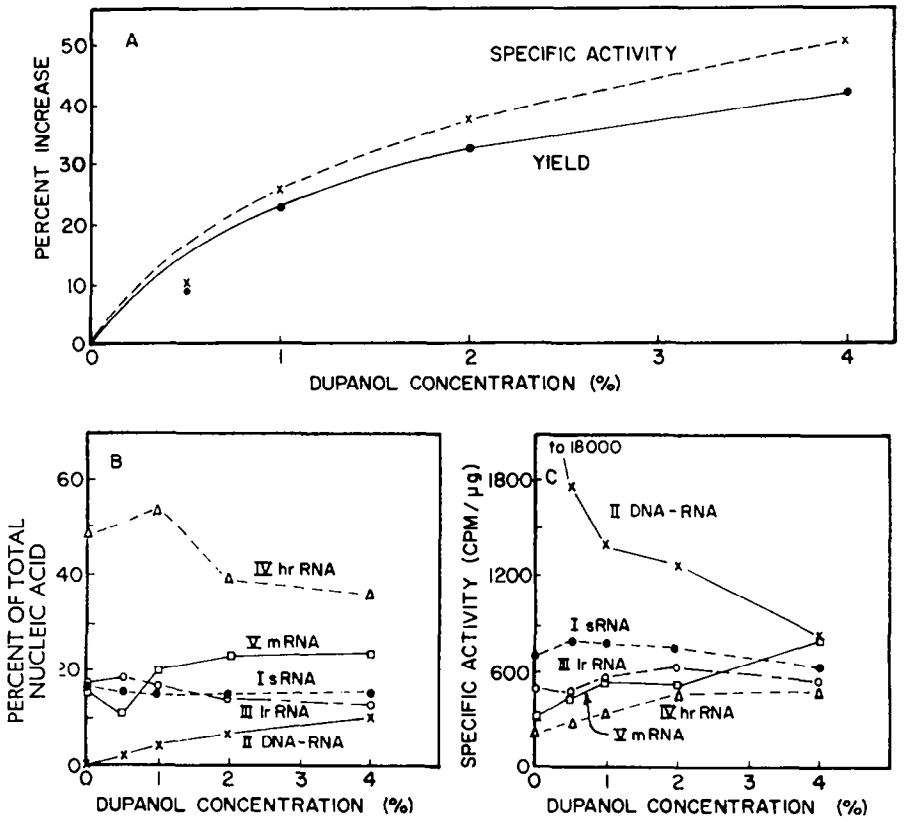

FIG. 4. EFFECT OF DUPANOL IN THE EXTRACTION MEDIUM ON THE EXTRACTABILITY OF NUCLEIC ACIDS.
A Yield and specific activity of the dialyzed nucleic acids (total) extracted with the aid of various concentrations of dupanol; B Relative amounts (per cent of total O.D.) of each of the five fractions of nucleic acids obtained from chromatography on MAK columns; C Specific activities of each of the fractions of nucleic acids obtained from chromatography on MAK columns.

[15] K. S. KIRBY, *Biochem. J.* **66**, 495 (1957).

tration of dupanol ranging from 0·5 to 2 per cent. In order to determine whether dupanol preferentially improves the extractibility of DNA and various types of RNAs, we extracted nucleic acids from peanut cotyledons using phenol and various concentrations of dupanol. Samples of peanut cotyledons labelled with ^{32}P were homogenized in media containing buffered phenol, pH 7·6; and dupanol to give concentrations of 0, 0·5, 1, 2 and 4%. The total yield and specific activities of the nucleic acids are shown in Fig. 4. The use of dupanol increases the total yield of nucleic acids by up to at least 40 per cent (Fig. 4A). It is also evident that dupanol improves the extractibility of rapidly labelled nucleic acid as the specific activity of the nucleic acids is increased with additions of dupanol even more than the yield. Thus, the additional nucleic acids extracted in the presence of dupanol contains a greater proportional amount of ^{32}P-labelled RNA. From results obtained by fractionating on MAK columns nucleic acids extracted in phenol with various concentrations of dupanol, it is clear that dupanol also greatly improves the extraction of DNA. Calculation of the relative amounts of nucleic acids extracted in the various concentrations of dupanol are presented in Fig. 4B. The per cent of DNA in the nucleic acid extract is increased from 0·1 per cent with no dupanol to 10 per cent when dupanol is added to give a final concentration of 4 per cent. The relative amount of hrRNA decreased and the amount of mRNA increased with increasing amounts of dupanol. The amount of dupanol in the extraction medium has no affect on the relative amount of sRNA and lrRNA.

Noteworthy, the specific activity of the mRNA fraction may be increased more than two-fold by extracting the tissue with tris–phenol containing 2 per cent dupanol (Fig. 4C). The specific activity (cpm/μg) of the mRNA increases with the amount of dupanol in the extraction medium. This occurs even though the relative amount of mRNA is also increased. The specific activity of sRNA, lrRNA, and hrRNA is highest when the extraction medium contained 2 per cent dupanol. Contrary to these results, the specific activity of the DNA–RNA fraction is very high when no dupanol is used and where only 1 to 10 per cent of the DNA is extracted. When the yield of DNA is improved by adding dupanol to the extraction medium, the specific activity is greatly reduced.

Differential Extraction Techniques

As mentioned above, several investigators[1–3] have used differential extraction techniques to preferentially extract two or more groups of nucleic acids. Georgiev[1] obtained three classes of RNA by varying the extraction temperature. Brawerman[3] and Georgiev[7] have fractionated nucleic acids into two classes by adjusting the pH of the extraction medium. We have examined the types of nucleic acids obtained by differential extraction techniques involving temperature, pH and dupanol, in order to assess their value.

As shown in Fig. 2, the specific activity of the nucleic acid increases with the extraction temperature. This implies that a tightly bound fraction of RNA which has a high specific activity is removed at high temperature. To determine the nature of this RNA, ^{32}P-labelled peanut cotyledons were homogenized and the bulk of the nucleic acids removed at 2°. Subsequently, the remaining nucleic acids were differentially extracted at 30° and 60°, respectively (see Fig. 5). Elution profiles on MAK columns of these three nucleic acid extracts show that a small fraction of RNA is differentially extracted at 30° which is mostly of the ribosomal and messenger type and which has a very high specific activity as compared to the RNA extracted at 2°. The RNA differentially extracted at 60° is badly degraded. The total yield and the relative amounts of each type of nucleic acid is summarized in Table 1. Although, the data show that the 30°-RNA is mostly hrRNA (45 per cent), it is apparent

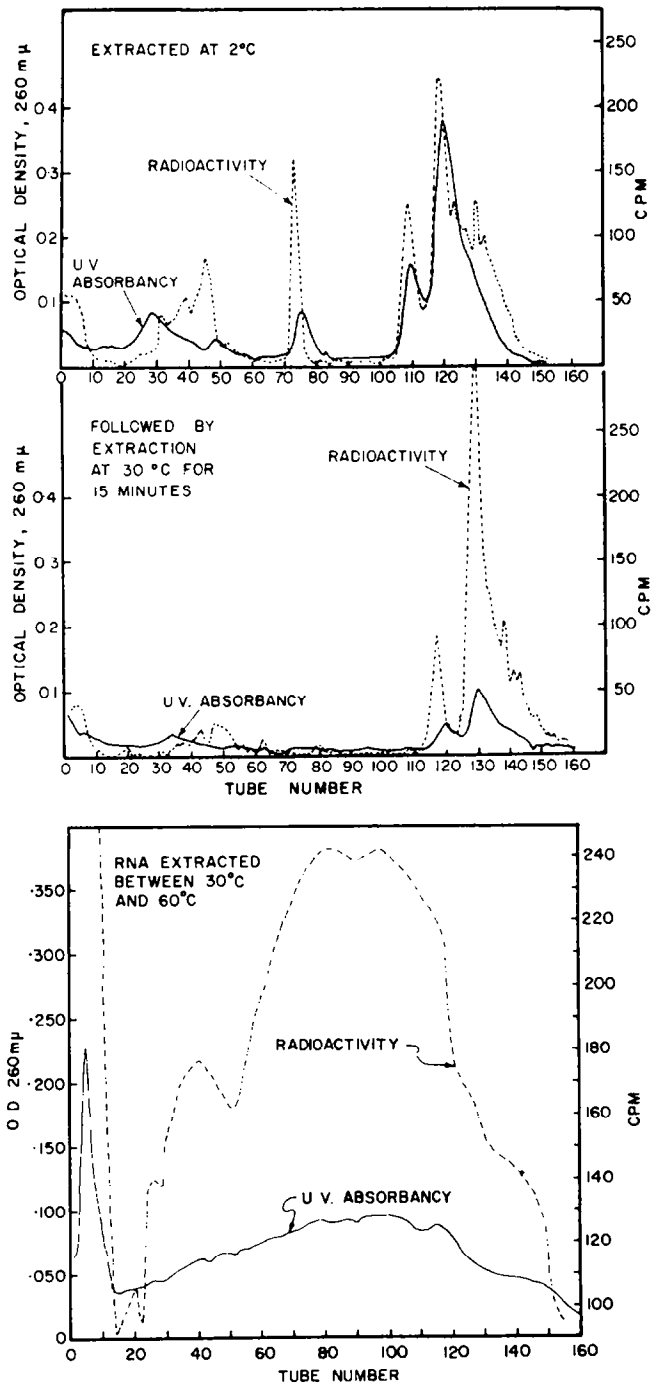
EXTRACTED AT 2°C
RADIOACTIVITY
U V ABSORBANCY
OPTICAL DENSITY, 260 mμ
CPM
FOLLOWED BY EXTRACTION AT 30 °C FOR 15 MINUTES
RADIOACTIVITY
U V. ABSORBANCY
TUBE NUMBER
RNA EXTRACTED BETWEEN 30°C AND 60°C
RADIOACTIVITY
U V. ABSORBANCY
O D 260mμ
TUBE NUMBER

FIG. 5.

that hrRNA has a more pronounced shoulder in the mRNA region. The specific activities of the total nucleic acid differentially extracted at 30° and the specific activities of each type of RNA or DNA are about 2 to 3 times greater than that extracted at 2° (Table 1). While the 30°-RNA represents only about 6% by weight of the 2°-nucleic acids, the marked increase in the specific activities of the ribosomal and messenger RNAs by differential extraction indicate that the most recently synthesized fractions are the hardest to extract.

TABLE 1. THE EFFECT OF A DIFFERENTIAL EXTRACTION TECHNIQUE EMPLOYING TEMPERATURE ON THE NUCLEIC ACIDS OBTAINED FROM PEANUT COTYLEDONS*

Extraction technique†	Total nucleic acid	Nucleic acid fractions				
		I sRNA	II DNA–RNA	III lrRNA	IV hrRNA	V mRNA
	Yield (μg/g tissue)	% of total extract				
Phenol, pH 7·6. 2° C	443	18·1	6·0	14·2	31·1	30·7
Followed by extraction at 30° C	27	23·6	0	15·6	45·1	15·8
		Specific activity (cpm/μg)				
Phenol, pH 7·6, 2° C	71	49	56	38	28	41
Followed by extraction at 30° C	141	45	0	92	130	134

* The nucleic acids were differentially extracted by the use of temperature and the total nucleic acid yield was determined. A sample of each was separated on a MAK column and the relative amount (per cent of total) of each type of nucleic acid was determined. The radioactivity of each fraction was determined and the specific activity calculated.

† The sample obtained at 60° (wt. 117 μg/g, specific activity 114 cpm/μg) was degraded and could not be fractionated.

Since Brawerman[3] has been able to differentially extract an RNA fraction, referred to as D-RNA, at pH 8 or 8·5 after removal of the bulk RNA at pH 7·6, we examined the type of RNAs that can be differentially extracted from peanut cotyledons by adjusting the pH. Labelled peanut cotyledons were homogenized in tris–buffer, pH 6·0, phenol and 2% dupanol to remove the bulk of the nucleic acids. After the unextracted fraction was washed once, additional tris–buffer, pH 8·5 was added and the pH of the entire solution was adjusted to 8·5. The two fractions of nucleic acid, that obtained at pH 6·0 and that differentially extracted at pH 8·5, were separated on MAK columns. Elution profiles of the two samples are similar to those differentially extracted by adjusting the temperature (Fig. 5), and is

FIG. 5. SEPARATION ON MAK COLUMNS OF NUCLEIC ACIDS DIFFERENTIALLY EXTRACTED EMPLOYING TEMPERATURE.

Peanut cotyledon slices labelled with ^{32}P were homogenized in a medium containing 0·01 M tris–HCl, pH 7·6, 0·06 M, KCl, 0·01 M $MgCl_2$, bentonite, phenol and 1% dupanol at 2°. The homogenate was centrifuged and the aqueous solution collected. Subsequently, tris-buffer was added to the phenol-sediment, mixed well and incubated at 30° for 15 min. Again the mixture was centrifuged and the aqueous solution collected. Next, more tris-buffer was added to the phenol and sediment, mixed and incubated at 60° for 15 min. The mixture was centrifuged and the aqueous solution collected. Between each step involving a change in temperature, the phenol and sediment was washed with tris-buffer at 2° and added to its corresponding aqueous solution. Each of the three aqueous solutions was treated twice with cold phenol (about 2°) and finally the nucleic acids were precipitated with ethanol. The dialyzed nucleic acids were separated on MAK columns.

TABLE 2. THE EFFECT OF A DIFFERENTIAL EXTRACTION TECHNIQUE EMPLOYING pH ON THE NUCLEIC ACIDS OBTAINED FROM PEANUT COTYLEDONS*

Extraction technique	Total nucleic acid	Nucleic acid fractions I sRNA	II DNA–RNA	III lrRNA	IV hrRNA	V mRNA
	Yield (μg/g tissue)	% of total extract				
Phenol, pH 6·0, 2°	923	15·5	3·3	18·2	46·6	16·3
Followed by extraction at pH 8·5, 2°	36	25·0	7·0	13·5	41·5	13·0
		Specific activity (cpm/μg)				
Phenol, pH 3·0, 2°	237	58	116	56	38	43
Followed by extraction at pH 8·5, 2°	437	37	192	333	231	153

* The nucleic acids were differentially extracted by the use of pH and the total nucleic acids yield was determined. A sample was fractionated on a MAK column and the relative amount (per cent of total) of each type of nucleic was determined. The radioactivity of each fraction was determined and the specific activity calculated.

therefore not shown. Only a small percentage (4%) of nucleic acids was obtained by re-extracting at pH 8·5 (Table 2). However, the nucleic acids obtained in this manner contains heavily labelled ribosomal and messenger RNAs. As shown in Table 2, the lr and hrRNAs, and mRNA of the pH 8·5 extract has specific activities about six and four times greater than that of the corresponding RNAs of the pH 6·0 extract. It is therefore apparent that a tightly

TABLE 3. THE EFFECT OF A DIFFERENTIAL EXTRACTION TECHNIQUE EMPLOYING DUPANOL ON THE NUCLEIC ACID OBTAINED FROM PEANUT COTYLEDONS*

Extraction technique	Total nucleic acid	Nucleic acid fractions I sRNA	II DNA–RNA	III lrRNA	IV hrRNA	V mRNA
	Yield (μg/g tissue)	% in total extract				
Phenol, pH 8·5, 2° C (No dupanol)	465	15·8	3·2	19·8	39·8	21·5
Followed by extraction with phenol, pH 6·0 and 2% dupanol	33	11·4	40·0	8·0	23·0	17·5
		Specific activity (cpm/μg)				
Phenol, pH 8·5, 2° C (No dupanol)	80	61	587	65	47	78
Followed by extraction with Phenol, pH 3·0 and 2% dupanol	187	67	97	91	89	93

* The nucleic acids were differentially extracted by the use of dupanol and the total nucleic acid yield was determined. Samples were fractionated on MAK columns and the relative amount (per cent of total) of each type of nucleic acid was determined. The radioactivity of each fraction of nucleic acid determined and the specific activity was calculated.

bound fraction of RNA, probably the most recently synthesized, can be differentially extracted by raising the pH from 6·0 to 8·5. From the elution profiles on MAK columns, it is not possible to determine whether the ribosomal and messenger RNA of the two extracts are different, other than in specific activity.

As shown in Fig. 4A, the addition of dupanol (4%) to the extraction medium increases the total nucleic acid yield by about 40 per cent. As the increase in specific activity by dupanol is greater than the increase in total yield it appears that dupanol brings about the extraction of highly labelled RNA. To test this hypothesis, the nucleic acids were differentially extracted from ^{32}P-labelled tissue with phenol followed by phenol containing dupanol and separated on MAK columns (Table 3). The addition of dupanol to the phenol-extracted residue extracts more of the DNA and its associated RNA fraction since the nucleic acid extract contains 40 per cent DNA and associated RNA (Table 3). The specific activities of ribosomal and messenger RNAs were at least 50 per cent higher in the case of the differentially extracted RNAs (Table 3). In the absence of dupanol and at pH 6·0, a fraction of nucleic acid can be extracted which contains mainly bulk RNA. However, when dupanol is added and the pH raised to 8·5 nearly all the DNA and highly labelled ribosomal and messenger RNAs are extracted.

DISCUSSION

While it would be desirable to differentially isolate two or more classes of RNA from tissue in which one would contain mostly messenger RNA, it appears that none of the three techniques employed in this paper does this entirely. In all three techniques tested, a small fraction of RNA with specific activities two to four times higher than the bulk RNA was isolated. Since extracting nucleic acids at 30° or higher leads to degradation of the ribosomal and messenger RNAs, differential extraction techniques employing changes in pH or the addition of dupanol appear to be most useful. In the case of peanut cotyledons, mostly bulk RNA can be extracted if the tissue is homogenized in tris buffer and phenol at pH 6·0. Following the removal of this RNA with the aqueous phase, highly labelled ribosomal and messenger RNAs and practically all the DNA can be removed by adding tris buffer, pH 8·5 and 2% dupanol. The extraction of DNA from peanut cotyledons in this manner is easier and faster than the method of extracting the tissue with 5% dupanol according to Marmur.[16] In either case (differential extraction or Marmur's procedure), we routinely found that the nucleic acid extracts from peanut cotyledons should be separated on MAK columns and the DNA peak isolated. When the DNA is extracted by Marmur's method[16] a great deal of protein cannot be easily removed from the extract. This protein clogs the MAK column unless it is removed by papain (E.C. 3.4.4.10). Therefore, we are using the differential extraction technique involving both pH and dupanol to extract DNA from plant tissue.

Differential extraction techniques have been previously employed in radioactive experiments to isolate a small fraction of nucleic acid which is highly labelled and apparently rich in messenger RNA. Since the base composition of this fraction of RNA is different from the bulk RNA and is similar to the DNA, it has been termed D-RNA.[1,3] As we have shown in this paper, the relative amount of mRNA or D-RNA present in the differential extract is much higher than that of the bulk nucleic acid extract. However, the differentially extracted nucleic acids are far from being mostly D-RNA. The fact that the nucleic acids extracted in this manner are heavily labelled (several-fold higher than bulk nucleic acids), indicates that

[16] J. MARMUR, *J. Mol. Biol.* 3, 208 (1961).

by using this technique one may extract the newly synthesized ribosomal and messenger RNAs. However, it appears to be incorrect to call the differentially extracted RNA specifically D-RNA. In cases where a relatively high amount of messenger RNA is desired this technique is very useful. Ingle *et al.*[17] has employed a technique similar to the one described to obtain fractions of RNA rich in the D-RNA type in a study of RNA metabolism in plants.

EXPERIMENTAL

Peanut seeds were germinated on moist absorbant paper or on moist vermiculite in the dark in a chamber kept at 29° and about 90% relative humidity. At the desired time after planting the germinated seeds were separated from the growing axis and washed several times in distilled water. The cotyledons were sliced into 1–2-mm slices and 10 g were incubated in a medium containing 1% sucrose, 10^{-4} M citric acid, pH 6·0 and about 0·5 mc carrier-free $NaH_2{}^{32}PO_4$. After two hours of incubation, the ^{32}P-labelled tissue was washed with distilled water and homogenized in a medium containing 20 ml 0·01 M tris–HCl, pH 7·6, 0·06 M KCl, 0·01 M $MgCl_2$; 1 ml bentonite (40 mg); an appropriate amount of 11% dupanol (sodium lauryl sulfate) to give the desired concentration in regards to the aqueous phase (1 g tissue arbitrarily considered 1 ml); and one volume of cold phenol washed with tris buffer. All samples were homogenized for two minutes with a VirTis homogenizer in an ice-bath. All subsequent steps were performed near 2° unless otherwise noted. In some experiments, the homogenates were made without dupanol, others without dupanol but at different pHs, and still other homogenates were made with dupanol and subsequently heated to various temperatures. Each of these changes are explained in the legend of the corresponding figure. In all cases, the homogenates were centrifuged at 20,000*g* for 10 min to separate the phenol from the aqueous phase. Then, the aqueous solutions which contained the nucleic acids were removed and subsequently extracted twice with an equal volume of cold phenol in the presence of bentonite. Finally, the nucleic acids were precipitated from the aqueous solution with two volumes of cold ethanol in the presence of 0·2 M potassium acetate. The precipitated nucleic acids were dissolved in 0·05 M sodium phosphate buffer, pH 6·7 and dialysed against the same buffer for two days.

Two mg samples of the nucleic acid extracts were placed on columns of methylated albumin coated onto kieselguhr (MAK) according to the method of Mandell and Hershey[18] and eluted with a linear gradient of NaCl from 0·35 M to 1·00 M in 0·05 M sodium phosphate, pH 6·7.

[17] J. INGLE, J. L. KEY and R. E. HOLM, *J. Mol. Biol.* **11**, 730 (1965).
[18] J. P. MANDELL and A. D. HERSHEY, *Anal. Biochem.* **1**, 66 (1960).