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Mitochondrial and Cytoplasmic Ribosomes from Mammalian Tissues

FURTHER CHARACTERIZATION OF RIBOSOMAL SUBUNITS AND VALIDITY OF BUOYANT-DENSITY METHODS FOR DETERMINATION OF THE CHEMICAL COMPOSITION AND PARTIAL SPECIFIC VOLUME OF RIBONUCLEOPROTEIN PARTICLES

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1. At 0–4°C mitochondrial ribosomes (55S) dissociate into 39S and 29S subunits after exposure to 300mM-K^+ in the presence of 3.0mM-Mg^{2+}. When these subunits are placed in a medium containing a lower concentration of K^+ ions (25mM), approx. 75% of the subparticles recombine giving 55S monomers. 2. After negative staining the large subunits (20.3nm width) usually show a roundish profile, whereas the small subunits (12nm width) show an elongated, often bipartite, profile. The dimensions of the 55S ribosomes are 25.5nm×20.0nm×21.0nm, indicating a volume ratio of mitochondrial to cytosol ribosomes of 1:1.5. 3. The 39S and 29S subunits obtained in high-salt media at 0–4°C have a buoyant density of 1.45g/cm^3; from the rRNA content calculated from buoyant density and from the rRNA molecular weights it is confirmed that the two subparticles have weights of 2.0×10^6 daltons and 1.20×10^6 daltons; the weights of the two subunits of cytosol ribosomes are 2.67×10^6 and 1.30×10^6 daltons. 4. The validity of the isodensity-equilibrium-centrifugation methods used to calculate the chemical composition of ribosomes was reinvestigated; it is confirmed that (*a*) reaction of ribosomal subunits with 6.0% (v/v) formaldehyde at 0°C is sufficient to fix the particles, so that they remain essentially stable after exposure to dodecyl sulphate or centrifugation in CsCl, and (*b*) the partial specific volume of ribosomal subunits is a simple additive function of the partial specific volumes of RNA and protein. The RNA content is linearly related to buoyant density by the equation RNA (% by wt.) = $349.5-(471.2\times1/\rho_{CsCl})$, where $1/\rho_{CsCl}=\bar{v}_{RNP}$ (partial specific volume of ribonucleoprotein). 5. The nucleotide compositions of the two subunit rRNA species of mitochondrial ribosomes from rodents (42% and 43% G+C) are distinctly different from those of cytoplasmic ribosomes.

We have reported (Sacchi *et al.*, 1973) that the 55S ribosomes of mammalian mitochondria are no smaller than the 70S ribosomes of bacteria, but differ from the latter in that about two-thirds of their mass is accounted for by protein. The particle mass was simply computed from the relative rRNA content and the known weights of the two subunit rRNA species. The chemical composition of the ribonucleoprotein was estimated from buoyant-density measurements, based on the demonstration (Hamilton & Ruth, 1969) that for formaldehyde-fixed ribosomes (*a*) the RNA content is linearly related to the reciprocal of their buoyant density in CcCl (ρ_{CsCl}), and (*b*) $1/\rho_{CsCl}$ is numerically identical with the partial specific volume calculated from the chemical composition, assuming additivity of the partial specific volumes of RNA and protein. Essentially similar results were reported by Hamilton & O'Brien (1973), who estimated the particle mass of bovine 55S ribosomes by the sedimentation-diffusion method, using a partial specific volume deduced from buoyant-density measurements.

However, the reliability of data based on isodensity equilibrium centrifugation in CsCl (Hamilton & Ruth, 1969; Hamilton *et al.*, 1971; Cammarano *et al.*, 1972; Sacchi *et al.*, 1973) has been questioned by the claim (McConkey, 1974) that (*a*) fixation of ribosomes with formaldehyde does not prevent the loss of a large proportion of protein when the formaldehyde-treated particles are banded in a CsCl gradient, and

(*b*) the buoyant density of ribosomes has no obvious correlation with their chemical composition. Therefore previous results (Sacchi *et al.*, 1973; Cammarano *et al.*, 1972) based on buoyant-density measurements could not be considered conclusive unless it is firmly established that (*a*) the partial specific volume of ribosomes is a simple additive function of the partial specific volumes of RNA and protein, and (*b*) formaldehyde-treated ribosomes remain stable during isodensity equilibrium centrifugation in CsCl.

The results in the present paper show that isopycnic centrifugation in CsCl is a basically valid tool for determination of the relative RNA content of ribosomes and for providing additional information on the physical and other structural properties of the subunits of mammalian mitochondrial ribosomes.

Methods

Media

The buffer compositions were as follows: buffer A, 25mM-KCl/3.0mM-magnesium acetate/1.0mM-dithiothreitol/20mM-Tris/HCl, pH7.6; buffer B (termed low-salt buffer), 150mM-KCl/3.0mM-magnesium acetate/50mM-Tris/HCl, pH7.6; buffer C (high-salt buffer I), 300mM-KCl/3.0mM-magnesium acetate/1.0mM-dithiothreitol/20mM-Tris/HCl, pH7.6; buffer D (high-salt buffer II), 500mM-KCl/5.0mM-magnesium acetate/1.0mM-dithiothreitol/10mM-Tris/HCl, pH7.0.

Isolation of ribosomes

Crude and highly purified 55S ribosomes were isolated from rat liver mitochondria as described previously (Sacchi *et al.*, 1973). Cytoplasmic ribosomes (rat and mouse liver, mouse Sarcoma 180 cells, mouse Ehrlich ascites-tumour cells, rabbit reticulocytes) were isolated by the method of Falvey & Staehelin (1970), or by the procedure described by McConkey (1974), depending on whether the 60S and the 40S subunits were to be dissociated by the 'run-off' method (Falvey & Staehelin, 1970) or by puromycin treatment (McConkey, 1974).

Isolation of high-speed-supernatant fractions, pH5 enzymes, initiation factors and globin mRNA

Cytoplasmic supernatant fractions (termed S-100) were isolated as described by Falvey & Staehelin (1970) from rat liver and rabbit reticulocytes. A pH5-enzyme fraction was prepared from reticulocytes as described by Schreier & Staehelin (1973). To obtain a high-speed mitochondrial supernatant, purified mitochondria were suspended in buffer B at 15mg of protein/ml, and lysed by ten freeze–thawing cycles in a solid-CO_2/acetone bath; the lysates were centrifuged for 4.0h at 150000*g* and the resulting supernatants were stored at −80°C. Initiation factors (IF_1 and IF_2) and globin mRNA were isolated from rabbit reticulocytes as described by Schreier & Staehelin (1973).

Isolation of '80S couples' and ribosomal subunits

80S couples. (These are used for the assay of globin synthesis *in vitro*.) Polyribosomes were incubated in a complete mixture for protein synthesis exactly as described by Falvey & Staehelin (1970). After 55min incubation at 37°C, the mixtures were chilled and centrifuged for 12h at 105000*g* in a type 40 Spinco rotor at 4°C through a cushion of 1.8M-sucrose in buffer A to sediment the '80S couples' released in synchrony with polypeptide-chain termination.

'*Run-off subunits*'. Polyribosomes prepared as described by Falvey & Staehelin (1970) were incubated in the complete mixture for protein synthesis at 37°C for 55min (Falvey & Staehelin, 1970). The mixtures were chilled and adjusted to 400mM-KCl and 3.0mM-magnesium acetate to dissociate the '80S couples'. The subunits were separated by zonal centrifugation of the mixtures for 15h at 24000rev./min in 15–40% (w/v) linear sucrose gradients made up in buffer C (300mM-K^+/3.0mM-Mg^{2+}), in an SW27 Spinco rotor operated at 4°C (see Cammarano *et al.*, 1972).

'*Puromycin subunits*'. Polyribosomes prepared as described by McConkey (1974) were suspended in 50mM-Tris/HCl, pH7.6, containing 100mM-KCl, 1.0mM-magnesium acetate, 1.0mM-dithiothreitol and 0.5mM-puromycin hydrochloride. The suspensions were incubated for 15min at 37°C and the subunits were separated by zonal centrifugation at 24000rev./min for 15h in 15–40% (w/v) linear sucrose gradients made up in buffer D (500mM-KCl/5.0mM-magnesium acetate), in an SW27 Spinco rotor operated at 4°C (McConkey, 1974).

'*Native subunits*'. Ribosomes prepared as described by Falvey & Staehelin (1970) were suspended in buffer C, to dissociate the naturally occurring subunit couples present as 80S monomers in the ribosome population; the subunits were separated by zonal centrifugation of the ribosome suspension in 15–40% (w/v) linear sucrose gradients made up in buffer C; the gradients (37ml) were centrifuged for 15h at 24000rev./min in the SW27 Spinco rotor.

'*Highly purified subunits*'. Subunits prepared by the 'run off' method of Falvey & Staehelin (1970) were subjected to an additional 12h centrifugation at 105000*g* through a 2.5ml cushion of 40% (w/v) sucrose in buffer C, in a type 40 Spinco rotor operated at 4°C.

In all preparative conditions listed, the subunits were collected by high-speed centrifugation (12h at 105000*g*) of the sucrose-gradient fractions corre-

sponding to the 60S and the 40S peaks of u.v. absorbance.

Chemical determinations

RNA was routinely assayed by the orcinol method of Schneider (1957), with AMP as the standard, after correction for the purine content of the rRNA being investigated (see Cammarano *et al.*, 1972); the RNA content determined by the orcinol method agreed, within 1% or less, with that determined by the method of Fleck & Munro (1962), by using appropriate absorption coefficients computed from the nucleotide composition. Ribosomal protein was measured by a modified biuret method (see legend to Fig. 1) or by the method of Lowry *et al.* (1951), with as standard either bovine serum albumin or ribosomal proteins isolated from reticulocyte 80S ribosomes and from mitochondrial 55S particles. As shown in Fig. 1, bovine serum albumin and ribosomal proteins display the same reactivity when assayed by the biuret reaction, but yield two distinctly different curves when the method of Lowry *et al.* (1951) is used, the reactivities of mitochondrial and cytosol ribosomal proteins being identical and higher than that of bovine serum albumin. Evidently, albumin and ribosomal protein have different phenylalanine and tyrosine contents. Accordingly, when bovine serum albumin was the standard only the biuret reaction was used. Ribosomal proteins were extracted from reticulocyte ribosomes as described by Collatz *et al.* (1975) and dissolved in 25mM-formic acid/0.1mM-EDTA. The absorption coefficient of the purified protein, $A_{280}^{1mg/ml}$ (1cm light-path) = 0.62.

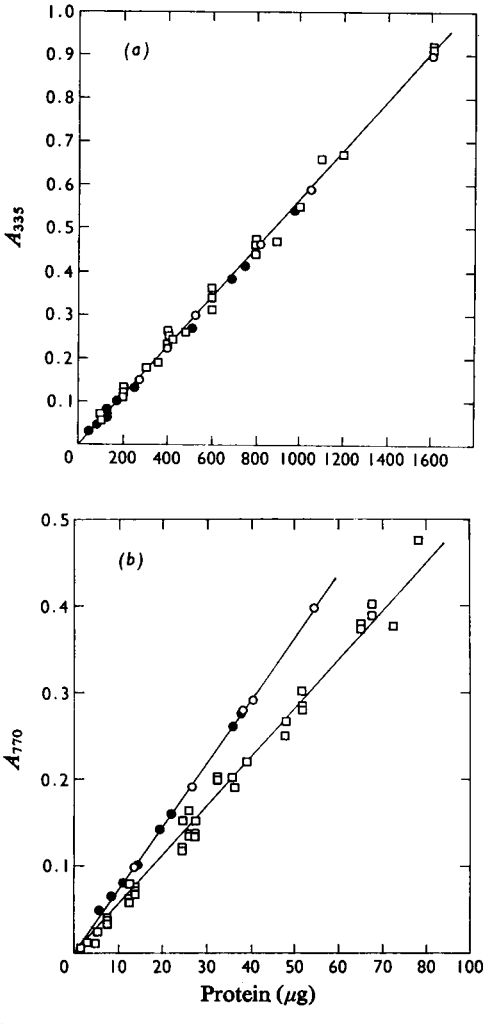


Fig. 1. *Reactivity of ribosomal protein and bovine serum albumin with the Lowry reagent (b) and in the biuret reaction (a)*

For the biuret assay, 0.5ml of the protein solution (100–150μg) in water was supplemented with 0.5ml of 2M-NaOH and with 50μl of the biuret reagent (0.6M-trisodium citrate / 0.95M-Na_2CO_3 / 0.08M-$CuSO_4$); the absorbance was measured 15min later at 335nm (Mehl, 1945). □, Bovine serum albumin; ●, reticulocyte ribosomal protein; ○, mitochondrial ribosomal protein.

Isodensity sedimentation in CsCl

Ribosomal subunits were treated with 6% (v/v) formaldehyde (final concn.) at 0°C for at least 30min and centrifuged to their equilibrium position in CsCl gradients containing 1% (v/v) formaldehyde as described earlier (Cammarano *et al.*, 1972). After a 10h centrifugation at 35000rev./min in the SW39 Spinco rotor operated at 4°C, the ribonucleoprotein material was precipitated with trichloroacetic acid (10%, w/v, final concn.) for RNA and protein determinations. The weight fraction of RNA was also calculated from ρ_{CsCl} by the expression:

$$\text{RNA (\% by wt.)} = 100\,\frac{\bar{v}_{RNP}}{\bar{v}_n}\,\frac{\left(\frac{1}{\bar{v}_{RNP}}-\frac{1}{\bar{v}_p}\right)}{\left(\frac{1}{\bar{v}_n}-\frac{1}{\bar{v}_p}\right)} \qquad (1)$$

where $\bar{v}_{RNP} = 1/\rho_{CsCl}$; $\bar{v}_n$ and $\bar{v}_p$ are the partial specific volumes of RNA and protein, taken as 0.53cm³/g and 0.74cm³/g respectively (Hamilton & Ruth, 1969).

Nucleotide composition

RNA was isolated from purified ribosomal subunits by two phenol/sodium dodecyl sulphate extractions at 45°C and hydrolysed with 0.3M-KOH; the

nucleotide composition was obtained as described by Sebring (1969).

Radioactivity measurements and radiochemicals

Radioactive material was precipitated with trichloroacetic acid (10%, w/v, final concn.) and collected by suction on to Millipore discs (HWA, 0.45 μm pore size); radioactivity was measured in a Packard Tri-Carb liquid-scintillation spectrometer with the standard 2,5-diphenyloxazole (PPO)/1,4-bis-(5-phenyloxazol-2-yl)benzene (POPOP) scintillation fluid (10 g of PPO and 0.5 g of POPOP in toluene to a volume of 1 litre). All radiochemicals were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

Electron microscopy

Ribosomes were suspended in buffer B at 0.2 A_{260} unit/ml; the particles were fixed with glutaraldehyde and negatively stained with uranyl acetate as described by Kleinow *et al.* (1974). Micrographs were taken in a JEOL B electron microscope at a direct magnification of ×100000; the magnification was calibrated with a cross-linked grating replica with 2160 lines/mm (E. Fullam, Schenectady, NY, U.S.A.).

Results

Dissociation and reassociation of mitochondrial ribosomes

In previous work (Sacchi *et al.*, 1973), 39S and 29S subunits were produced by incubating 55S ribosomes at 37°C in a medium containing 150mM-K^+ and 3.0mM-Mg^{2+}. The sedimentation profiles presented in Fig. 2 show that mitochondrial ribosomes are converted into their component subunits at 0–4°C when exposed to higher concentrations of univalent cations (300mM-K^+ in the presence of 3.0mM-Mg^{2+}).

For the experiment of Fig. 2, radioactively labelled crude ribosomes were derived from mitochondria that had been incubated under conditions of protein synthesis with [^{14}C]leucine; the labelled ribosomes were suspended in either buffer A (25mM-K^+/3.0mM-Mg^{2+}) or in buffer C (300mM-K^+/3.0mM-Mg^{2+}) and then centrifuged in sucrose gradients with the same ionic composition as the suspending medium. As the sedimentation profiles show, at 4°C the 55S ribosomes were essentially stable in buffer A (Fig. 2*a*), but were almost completely dissociated to free 39S and 29S subunits in the high-salt solution (Fig. 2*b*); essentially all the labelled peptide chains remained associated, with a minor fraction of 55S monomers resisting dissociation (Fig. 2*b*): the ^{14}C label was released from these residual monomers when the mitochondrial-ribosome fraction was incubated under conditions of protein synthesis in the presence of a mitochondrial-supernatant fraction, or with puromycin (Fig. 2*c*). The significance of the fast-sedimenting material appearing when crude mitochondrial ribosomes are centrifuged in sucrose gradients containing the high-salt medium has been analysed previously (Sacchi *et al.*, 1973).

Table 1. *Base composition of rRNA from mammalian mitochondrial ribosomes*

	Content (mol/100 mol)				
	A	U	G	C	G+C
Subunit					
Large subunit (39S)	32.4	24.0	23.2	20.4	43.6
Small subunit (29S)	33.6	23.8	25.2	17.4	42.6

Fig. 3 shows that when the free 39S and 29S subunits, reisolated from the high-salt sucrose gradients, were again mixed in buffer A at a 2:1 A_{260} ratio, approx. 75% of the subunits recombined to give 55S monomers; thus the vast majority of the 55S ribosomes obtained by standard procedures consist of 'subunit couples', free of growing peptide chains, which dissociate reversibly in high-salt solutions at 0–4°C.

The notion that the subunit particles obtained in buffer C at 4°C are genuine subunits of mitochondrial ribosomes is documented by the base analysis of the two subunit rRNA species (Table 1); the 39S and 29S subunits contain respectively 43% and 42% G+C; under identical analytical conditions the two subunit rRNA species of cytoplasmic ribosomes were found to contain 67% and 59% G+C.

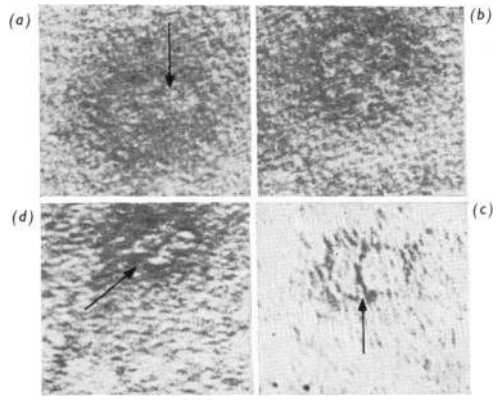
Electron microscopy of mitochondrial ribosomes

The morphology and dimensions of the ribosomal subunits produced by high-salt treatment at 4°C and those of the parent 55S particles are illustrated in Plates 1 and 2 and in Table 2.

The isolated large subunits (39S) most frequently display a roughly circular profile about 20nm in diameter (Plate 1*b*); the presence of a central channel or depression cannot be identified. Triangular profiles with curved flanks, similar to those usually seen in electron micrographs of cytoplasmic ribosomes, were only occasionally detected (Plate 1*a*).

The isolated small subunits (29S) reveal a slightly elongated profile or an approximately rectangular shape, which frequently appears as a bipartite structure (Plates 1*c* and 1*d*) similar to that of the corresponding subunit of the 80S ribosomes.

Among the variety of monomer (55S) profiles the F forms (frontal view) and the L forms (lateral view) were easily identified.



EXPLANATION OF PLATE 1

Selected images of negatively stained isolated mitochondrial ribosome subunits (*a*, *b*, 39*S*; *c*, *d*, 29*S*) (*magnification* ×500000) (*a*) Frontal view of a large subunit displaying a triangular profile with curved flanks. Arrow indicates a depression or dense spot located off-centre at the right-hand side. (*b*) More usual view of the large subunit, characterized by a roundish profile and by an irregular inner structure. (*c*) Lateral view of a small subunit in which the cleft subdividing the elongated rectangular profile into two parts is evident (arrow). (*d*) Up-side view of a small subunit showing the unequal subdivision. The arrow indicates the cleft.

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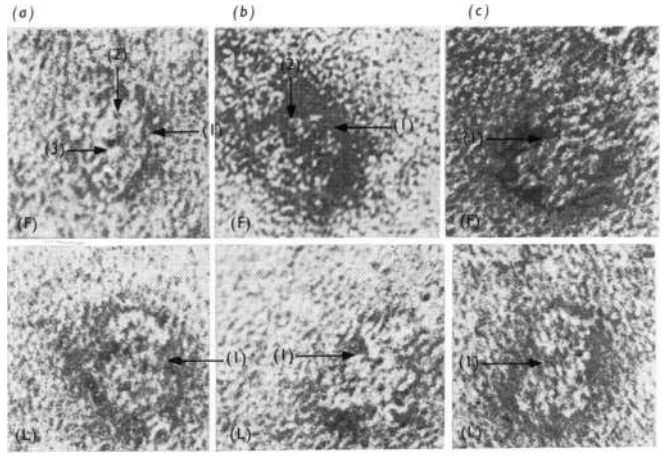

EXPLANATION OF PLATE 2

Selected images of negatively stained mitochondrial ribosome monomer

Three projections (*a*, *b*, *c*) are shown both for frontal views (F) and for lateral views (L). Large arrows (1) indicate the band between small and large subunits, intermediate arrows (2) indicate the bipartition of the small subunit, and the small arrow (3) indicates the dense spot located off-centre in the large subunit. Magnification ×500000.

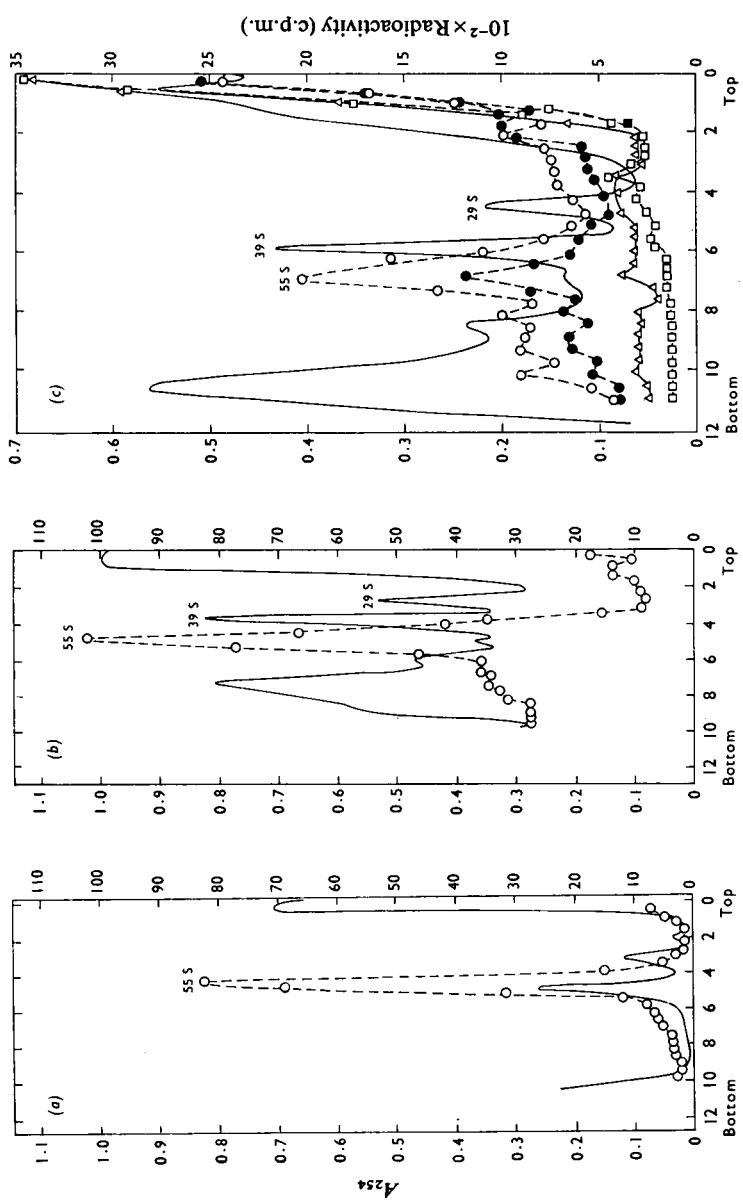

Fig. 2. *Effect of the high-salt buffer on the dissociation of 55S ribosomes (b); effect of different incubation conditions on the release of labelled polypeptide chains from 55S ribosomes (c)*

Intact mitochondria were incubated in a system *in vitro* containing (per ml): mitochondria, 1.5mg of protein (equivalent to 0.1g wet wt. of tissue); KCl, 90μmol; EDTA, 1.3μmol; NAD^+, 0.5μmol; each of 20 amino acids except leucine, 50μmol; $(NH_4)_2SO_4$, 35μmol; Bicine [*N,N*-bis-(2-hydroxyethyl)glycine] (pH7.6), 30μmol; cytochrome *c* (Calbiochem, Los Angeles, CA, U.S.A.), 0.2mg; bovine serum albumin, 1.0mg; [^{3}H]leucine (sp. radioactivity 37Ci/mmol), 2.5μCi. The reaction mixtures were incubated at 30°C for 15min. The crude particles were isolated from 25ml of the mixture and centrifuged at 4°C in sucrose gradients containing buffer A (25mM-K^+/3.0mM-Mg^{2+}) (*a*) or the high-salt buffer I (300mM-K^+/3mM-Mg^{2+}) (*b*). In (*c*) the particles were incubated under different conditions for 20min at 37°C before sedimentation analysis. ——, A_{254} profile of one representative gradient; ○, radioactivity of control ribosomes incubated in high-salt buffer; ●, radioactivity of ribosomes incubated in the complete mixture for protein synthesis (see the Methods section) in the presence of a cytosol (S-100) supernatant; □, radioactivity of ribosomes incubated in the complete mixture for protein synthesis in the presence of a mitochondrial high-speed supernatant; △, radioactivity of ribosomes incubated in th epresence of 0.5mM-puromycin. The sucrose gradients (12.5ml, linear, 15–40%, w/v) were centrifuged for 10h at 4°C at 28000rev./min in the SW40 Spinco rotor. The A_{254} distribution was monitored at a constant flow rate by using the flow-through cell of a LKB Uvicord u.v. analyser; trichloroacetic acid-precipitable radioactivity was assayed as described in the Methods section.

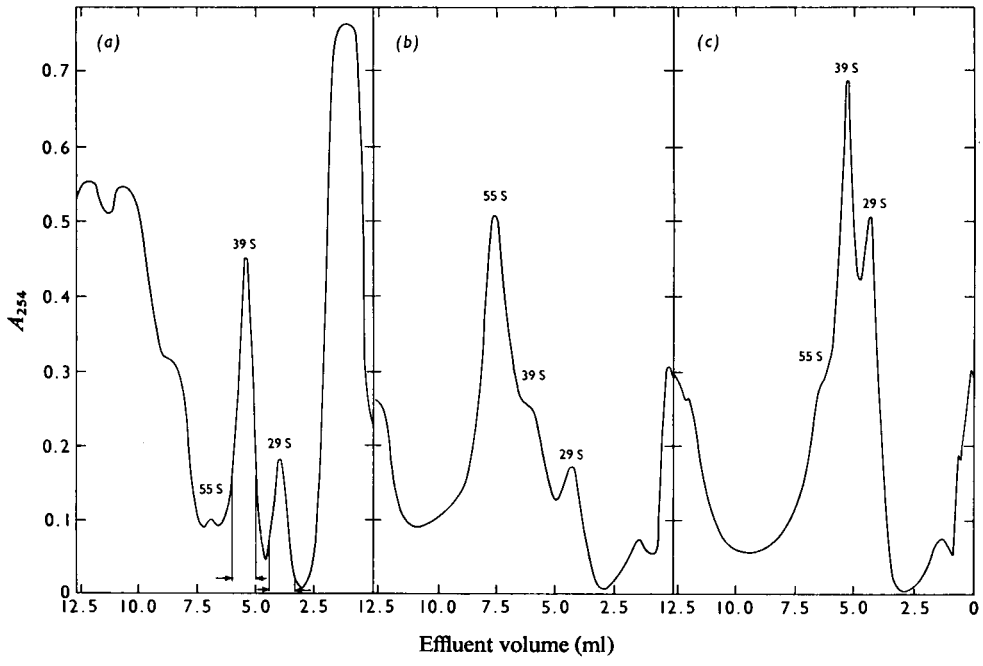

Fig. 3. *Formation of 55 S monomers from 39 S and 29 S subunits reisolated from a high-salt buffer*
(*a*) Sedimentation analysis of crude mitochondrial particles in sucrose gradients containing buffer C. The fractions between pairs of arrows were separately pooled and the particles were harvested by high-speed centrifugation (12h at 105000*g* at 4°C). (*b*) Sedimentation pattern of 39S and 29S particles (2:1 A_{254} ratio) suspended in buffer A and centrifuged in a sucrose gradient made up in buffer A. (*c*) Sedimentation pattern of 39S and 29S particles suspended in buffer C and centrifuged in a sucrose gradient made up in buffer C. The sucrose gradients (linear, 15–40%, w/v) were centrifuged for 10h at 4°C at 28000rev./min in the SW40 Spinco rotor.

The F forms (Plate 2) exhibit a horizontal band between the large and small subunit which is apparent in projections shown in Plates 2(*a*) and 2(*c*). A bipartition of the small subunit and the presence of a dense spot always located off-centre (usually at the left, being the images 'left-featured'; see Nonomura *et al.*, 1971) are visible in the projections shown in Plates 2(*a*) and 2(*b*). 'Right-featured' (Nonomura *et al.*, 1971) forms were rarely present. In the L forms (Plates 2*b* and 2*c*) both the different dimensions of the subunits and the cleft between them are clearly discernible. The dimensions of the negatively stained mammalian 55S particles are 26.5nm×20.8nm×20.3nm (Table 2).

Table 2. *Dimensions of negatively stained mitochondrial ribosomes*
Dimensions are averages ± S.D. from 30 measurements.

	Frontal view (nm)	Lateral view (nm)
Total height	26.5 ± 2.1	27.1 ± 2.5
Height of large subunit	15.9 ± 1.6	15.5 ± 1.9
Height of small subunit	10.6 ± 1.5	10.3 ± 1.8
Width along the cleft	20.8 ± 2.2	—
Width of large subunit	—	20.3 ± 1.7
Width of small subunit	—	12.1 ± 2.0

Buoyant density of 39 S and 29 S subunits dissociated in high salt

Fig. 4 illustrates isodensity-equilibrium-centrifugation profiles of 39S and 29S subunits (labelled with [^{14}C]orotic acid); after fixation with formaldehyde at 0°C, the 39S particles produced in 300mM-K^+ at 0–4°C have exactly the same buoyant density ($\rho = 1.45$g/cm^3) as those obtained by heat-treatment of 55S ribosomes in media containing a lower concentration of univalent cations (150mM-K^+/3.0mM-Mg^{2+}) (see Sacchi *et al.*, 1973). The sedimentation profile of the small subunits (not analysed in previous work) reveals some low-density and high-density components, in addition to a main band of density $\rho = 1.45$g/cm^3. The material with a buoyant density higher than 1.45g/cm^3 presumably represents particles that have lost part of their protein during

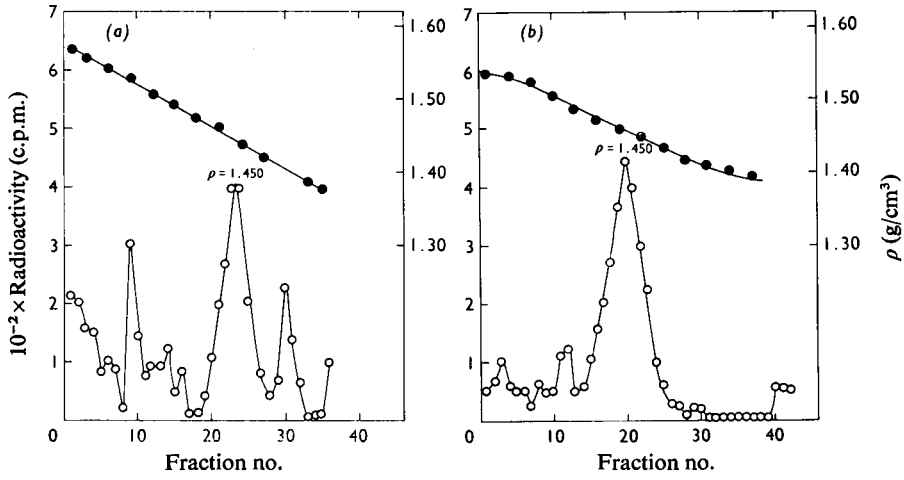

Fig. 4. *Isodensity-sedimentation profiles of* 39*S subunits* (*b*) *and* 29*S subunits* (*a*) *isolated by the high-salt procedure at* 4°*C, and further purified by re-centrifugation through a cushion of* 1.0M-*sucrose made up in buffer C, containing* 0.3% *Brij*-58 (*Serva Feinbiochemica, Heidelberg, Germany*)

The particles were dissolved in 50mM-triethanolamine/HCl buffer (pH7.6)/25mM-KCl/5.0mM-magnesium acetate and were fixed for 1h at 0°C with formaldehyde (6% final concn.) buffered with 200mM-triethanolamine/HCl, pH7.6. The fixed material was mixed with 4.5ml of a CsCl solution containing 25mM-triethanolamine/HCl (pH7.4)/50mM-KCl and 1% buffered formaldehyde, pH7.4. The solutions were centrifuged for 20h at 40000rev./min in the SW50.1 Spinco rotor, and fractions of volume 0.12ml each were collected dropwise. Fractions corresponding to the main band were precipitated with trichloroacetic acid (10% final concn.) for radioactivity measurements; 1μl portions of chosen fractions were withdrawn to measure the refractive index, corrected by subtracting the refractive index of a solution containing 25mM-triethanolamine/50mM-KCl/1% formaldehyde. The radioactively labelled subunits used for these experiments were isolated from the mitochondria of rats given one daily injection of [^{3}H]orotic acid (sp. radioactivity 27Ci/mol; 100μCi/100g body wt.) for 3 consecutive days before death; ○, acid-precipitable ^{3}H radioactivity; ●, density of CsCl.

exposure to high-salt buffer; it seems possible that the presence of some protein-depleted 29S subunits accounts for the incomplete reassociation of the subparticles in buffer A (see Fig. 3).

These results confirm that the two subunits of mammalian mitochondrial ribosomes have an exceptionally high protein content (about 75%) (eqn. 1), inasmuch as isodensity sedimentation in CsCl is a valid tool for determining the chemical composition of ribonucleoprotein particles. Accordingly, experiments were done to ascertain whether (*a*) fixation with formaldehyde at 0°C is sufficient to stabilize ribosomes, and whether (*b*) the buoyant-density values are truly related to RNA content. Since 55S material was available in limited amounts, cytosol ribosomes were used for most experiments.

Stability of formaldehyde-fixed ribosomes

The results presented in Fig. 5 show that ribosomes treated with 6% formaldehyde for 90min at 0°C remain stable on exposure to detergent treatments designed to release RNA completely from protein; when formaldehyde-treated 60S and 40S subunits were adjusted to 1.0% with lithium dodecyl sulphate and subjected to zonal centrifugation at 20°C in sucrose gradients containing lithium dodecyl sulphate (0.5%), the sedimentation diagrams of the detergent-treated particles were superimposable on those of untreated control particles (both fixed and unfixed) run in parallel gradients lacking detergent. As expected, incubation of unfixed subunits in the presence of lithium dodecyl sulphate (1%) led to the complete separation of RNA from protein (results not shown). Since the absorption coefficient of protein at 260nm is much less than that of RNA, it seemed possible that some ribosomal proteins could be released by lithium dodecyl sulphate without their loss being apparent. To elucidate this point, Ehrlich ascites-cell ribosomes were labelled *in vivo* with a ^{14}C-labelled protein hydrolysate (see inset to Fig. 6); the labelled particles (13A_{260} units, 5810c.p.m.) were fixed with formaldehyde and banded in a CsCl gradient. The isodensity-sedimentation profile in Fig. 6 shows that essentially all the ^{14}C label (5400c.p.m.) accompanies the particles to their equilibrium position; no radioactivity was detected on the top of the gradient

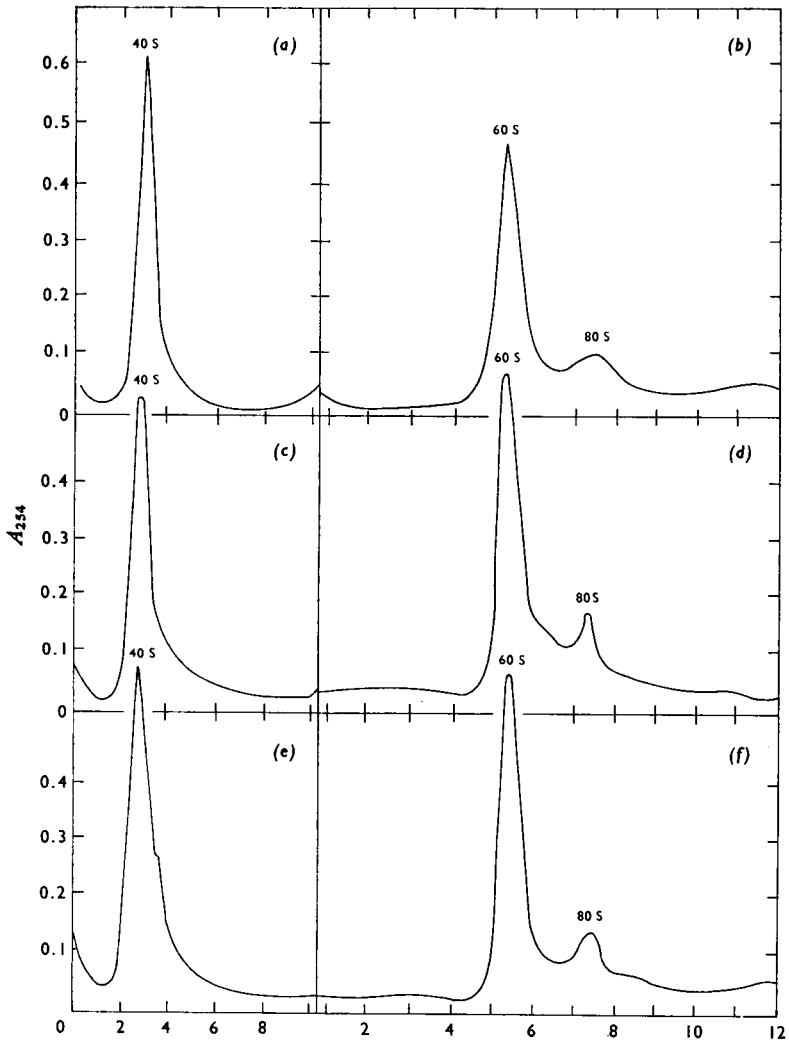

Fig. 5. *Stability in lithium dodecyl sulphate of 60S and 40S subunits fixed with formaldehyde at 0°C*
(*a*) and (*b*), Sedimentation profiles of subunits not fixed with formaldehyde and centrifuged in sucrose gradients lacking detergent. (*c*) and (*d*), Sedimentation profiles of formaldehyde-fixed 40S and 60S subunits treated with lithium dodecyl sulphate and centrifuged in sucrose gradients containing detergent. (*e*) and (*f*), Sedimentation profiles of formaldehyde-fixed 40S and 60S subunits not exposed to detergent and directly centrifuged in sucrose gradients lacking detergent. The subunits (about 3.0 A_{260} units) were dissolved in 270 μl of a solution containing 20mM-triethanolamine/HCl, pH7.6, 50mM-NaCl and 5.0mM-magnesium acetate. In (*a*) and (*b*) the solutions were directly centrifuged in sucrose gradients lacking detergent. In (*c*) and (*d*) the subunit solutions were supplemented with 50 μl of 36% formaldehyde containing 200mM-triethanolamine/HCl buffer (pH7.4) to fix the particles; after standing at 0°C for 90min the solutions were adjusted to 1.0% with lithium dodecyl sulphate and centrifuged in sucrose gradients with lithium dodecyl sulphate. Under all conditions the sucrose gradients contained 20mM-triethanolamine/HCl, pH7.6, 50mM-NaCl and 0.5mM-magnesium acetate. The gradients (linear, 10–30%, w/v) were centrifuged at 20°C for 3h at 35000rev./min in the SW41 Spinco rotor.

where proteins ($\rho = 1.35$ g/cm^3) are expected to band. The residual radioactivity (400c.p.m.) was recovered by washing the tube walls after collecting the gradient fractions dropwise.

The degree of conservation of ribosomal protein is further illustrated in Table 3, by comparing the chemical composition of the fixed ribonucleoprotein particles before and after isodensity-equilibrium

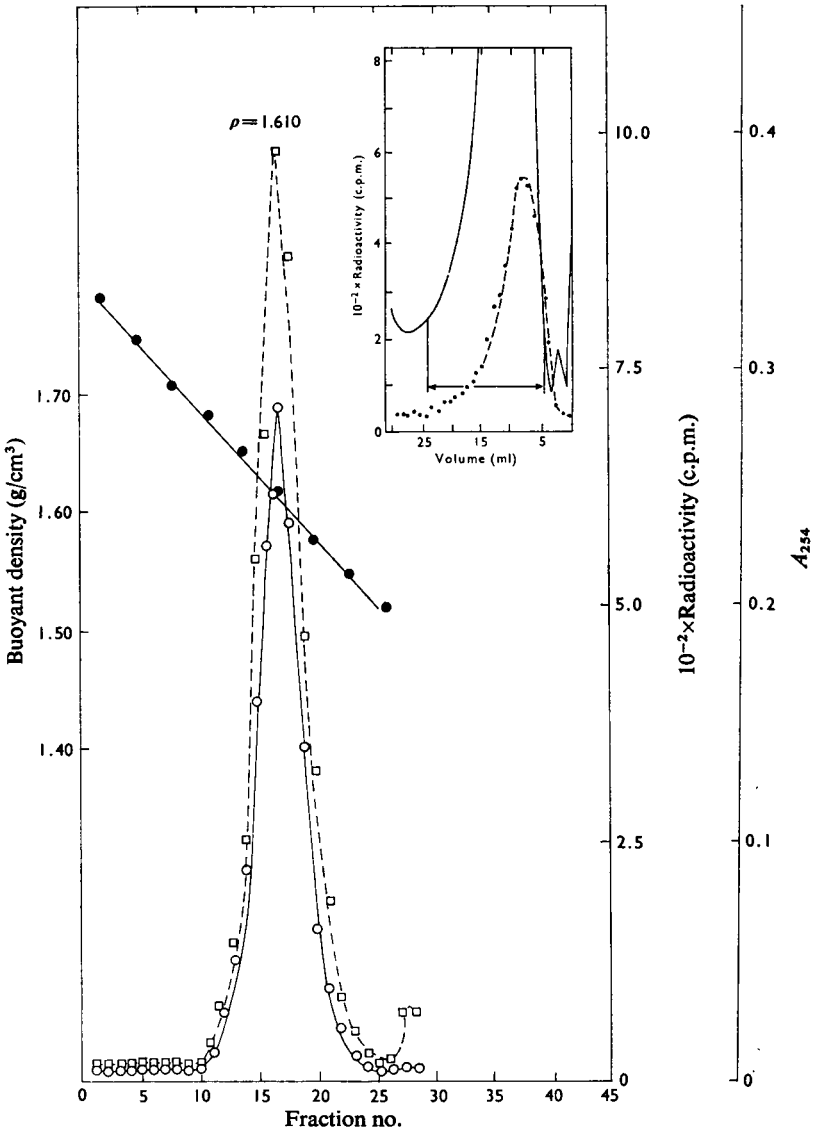

Fig. 6. *Isodensity-sedimentation profile of ribosomes labelled with a ^{14}C-labelled protein hydrolysate*

Swiss mice were given 25 μCi of a uniformly ^{14}C-labelled protein hydrolysate (sp. radioactivity 45 mCi/mg-atom of C) on days 2, 3 and 4 after the transplantation of Ehrlich ascites-tumour cells; the animals were killed 3 days after the last injection. The crude ribosomes isolated as described by Falvey & Staehelin (1970) were further purified by zonal centrifugation in a preparative linear sucrose gradient (35 ml, 10–30%, w/v) containing 300 mM-KCl, 3.0 mM-magnesium acetate and 20 mM-triethanolamine/KCl, pH 7.4 (see the inset). The ribosomes in the gradient fractions included between the arrows (inset) were harvested by centrifugation at 105000*g* for 12 h and were fixed with formaldehyde as described in the legend to Fig. 4. One sample of the solution of fixed ribosomes (5813 c.p.m.) was banded in a CsCl gradient (cf. Fig. 4). □, ^{14}C radioactivity; ○, A_{260}. Continuous line and dots in the inset panel represent A_{260} and ^{14}C radioactivity respectively.

centrifugation in CsCl gradients. The RNA content of the mitochondrial 55S ribosomes recovered from the CsCl gradients (23%) was essentially the same as that of control fixed particles not exposed to CsCl (24.5%). The RNA content of the 60S and 40S subunits of cytosol ribosomes recovered after

Table 3. *RNA content of ribosomes and ribosomal subunits determined by buoyant density in CsCl, and by colorimetry*
Numbers of experiments are given in parentheses. Each experiment includes at least three determinations with different samples of the alkali hydrolysate. The final values reported in the two last columns are the averaged RNA contents (determined by colorimetry) of ribosomes with the same buoyant density in CsCl regardless of source of ribosomes and mode of preparation.

Type of particle		RNA content by colorimetry: Before CsCl	RNA content by colorimetry: After CsCl	Buoyant density (g/cm^3)±S.D.	Average RNA content (from ρ_{CsCl})	Averaged RNA content (colorimetry) of ribosomes with the same density (±S.D.): Before CsCl	Averaged RNA content (colorimetry) of ribosomes with the same density (±S.D.): After CsCl
Mitochondrial 55S		24.5 (3)	23.0 (3)	1.450±0.003 (10)	25%	24.5	23.0
Native 40S	(rodents)	45.0 (1)	47.0 (1)	1.557±0.003 (10)	47%	45.3±0.5	47.5±0.6
	(rabbit)	45.0 (2)	47.0 (2)	1.557±0.003 (5)			
'Run-off' 40S	(rodents)	45.0 (1)	48.0 (1)	1.557±0.003 (13)			
	(rabbit)	46.0 (2)	48.0 (2)	1.557±0.003 (5)			
'Puromycin' 40S	(rodents)	47.0 (1)	49.0 (1)	1.575±0.005 (2)	50%	46.8±0.4	48.8±0.4
	(rabbit)	46.5 (2)	48.5 (2)	1.575±0.005 (5)			
Native 60S	(rodents)	55.0 (1)	59.0 (1)	1.615±0.003 (13)	57%	56.0±1.0	58.5±0.8
	(rabbit)	57.0 (3)	58.0 (3)	1.615±0.003 (5)			
'Run-off' 60S	(rodents)	61.0 (1)	62.0 (2)	1.635±0.005 (15)	61%	59.5±2.0	62.0±0.8
	(rabbit)	59.0 (1)	63.0 (1)	1.635±0.005 (15)			
'Puromycin' 60S	(rodents)	61.0 (1)	62.0 (2)	1.635±0.005 (2)			
	(rabbit)	59.0 (1)	61.0 (2)	1.635±0.005 (2)			
Highly purified 60S	(rodents)	63.0 (3)	64.0 (1)	1.650±0.005 (5)	63%	63.5±1.0	63.8±0.4
	(rabbit)	64.0 (1)	63.5 (1)	1.650±0.005 (6)			

isodensity-equilibrium centrifugation was no more than 2% higher than that of the control particles before centrifugation.

Relationship between buoyant density and chemical composition of ribonucleoprotein particles

In Table 3 the RNA content estimated by colorimetry is compared with that calculated from buoyant-density measurements from eqn. (1) by assuming $\bar{v}_n = 0.53\,cm^3/g$ and $\bar{v}_p = 0.74\,cm^3/g$; various different particles were used so as to cover the widest range of density values possible: (*a*) 55S mitochondrial ribosomes, (*b*) native 60S and 40S subunits (0.3 M-K^+, 3.0 mM-Mg^{2+}), (*c*) derived 'run-off' subunits (0.3 M-K^+, 3.0 mM-Mg^{2+}), (*d*) highly purified 'run-off' subunits (see the Methods section).

The 55S ribosomes, with the lowest buoyant density ($1.45\,g/cm^3$), contain only 23–24% RNA.

The 40S 'puromycin subunits' (49% RNA by colorimetry) contain 2–3% less protein and have a higher buoyant density ($\rho = 1.575\,g/cm^3$) than those produced by the 'run-off' method (47.5% RNA and $\rho = 1.557\,g/cm^3$).

The 60S subunits prepared by either procedure (ribosome 'run-off' or puromycin treatment) display an identical buoyant density ($\rho = 1.635\,g/cm^3$) and contain an identical amount of RNA (62% by colorimetry); this latter estimate is substantially lower than reported by McConkey (1974) for the 'puromycin' 60S subunits (67% RNA).

The native 60S subunits contained consistently less RNA (58% by wt.) than the derived subunits and had a correspondingly lower buoyant density ($\rho = 1.615\,g/cm^3$), regardless of ribosome source (tissue of rodents, ascites-tumour cells, rabbit reticulocytes). The highly purified 'run-off' subunits contained 63% RNA and had the highest buoyant density.

The buoyant density of the small (40S) subunits produced by the puromycin procedure was previously reported as $1.55\,g/cm^3$ (McConkey, 1974), i.e. the same value as found for the 'run-off' subunits (Cammarano *et al.*, 1972). That the subparticles prepared by the 'puromycin' and the 'run-off' methods show an identical buoyant density of the large subunit but distinctly different densities of the small subunits ($\rho = 1.575$ and $1.555\,g/cm^3$ respectively) is documented by the isodensity-sedimentation profiles of Fig. 7.

The results in Table 3 were then used to determine the dependence of buoyant density in CsCl on RNA content. By rearranging eqn. (1) (see the Methods

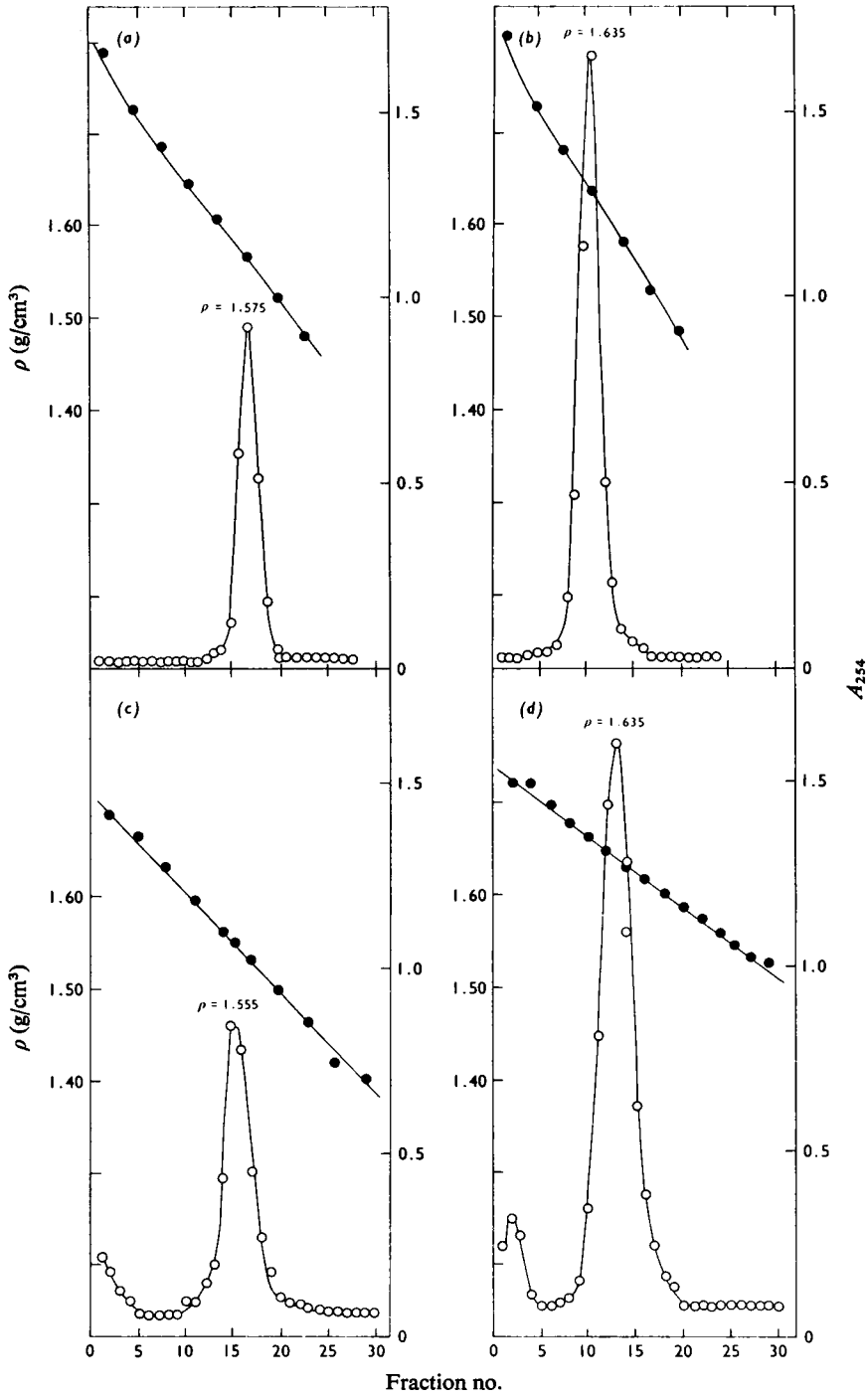

Fig. 7. *Isodensity-sedimentation profiles of* 40*S* (*a*, *c*) *and* 60*S* (*b*, *d*) *subunits isolated as described by McConkey* (1974) (*a*, *b*) *and Falvey & Staehelin* (1970) (*c*, *d*)

The subunits were fixed at 0°C for 1 h and centrifuged to their equilibrium position in CsCl gradients as described in the Methods section; ○, A_{254}; ●, ρ_{CsCl} (g/cm³).

section), the slope and the intercept of the straight line relating RNA content to reciprocal of the buoyant density are given explicitly by the expression:

$$\text{RNA (\% by wt.)} = 100\left[\frac{\bar{v}_p}{\bar{v}_p - \bar{v}_n} - \left(\frac{1}{\bar{v}_p - \bar{v}_n} \cdot \bar{v}_{RNP}\right)\right] \quad (1a)$$

where $\bar{v}_{RNP} = (1/\rho_{CsCl})$ is assumed to be a simple additive function of $\bar{v}_n$ and $\bar{v}_p$. Solving eqn. (1*a*) for $\bar{v}_n = 0.53$ and $\bar{v}_p = 0.74$ (Hamilton & Ruth, 1969):

$$\text{RNA (\% by wt.)} = 352.4 - (476.2 \times \bar{v}_{RNP}) \quad (2)$$

From the values in Table 3, the reciprocals of buoyant density have been plotted versus the RNA content determined by colorimetry (Fig. 8); the straight line fitted by the least-squares method through the experimental points over the density range 1.45–1.65 g/cm^3 obeys eqn. (3), which is similar to eqn. (2):

$$\text{RNA (\% by wt.)} = 349.5 - (471.2 \times \bar{v}_{RNP}) \quad (3)$$

From eqn. (3), for RNA = 100, $\bar{v} = 0.529$, and for RNA = 0, $\bar{v} = 0.742$, in agreement with the partial specific volumes of RNA (0.53 cm^3/g) and protein (0.74 cm^3/g) proposed by Hamilton & Ruth (1969).

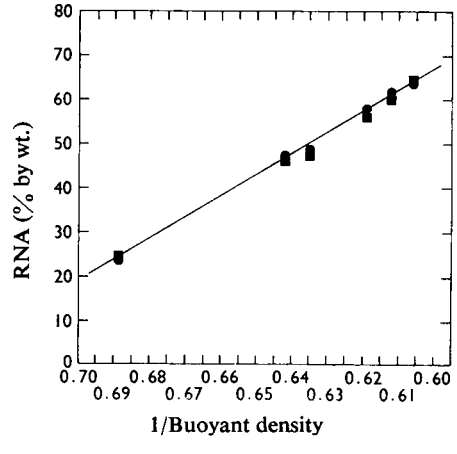

Fig. 8. *Plot of reciprocal of buoyant density versus RNA content estimated by colorimetry*

Each point is the averaged RNA content of particles of identical buoyant density (data from Table 3). ■, RNA content of unfixed ribosomes before centrifugation; ●, RNA content of fixed ribosomes recovered from the CsCl gradients after centrifugation. The dimensions of the symbols encompass the standard deviations of the averaged values (cf. Table 3).

Comparative activity of 'run-off' and 'puromycin' subunits

From buoyant-density measurements (Table 3), 40S subunits prepared by the 'puromycin' method ($\rho = 1.575$ g/cm^3) contain 3% less protein than the corresponding particles prepared by the 'run-off' method ($\rho = 1.555$ g/cm^3). To test whether the lost proteins are structural components (permanently assembled with their rRNA) or adventitious material cross-linked by aldehyde, the activities of the subparticles prepared by the two procedures were compared in a cell-free system programmed with globin mRNA. As Table 4 shows, the subunits isolated by the 'run-off' and the 'puromycin' methods are equally active in globin synthesis. This result allows the conclusion that the 40S subunits prepared by ribosome 'run-off' contain about 3% of extrinsic proteins, which are removed when the harsher ionic conditions adopted by McConkey (1974) are used to dissociate ribosomes (500mM-K^+ and 5.0mM-Mg^{2+}).

Discussion

Validity of buoyant-density methods for the determination of the chemical composition of ribonucleoprotein particles

Isodensity-equilibrium centrifugation in CsCl has been used in the past to determine the chemical composition and the partial specific volume of ribosomes (Perry & Kelley, 1968; Hamilton & Ruth, 1969). The validity of the method has been questioned by McConkey (1974), since it was found that (*a*) as much as 10% of the ribosomal proteins were lost during isopycnic centrifugation of aldehyde-fixed ribosomes, and (*b*) no simple relationship existed between the chemical composition of the ribonucleoprotein particles recovered from the CsCl gradients and the buoyant-density value; as an example, in McConkey's (1974) work 40S subunits, banding at $\rho = 1.55$ g/cm^3, were estimated to contain 61% RNA (by colorimetry), instead of 47% as predicted by the relationship proposed by Hamilton & Ruth (1969). It was implied that differences in conformation and/or hydration of the particles may affect their partial specific volume, and the conclusion was drawn that the partial specific volume of ribosomes is not a simple additive function of the partial specific volumes of RNA and protein.

The results in the present report show that: (*a*) ribosomes fixed with 6% formaldehyde at 0°C remain formally intact after exposure to detergent treatments normally designed to release RNA completely from protein, in agreement with data by Cox (1969), who used 0.5% formaldehyde at 20°C for 2h to stabilize the particles; essentially all the ^{14}C-

Table 4. *Haemoglobin-synthesizing activity of subunits prepared by the methods of McConkey* (1974) *and of Falvey & Staehelin* (1970)

The standard reaction mixture contained, per ml: 20 μmol of phosphocreatine, 3.75 units of creatine kinase (Boehringer/Mannheim G.m.b.H., Mannheim, Germany; activity 25 units/mg), 1.0 μmol of ATP, 0.4 μmol of GTP, 70 μmol of KCl, 4.3 μmol of $MgCl_2$, 30 μmol of Tris/HCl, pH 7.6, 1 μmol of dithiothreitol, 30 μmol of each L-amino acid, 5 μCi of [^{3}H]leucine (sp. radioactivity 50 Ci/mmol) and 0.25 ml of pH 5 enzyme; each assay (0.1 ml) also contained: 0.05 A_{260} unit of globin mRNA ($\simeq$2 μg); 7 pmol of '80S couples' (calculated from the particle weight of 4.0×10^6 daltons and $A_{260}^{1mg/ml} = 13.5$); 45 μg of IF_1 and 60 μg of IF_2. These last two values correspond to the amount of initiation factors yielding maximum incorporation. Initiation factors were prepared by the method of Schreier & Staehelin (1973). Incubations were carried out at 30°C for 40 min and the reaction was stopped by chilling in ice and addition of cold water (0.5 ml). After the addition of 3 ml of 10% trichloroacetic acid the samples were heated for 15 min at 90°C and the hot-trichloroacetic acid precipitate was collected by suction on Whatman GF/A glass-fibre filters. Filters were washed with 3 × 5 ml of 5% trichloroacetic acid and dried before counting for radioactivity in a liquid-scintillation spectrometer at 35% efficiency: 1000 c.p.m. correspond to approx. 8 pmol of leucine incorporated.

Type of particles	mRNA added (μg)	Initiation factors added (μg) IF_1		IF_2	[^{3}H]Leucine incorporated (pmol/7 pmol of ribosomes)
'80S couples'	2	45	+	60	68
	None	45	+	60	23.1
	2	None		None	18
	None	None		None	4
Subunits prepared by McConkey (1974) method					
40S+60S (rat liver)	2	45	+	60	64.2
40S+60S (rabbit reticulocytes)	2	45	+	60	59.5
Subunits prepared by Falvey & Staehelin (1970) method					
40S+60S (rabbit reticulocytes)	2	45	+	60	69.8

labelled ribosomal protein accompanies the fixed ribosomes to their buoyant density, and the protein loss during centrifugation in CsCl ranges from none (mitochondrial ribosomes) to no more than 2% (cytosol ribosomes); (*b*) a linear relationship exists between the RNA content (estimated by colorimetry) and the reciprocal of the buoyant density of ribosomes over a wide range of density values ($\rho = 1.45$–1.65 g/cm^3); from the parameters of the plot in Fig. 8 it is calculated that $\bar{v}_n = 0.53$ and $\bar{v}_p = 0.74$, in striking agreement with the $\bar{v}$ values proposed previously (Hamilton & Ruth, 1969); (*c*) the partial specific volume of formaldehyde-fixed ribosomes is actually a simple additive function of the partial specific volumes of RNA and protein:

$$\text{RNA (\% by wt.)} = \left(0.53 \times \frac{\text{RNA wt.}}{\text{particle wt.}} \times 100\right) + \left(0.74 \times \frac{\text{protein wt.}}{\text{particle wt.}} \times 100\right)$$

i.e., conformational differences among various particles do not affect their partial specific volume, the buoyant density of ribosomes being equal to the weighted average of the buoyant densities of RNA and protein.

The small amount of protein that appears to be lost during centrifugation of ribosomes in CsCl (no more than 2%) introduces a minor element of ambiguity in estimating the chemical composition from buoyant-density measurements: if the lost proteins are structural components permanently associated with RNA (rather than adventitious protein not fixed by aldehyde), the actual RNA content of the particles may be 2% lower than that computed from the buoyant density.

The massive loss of protein observed by McConkey (1974) for ribosomal subunits after centrifugation in CsCl most probably reflects inadequate fixation of the particles. This contention is supported by the results of double-labelling experiments of Kumar & Subramanian (1975): when doubly labelled ribosomes (^{14}C for RNA, ^{3}H for protein) were fixed with aldehyde and banded to their equilibrium position in a CsCl gradient, the ^{14}C/^{3}H ratio of the ribonucleoprotein band was identical with that of control particles not exposed to CsCl, over a wide range of density values; higher ^{14}C/^{3}H ratios were only detected when inadequately fixed ribosomes were deliberately used.

Thus we conclude that isodensity equilibrium centrifugation is a basically valid tool with which to determine accurately the RNA content of ribonucleoprotein particles, the uncertainty limits lying within 2% depending on the nature of the lost proteins. Nevertheless, major sources of error may arise from (*a*) inadequate fixation of the particles,

and/or (*b*) the use of protein standards, such as serum albumin, with a different reactivity from that of ribosomal protein with the Lowry reagent (see the Methods section).

Particle weights of cytoplasmic and mitochondrial ribosomes deduced from buoyant density

Table 5 lists ribosome weights computed from the relative RNA content and rRNA molecular weights. The molecular weights of the two subunit rRNA molecules of cytosol ribosomes were taken as 1.65×10^6 and 0.65×10^6, on the basis of the electrophoretic mobility of the two rRNA species in formamide gels (Cammarano *et al.*, 1975).

It is particularly relevant to compare the weights of cytosol ribosomes calculated from the chemical composition reported by McConkey (1974) (for subunits not exposed by CsCl) and from the composition obtained in the present work. From McConkey's data (1974) the 'puromycin' 40S particles (56% RNA by colorimetry) and 60S particles (67% RNA by colorimetry) would constitute a ribosome with a weight of 3.60×10^6 daltons. From the present results the 'puromycin' 40S and 60S subunits (respectively 50% and 61% RNA from buoyant density) would have to be in a particle of 4.0×10^6 daltons. The difference in RNA content for similarly prepared subunits in our two laboratories is striking. Nevertheless, in our experiment excellent agreement exists between the RNA content estimated from buoyant density and that determined by colorimetry.

The particle weight of the 80S ribosomes deduced from the weights of the 'run-off' subunits is only 0.1×10^6 daltons larger than that deduced from the weights of the 'puromycin' subunits. The difference is entirely accounted for by the greater degree of purity of the small subunit when the ionic conditions adopted by McConkey (1974) are used to isolate ribosomes and ribosomal subunits.

The mass of mitochondrial ribosomes reported previously (Sacchi *et al.*, 1973) is confirmed by the present results; from the chemical composition (23–24% RNA) and the known molecular weights of the two major components of rRNA, the 55S monomer (3.2×10^6 daltons) of mammalian mitochondria is composed of two subunits with weights of 2.0×10^6 and 1.2×10^6 daltons, i.e. mitochondrial ribosomes would be about 20% smaller in mass than cytosol ribosomes.

The dimensions of the negatively stained mitochondrial ribosomes (cf. Table 1) are 26.5nm× 20.8nm×20.3nm; those of the corresponding cytosol ribosomes are 30.0nm×25.0nm×25.0nm (Nonomura *et al.*, 1971), indicating a volume ratio of mitochondrial to cytoplasmic ribosomes of about

Table 5. *Particle weights of 80S ribosomes calculated from the weights of the subunits isolated by the 'puromycin' method and by the 'run-off' method*

In the McConkey (1974) data, RNA content of subunits after CsCl centrifugation is not available because of the reported loss of protein during isodensity sedimentation in these experiments. Particle weights were calculated as:

$$\text{particle wt.} = \frac{\text{rRNA mol.wt.}}{\text{RNA (\% by wt.)}}$$

rRNA weights were taken as 1.65×10^6 and 0.65×10^6 daltons (Cammarano *et al.*, 1975).

Mode of preparation ...			Puromycin					'Run-off'			
Source of data ...	McConkey (1974)		Present paper					Present paper			
Type of particle	RNA (%) (by colorimetry) Before CsCl	$10^{-6}\times$ Particle wt. (daltons)	RNA (%) (by colorimetry) Before CsCl	After CsCl	RNA (%) (from ρ_{CsCl})	$10^{-6}\times$ Particle wt. from ρ_{CsCl} (daltons)	RNA (%) (by colorimetry) Before CsCl	After CsCl	RNA (%) (from ρ_{CsCl})	$10^{-6}\times$ Particle wt. from ρ_{CsCl} (daltons)
40S	56	1.16	47.8	48.8	50	1.30	46.8	48.8	47	1.38
60S	67	2.46	59.5	62.0	61	2.67	59.5	62.0	61	2.71
80S	—	3.60	—	—	—	3.97	—	—	—	4.09

1:1.67. The mitochondrial ribosomes from such distantly related organisms as the Arthropoda (*Locusta migratoria*) have dimensions of 27.0nm× 21.0nm×21.5nm (Kleinow *et al.*, 1974); it is inferred that the size of these particles has been largely conserved during animal evolution.

In positively stained tissue sections the volume ratio of mitochondrial to cytoplasmic ribosomes has been estimated to be 1:3 (Andrè & Marinozzi, 1965). The discrepancy in volume ratio between mitochondrial and cytosol ribosomes in negatively stained specimens (1:1.5) and positively stained sections (1:3) presumably reflects the peculiar chemical composition of mitochondrial ribosomes. In the positively stained sections the stain (uranyl acetate) binds principally to RNA, whereas in the negatively stained specimens essentially all the ribosome profile is seen; since RNA accounts for only 25% of the particle mass of mitochondrial ribosomes but for more than 55% of the mass of cytosol ribosomes, in the sections the 55S particles might appear considerably smaller than the 80S ribosomes.

The overall structure of the 55S ribosomes revealed by electron microscopy is basically similar to that of cytoplasmic ribosomes (80S); as reported by Aaji *et al.* (1972) the particles exhibit a cleft between two unequal subunits and a bipartition of the small subunits. However, some aspects of the structural arrangements of mitochondrial ribosomes are quite peculiar; the profiles of the isolated large subunits are almost always of the roundish type and slightly irregular, whereas triangular profiles with curved flanks, which are the predominant species in electron micrographs of cytoplasmic ribosomes, are rarely detected. Moreover, it has never been possible to identify knob-like projections similar to those seen in cytoplasmic ribosomes and in yeast mitochondrial ribosomes (Vignais *et al.*, 1971).

The nucleotide composition of the rRNA species of the two subunits of mammalian mitochondria resembles closely that of *Xenopus* mitochondria (Dawid & Chase, 1972) and differs from that of both the Fungi (*Neurospora crassa*) (Rifkin *et al.*, 1967) and the Protozoa (*Tetrahymena pyriformis*) (Chy & Suyama, 1970).

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