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The Chemical Composition of Microsomes and Mitochondria from Silver Beet

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Chemical analyses of the 'small granules' or microsomes isolated by the differential centrifuging of dispersions of animal tissues have been reported by a number of workers (see review by Schneider & Hogeboom, 1951). The results show that such microsomes contain a high proportion of ribonucleic acid (RNA) and lipid in addition to protein. About 50% of the total cellular RNA occurs in these particles. Mitochondria from animal tissues such as rat liver also contain a large amount of lipid (about 30% of the dry weight) but usually much less RNA than do microsomes (see Ada, 1949; Schneider & Hogeboom, 1951).

However, there are few published analyses of the cytoplasmic particles isolated from dispersions of plant tissues. Stafford (1951) found that the proportion of RNA in particles isolated from disrupted pea seedlings varied from 0·10% of RNA phosphorus in the larger particles to 0·24% in those which sedimented only when centrifuged at 60000 *g* for 30 min. The 'mitochondria' contained 30% of lipid and some deoxyribonucleic acid (DNA) (0·07–0·1% of DNA phosphorus), which was considered to arise as a contamination from disrupted nuclei. Brown, Jackson & Chayen (1953) analysed particles sedimented from dispersions of bean roots. The RNA (as mg./mg. of total nitrogen) varied from a value of 0·5 for particles sedimented at 1400 *g* for 1 hr. to 1·01 for particles sedimented between 25000 *g* for 1 hr. and 130000 *g* for 2 hr.

In a previous paper (Martin & Morton, 1956*a*) the isolation of microsomes and mitochondria from silver beet was described. These cytoplasmic particles were characterized in terms of their enzymic properties. The present paper describes analyses of these isolated cytoplasmic components to determine the lipid, RNA, DNA and protein contents.

To obtain a reliable estimation of both RNA and DNA, several different analytical procedures were compared. In agreement with Stafford (1951) and McClendon (1952), it has been found that procedures used by earlier workers do not give satisfactory values for RNA and DNA in the particles isolated from plant tissues. An improved analytical procedure is described. It has been found that the

microsomes and mitochondria of silver beet contain similar amounts of nucleic acids but differ markedly in the content of lipids.

MATERIALS AND METHODS

Nucleic acids. RNA and DNA standards for the ribose and deoxyribose estimations were semi-purified preparations kindly supplied by Mr G. L. Ada. The RNA (from yeast) was purified by the method of Smith & Markham (1950), and the DNA (from thymus gland) by the method of Signer & Schwander (1949). The preparations contained 8·3 and 7·9% of P respectively. Hydrolysates of RNA (Schwartz Laboratories Inc., New York, U.S.A.) and of DNA (British Drug Houses Ltd.) were used as markers for chromatography of bases.

Lipid solvents. Redistilled laboratory-reagent-grade *n*-butanol, A.R.-grade methanol and chloroform, and anaesthetic ether were used.

Buffered NaCl *and buffered* KCl. Sodium chloride (100 g.) was dissolved in 1 l. of 0·1 M sodium acetate buffer at pH 4·0. In some cases potassium salts were used in place of the sodium salts.

Perchloric acid. Analytical reagent (72%, w/v) was diluted and adjusted to N, 0·5 N and 0·2 N.

Protamine. A saturated solution of salmine sulphate (British Drug Houses Ltd., laboratory-reagent grade) in water was adjusted to pH 4·0 with N-H_2SO_4. This solution contained 1·8 mg. of protein N/ml.

Tissue preparations

Mitochondria and microsomes. These were isolated from dispersions of white petioles of silver beet (*Beta vulgaris*) by differential centrifuging as described previously (Martin & Morton, 1956*a*). The isolated particles were generally washed with 0·15 M-NaCl (see Fig. 1, Martin & Morton, 1956*a*) and the resedimented material was dried over P_2O_5 at room temperature. The products from several different preparations were combined to provide sufficient material for analysis. The mitochondria and microsomes for preparation I (see Table 4) were isolated from ten preparations between July and November 1953; for preparation II, from six preparations between March and May 1954; and for preparation III, from eight preparations in July 1955.

Whole beet petiole. Approx. 100 g. of white petioles of beet was dispersed in 100 ml. of water in a Waring Blendor for 2 min., frozen and dried *in vacuo*, and then ground to a fine powder. This was finally dried to constant weight *in vacuo* over P_2O_5 at room temperature.

Heart-muscle preparation. Disrupted sarcosomes from pig's heart muscle were obtained by the procedure of Keilin & Hartree (1947) as modified by Slater (1949), and dried over P_2O_5 *in vacuo* at room temperature.

Extraction of lipids, acid-soluble phosphates, nucleic acids and protein

The following extraction procedures have been used in the analyses of whole beet petiole and isolated cytoplasmic particles. The suitability of the procedures for extraction of RNA and DNA is considered in the Experimental section.

Dispersion of dried material. The dried materials were weighed into 1 cm. × 10 cm. centrifuge tubes, ground internally, into which a ground-glass pestle could be fitted. In some later work, smooth-walled centrifuge tubes fitted with a Nyathene (polyethylene plastic) pestle, as described by Kamphausen & Morton (1956), were used. The pestle was previously thoroughly extracted at room temperature with the solvents to be used.

After dispersion of the material either mechanically or by hand (as necessary), the pestle was washed clean with excess of the solvent applied as a jet from a syringe.

Lipids. The following treatments were all carried out at room temperature (16–22°).

The dried material (50–100 mg.) was dispersed (see above) in 3 ml. of methanol, extracted for 30 min. and centrifuged. The residue was extracted for 30 min. by dispersing in 6 ml. of 1:1 chloroform–methanol, followed by a similar treatment with 6 ml. of *n*-butanol. The residue was washed two or three times with 5 ml. quantities of diethyl ether, which was discarded. The lipid extract was made up to 20 ml. with methanol. Portions of 0·3 ml. were used for estimation of lipid P. For the estimation of total lipid by dry weight, 5 ml. portions of this extract were evaporated to dryness at the water pump and then dried to constant weight *in vacuo* over P_2O_5, $CaCl_2$ and paraffin chips at room temperature.

The remaining ether in the lipid-free material was removed by holding the material in a water bath at about 60° for approx. 15 min.

Acid-soluble phosphates. The lipid-free residue was extracted by dispersion in 4 ml. of 0·2 N-$HClO_4$ at 0° and immediately centrifuged at 0–2°. The residue was rapidly washed twice with 3 ml. portions of 0·2 N-$HClO_4$ at 0°, and the combined extracts were made up to 10 ml. Portions (0·2 ml.) were used for the estimation of total and organic P.

RNA. The residue from the treatment described above was dispersed in 6 ml. of N-$HClO_4$. The tubes were sealed with $HClO_4$-extracted plastic sheet (secured with rubber bands) and mechanically shaken for 24 hr. at 2–4°. After centrifuging, the residue was twice extracted with 4 ml. portions of N-$HClO_4$ for 3 hr. at 2–4°. The combined extracts were made up to 15 ml. with N-$HClO_4$ and centrifuged at 10000 *g* for 15 min. The small precipitate was then returned to the main residue material. Generally, 2 ml. portions of the combined extracts were used for estimation of total and inorganic P.

DNA. The remaining residue was washed once with 4 ml. of water at about 2° to remove residual $HClO_4$, dispersed in 5 ml. of buffered NaCl (or KCl) and then held for 20 min. in a boiling-water bath. After centrifuging, the residue was further extracted with 3 ml. and 2 ml. portions of buffered NaCl (or KCl) at about 100° for 2–5 min. each. The combined extracts were made up to 10 ml. and clarified as above when necessary. Portions of 2 ml. were used for estimation of total P and deoxyribose. As shown later, this material may consist largely of RNA.

Total nucleic acids (NA). In some cases, the extraction of RNA was omitted and the total NA was extracted with buffered NaCl (or KCl), as described above for extraction of DNA.

Protein. The residue remaining after removal of RNA and DNA was successively extracted at about 100° for 15 min. with 10 ml. of N-NaOH and 5 ml. of N-H_2SO_4. The combined extracts were made up to 20 ml. with water. Portions of 0·1 and 1 ml. were taken for the estimation of protein N and protein P respectively.

The residue remaining after the protein extraction, mainly polysaccharide with varying proportions of glass abraded from the pestle or homogenizer tube, was not examined.

Chromatographic procedures

Hydrolysis of nucleic acids. The dried, desalted nucleic acid extracts, containing 20–30 μg. of P, were taken up in 1 ml. of 88% (v/v) formic acid, and hydrolysed in a sealed tube in an electric oven at 180° for 90 min. The formic acid was removed from the opened tube by drying *in vacuo* over NaOH pellets at room temperature, and the residue taken up in 0·1 ml. of N-HCl and centrifuged. Portions of 6–12 μl. were used for the estimation of P, and 20–50 μl. for chromatography.

Paper chromatography. Whatman no. 1 filter papers were washed as described by Hanes, Hird & Isherwood (1952). Descending chromatograms were run for 20 hr. at 20–22° after equilibration with the solvent for about 1 hr. Unless otherwise stated, the propan-2-ol–HCl solvent of Wyatt (1951) was used. The chromatograms were dried in warm air and printed photographically as described by Markham & Smith (1949), the light from a 15 w General Electric Edison germicidal tube being used, filtered through a solution containing 10 g. of $CoSO_4,7H_2O$ and 35 g. of $NiSO_4,7H_2O$/100 ml. Kodak standard contact paper was used.

Elution of purines and pyrimidines. The positions of the bases on the chromatograms were traced from the photographic prints. Strips of 2–3 cm. length containing the spot were cut out, together with a similar blank cut from a free lane adjacent to the substance to be estimated. At one end the strip was cut to a tapered point, on which a glass clip was placed to direct the flow of the eluate into a collecting tube graduated at 1·2 and 2·5 ml. By means of a similar glass clip, the other end was attached to another strip of washed Whatman no. 1 filter paper, approx. 4–5 cm. long, the free end of which dipped into a glass trough containing 0·1 N-HCl. The paper was eluted until no material absorbing ultraviolet light could be detected in it. This usually took 30–45 min., 0·2–0·3 ml. of eluate being collected. The absorption of the eluates between 225 and 290 mμ. was measured after making up to volume (1·2 or 2·5 ml.) with 0·1 N-HCl.

Spectrophotometry

All absorption measurements were made with a Beckman spectrophotometer, model DU, with 1 cm. quartz cuvettes. Where required, the volume (approx. 3 ml.) of the cuvette was reduced to 1·1 ml. by using a displacer. This consisted of a solid Perspex block (1·0 cm. × 0·95 cm. × 4·6 cm.), slightly rounded at the corners so as to fit closely into the cuvette. About 0·5 cm. projected above the top of the cuvette. In the direction of the light path, a slot (0·64 cm. × 1·43 cm.) was cut through the block at 1·05 cm. from the base and centrally to the face of the displacer. A hole (0·45 cm. diam.), drilled from the top of the block, allowed material to be introduced into the cuvette. The displacer was extensively extracted with dil. HCl at room temperature to remove material absorbing u.v. light. RNA phosphorus and DNA phosphorus were estimated from the absorptions at $\lambda_{max.}$, with the extinction coefficients given by Ogur & Rosen (1950). Measurements were made with appropriate blanks.

The concentrations of bases in eluates from chromatograms were calculated from the absorption data given by Beavan, Holiday & Johnson (1955).

Calf-thymus DNA and yeast RNA, treated similarly to the plant material, were used as standards for estimation of DNA phosphorus and RNA phosphorus from the colorimetric determinations of deoxyribose and ribose respectively.

Other analytical methods

Dry weight. This was determined after drying to constant weight over P_2O_5 *in vacuo* at room temperature.

Deoxyribose. The colorimetric method of Ceriotti (1952) was used. Purification of chloroform as described by Ceriotti (1952) caused variable results. It was therefore used either as obtained commercially, or, in later work, after distillation over MgO in a fractionating column, the fraction obtained between 61·0 and 61·5° at atmospheric pressure being retained.

Ribose. The orcinol method of Mejbaum (1939) as modified by Hurlbert, Schmitz, Brumm & Potter (1954) was used.

Phosphorus. Inorganic P was estimated by the procedure of Martin & Doty (1949) as modified by Weil-Malherbe & Green (1951). Total P was determined after digestion of the material for 30 min. with 10N-H_2SO_4 and 1 drop of conc. $HClO_4$ (if not already present in the sample). The amount of 10N-H_2SO_4 (normally 0·5 ml.) was increased by 0·2 ml. for each 1 ml. of buffered NaCl (or KCl) present in the sample.

Nitrogen. Initially, the method of Harvey (1951) was used. In later studies, it was estimated colorimetrically with Nessler's reagent as previously described (Morton, 1955).

RESULTS

Extraction of nucleic acids

If the material extracted in the analytical procedure is predominantly NA, then good correlation should be obtained between estimates based on absorption at or near 260 mμ. (due to purines and pyrimidines), NA sugar, and organic P. This criterion was used to assess the suitability of procedures for estimation of RNA and DNA.

Comparison of the procedures of Schmidt & Thannhauser (1945) *and Ogur & Rosen* (1950). Initially, experience was gained with a preparation of disrupted sarcosomes from pig's heart. A summary of the results obtained with the Schmidt & Thannhauser (1945) and Ogur & Rosen (1950) procedures for fractionation of RNA and DNA is given in Table 1. Estimates of RNA phosphorus and DNA phosphorus were based on measurements of organic P, absorption of ultraviolet light and of the appropriate sugars. Table 1 shows that the value for RNA phosphorus obtained by the Schmidt & Thannhauser (1945) method is greatly in excess of the total NA phosphorus obtained by the Ogur & Rosen (1950) procedure. Moreover, the RNA extract obtained with the former procedure showed no absorption maximum between 250 and 270 mμ., thus indicating the presence of compounds other than RNA which interfere with the typical absorption of RNA. Hence the Schmidt & Thannhauser (1945) procedure was abandoned and attention was directed to the Ogur & Rosen (1950) procedure, which was developed specifically for estimation of NA in plant tissues.

Table 1. *Comparison of two procedures for determination of nucleic acids*

Results are means of duplicate determinations on a preparation of disrupted sarcosomes obtained from pig's heart (see under Materials and Methods). All results are expressed as percentages of dry weight. TCA, trichloroacetic acid.

	Ogur & Rosen (1950) method		Schmidt & Thannhauser (1945) method	
Estimation	Extraction procedure	Result	Extraction procedure	Result
Total lipid	*n*-Butanol at 60° for 15 min.	24·0	*n*-Butanol at 60° for 15 min.	22·4
Acid-soluble phosphates	0·2 N-$HClO_4$ at 0°, rapidly	—	10% (w/v) TCA at 0°, rapidly	—
Total NA phosphorus	—	—	N-NaOH at 37° for 18 hr.	—
RNA phosphorus				
(i) Direct	N-$HClO_4$ at 2° for 18 hr.	(i) 0·061	Supernatant remaining after	(i) 0·421
(ii) From absorption		(ii) 0·065	addition of 30% (w/v) TCA	(ii) *
DNA phosphorus				
(i) Direct	0·5 N-$HClO_4$ at 70° for 30 min.	(i) 0·089	Precipitate obtained from	(i) 0·113
(ii) From absorption		(ii) 0·095	total NA extract with TCA	(ii) 0·106
(iii) From deoxyribose		(iii) 0·090	(as above)	(iii) 0·083

* No absorption maximum in the range 250–270 mμ.

Extraction of nucleic acids from beet petiole. Ogur & Rosen (1950) established that RNA is quantitatively extracted from maize roots by N-$HClO_4$ in 18 hr. at about 2°. With whole beet petiole, however, this treatment continued to extract material absorbing at or near 260 mμ. up to 24 hr. (but none subsequently up to 30 hr.), even when the material was thoroughly dispersed as described under Methods. All extractions of RNA with the Ogur & Rosen (1950) procedure (summarized in Table 1) were therefore carried out for a total period of approx. 30 hr. at about 2–4°.

As shown in Fig. 1, the absorption spectrum of the extract of RNA (curve *A*) so obtained from beet petiole has a well-defined maximum at about 258 mμ. By contrast, however, an extract of total NA obtained by treatment of the same tissue with 0·5 N-$HClO_4$ at 70° for 30 min. shows a minimum absorption between 250 and 270 mμ. (curve *D*). It would be expected, therefore, that an extract of DNA (obtained by similar treatment) would show an equally unsatisfactory absorption curve. Hence DNA phosphorus as estimated from absorption could not be expected to correlate well with values determined as organic P.

The residue remaining after extraction of the total NA from whole beet petiole with buffered NaCl (see below) was subsequently extracted with 0·5 N-$HClO_4$ at 70° for 30 min. Like the extract of total NA obtained with hot 0·5 N-$HClO_4$ (curve *D*), this extract also showed a minimum absorption between 250 and 270 mμ. (Fig. 1, curve *C*). It contained negligible organic P (Table 2). Thus treatment of beet petiole with hot 0·5 N-$HClO_4$ extracts compounds which absorb ultraviolet light but which are not NA, a finding similar to that of Stafford (1951) with pea seedlings.

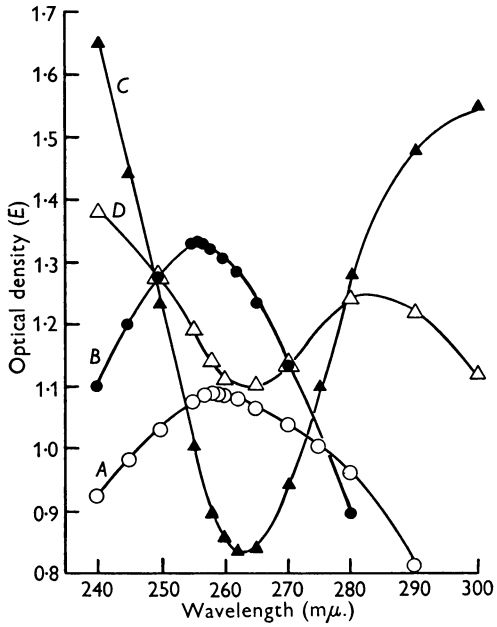

Fig. 1. Absorption spectra of extracts of beet-petiole preparations obtained with N-$HClO_4$ at 2–4° for 30 hr. (curve *A*); with buffered NaCl (10%, w/v) at 100° for 20 min. (curve *B*); with 0·5N-$HClO_4$ at 70° for 30 min., after treatment with buffered NaCl (curve *C*); and with 0·5N-$HClO_4$ at 70° for 30 min. (curve *D*).

While treatment with N-$HClO_4$ in the cold may therefore be satisfactory for extraction of RNA, clearly the use of hot 0·5 N-$HClO_4$ is not suitable for analytical determination of DNA in plant material.

Extraction of nucleic acids by NaCl. When whole beet petiole was extracted with 10% (w/v) NaCl in

a boiling-water bath for 20 min., the solution showed a well-defined absorption maximum at 260–262 mμ. However, the estimate of total NA phosphorus based on the absorption at $\lambda_{max.}$ exceeded the direct estimation of organic P by about 30 %. Better agreement between the two estimates of total NA phosphorus was obtained by extracting as above with NaCl buffered at pH 4·0 (see Materials and Methods) in order to minimize the extraction of non-nucleic acid material. Fig. 1 (curve *B*) shows the absorption spectrum of the total NA extracted with buffered NaCl. The failure to remove any additional NA by further treatment of the residue with hot 0·5N-$HClO_4$ (see p. 224, and Table 2) indicates that the NaCl treatment is adequate for quantitative extraction of NA from beet petiole.

These results suggested that more satisfactory estimates would be obtained by extracting first RNA with cold N-$HClO_4$ and subsequently DNA with hot buffered NaCl. Where required, total NA could be extracted with hot buffered NaCl only. This is the basis of the procedure described under Materials and Methods.

Colorimetric estimation of NA sugars in extracts of beet petiole

Table 3 shows that estimation of ribose in extracts of particulate preparations from silver beet give values for RNA far higher than those obtained either by measurements of absorption at $\lambda_{max.}$ or of organic P. The absorption curves of the coloured complexes obtained by the method of Hurlbert *et al.* (1954) suggested that most of the material responsible for the colour in extracts of mitochondria was, in fact, ribose, but considerable amounts of other interfering substances were present in the extracts of whole beet petiole. As shown below, separation of the NA from interfering compounds could not be achieved. Hence the determination of ribose was not used to estimate RNA.

Determination of deoxyribose in extracts of mitochondria and microsomes from beet petiole by the Ceriotti (1952) procedure gave values of DNA phosphorus somewhat greater than those obtained by chromatographic estimation of thymine (see below).

Table 2. *Comparison of perchloric acid and sodium chloride for extraction of nucleic acids from whole beet petiole*

Lipids and acid-soluble phosphates were extracted from dried beet petiole as described under Materials and Methods. In Expt. 1, the residue was then treated to extract RNA and DNA separately. In Expt. 2, a second portion was treated to extract total NA, and then the remaining material was further extracted with $HClO_4$. The results are the means of duplicate determinations and are expressed as percentages of dry weight.

Expt.	Determination	Extraction procedure	Organic P	NA phosphorus (from u.v. absorption)	Characteristics of absorption curve
1	RNA	N-$HClO_4$ at 2–4° for 30 hr.	0·036	0·048	Fig. 1, curve *A*
	DNA	10 % (w/v) NaCl* at 100° for 20 min. (after extraction of RNA)	0·030	0·037	Sharp peak at 256 mμ.
2	Total NA	10 % (w/v) NaCl* at 100° for 20 min.	0·063	0·060	Fig. 1, curve *B*
	Remaining NA	0·5N-$HClO_4$ at 70° for 30 min. (after NaCl extraction)	0·002	0	Fig. 1, curve *C*

* Buffered at pH 4·0 as described under Methods.

Table 3. *Comparative values for RNA in particulate fractions from silver-beet petiole, based on measurement of phosphorus, absorption of ultraviolet light, and ribose measurements*

Fractions were obtained from dispersions of whole beet petiole by differential centrifuging as described under Materials and Methods. The extracts were prepared as described in the text. The results are expressed as RNA phosphorus/ml. of extract, and may be compared only within the one extract.

Particulate fraction	Solvent	RNA phosphorus: From organic P	From u.v. absorption	From ribose
Mixed plastids, nuclei and mitochondria*	Cold N-$HClO_4$	2·8	2·9	10·1
	Hot 10 % (w/v) KCl	6·0	5·8	39·5
Mitochondria	Cold N-$HClO_4$	1·8	1·8	3·9
	Hot 10 % (w/v) KCl	4·2	4·1	11·7

* Obtained by centrifuging whole dispersion at 10000 *g* for 15 min.

Precipitation of nucleic acids from extracts of beet petiole

To improve the agreement between estimates based on measurements of ultraviolet absorption, NA sugar and organic P, an attempt was made to precipitate both RNA and DNA quantitatively and free from contaminant compounds. The extent of precipitation was estimated by measuring the absorption at $\lambda_{max.}$ of either the supernatant, obtained by centrifuging, or of the dissolved precipitate, as appropriate.

Organic solvents, including dioxan, methanol, ethanol and propanol, and methanol–butanol, methanol–diethyl ether and methanol–chloroform mixtures were used to precipitate the RNA extracted from lipid-free baker's yeast by hot buffered NaCl (10%, w/v). No more than 70% of the RNA was precipitated by any of these solvents.

The conditions for precipitation with protamine were more extensively investigated. For precipitation of yeast RNA and calf-thymus DNA from solutions of low ionic strength, the optimum pH values were 7·5 and 4·5 respectively, and the optimum proportions were 35 mg. of protamine/mg. of RNA or DNA. Under these conditions, about 96% of the RNA and 85% of the DNA were precipitated.

However, the extent of precipitation of RNA fell considerably when the concentration of potassium phosphate buffer exceeded 0·08 M at pH 7·6. Increasing the concentration of sodium acetate buffer (pH 4·3) to 0·5 M decreased by half the amount of DNA precipitated, the effect being almost linear with increasing salt concentration up to 0·5 M.

When yeast RNA was treated for 30 hr. with N-$HClO_4$ at 2–4°, only 10–20% was precipitated with protamine, even in the presence of added methanol. There was little or no precipitation of NA from buffered NaCl extracts of whole beet petiole unless the salt was removed by dialysis against water (Visking cellophan tubing was used). However, up to 80% of the NA in the extract was lost through the sac during this dialysis.

It appears therefore that NA is so depolymerized by both these treatments (which are used for extraction) that quantitative precipitation with protamine cannot be obtained.

Analyses of whole tissue, mitochondria and microsomes

The results obtained with the procedures described under Materials and Methods for estimation of lipids, nucleic acids and proteins are given in Table 4. All results are the means of duplicates, except that triplicates were used for microsome preparation III. The maximum variation between replicates was 7%, and usually did not exceed 4%.

Table 4 shows that, both in the $HClO_4$ and NaCl extracts, there is good agreement between estimates of NA phosphorus based on measurements of absorption of ultraviolet light and of organic P. However, except in whole beet petiole, the values of DNA phosphorus obtained by determination of deoxyribose in the NaCl extracts are considerably below those obtained from either of the other two measurements.

Analyses of nucleic acids by chromatographic estimation of bases in extracts from cytoplasmic particles

Since the analyses (Table 4) showed a marked discrepancy between the estimate of DNA based on deoxyribose as compared with those obtained by other methods, it appeared essential to examine the nature of the components in the separate extracts. Quantitative estimation of the bases in the $HClO_4$ and salt extracts were therefore carried out chromatographically.

Since previous experience had shown that the removal of NaCl from the extracts containing DNA was difficult without loss of material, buffered KCl was used in place of buffered NaCl for extraction of DNA for the analyses of preparation III.

Concentration and chromatography of extracts. The material remaining from the chemical analyses of microsome preparation III (see Table 4) was used. The RNA extract (in N-$HClO_4$) was neutralized with 5N-KOH to pH 7·0 with bromothymol blue as an external indicator. The solution was cooled to 0° and the $KClO_4$ removed by centrifuging. The supernatant, in a small tube in a water bath at 60°, was evaporated to dryness by a stream of air directed on to the liquid surface. To remove the last traces of $KClO_4$, the residue was extracted with approx. 1 ml. of water at 0° and centrifuged, and the supernatant re-evaporated. With freshly prepared $HClO_4$ extracts about 90% of the NA was always recovered, indicating little or no adsorption of nucleic acid by the precipitates of $KClO_4$.

Approx. 11N-$HClO_4$ was added to the DNA extract (in buffered KCl) at 0° until no further precipitate of $KClO_4$ appeared. The HCl produced by precipitation of the K^+ ion was then removed by evaporating the supernatant to a constant volume at 40° and then freezing and drying *in vacuo*. The residue (containing slight excess of $HClO_4$) was diluted with 1 ml. of water and the $HClO_4$ removed as described above by neutralization with 5N-KOH. The recovery of NA varied from 75 to 90%.

Chromatograms obtained after hydrolysis with 11N-$HClO_4$ at 100° for 1 hr. (Marshak & Vogel, 1950) showed variation in the proportion of purines to pyrimidines. Often all spots were partially obscured by fluorescent streaks, possibly due to

Table 4. *Analysis of whole petiole dispersion, mitochondria and microsomes from silver beet*

Materials were prepared and analysed as described under Materials and Methods. Numbers I, II and III refer to different preparations as described under Methods. All results are means of duplicate or triplicate determinations and are expressed as percentage of dry weight.

		Whole petiole dispersion		Mitochondria		Microsomes		
Extraction procedure	Determination	I	II	I	II	I	II	III
Methanol, chloroform–methanol, *n*-butanol, and ether, at room temp.	Total lipid	14·2	—	38·7	38·7	48·6	50·2	56·9
	Lipid P	0·050	0·077	0·273	0·266	0·760	0·687	0·752
	P as % of total lipid	0·35	—	0·71	0·69	1·56	1·37	1·32
0·2 N-$HClO_4$ at 0°, rapidly	Acid-soluble P							
	Total P	0·170	0·165	0·570	0·577	0·440	0·473	—
	Organic P	—	—	—	0·368	—	0·318	—
	Inorganic P	—	—	—	0·209	—	0·155	—
N-$HClO_4$ at 2–4° for 30 hr.	RNA phosphorus							
	Direct	0·0142	0·0152	0·0165	0·0252	0·0245	0·0386	0·0504
	From absorption	0·0150	0·0167	0·0176	0·0230	0·0232	0·0417	0·0510
10% (w/v) NaCl (or KCl) at 100° for 20 min.	DNA phosphorus							
	Direct	0·0140	0·0128	0·0465	0·0327	0·0456	0·0310	0·0450
	From absorption	0·0186	0·0159	0·0485	0·0361	0·0475	0·0382	0·0400
	From deoxyribose	0·026	—	0·0142	0·0086	0·0099	0·0085	0·0098
N-KOH at 100° for 15 min. then N-H_2SO_4 at 100° for 15 min.	Protein							
	Total P	0·0225	0·0230	0·075	0·211	0·102	0·111	0·360
	Nitrogen	—	—	6·10	7·28	4·97	6·44	3·64
	Protein (N × 6·25)	—	—	38·1	45·5	31·1	40·2	22·7
Total P (by direct determination)		0·280	0·285	0·94	1·12	1·11	1·35	—
Total P (by addition)		0·271	0·293	0·98	1·11	1·37	1·34	—

protein in the original extracts (Wyatt, 1952). Far better results were obtained with the formic acid procedure of Wyatt & Cohen (1953).

Duplicate spots (20–50 μl.) of the hydrolysates of the extracts, together with markers consisting of similar hydrolysates of authentic samples of RNA and DNA, were applied to washed Whatman no. 1 filter papers (approx. 35 cm. × 35 cm.). The bases were then separated and estimated as described under Materials and Methods.

Purines and pyrimidines in $HClO_4$ *and* KCl *extracts.* The chromatograms of the N-$HClO_4$ extract from beet microsomes III showed four spots corresponding in position with guanine, adenine, cytosine and uracil in the hydrolysate from authentic RNA. Moreover, as shown in Table 5, the absorption spectra corresponded closely with the spectra of these bases as given by Beavan *et al.* (1955). No thymine was detected in any chromatograms of N-$HClO_4$ extracts of microsomes or of mitochondria. Table 5 shows the relative proportions of bases (in relation to applied NA phosphorus) in the $HClO_4$ extract of microsome preparation III.

Chromatograms of the hydrolysate of the buffered KCl extract of microsome preparation III showed four spots, identified as guanine, adenine, cytosine and uracil by criteria similar to those described above. A much weaker fifth spot corresponded in position with thymine from the hydrolysate of authentic DNA. However, the amount of material in the eluate of this spot was insufficient for adequate identification by the absorption spectrum or by rechromatography in other solvents. However, the absorption at $\lambda_{max.}$ (268 mμ.) was used to obtain an estimate of the maximum possible concentration of this base. The purine and pyrimidine composition of the KCl extract is given in Table 6.

Table 6 shows that the KCl extract contains a large proportion of uracil. As shown in Table 5, the proportion of uracil in the RNA extracted by N-$HClO_4$ is 0·247 mole/0·913 mole of material (i.e. 0·27 mole/mole of NA phosphorus). If it is assumed that the proportion of uracil in the RNA extracted by buffered KCl is the same, then the amount of RNA phosphorus in this extract may be calculated from the uracil estimate (0·243 mole; Table 6) to be approx. 90 % of the applied NA phosphorus. The proportion of thymine in wheat-germ DNA is approx. 0·27 mole of thymine/mole of P, as calculated from figures given by Chargaff (1955). If it is

Table 5. *Purine and pyrimidine composition of nucleic acid extracted from beet petiole microsomes by extraction with cold* N-$HClO_4$

Extracts were hydrolysed by 88 % (v/v) formic acid at 180° for 90 min. and chromatographed according to Wyatt & Cohen (1953). Spots were eluted in 0·1 N-HCl and the absorption curve was determined in a Beckman spectrophotometer. The absorption data of Beaven *et al.* (1955) were used for the calculation of concentrations of bases. Results are the means of duplicate determinations and are expressed as μg. (or mole) of base recovered/μg. (or mole) of organic P applied. Wavelengths are expressed in mμ.

		Guanine	Adenine	Cytosine	Uracil
$\lambda_{max.}$	Observed	250, 268	262	275	261
	From Beaven *et al.* (1955)	248·5, 275	262·5	276	260
$\lambda_{min.}$	Observed	225	230	238	230
	From Beaven *et al.* (1955)	224	229	238·5	228·5
μg. of base/μg. of P		1·12	0·867	0·853	0·895
Mole of base/mole of P		0·229	0·199	0·238	0·247

Recovery (%) = 91·3

Ratios of bases: $\frac{\text{Purines}}{\text{Pyrimidines}} = 0{\cdot}88$; $\frac{\text{Adenine + uracil}}{\text{Guanine + cytosine}} = 0{\cdot}95$.

Table 6. *Purine and pyrimidine composition of nucleic acid extracted from beet petiole microsomes by treatment with hot* KCl

Extraction procedure is described under Materials and Methods. Other details are as shown in Table 5.

	Guanine	Adenine	Cytosine	Uracil	Thymine*
$\lambda_{max.}$	249, 270	262	275	260	—
$\lambda_{min.}$	225	230	238	232	—
μg. of base/μg. of P	1·10	0·895	0·815	0·880	0·020
Mole of base/mole of P	0·226	0·205	0·227	0·243	0·005

Recovery (%) = 90·6.

* The spot running in an identical position with that of thymine showed an unsatisfactory u.v. absorption spectrum, suggesting contamination of thymine with other material. Calculations based on this result give a maximum possible figure for thymine.

assumed that the proportion of thymine in the DNA extracted by buffered KCl is about the same as in wheat germ, then the amount of DNA phosphorus in the salt extract may be calculated from the thymine figure (0·005 mole, Table 6) to be approx. 2 % of the applied NA phosphorus.

Only 91 % of the total NA phosphorus of the KCl extract could be accounted for by purines and pyrimidines found on chromatography (Table 6). Even if the whole of the unrecovered organic P were DNA phosphorus, this could not exceed 8–9 % of the total NA phosphorus of the KCl extract. The estimate of DNA phosphorus in microsome preparation III by chromatographic methods is therefore between 2 and 9 % of the total NA phosphorus of the buffered KCl extract. However, Table 4 shows that the estimate of DNA phosphorus (0·0098 % dry weight) based on deoxyribose is approx. 22 % of the total NA phosphorus (0·045 % dry weight) in the salt extract of the same preparation. The estimate based on deoxyribose therefore considerably exceeds that obtained from measurement of thymine.

In similar studies of extracts of the mitochondrial preparations, the estimate of DNA phosphorus based on recovered thymine was always less than that obtained from measurement of deoxyribose.

Therefore, the estimate of DNA phosphorus in the particle preparations obtained by measurement of deoxyribose is probably a maximum and may exceed the true figure. The excess NA phosphorus in the salt extracts is RNA phosphorus, which may be estimated by subtracting the DNA phosphorus (estimated from deoxyribose) from the total organic P. The true RNA phosphorus figure for any preparation is the sum of that extracted by N-$HClO_4$ and by salt. Table 7 shows the corrected nucleic acid composition of the petiole microsomes and mitochondria.

Percentage distribution of nitrogen, lipid phosphorus and NA *phosphorus*

Table 8 shows the percentage distribution of total dried material, nitrogen, lipid P and total NA phosphorus in the various fractions isolated from beet petiole. For these analyses, the total NA phosphorus was extracted with buffered KCl as described under Materials and Methods.

DISCUSSION

Analytical procedures

Lipid analysis. Various workers (for example, Ada & Perry, 1954) have shown that failure to extract phospholipids completely is a common fault of some procedures, such as that of Schmidt & Thannhauser (1945), designed to estimate RNA and DNA as organic phosphorus.

Many phospholipids are poorly soluble in solvents such as ether and ethanol, frequently used by other workers (for example, Ogur & Rosen, 1950). Folch, Ascoli, Lees, Meath & LeBaron (1951) found that methanol–chloroform quantitatively removes phospholipids from brain and spinal cord with minimal co-extraction of non-lipid material. Morton (1950) found *n*-butanol to be particularly effective in splitting lipoprotein complexes. Hence the choice of these solvents in the analytical procedure. Final washing with ether is necessary, particularly to remove *n*-butanol.

The use of an homogenizer to disperse the dried material greatly facilitates extraction of both lipids and nucleic acids. Moreover, the extraction of any given fraction is greatly improved, thereby minimizing the contamination of one fraction with another.

Nucleic acid analysis. There are three methods in common use. In that described by Schneider (1945),

Table 7. *Nucleic acid composition of beet petiole mitochondria and microsomes, corrected for RNA occurring in the hot salt extracts*

This table is calculated from Table 4 by assuming that the hot salt (KCl or NaCl) extract contains RNA phosphorus equivalent to the difference between organic P and DNA phosphorus (as estimated from deoxyribose). All results are expressed as percentage of dry weight.

	Mitochondria		Microsomes		
	I	II	I	II	III
RNA phosphorus (direct)					
(*a*) Extracted by cold $HClO_4$	0·0165	0·0252	0·0245	0·0386	0·0504
(*b*) Extracted by hot salt (corrected for DNA phosphorus)	0·0323	0·0241	0·0357	0·0225	0·0352
Total RNA phosphorus					
As organic P [sum of (*a*) and (*b*)]	0·049	0·049	0·060	0·061	0·086
From absorption	0·045	0·045	0·053	0·064	0·076
DNA phosphorus					
From deoxyribose	0·0142	0·0086	0·0099	0·0085	0·0098
Sum of RNA phosphorus and DNA phosphorus	0·063	0·058	0·070	0·070	0·096

hot trichloroacetic acid is used to extract both nucleic acids together, RNA and DNA being estimated by colorimetric determinations of ribose and deoxyribose. Although apparently satisfactory as applied to animal material (Schneider, 1945), several workers with plant material (Stafford, 1951; McClendon, 1952; Oota, Fujii & Osawa, 1953) have failed to obtain good correspondence between RNA as based on such sugar estimates and as determined by other procedures. The large amounts of pentoses (possibly derived from pentosans and polyuronides) which occur in the nucleic acid extracts from silver beet (Table 3) confirm that this method is unsuitable for analysis of plant material.

The method of Schmidt & Thannhauser (1945) is unreliable, since organic phosphorus compounds other than RNA occur in the RNA fraction (Davidson, Frazer & Hutchison, 1951; Mitchell & Moyle, 1951; Davidson & Smellie, 1952; Drasher, 1953). Moreover, there is very poor correlation between determinations of RNA based on absorption of ultraviolet light and on direct estimation of phosphorus (Table 1).

However, with the Ogur & Rosen (1950) procedure, Stafford (1951) obtained satisfactory estimates of RNA in whole tissue and in cytoplasmic particles from pea seedlings, with good agreement between estimates based on absorption and on direct determination of phosphorus. McClendon (1952) made similar observations with whole tissue and particles separated from tobacco leaves. All these workers have shown that no further nucleic acid is obtained by extraction with cold perchloric acid for longer than 18–24 hr. This has been confirmed in the present study with beet petiole (see Results).

Although Ogur, Minckler, Lindegren & Lindegren (1952) found slight traces of DNA in cold perchloric acid extracts of yeast, no DNA was found in similar extracts obtained from pea seedlings by Stafford (1951), from tobacco leaf by McClendon (1952) and from beet petiole (Table 5). These findings suggested that the cold perchloric acid treatment would be adequate for the extraction of RNA from beet petiole and that any remaining nucleic acid would be DNA. However, Tables 5–7 show that, although cold perchloric acid extracts only RNA, it does not quantitatively remove it. About half of the RNA remains in the tissue. Cold perchloric acid also fails to extract quantitatively the RNA from influenza virus (Ada & Perry, 1945) or from mammary gland microsomes (Bailie & Morton, in preparation).

After removal of RNA, Ogur & Rosen (1950) extracted the remaining nucleic acid by treatment for 20–30 min. with perchloric acid (0·5N) at 70°. However, Stafford (1951) showed that material absorbing ultraviolet light was still removed in the third wash with hot perchloric acid, and both

Table 8. *Distribution of dry weight, nitrogen, lipid P and nucleic acid P in cytoplasmic fractions isolated from silver-beet petiole*

Fractions were isolated from dispersions of beet petioles (see under Materials and Methods; also Martin & Morton, 1956*a*). The analyses were carried out as described under Materials and Methods, the total NA being extracted with hot NaCl, and the extraction of RNA with cold N-$HClO_4$ being omitted. 'Washed' particles refer to those 'washed' by sedimentation from 0·15M-NaCl (see Martin & Morton, 1956*a*). Results are the means of duplicate estimates.

Fraction	Dry wt. (mg./100 g. fresh wt.)		Distribution as percentage of dry wt.						Distribution as percentage of whole dispersion*		
			Nitrogen		Lipid P		NA phosphorus				
	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	Nitrogen	Lipid P	NA phosphorus
Whole dispersion	—	—	—	—	—	—	—	—	100	100	100
1500 *g*, 15 min.	74·3	—	6·95	—	0·41	—	0·09	—	8·1	43·6	30·2
10000 *g*, 15 min. (mitochondria)	32·3	22·8	14·2	4·6	0·40	—	0·21	0·04	6·8	18·5	30·3
20000 *g*, 15 min. (intermediate fraction)	28·5	—	5·61	—	0·22	—	0·04	—	2·4	9·2	5·1
50000 *g*, 90 min. (microsomes)	23·9	27·0	10·2	4·9	0·65	0·71	0·13	0·07	3·6	22·5	13·7
Supernatant	—	—	—	—	—	—	—	—	72	3·9	23·5
Recovery (%)	—	—	—	—	—	—	—	—	93	98	103

* Based on figures for unwashed particles.

Stafford (1951) and McClendon (1952) found the absorption of the hot perchloric acid extract unreliable for the estimation of nucleic acid. This has been confirmed in the present investigation (see Results and Fig. 1). This defect is most apparent with whole plant material.

Numerous workers have employed hot sodium chloride solutions for extracting nucleic acids (see Volkin & Cohn, 1954), but the extraction has not been considered sufficiently quantitative for analytical purposes (Davidson & Smellie, 1952). However, Ada & Perry (1954) have shown that extraction of lipid-free influenza virus with hot 10% (w/v) sodium chloride completely removes the nucleic acid (RNA). The extraction of DNA with buffered sodium chloride at about 100° in place of the treatment with hot perchloric acid was therefore investigated. Fig. 1 shows that the material extracted by hot sodium chloride is essentially nucleic acid. Moreover, there is good agreement between estimates based on $\lambda_{max.}$ and on phosphorus (see Results and Table 2). Fig. 1 and Table 2 also establish that the extraction of nucleic acid is complete. However, by use of paper chromatography it has been shown that the extract obtained by hot salt after treatment with cold perchloric acid contains predominantly RNA with some DNA (see Table 6).

The estimation of DNA in the hot sodium chloride extract by measurement of deoxyribose exceeds that based on the concentration of thymine (Tables 4 and 6). For the latter estimate, however, the base composition of the particular DNA is required. Among plant tissues, this has been established only for wheat-germ DNA. The estimate based on deoxyribose is therefore taken as giving a maximum figure for DNA, which probably somewhat exceeds the true value.

It seems evident that none of the methods so far investigated for the differential extraction of RNA and DNA can be applied to any tissue without prior investigation of the validity of the separation. Estimates of RNA and DNA based on the quantitative chromatographic isolation of purines and pyrimidines would appear to be the most reliable method for differentiating RNA and DNA, but the application of such procedures is limited by the lack of information about the base composition of the nucleic acids in the particular tissues being studied. Where an estimate of total NA only is required, extraction with hot sodium chloride solution and correction of the ultraviolet absorption with bovine serum albumin as described by Ada & Perry (1954) has much to recommend it.

Comparative chemical properties of microsomes and mitochondria from petiole of silver beet

The analyses reported here (Tables 4, 7 and 8) have been carried out on particles isolated by differential centrifuging from buffered 0·2 M sucrose. The difference in enzymic properties clearly indicates that the isolated particles are derived from distinct cytoplasmic components (Martin & Morton, 1956*a*). The chemical analyses of the washed microsomes and mitochondria also reflect this difference, although the chemical distinctions are not as marked as the enzymic differences.

As in the similar particles from various animal tissues (see Ada, 1949), the lipid content of the beet microsomes (49–57%) is considerably higher than that of the mitochondria (about 39% of the dry weight; Table 4). The results for beet mitochondria are in agreement with Stafford's (1951) finding that mitochondria from pea seedlings contain 25–38% of dry weight as lipid. However, Levitt (1954*a*, *b*) has reported lipid of 'microsomes' and mitochondria from potato tubers as 12 and 22% of the dry weight respectively.

The microsomes and mitochondria of silver-beet petiole differ even more markedly in the content of phospholipids (Table 4), the microsomes containing almost three times the amount of lipid phosphorus found in the mitochondria. Lipid and protein account for over 90% of the dry weight of the beet microsomes. These results indicate the quantitative importance of the lipid component, which is probably an essential structural requirement for the endoplasmic reticulum from which microsomes are derived.

Table 9 compares the nucleic acid composition of microsomes and mitochondria from silver beet with the composition of the similar components from some animal and plant tissues. Whereas microsomes from animal tissues contain up to about 150 μg. of RNA phosphorus/mg. of protein nitrogen, those from silver beet contain about 10–24 μg. of RNA phosphorus/mg. of protein nitrogen. However, mitochondria from beet petiole resemble mitochondria from some animal tissues. Microsomes and mitochondria from silver beet do not differ markedly in their nucleic acid contents (Tables 7 and 9), in contrast to the marked difference between these particles as isolated from animal tissue (Table 9).

The amount of RNA/mg. of protein nitrogen found for beet microsomes and mitochondria is, in each case, lower than the figures reported for the other plant tissues. The higher figures obtained by Stafford (1951) may possibly reflect the higher metabolic activity of pea seedlings as compared with the mature tissue in beet petiole. The results reported by Levitt & Millikan (1954) for mitochondria and microsomes of potato tuber are based entirely on estimation of ribose with the orcinol procedure. As shown above, this procedure is unreliable as applied to silver-beet and some other plant tissues.

Using a method that combines features of that of Schneider (1945) with that of Ogur & Rosen (1950), Oota & Osawa (1954) determined the nucleic acid composition of particles from germinating bean hypocotyls. The very high figures obtained by these workers (Table 9) may reflect the greater metabolic activity of bean hypocotyls as compared with other plant material. Since these workers have claimed to establish a relationship between microsomal nucleic acid and protein synthesis, some evidence for the reliability of the estimations would have been desirable.

Table 7 shows that both washed mitochondria and microsomes from beet petiole contain DNA, identified by the specific Ceriotti (1952) reaction for deoxyribose and by the chromatographic detection of thymine. Schneider, Hogeboom & Ross (1950) and Bailie & Morton (in preparation) have found small amounts of DNA in microsomes and mitochondria isolated from animal tissues, and Stafford (1951) reported some DNA in particles isolated from pea seedlings. This small amount of DNA could possibly arise from damaged nuclei. However, since the procedure used for disruption of the beet petiole caused very little damage to fragile chloroplasts (Martin & Morton, 1956*a*), the damage to the more robust nuclei should be negligible. A similar amount of DNA was found in microsomes isolated from wheat roots (Martin & Morton, 1956*b*). Hence it seems improbable that the DNA is a nuclear contaminent, but rather that it is an integral component of some cytoplasmic particles. Chayen & Norris (1953) demonstrated the presence of DNA in the cytoplasm of bean-root cells by histological methods. In the light of these findings, it would seem important to determine whether the cytoplasmic DNA has the same base composition as the nuclear DNA from the same cells.

The low nucleic acid content of plant microsomes

Washed microsomes from beet petiole (and from most other plant tissues) have a relatively low nucleic acid content as compared with microsomes from animal tissue (Table 9). It is unlikely that the low figure results from the preparative procedure, even though the isolated particles were generally rapidly washed twice with 0·15 M sodium chloride (see Martin & Morton, 1956*a*) to remove sucrose for estimation of dry weights. Dianzani (1953), for example, showed that only about 5 % of the RNA was lost when liver mitochondria were washed with 0·15 M sodium chloride. Although on a dry-weight basis washing removed an appreciable amount of nucleic acids, both from beet mitochondria and microsomes (Table 8), when expressed as μg. of nucleic acid phosphorus/mg. of protein nitrogen (Table 9), the loss is only 30 % for mitochondria and is negligible for microsomes. This suggests that the

Table 9. *Comparison of nucleic acid compositions of mitochondria and microsomes from silver-beet petiole with those of similar particles from other tissues*

Results are expressed as μg. of nucleic acid P/mg. of protein nitrogen (or, where indicated, per mg. of total nitrogen).

				Mitochondria			Microsomes		
Tissue	Analytical procedure	Reference	Treatment of particles	Total NA phosphorus	RNA phosphorus	DNA phosphorus	Total NA phosphorus	RNA phosphorus	DNA phosphorus
Beet petiole	This paper	Tables 4 and 7, this paper	Unwashed	15·2	—	—	12·2	—	—
			Washed	8·0–10·3	6·8–8·0	1·2–2·3	10·9–26·7	9·6–24·0	1·3–2·7
Germinating wheat root	This paper	Martin & Morton (1956*b*)	Washed	12·8–13·9	9·2–9·5	3·6–4·4	41·1	38·7	2·4
Pea seedlings	Ogur & Rosen (1950)	Stafford (1951)	Unwashed	29·3	27·0	2·3	87·7	82·5	5·2
			Washed	12·0	10·5	1·5	—	—	—
Germinating bean hypocotyl	Schneider (1945) with Ogur & Rosen (1950)	Oota & Osawa (1954)	Washed	—	37–80	—	—	48–123	—
Potato tuber	Schneider (1945)	Levitt & Millikan (1954)	Unwashed	—	136–207*†	—	—	198–243*†	—
Rat liver	Schneider (1945)	Hogeboom (1949)	Unwashed	—	17·9*	—	—	40·6*	—
			Washed	—	13·1*	—	—	44·2*	—
Cow mammary gland	This paper	Bailie & Morton (in preparation)	Washed	—	—	—	151	148	3·0

* μg. of nucleic acid P/mg. of total nitrogen.

† Figures calculated from nucleic acid figures reported by Levitt & Millikan (1954), together with lipid and protein analyses of Levitt (1954*a*, *b*).

material removed in the washing treatment may be merely adsorbed to the particles and is not an integral component of them. The microsomes are largely derived by rupture of the endoplasmic reticulum of plant cells, and consist of vesicular elements comprising a distinct membrane enclosing some ill-defined material (Hodge, Martin & Morton, in preparation). Components of the 'cell sap' or 'supernatant fraction' may well be occluded by the microsomes on rupture of the endoplasmic reticulum, and subsequently lost in washing treatments. Hence the washing treatments must be a compromise between removal of adsorbed material, and loss of components integrally associated with the endoplasmic reticulum *in vivo*. The same arguments apply to the mitochondria which swell osmotically in hypotonic solutions (Slater & Cleland, 1953; Farrant, Robertson & Wilkins, 1953) possibly with loss of some components such as nucleic acids.

However, comparable analyses of microsomes from cow mammary gland similarly washed show that these contain about 0·7 % total nucleic acid phosphorus (Bailie & Morton, in preparation), whereas those from beet petiole contain less than 0·1 % (Table 7). Moreover, the analyses of unwashed beet particles leave no doubt of the low nucleic acid content in beet microsomes. The good agreement obtained between estimates based on organic phosphorus, absorption of ultraviolet light and chromatographic determination of uracil and of thymine establishes the validity of the figures reported.

Electron-micrographs of thin sections of fixed material show that, both in animal (Palade & Porter, 1954; Palade, 1955) and plant (Hodge, Martin & Morton, in preparation) cells, the endoplasmic reticulum has associated with it electron-dense granules of about 5–15 mμ. diameter. Similar observations of thin sections of isolated microsomes show that these are derived from the endoplasmic reticulum and consist of a mixture of vesicular elements and the electron-dense granules (Hodge, Martin & Morton, in preparation). Preliminary findings of Palade & Seikevitz (1955) indicate that the latter granules contain most of the nucleic acid of microsomes, and the less dense vesicles consist mostly of protein and lipid material. Comparison of electron-micrographs of the microsome fractions isolated from various tissues (Hodge & Morton, 1956) shows that the number of electron-dense granules is greatest in the microsomes fraction from cow mammary gland, less in that from wheat root and least in microsomes from beet petiole. The nucleic acid contents of the isolated microsomes show a similar gradation (Table 9). Although strictly quantitative information is not available, these observations confirm that most of the nucleic acid of the microsomes occurs in the Palade granules. Thus the low nucleic acid content of beet microsomes reflects the difference in the endoplasmic reticulum of the mature petiole cells as compared with that of young wheat-root cells or of secretory animal cells.

Although treatment with cold perchloric acid extracts between 40 and 60 % of the RNA of microsomes and mitochondria, no further RNA is extracted even by prolonged treatment with this solvent. Hot sodium chloride extracts the remaining RNA (Tables 2 and 7). Hence beet petiole appears to contain two types of RNA. Stafford (1951) suggested such a possibility after her study of the RNA content of pea seedlings.

The estimations of deoxyribose, as well as of thymine, establish that only a small portion of the nucleic acid in the hot sodium chloride extract is DNA (Tables 4, 6 and 7). Similar observations on microsomes from cow mammary gland show that most of the RNA of these particles is extractable with cold perchloric acid but some is extractable only with hot sodium chloride (Bailie & Morton, in preparation). While it has not yet been ascertained whether these solubility differences are due to differing degrees of polymerization or to a difference in the strength of the bonds holding the RNA in the nucleoprotein complex, it is possible that one type of RNA is concerned in structural elements of the particles and the other is functional in metabolic processes. Comparison of the results obtained with the microsome fractions from animal and plant tissue, together with the electron-micrographs already discussed, suggest that the RNA extractable with cold perchloric acid may be associated mainly with the Palade granules, and the other type of RNA may be an integral structural component of the lipoprotein complex. It is hoped to be able to clarify this point by studies with radioactive phosphorus.

Distribution of nitrogen, phospholipid and nucleic acids in beet petiole

The distribution studies (Table 8) show that much of the cellular dispersion appeared in the fraction isolated at low centrifugal force (1500 *g* for 15 min.). This contains nuclei and plastids, as well as 'debris' from broken cells. Microsomes constitute a relatively low proportion of the total cytoplasmic constituents in this mature and comparatively dormant tissue.

No special significance is claimed for the percentage distribution of phospholipid and nucleic acid among the various cytoplasmic components. The microsome fraction from animal cells contains up to 60 % of the total RNA of the cell (Schneider & Hogeboom, 1951). When only the results based on unwashed particles (Table 8) are considered, it is

seen that much less of the total nucleic acid of beet petiole is localized in the microsome fraction. Much of the nucleic acid (about 23 % of the total) and most of the nitrogen (about 72 % of the total) is found in the supernatant fraction remaining after centrifuging at 50 000 *g* for 90 min. Undoubtedly more material would have been sedimented at higher centrifugal forces. It is likely that some, if not all, of the nucleic acid of the supernatant fraction is associated with Palade granules separated from the endoplasmic reticulum and the derived microsomes during the isolation of the cytoplasmic particles.

SUMMARY

1. Dispersions of whole beet petiole, and microsomes and mitochondria separated from such dispersions by differential centrifuging, have been analysed to determine the lipid, nucleic acid and protein contents.

2. To establish the validity of the analytical procedures for determination of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), agreement between estimates based on absorption of ultraviolet light, organic phosphorus and ribose (or deoxyribose) was sought. Several published analytical procedures were shown to be unsatisfactory. Estimates of RNA based on determination of ribose were considerably above the true value.

3. An improved analytical procedure for determination of RNA and DNA in plant materials is described. The validity of the separation of RNA and DNA was examined by estimation of uracil and thymine isolated from hydrolysates with paper chromatography. No method of differential extraction of RNA and DNA was found to be satisfactory.

4. The microsomes and mitochondria from beet petiole contain two types of RNA: one extractable with cold N perchloric acid, and the other not extractable with this solvent but extractable with 10 % (w/v) sodium chloride at 100° for 20 min. It is suggested that the former type is associated predominantly with Palade granules, and the latter type with structural lipoprotein material.

5. Mitochondria and microsomes of beet petiole differ considerably in their content of lipid, particularly of phospholipid. Lipid and protein comprise over 90 % of the dry weight of beet microsomes. The mitochondria and microsomes differ only slightly in their content of nucleic acids.

6. The distribution of nitrogen, phospholipid and nucleic acids among the fractions from dispersions of beet petiole was examined. About 72 % of the nitrogen and about 23 % of the nucleic acid remained in the supernatant after centrifuging at 50 000 *g* for 90 min.

7. There are fewer Palade granules associated with microsomes from beet petiole than with the microsomes from animal tissues. This probably accounts for the lower nucleic acid content of beet microsomes as compared with microsomes from animal tissues.

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Transaminations with L-Glutamate and α-Oxoglutarate in Fresh Extracts of Animal Tissues

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When the present work was begun current opinion was that biological transamination involved only glutamic, aspartic and cysteic acids and alanine (Cohen & McGilvery, 1949); subsequently Feldman & Gunsalus (1950), using bacterial extracts, demonstrated syntheses of phenylalanine and tyrosine by transaminations with L-glutamate, and Cammarata & Cohen (1950) observed transamination to α-oxoglutarate, with soluble extracts of freeze-dried pig tissues, from a wide range of L-α-amino acids.

We first investigated the possibility that L-glutamine, rather than L-glutamate, might function as an amino donor to some α-keto acids. With homogenates and washed sedimentable particles of rat liver, the formation of phenylalanine was observed chromatographically from phenylpyruvate and L-glutamine, but greater formation was indicated in L-glutamate controls. Transamination with *p*-hydroxyphenylpyruvate and L-glutamate, forming tyrosine, was also observed, and from some L-α-amino acids to α-oxoglutarate. Transaminases which catalyse the formation of phenylalanine and tyrosine were located in the sedimentable particles of fresh rat-liver homogenates, in particular in mitochondria. A preliminary report of this work has been published (Hird & Rowsell, 1950), and a detailed account is now presented.

The formation of leucine by transamination is also described. Transamination in other fresh tissue extracts is reported, and more quantitative data have been obtained with a recording photodensitometer on paper chromatograms, and with bacterial amino acid decarboxylases. Observations with the same tissue preparations indicate that transamination to α-oxoglutarate is a more powerful mechanism than aerobic oxidation, for the deamination of L-α-amino acids.

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MATERIALS AND METHODS

Phenylpyruvic acid. This was synthesized from freshly distilled benzaldehyde via β-acetamidocinnamic acid (Herbst & Shemin, 1939). It was recrystallized from benzene.

p-*Hydroxyphenylpyruvic acid* was synthesized by the same procedure, with *p*-hydroxybenzaldehyde as starting material. The solid acids were kept in sealed tubes at −15°.

α-*Oxoisocaproic acid* was prepared by the enzymic deamination of D-leucine. An acetone-dried powder of sheep-kidney cortex was prepared and extracted with 0·1M sodium pyrophosphate buffer, pH 8·3, according to the method of Keilin & Hartree (1936). After dialysis against buffer, observations were made, with Warburg manometers, to determine conditions for a large-scale oxidative deamination. Then 1 g. of DL-leucine was dissolved in water and added to the bulk of the extract. Incubation was at 30° for 12 hr. with continuous aeration. (Samples were withdrawn for examination in manometers, and it was observed that a high level of oxidation was maintained for the whole period.) At the end of the incubation proteins were precipitated by heating, after adjusting to pH 3·0 with conc. HCl. Denatured protein was centrifuged down and the supernatant evaporated to dryness at 60° under reduced pressure. From the solid residue α-oxo*iso*caproic acid was brought into ether solution in a Soxhlet extractor. Evaporation of the ether solution produced a syrup which solidified *in vacuo*. The yield was poor (about 50 mg.) and it was therefore decided to use this crude product without further purification.