Preparation of Ribosome-Free Membranes from Rat Liver Microsomes by Means of Lithium Chloride

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1. Treatment of washed rat liver microsomes in a medium containing 0.12M-sucrose, 12.5mM-potassium chloride, 2.5mM-magnesium chloride and 25mM-tris-hydrochloric acid buffer, pH 7.6, with 2M-lithium chloride at 5° for 16 hr. leads to the formation of membranes free of ribosomes and ribosomal subunits. 2. Confirmation of the absence of ribosomes from lithium chloride-prepared membranes was obtained by treatment of the membranes with sodium deoxycholate, followed by sucrose-density-gradient centrifugation, which showed the complete absence of ribosomes. 3. Treatment of membranes with phenol, followed by sucrose-density-gradient analysis of the isolated RNA, showed the presence of a small amount of 4s material. Repetition of the phenol extraction procedure in the presence of liver cell sap as a ribonuclease inhibitor again showed the presence of only 4s material. The 4s RNA was shown to be transfer RNA by the fact that it had the same capacity for accepting ¹⁴C-labelled amino acids as isolated transfer RNA from rat liver pH 5 enzyme. 4. Analysis showed that microsomes and membranes possessed similar glucose 6-phosphatase, NADH-2,6-dichlorophenol-indophenol reductase, NADH-neo-tetrazolium reductase, NADH-cytochrome c reductase and ribonuclease activities. 5. ³H-labelled ribosomal RNA binds to membranes. However, isolation of the bound RNA by the phenol extraction procedure, followed by sucrose-density-gradient analysis, shows the RNA to be degraded to 7s material. Very little breakdown of ³H-labelled ribosomal RNA bound to membranes occurs if the binding and isolation are carried out in the presence of liver cell sap.

The importance of the endoplasmic reticulum in protein synthesis has been realized for some time and it has been shown that microsomes are a more efficient protein-synthesizing system *in vivo* than unattached ribosomes (Siekevitz & Palade, 1960). Various methods have been used in attempting to release ribosomes from the membranes of the endoplasmic reticulum. Sodium deoxycholate appears to dissolve or disperse the lipoprotein membranes while leaving the ribosomes and polyribosomes apparently intact (Korner, 1959); Takanami, 1961; Wettstein, Staehelin & Noll, 1963). On the other hand, fairly high concentrations of EDTA release ribosomes and ribosomal subunits, leaving the membrane intact but still having some attached ribosomes that are not released with this chelating agent (Sabatini, Tashiro & Palade, 1966). A previous attempt to prepare membranes free of ribosomes from rat liver microsomes through the use of iso-octane (Hawtreys & Schirren, 1962) was not successful, as it was shown later by Campbell, Cooper & Hicks (1964) that the membrane fraction obtained by the iso-octane procedure still had considerable

amounts of attached ribosomes. Hallinan & Munro (1965) have used the iso-octane procedure to prepare smooth-surfaced membranes of rat liver endoplasmic reticulum.

All the methods described above, however, give little information as to the mechanism or mode of attachment of the ribosome to the membranes of the endoplasmic reticulum. To gain some insight into this problem, it appeared to us that possibly a more promising approach would be to prepare membranes free of ribosomes from microsomes for the purpose of designing experiments aimed at re-attaching ribosomes or alternatively ribosomal components such as structural RNA or protein to the membranes.

In the present paper we describe a method for preparing membranes free of ribosomes from microsomes by means of lithium chloride.

MATERIALS AND METHODS

Chemicals. LiCl was supplied by the Fisher Scientific Co., Pittsburg, Pa. U.S.A. Sodium deoxycholate was purchased from General Biochemicals Inc., Chagrin Falls,

Ohio, U.S.A. Dodecyl sulphate (sodium salt) was supplied by Serva, Heidelberg, Germany. Yeast RNA was supplied by British Drug Houses Ltd., Poole, Dorset.

Radioactive chemicals. [^3H]Orotic acid (16.8 c/m-mole) and ^{14}C -labelled yeast-protein hydrolysate (1500 $\mu\text{C}/\text{mg}$.) were obtained from Schwarz BioResearch Inc., Orangeburg, N.Y., U.S.A.

Scintillation chemicals. 2,5-Diphenyloxazole and 1,4-bis-(5-phenyloxazol-2-yl)benzene were obtained from Packard Instrument Co. Inc., Le Grange, Ill., U.S.A.

Solvents. Methanol and chloroform were of the highest analytical-reagent grade and were obtained from E. Merck A.-G., Darmstadt, Germany.

Animals. The livers of albino rats (120–180 g. body wt.) were used throughout these studies. Animals were starved for 24 hr. before being killed by decapitation. Preparation of radioactive material, when required, was carried out by intraperitoneal injection of 30 μC of [^3H]orotic acid in water/animal 14–15 hr. before death.

Preparation of homogenate. After decapitation of rats, livers were dissected out rapidly and placed on ice in a tared beaker. The weight of the livers was recorded and they were washed four times with medium A [0.25 M-sucrose in TKM buffer containing (final concns.) 5 mM-MgCl₂, 25 mM-KCl and 50 mM-tris-HCl buffer, pH 7.60]. The washed livers were blotted dry with Whatman no. 1 filter paper and broken up by mincing and grinding with pestle and mortar. The mince was transferred with 2 vol. of medium A into a glass homogenizer (Dounce) and homogenized with 14 passes of the loose plunger.

Preparation of microsomes. The homogenate was centrifuged at 15000g for 20 min. (Spinco no. 30 rotor) and the resulting 15000g supernatant then centrifuged at 100000g for 90 min. (Spinco no. 30 rotor) to yield a 100000g supernatant and a microsome pellet. The 100000g supernatant was recentrifuged at 100000g for 90 min. to yield the cell sap containing a ribonuclease inhibitor. The microsome pellet was resuspended in medium A by homogenization (equal volume of medium A to original weight of liver taken) and centrifuged at 100000g for 90 min. (Spinco no. 30 rotor). The washing procedure by centrifugation was repeated once more. The washed microsome pellet was finally resuspended in medium A by homogenization (equal volume of medium A to original weight of liver taken) and stored in ice.

Preparation of membrane. Suspensions of microsomes (in medium A) as prepared above were treated with an equal volume of 4 M-LiCl to give a final concentration of 2 M with respect to LiCl. The treated microsomes were kept in ice for 16 hr. with continuous slow stirring (magnetic stirrer). At the end of this time the precipitated RNA was centrifuged down at 1400g for 20 min. The 1400g supernatant was centrifuged at 100000g for 90 min. (Spinco no. 30 rotor). The resulting gelatinous red pellet of LiCl-treated microsomes was resuspended by homogenization in medium A and recentrifuged at 100000g for 90 min. (Spinco no. 30 rotor). The pellet obtained after this centrifugation was suspended in medium A or in an appropriate buffer system, depending on the experiment intended. Membrane preparations were stored in ice and discarded after 3 days.

Preparation of membranes for electron microscopy. Membranes were prepared from microsomes by means of LiCl as described. After washing with medium A by high-speed centrifugation, the pellets were fixed with 5% glutaraldehyde in 0.1 M-sodium phosphate buffer, pH 7.3, at 0° for

4 hr. The fixed pellets were then washed four times successively with 0.1 M-sodium phosphate buffer, pH 7.3. Post-fixation of the pellets was carried out in OsO₄. After dehydration, the pellets were embedded in Araldite.

Preparation of ribosomes. Washed microsomes suspended in medium A were treated with a 10% (w/v) solution of sodium deoxycholate to give a final concentration of 1% and kept for 15 min. in ice. The resulting clear solution was layered over 1 M-sucrose in TKM buffer (15 ml.) and centrifuged at 100000g for 3 hr. (Spinco no. 30 rotor). The ribosome pellet obtained was suspended in medium A by gentle homogenization and dialyzed exhaustively against the same medium. Finally, before use, ribosome suspensions were centrifuged at 1400g for 10 min. to remove any denatured material. All preparations were stored in ice.

Preparation of ^3H -labelled 28S and 18S RNA. Microsomes were prepared as described above from animals previously injected with [^3H]orotic acid. They were diluted to 2 vol. with a mixture of water and cell sap (1:1, v/v) and then treated with a solution of sodium dodecyl sulphate at a final concentration of 1% (w/v). The whole mixture was shaken for 1 hr. at room temperature in the presence of an equal volume of 90% (w/v) phenol. The two phases were separated by centrifugation at 1400g for 20 min. and the top aqueous layer was collected. RNA was precipitated from this aqueous phase by the addition of 0.1 vol. of 20% (w/v) potassium acetate and 2.5 vol. of 96% (w/v) ethanol and left for 24 hr. at -15°. The precipitated RNA was dissolved in a small volume of water and dialysed against water to remove all trace of phenol. Preparations of RNA were characterized on 5–20% (w/v) sucrose density gradients and stored at -15° until required.

Aminoacyl-tRNA* synthetases. The synthetase enzymes were prepared from rat liver by the method of Hele (1961) and used within 2 days of preparation.

Incubation of membrane with ^3H -labelled RNA. A sample of membrane (20 ml., containing 8.6 mg. of protein/ml.) and ^3H -labelled rRNA (5 ml., containing 4.5 mg. of RNA and carrying 386000 c.p.m.) was incubated in a buffer medium containing 5 mM-tris-HCl buffer, pH 7.6, 1 mM-KCl, 12 mM-sucrose and 1 mM-MgCl₂ for 10 min. at 0°. Samples of the incubation mixture were then layered over 1 M-sucrose (25 ml.) in TKM buffer and centrifuged for 1.5 hr. at 100000g in the Spinco no. 30 rotor. The above washing procedure through 1 M-sucrose was repeated once more and the final pellets of membrane were suspended in medium A. For extraction of ^3H -labelled rRNA from the washed membranes, the phenol-dodecyl sulphate method used for the preparation of ^3H -labelled 28S and 18S RNA from microsomes was used (see above).

Preparation and monitoring of sucrose density gradients. All gradients were prepared by the method of Henshaw, Bojarski & Hiatt (1963) as modified by Stead, Nourse & Hawtrey (1964). Fractions (1 ml.) were collected by using a hypodermic needle, diluted to 2 ml. with water and their E_{260} was measured in a spectrophotometer (Unicam model SP. 500).

Enzyme assays. The following assays were carried out on microsome and membrane preparations.

(a) Ribonuclease activity. An incubation mixture (final vol. 2 ml.) containing the sample under test (microsome or

*Abbreviations: tRNA, transfer RNA; rRNA, ribosomal RNA.

membrane suspension) and 2 mg. of yeast RNA was incubated at 37° for 40 min. The incubation mixture was brought to 37° and the yeast RNA added at zero time. At 10, 20 and 30 min., 0.5 ml. samples of the reaction mixture were withdrawn and pipetted into 5 ml. of ice-cold 0.4 M-perchloric acid. Perchloric acid was added to the 40 min. sample and this was also placed in ice. This procedure was repeated for a sample of RNA incubated in the absence of membrane or microsome. After standing for 15 min. in ice, precipitated material was centrifuged down at 1400g and the E_{260} of the clear supernatant measured for release of acid-soluble nucleotides. The supernatants from the RNA sample incubated alone were used as blanks in the assay.

(b) Glucose 6-phosphatase activity. This procedure followed that of Swanson (1955).

(c) NADH-2,6-dichlorophenol-indophenol reductase activity. This assay was carried out in 1 cm. glass cuvettes. Each reaction cuvette contained 100 mM-tris-acetate buffer, pH 7.6, 1 mM-KCN, 3 mM-MgCl₂, 20 mM-nicotinamide, 2 mg. of NADH, 36 mM-2,6-dichlorophenol-indophenol and a portion (1.5 ml.) of the test sample suitably diluted to give a reasonable assay value. Reduction of the 2,6-dichlorophenol was followed at 660 nm. Assays were performed at room temperature.

Determinations. Protein was determined by the method of Gornall, Bardawill & David (1949), with crystalline bovine serum albumin as standard.

RNA was determined essentially as described by Hawtrey, Schirren & Dijkstra (1963).

Total phospholipid was extracted from microsomes and membranes by the method of Folch, Lees & Sloane-Stanley (1957). Phospholipid phosphate was then determined by the method of Bartlett (1959).

Counting of samples. In experiments in which ¹⁴C-labelled aminoacyl-tRNA was used, the radioactivity of trichloroacetic acid-washed precipitates on Millipore filters was counted in toluene containing 0.5% (w/v) of 2,5-diphenyloxazole and 0.03% (w/v) of 1,4-bis-(5-phenyloxazol-2-yl)benzene. Samples from sucrose gradients (2 ml.) were counted in Bray's (1960) solution.

RESULTS

Properties of membrane prepared from microsomes by means of 2M-lithium chloride. Treatment of microsomes with 2M-lithium chloride at 5° for 16 hr. results in the formation of a membrane fraction (lipoprotein) with virtually no RNA present on

analysis (Table 1). Apart from the loss of ribosomal structural RNA through precipitation with lithium chloride, there is also a corresponding loss of ribosomal protein that is solubilized by this treatment. However, lithium chloride does not appear to have seriously affected the membrane, which appears to be relatively intact. Supporting evidence for this

Table 2. *Comparison of certain enzyme activities in microsomes and membranes prepared with lithium chloride*

Details of the individual enzyme assays are given in the Materials and Methods section. Glucose 6-phosphatase specific activity is given as μ g. of P_i released/10 min./mg. of protein at 37°. NADH-2,6-dichlorophenol-indophenol reductase specific activity is given as ΔE_{660} /min./mg. of protein during the initial stage of the reaction at room temperature. NADH-neotetrazolium reductase units are given as formazan produced (ΔE_{510})/5 min./mg. of protein at 37°. Assays for neotetrazolium reductase were carried out in an incubation medium containing: 75 mM-tris-HCl buffer, pH 7.4, 0.3 mM-NADH, 0.5 mg. of neotetrazolium chloride, membrane (0.32 mg. of protein) and microsomes (0.63 mg. of protein). Incubation was for 5 min. at 37°. Reactions were stopped with 1.0 ml. of 10% (w/v) trichloroacetic acid and the formazan was extracted with ethyl acetate. NADH-cytochrome *c* reductase units are given as ΔE_{550} /min./mg. of protein at room temperature. In the cytochrome *c* assays incubation conditions were as described for 2,6-dichlorophenol-indophenol reductase, except that cytochrome *c* was present as the electron acceptor (2 mg. of cytochrome *c* per assay). Ribonuclease units are defined by release of perchloric acid-soluble nucleotides from RNA (ΔE_{260})/10 min./mg. of protein at 37°.

Enzyme system	Sp. activity of enzyme assayed	
	Microsomes	Membrane
Glucose 6-phosphatase	17.71	18.17
NADH-2,6-dichlorophenol-indophenol reductase	2.11	2.36
NADH-neotetrazolium reductase	1.14	1.38
NADH-cytochrome <i>c</i> reductase	0.38	0.48
Ribonuclease	0.020	0.025

Table 1. *Analysis of microsomes and membranes prepared from microsomes by means of 2M-lithium chloride*

Preparation of washed microsomes and membranes, as well as the analytical procedures for the determination of protein, RNA and phospholipid, were carried out as described in the Materials and Methods section. Microsomes refer to that fraction of rat liver homogenate sedimented at 100000g for 90 min. (Spinco no. 30 rotor) and washed twice by repeated centrifugation under the same conditions. Membrane refers to that fraction obtained from washed microsomes by treatment with 2M-LiCl.

Fraction	Analysis				
	Protein (mg./g. of liver)	RNA (mg./g. of liver)	Phospholipid (mg./g. of liver)	RNA/protein ratio	Phospholipid/protein ratio
Microsomes	6.85	0.70	2.80	0.12	0.41
Membrane	3.65	0.04	1.53	0.01	0.42

Fig. 1. Sucrose-density-gradient centrifugation of LiCl-prepared membranes and membranes treated with sodium deoxycholate. Linear sucrose gradients (16–34%, w/v) in medium A buffer were overlaid on a 5 ml. cushion containing 2M-sucrose. Centrifugation was in the Spinco SW 25.1 rotor at 88000g for 2 hr. The temperature of the gradient was 5°. Fractions (1ml.) were collected, diluted to 2ml. with water and the E_{260} was read. (a) LiCl-prepared membranes (1.35mg. of protein). (b) LiCl-prepared membranes treated with sodium deoxycholate.

Fig. 2. Sucrose-density-gradient centrifugation of microsomes and microsomes treated with sodium deoxycholate. Details of the gradient centrifugation are identical with those given in Fig. 1. The unlabelled arrows indicate gradient cushion, (a) Microsomes (1.20mg. of protein). (b) Microsomes treated with sodium deoxycholate.

assumption was obtained by comparative enzyme assays presented in Table 2. On a protein basis it was found that the activities of enzymes tested were of a similar order for both microsomes and

membrane preparations. However, membrane enzyme activities were all found to be slightly higher than the corresponding activities for microsomes.

The result of sucrose-density-gradient analysis of the membrane prepared by means of lithium chloride is shown in Fig. 1(a). As evidenced by the gradient, no free ribosomes are present. Sucrose-density-gradient analysis of the membrane after treatment with sodium deoxycholate is shown in Fig. 1(b), which clearly illustrates the complete absence of any ribosomal material. Sucrose-density-gradient analysis of microsomes (Fig. 2a) was carried out as a control. Fig. 2(b) illustrates the effect of treating microsomes with sodium deoxycholate followed by sucrose-density-gradient analysis.

Further evidence that the membranes prepared from rat liver microsomes by means of lithium chloride are free of ribosomes was obtained via electron micrographs of the membranes. As shown in Plate 1, membranes appear to be relatively intact and free of attached ribosomes.

Phenol extraction of the membrane starting with over 2g. of material (measured as protein) resulted in a small 4s peak of RNA, as shown in the sucrose-density-gradient profiles in Fig. 3(a). The isolation was repeated in the presence of cell sap to prevent any possible degradation of larger species of RNA if present. The result shown in Fig. 3(b) gives no evidence of any RNA species larger than 4s. A large percentage of the E_{260} value of the 4s peak in Fig. 3(b) is due to the 4s RNA present in cell sap (Fig. 3c). It may be that the lithium chloride treatment has already precipitated any RNA present in the membrane of a size greater than 4s. Evidence of large RNA species has been found by other workers (King & Fitschen, 1968; Tata, 1967), but as yet no evidence has been found to show this in the membranes made by the lithium chloride procedure.

Characterization of 4s RNA found in lithium chloride-prepared membranes. The 4s RNA found in membranes prepared by the lithium chloride method could consist of degraded rRNA, tRNA or a mixture of both. Strong evidence that the membrane-bound RNA is tRNA is indicated by the results in Table 3, which shows that, on a weight basis, membrane-bound 4s RNA has the same capacity for accepting radioactive amino acids as tRNA isolated from rat liver pH 5 enzyme.

Characterization of ^3H -labelled RNA prepared from microsomes. Analysis of the RNA extracted from microsomes by the phenol procedure in the presence of cell sap was carried out by means of sucrose-density-gradient analysis. Fig. 4(a) shows the result of such an experiment. The 4s peak of material present in Fig. 4(a) is due largely to RNA derived from cell sap.

EXPLANATION OF PLATE I

Electron micrograph of membranes prepared from microsomes by means of 2M-lithium chloride as described in the text; magnification: $\times 50000$.

Fig. 3. Sucrose-density-gradient centrifugation of RNA extracted from LiCl-prepared membranes by the phenol procedure in the absence or presence of cell sap. Linear sucrose gradients (5–20%, w/v) contained 10 mM-potassium acetate, pH 5.2, 5 mM-EDTA, pH 5.2, and 50 mM-NaCl. Centrifugation was carried out in the SW 25.1 rotor at 88000g for 14 hr. The temperature of the gradient was 5°. Fractions were collected as in Fig. 1. (a) RNA from LiCl-prepared membranes. (b) RNA extracted from membranes in the presence of cell sap. (c) RNA of cell sap.

Table 3. *Aminoacylation of isolated membrane RNA with ^{14}C -labelled amino acids in the presence of aminoacyl-tRNA synthetases*

Incubation mixtures contained: 35 mM-tris-HCl buffer, pH 7.6, 17 mM-KCl, 6 mM-MgCl₂, 1.6 mM-GSH, pH 7.6, 3 mM-ATP, potassium salt, pH 7.6, ^{14}C -labelled yeast protein hydrolysate (1.0 μC), 50 μg . of membrane RNA or deacylated tRNA, and aminoacyl-tRNA synthetase (80 μg . of protein) in a final volume of 1 ml. Incubation was carried out at 37° for 20 min. Reactions were stopped with cold 10% trichloroacetic acid. Samples were washed twice by low-speed centrifugation with cold 5% trichloroacetic acid and then transferred to Millipore filters where they were washed successively with cold 5% trichloroacetic acid (4 $\times$ 5 ml.), ethanol (1 $\times$ 5 ml.) and ethanol-ether (1:1, v/v) (1 $\times$ 5 ml.). The Materials and Methods section contains details on the preparation of aminoacyl-tRNA synthetase enzymes and counting procedure.

System	Radioactivity bound by RNA (c.p.m./100 μg . of RNA)
Membrane RNA	12702
Deacylated tRNA	12942

Binding of labelled rRNA to membranes. ^3H -labelled rRNA has been found to bind to membranes. Membranes containing bound ^3H -labelled

rRNA were washed by repeated centrifugation through 1M-sucrose in TKM buffer. Extraction of the labelled RNA from washed membranes by the phenol-dodecyl sulphate extraction procedure and analysis of the isolated RNA by sucrose-density-gradient centrifugation showed it to consist of degraded material, approx. 7s in size (Fig. 4b). The gradient profile of control ^3H -labelled rRNA is shown in Fig. 4(a). When on the other hand all operations in the binding and isolation experiments were carried out in the presence of cell sap, which is known to contain a ribonuclease inhibitor (Blobel & Potter, 1966; Lawford, Sadowski & Schachter, 1967), very little degradation of membrane-bound ^3H -labelled RNA occurred. These results are not shown.

DISCUSSION

Treatment of washed microsomes with 2M-lithium chloride for 16 hr. at 5° has been found to give a membrane fraction that is free of ribosomes and ribosomal subunits. The use of 2M-lithium chloride for dissociating liver ribosomes into structural RNA and proteins was first reported by Curry & Hersh (1962). Barlow, Mathias & Williamson (1963) applied the same technique for the dissociation of rabbit reticulocyte ribosomes. We have

Fig. 4. Sucrose-density-gradient centrifugation of ^3H -labelled rRNA and membrane-bound ^3H -labelled rRNA. Details of the binding of ^3H -labelled rRNA to washed membranes and the isolation of bound RNA from membranes are given in the Materials and Methods section. Details of the centrifugation are as given in Fig. 3. (a) ^3H -labelled rRNA run as a control: —, E_{260} ; ----, radioactivity. (b) Reisolated membrane-bound ^3H -labelled rRNA: —, E_{260} ; ----, radioactivity.

applied this method to rat liver microsomes and as shown by the results described, have been able to solubilize the ribosomes while leaving the membrane apparently intact.

According to Richardson, Hultin & Green (1963), membranes consist essentially of a structural protein backbone with lipids attached through hydrophobic bonds. Also present are a number of enzymes and cytochromes, which are the more soluble proteins. The results of the enzyme assays carried out on the membrane give no evidence of the solubilization of certain of these enzymes (Table 2). Although not shown, cytochrome b_5 contents were also found to be virtually the same in both microsomes and membranes. The behaviour of the membrane on sucrose-density-gradient centrifugation (Fig. 1) also supports the idea that the membrane is intact and is not in fact a structure depleted of protein. This latter result is supported further by chemical analysis of membranes and microsomes (Table 1). The results appear to indicate that lithium chloride-prepared membranes are a reasonable representation of the endoplasmic reticulum and therefore suitable

for a study of ribosome or ribosomal-component attachment.

Lithium chloride-prepared membranes have been found to contain very small amounts of 4s RNA (Fig. 3). Treatment of washed microsomes with lithium chloride in the presence of cell sap, which contains a ribonuclease inhibitor, showed no species of RNA other than 4s in size (Fig. 3b). Lithium chloride-prepared membranes possess ribonuclease activity (T. Scott-Burden & A. O. Hawtrey, unpublished work), which, although weak at 0° , may nevertheless be capable of appreciable degradation of RNA during the time required to prepare the membrane. The 4s RNA bound to membranes could be degraded rRNA or tRNA or a mixture of both. Strong evidence that 4s material is tRNA is shown by the fact that it has the same capacity for accepting amino acids as tRNA isolated from rat liver pH 5 enzyme (Table 3). At present it is not known whether the membrane-bound tRNA is a functional part of the membrane or whether it is simply an artifact of the preparative procedure.

Membranes prepared by the lithium chloride

procedure have been found to bind ^3H -labelled rRNA. The bound RNA is largely degraded to material of approx. 7s. The degradation observed is presumably due to the low ribonuclease activity exhibited by membrane preparations. However, it has been found that, if the binding of labelled RNA to membranes, the purification of such complexes and the final phenol extraction of bound RNA are carried out in the presence of cell sap at all stages, very little degradation of bound rRNA occurs.

It is concluded that the preparation of ribosome-free membranes from rat liver microsomes by means of lithium chloride offers a simple reproducible method for obtaining membranes. The membrane preparations obtained by this method may prove useful in studies aimed at reattachment of ribosomes or ribosomal components.

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REFERENCES

- Barlow, J. J., Mathias, A. P. & Williamson, R. (1963). *Biochem. biophys. Res. Commun.* **13**, 61.
- Bartlett, G. R. (1959). *J. biol. Chem.* **234**, 466.
- Blobel, G. & Potter, V. R. (1966). *Proc. nat. Acad. Sci., Wash.*, **55**, 1283.
- Bray, G. A. (1960). *Analyt. Biochem.* **1**, 279.
- Campbell, P. N., Cooper, C. & Hicks, M. (1964). *Biochem. J.* **92**, 225.
- Curry, J. B. & Hersh, R. T. (1962). *Biochem. biophys. Res. Commun.* **6**, 415.
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.
- Gornall, A. G., Bardawill, C. S. & David, M. M. (1949). *J. biol. Chem.* **177**, 751.
- Hallinan, T. H. & Munro, H. N. (1965). *Quart. J. exp. Physiol.* **50**, 93.
- Hawtrey, A. O. & Schirren, V. (1962). *Biochim. biophys. Acta*, **61**, 467.
- Hawtrey, A. O., Schirren, V. & Dijkstra, J. (1963). *Biochem. J.* **88**, 106.
- Hele, P. (1961). *Biochem. J.* **81**, 329.
- Henshaw, E. C., Bojarski, T. B. & Hiatt, H. H. (1963). *J. molec. Biol.* **7**, 122.
- King, H. S. W. & Fitschen, W. (1968). *Biochim. biophys. Acta*, **155**, 32.
- Korner, A. (1959). *Biochim. biophys. Acta*, **35**, 554.
- Lawford, G. R., Sadowski, P. & Schachter, H. (1967). *J. molec. Biol.* **23**, 81.
- Richardson, S. H., Hultin, H. O. & Green, D. E. (1963). *Proc. nat. Acad. Sci. Wash.*, **50**, 821.
- Sabatini, D. D., Tashiro, Y. & Palade, G. E. (1966). *J. molec. Biol.* **19**, 503.
- Siekevitz, P. & Palade, G. E. (1960). *J. biophys. biochem. Cytol.* **7**, 619.
- Stead, R., Nourse, L. D. & Hawtrey, A. D. (1964). *S. Afr. J. Med. Sci.* **29**, 79.
- Swanson, M. A. (1955). In *Methods in Enzymology*, vol. 2, p. 541. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Takanami, M. (1961). *Biochim. biophys. Acta*, **51**, 85.
- Tata, J. R. (1967). *Biochem. J.* **105**, 47P.
- Wettstein, F. O., Staehelin, T. & Noll, H. (1963). *Nature, Lond.*, **197**, 430.