The Dissociation of Rat Liver Ribosomes by Ethylenediaminetetraacetic Acid; Molecular Weights, Chemical Composition, and Buoyant Densities of the Subunits*

Mary G. Hamilton and Mary E. Ruth†

ABSTRACT: Rat liver ribosomes were dissociated to subunits by the addition of EDTA and the subunits were separated by sucrose gradient centrifugation in a zonal rotor. Transfer ribonucleic acid, 5S ribosomal ribonucleic acid, and a "7S" ribonucleic acid were found in a slowly sedimenting peak which also contained protein-like material, presumably nascent peptides. For molecular weight measurements with absorption optics by high-speed equilibrium centrifugation, the isolated subunits were fixed with 1% formaldehyde at pH 7.4. Partial specific volumes were calculated from the protein and ribonucleic acid contents of the subunits as determined by chemical analysis and from their buoyant densities in CsCl after formaldehyde fixation. The large subunit had a molecular weight of 3.1 million daltons and contained 45% protein; in 0.03 M KCl–0.001 M potassium phosphate (pH 7.0)–0.2 mM $MgCl_2$ it had a sedimentation coefficient, $s^0_{20,w}$, of 50 S. The small subunit had a molecular weight of 1.2 million daltons and contained 50% protein. Its sedimentation coefficient was 29 S.

The buoyant densities of the large and small subunits were 1.602 and 1.593 g per ml in CsCl and 1.442 and 1.425 g per ml in Cs_2SO_4.

Study of the internal organization of rat liver ribosomes requires precise values for their size and chemical composition. Although we (Hamilton *et al.*, 1962; Petermann and Pavlovec, 1966) and others (Tashiro and Yphantis, 1965) have measured the molecular weights of mammalian ribosomes, the solutions studied were usually not monodisperse. Moreover, being part of a complex protein-synthesizing system, the ribosomes may vary in composition depending upon the presence or absence of tRNA molecules, mRNA, and nascent peptides, among other things. The two subunits, on the other hand, may be more constant chemical entities than the intact ribosome and thus more suitable for physical measurements. Since they differ in size and in protein content, they must first be separated.

Several recent technical developments have improved the accuracy of molecular weight measurements: (1) the zonal rotor to facilitate obtaining monodisperse solutions (Anderson, 1966), (2) the photoelectric scanner attachment to the analytical ultracentrifuge which permits measurements at very low concentrations, and (3) the meniscus depletion method of equilibrium centrifugation which uses speeds where the ultracentrifuge does not vibrate (Yphantis, 1964). We have stabilized the subunits for the long period of centrifugation by fixing them with formaldehyde.

Despite the ease of measuring the concentration distribution with the photoelectric scanner, the measurement of partial specific volume, $\bar{v}$, required for calculating molecular weight, is still a problem (Van Holde, 1967). For the subunits, we have calculated $\bar{v}$ from the chemical composition by assuming additivity of the values for RNA and protein. We have also used the buoyant density in CsCl of formaldehyde-fixed particles as a measure of the protein content (Spirin *et al.*, 1965). This proves to be a useful method for ribosomes. At the end of this paper we shall discuss the relation of our isolated subunits to the physiologically active units.

Experimental Section

Isolation and Purification of Ribosomes. The method of Petermann and Pavlovec (1967) was used for isolating electrophoretically pure ribosomes from rat liver.

Dissociation of Ribosomes to Subunits and Separation of the Subunits by Zonal Centrifugation. The chief solvent used in this work was 0.03 M KCl–0.001 M potassium phosphate (pH 7.0) (buffer A).[1] About 15 ml of a solution of ribosomes (about 10 mg/ml) was adjusted to pH 8 by the addition of 0.1 M $KHCO_3$ to a final concentration of 0.02 M and trisodium EDTA (2 μmoles/mg of ribosomes) was added. The subunits were separated by centrifugation into a sucrose gradient contain-

* From the Sloan-Kettering Institute for Cancer Research and the Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University Medical College, New York, New York 10021. *Received October 21, 1968.* This work was supported by U. S. Public Health Service Grant CA 08748 and Atomic Energy Commission Contract AT(30-1)-910. It was presented in part at the 12th Annual Meeting of the Biophysical Society in Pittsburgh, Pa., in Feb 1968.

† Present address: Hoffmann-LaRoche Co., Nutley, N. J.

[1] Abbreviation used: buffer A, 0.03 M KCl–0.001 M potassium phosphate (pH 7.0).

ing buffer A (Hamilton and Ruth, 1967). The B-XIV zonal rotor with a capacity of 700 ml was used.

Chemical Analyses. To remove sucrose, the zonal fractions (see Figure 1) were passed through a 32 × 1 cm column of G-50 Sephadex in buffer A. Each sample was then analyzed for protein by the Lowry method with bovine serum albumin as the standard (Petermann, 1964). RNA was determined by the orcinol method (Petermann, 1964) with adenosine monophosphate as a standard and correction factors of 1.93 for the large subunit and 1.97 for the small subunit, calculated from the purine contents of their RNAs (Hirsch, 1966). All analyses were carried out in duplicate or triplicate and the standard errors of the means were 2.5% or less.

Formaldehyde Fixation of the Subunits. The samples were dialyzed for 24 hr or longer at 5° against buffer A adjusted to pH 7.4 and containing 1% formaldehyde. For isodensity equilibrium centrifugation KCl was omitted; in some experiments additional formaldehyde was added.

Ultracentrifugation. The samples were analyzed at temperatures below 10°, in most cases at low concentrations (about 50 μg/ml) with ultraviolet absorption optics with either film and densitometry or with the photoelectric scanner. With the scanner three samples could be simultaneously analyzed under identical conditions. The densities and viscosities of the formaldehyde-containing buffers were measured.

Sedimentation Velocity Studies. To study the concentration dependence of sedimentation, material pooled from the zonal peaks (see Figure 1) was pelleted, resuspended in a small volume of buffer A with 0.2 mM $MgCl_2$, and dialyzed for 5 hr against the same buffer. For these experiments schlieren optics were used. The concentration of the stock solution was computed from the schlieren pattern, and dilutions were made by weight. For each subunit four samples, with concentrations ranging from 0.5 to 3 mg per ml for the large subunit and from 0.3 to 1.7 mg per ml for the small subunit, were analyzed in one run by using suitable wedge windows. The values of $1/s_{20,w}$ were plotted against the average concentration during the time interval used in the calculation.

Isodensity Equilibrium Centrifugation. Saturated CsCl or Cs_2SO_4 was added to give the desired density. The samples were centrifuged to equilibrium (about 20 hr) at 44,000 or 44,770 rpm at 25° in analytical ultracentrifuges. The densities of the recovered solutions were measured in a calibrated 0.5-ml weighing pipet. To obtain the density of the protein in CsCl and Cs_2SO_4, unfixed ribosomes were centrifuged in solutions of densities appropriate for protein, because the isolated proteins were insoluble after fixation. Values for RNA in Cs_2SO_4 were obtained on samples of rRNA from rat liver and the rat tumor Jensen sarcoma (Petermann and Pavlovec, 1963; Hamilton, 1967) which had been fixed with formaldehyde (Lozeron and Szybalski, 1966).

Molecular Weight Measurements. Equilibrium centrifugation was carried out in short columns (2–3 mm) in 12-mm double-sector cells or in a multicompartment cell (Yphantis, 1964) in the An-J rotor. The absorption optical system was equipped with a monochromator and photoelectric scanner. The meniscus-depletion technique of Yphantis (1964) was used. After a 30-min overspeed period at 6000 rpm, the centrifuge was decelerated to 3000 or 4000 rpm and run until the patterns no longer changed with time, about 72 hr. The centrifuge was then accelerated to 5600 or 6000 rpm for an additional 24 hr. Postequilibrium velocity runs were made to check the stability of the samples. The fixed samples were stable for weeks in the cold, but were damaged by freezing.

Concentrations were measured on expanded traces. Base lines for the lower speed patterns were taken from those obtained at the higher speed. Molecular weights were calculated from the slopes of plots of log absorbance against the square of the radius (Schachman and Edelstein, 1966). The partial specific volume, $\bar{v}$, was calculated from the RNA:protein ratio, a $\bar{v}$ for protein of 0.739 computed from the amino acid composition of the whole ribosome (Crampton and Petermann, 1959), and a $\bar{v}$ of 0.53 for the RNA (Petermann and Pavlovec, 1966). It was assumed that formaldehyde fixation would not affect $\bar{v}$; that it would not contribute significantly to the molecular weight was verified experimentally. To test the technique, the molecular weights of the subunits of *Escherichia coli* ribosomes were measured. Single analyses gave values of 1.84 million daltons for the large and 0.84 million daltons for the small subunit, in very good agreement with published values (Tissières *et al.*, 1959).

Calculations. The programs of Trautman (1968) for the Olivetti-Underwood Programma 101 computer were used. These utilize the least-squares method to give unbiased values and to calculate the standard errors; quadratic least squares are used to test whether the data are linear, and linear least squares are used for processing the data. Where the mean values of replicate measurements are given, the mean and the standard error of the mean (*i.e.*, the standard deviation) are expressed as $a \pm b$.

Results

The Pattern of Dissociation of Rat Liver Ribosomes. Figure 1 illustrates the pattern obtained by sucrose gradient centrifugation of EDTA-dissociated ribosomes in the zonal rotor and shows how the material was sampled for the various measurements. It also shows that in addition to the expected two subunit peaks, a slowly sedimenting peak was present. Planimetry of three such patterns gave an average percentage distribution of absorbance at 260 mμ of 6, 27, and 67 for the three peaks. When the percentages are corrected[2] for the protein content of each peak (*i.e.*, 36, 50, and 45%), the distribution is 5, 29, and 66%. Since the ratio of large subunit to small subunit expected from their

[2] Extinction coefficients, $E^{0.1\%}_{1cm,260m\mu}$, were estimated for substances of various RNA: protein ratios by assuming values for RNA and protein of 27 and 1.

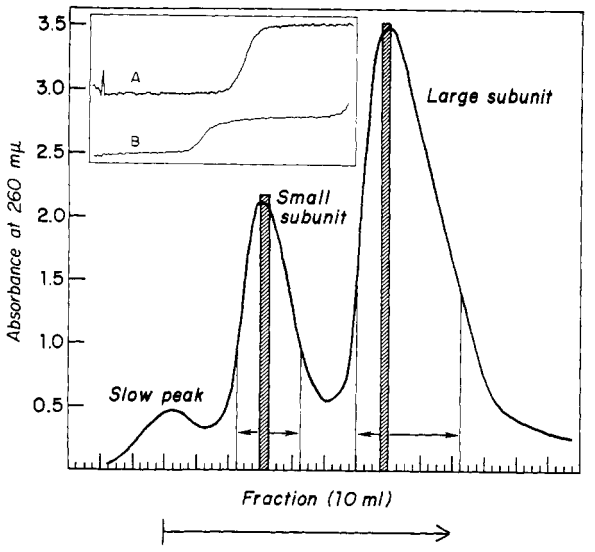

FIGURE 1: Separation of rat liver ribosomal subunits in the B XIV zonal rotor. Gradient: 5–20% sucrose in 0.03 M KCl–0.001 M potassium phosphate (pH 7.0). Centrifugation conditions: 23 hr at 20,000 rpm at 8°. The hatched areas indicate the fractions used in the molecular weight and density measurements and for chemical analysis, while the arrows indicate the pooled fractions used for the sedimentation studies, some of which are illustrated in Figure 2. The inset figure shows velocity sedimentation analyses of material from the hatched areas after dialysis against 0.03 M KCl–0.001 M potassium phosphate (pH 7.0)–1% HCHO. The absorption patterns were recorded by the photoelectric scanner. (A) The large subunit after 104 min at 20,000 rpm at 7°, scanned at 265 mμ. (B) The small subunit after 40 min at 40,000 rpm at 8°, scanned at 280 mμ.

molecular sizes (Table I) is 2.6, there seems to be an excess of small subunits.

Identification of the Slowly Sedimenting Peak. The sedimentation coefficient of material from the slow peak was about 6 S. When examined in 1% sodium dodecyl sulfate, to dissociate any protein that was not covalently bound to the RNA, the RNA had a sedimentation coefficient of 5 S. Both samples, however, were polydisperse. Chemical analysis showed that the material consisted of 64% RNA and 36% protein. The "protein" aggregated when attempts were made to concentrate it for disc electrophoresis, and it was insoluble in 6 M urea–0.03 M sodium acetate (pH 5.6), a good solvent for ribosomal proteins; it may represent incomplete peptide chains released when the ribosomes were dissociated to subunits. In gel filtration on G-100 Sephadex, under the conditions of Galibert *et al.* (1965), the phenol-extracted RNA consisted of tRNA, 5S RNA, and an RNA fraction which was excluded from the Sephadex and had an average sedimentation coefficient of 7 S. Electrophoretic analysis in a polyacrylamide gel (Peacock and Dingman, 1967) showed that the "7S" RNA contained bands at positions corresponding to 7S and 8S RNAs as well as polydisperse material of intermediate sizes (McPhie *et al.*, 1966). The distribution of components, calculated from densitometry of the methylene blue stained patterns, was 30% tRNA, 28% 5S RNA, and 42% of the larger species. Thus the slow peak contained 1.7 molecules of tRNA, 1.1 molecules of 5S RNA, and 0.7 molecule of the larger RNAs per ribosome. The values for tRNA and 5S RNA are close to the expected amounts and suggest that both RNAs were completely removed by EDTA treatment. In addition, no small RNA molecules were detected in gel electrophoresis of RNA obtained by phenol extraction of the large subunit.

Velocity Sedimentation of the Ribosomal Subunits. The sedimentation properties of the subunits in buffer A containing 0.2 mM $MgCl_2$ are given in Table I. For these experiments the factions were pooled (see Figure 1) and the subunits were not fixed.

The effects of $MgCl_2$ and formaldehyde on the sedimentation of the subunits can be illustrated by results obtained at very low concentrations (50 μg/ml) with absorption optics. In solvents containing 0.03 M KCl–0.001 M potassium phosphate (pH 7.4) but with either no $MgCl_2$ (*i.e.*, as isolated), 1 mM $MgCl_2$, or 1% formaldehyde, the sedimentation coefficients, $s_{20,w}$, for the large subunit were 44, 52, and 50 S and for the small subunit, 23, 29, and 25 S. Magnesium ions make the subunits more compact (Petermann and Pavlovec, 1969); fixation seems to have had a similar effect. Under various other ionic conditions the subunits tend to associate into dimers, etc. For example, the small subunit in buffer A with 5 mM $MgCl_2$ showed boundaries with sedimentation coefficients of 31, 48, and 63 S, whereas in 1 mM $MgCl_2$ it sedimented as a single boundary of 29 S. A schlieren run on the large subunit at a higher pH and $MgCl_2$ concentration showed 56S, 83S, 105S, and 123S components (Figure 2A).

TABLE I: Properties of the Isolated Subunits of Rat Liver Ribosomes.

Subunit	$s^0_{20,w}$ (S)	$(k)^a$	Protein Content Mean (%) Std Error [b]	$\bar{v}$ [c] (ml/g)	$1/\theta$ [d] (ml/g)	Mol Wt × 10^{-6} Mean Std Error [b]
Large	49.9	(0.066)	44.69 ± 0.97	0.623	0.624	3.12 ± 0.024
Small	28.6	(0.080)	49.50 ± 2.06	0.633	0.628	1.18 ± 0.043

[a] k is the constant in the expression, $1/s = 1/s^0(1 + kc)$, when c is given in milligrams per milliliter. These measurements were made in 0.03 M KCl–0.001 M potassium phosphate (pH 7.0)–0.2 mM $MgCl_2$. [b] Standard error of the mean. [c] Calculated from the protein and RNA content. [d] Reciprocal of θ, the buoyant density in CsCl.

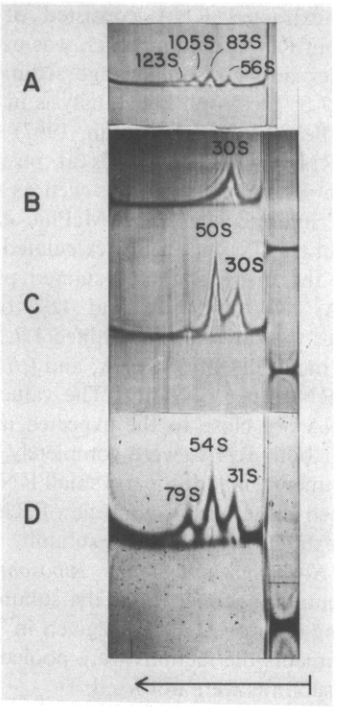

FIGURE 2: Velocity sedimentation analyses of the ribosomal subunits with schlieren optics. (A) The isolated large subunit in 0.025 M KCl–0.05 M Tris (pH 8.0 at 5°)–2.5 mM $MgCl_2$. (B) The isolated small subunit in 0.03 M KCl–0.001 M potassium phosphate (pH 7.0)–0.2 mM $MgCl_2$. (C) Mixture of the isolated large and small subunits, same solvent as B. (D) Mixture in the same solvent, but with 1.5 mM $MgCl_2$. For B and C the peaks are identified by their sedimentation coefficients at infinite dilution. For A and D the values given are those measured at initial concentrations of 1.7 mg/ml.

Reassembly of the Isolated Subunits. Figure 2 illustrates a test of the ability of the isolated subunits to re-form 80S particles. Figure 2B shows the ultracentrifugal pattern of the pooled small subunit after pelleting and resuspension in buffer A containing a low concentration of $MgCl_2$ (0.2 mM); and C shows a mixture of B with pooled large subunit. Little reassociation into 80S particles occurred. When the $MgCl_2$ concentration was increased to 1.5 mM, however, as in D, about one-third of the mixture sedimented as 80S particles. As described above, however, the subunits can themselves associate into faster sedimenting particles at high concentrations of $MgCl_2$.

Chemical Analysis of the Ribosomal Subunits. The results of analyses of peak fractions from three zonal runs, with the standard errors of the means, are given in Table I. The large subunit contains less protein than the small one; their respective RNA:protein ratios are 1.2 and 1.0.

Isodensity Equilibrium Centrifugation of Ribosomal Subunits. Isodensity centrifugation, a sensitive way to detect density differences between macromolecules (Vinograd and Hearst, 1962), was used to compare the subunits. To prevent the dissociation of protein that occurs on exposure to the high salt concentration required for the experiment, the subunits were fixed with formaldehyde (Perry and Kelley, 1966). For eight samples of peak fractions of the large subunit the mean buoyant density was 1.602 ± 0.002 g per ml; for nine samples of the small subunit the buoyant density was 1.593 ± 0.002 g per ml.

The bands formed by the subunits were broad. For a particle of 3 million daltons, the expected band width is 0.007 g/ml, but in a typical experiment on the large subunit twice the standard deviation of the curve was 0.021 g/ml. The breadth of the band arises from density heterogeneity which may be the result of incomplete fixation, although the presence of additional formaldehyde, up to 6%, had no effect on the band width. When samples from the two sides of the band formed in a preparative experiment were recentrifuged in the analytical ultracentrifuge, they formed sharper bands but at the same densities at which they had banded in the preparative experiment. The small subunit gave even broader bands (see Figure 3). Despite these complications the reciprocals of the measured values fall

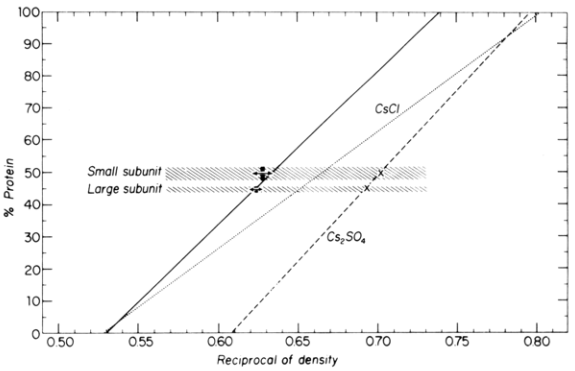

FIGURE 3: Comparison of the buoyant densities of the subunits with the densities calculated from their protein contents. The solid line is drawn from the partial specific volume of NaRNA to that of the anhydrous protein. The dotted line is drawn between the reciprocal of the buoyant density in CsCl of RNA (Bruner and Vinograd, 1965) and that of the unfixed protein, 1.245 ± 0.004 g per ml (seven measurements). The height of the shaded areas shows the range of protein contents from chemical analysis (see Table I). The small black rectangles represent the data for the two subunits in CsCl with the horizontal dimension calculated from the standard error of the mean of the density measurements. The arrows show the width of the bands, *i.e.*, twice the standard deviation in density units. The dashed line refers to measurements made in Cs_2SO_4. The buoyant density of formaldehyde-fixed CsRNA was 1.644 ± 0.001 g per ml (four measurements on various RNAs). No difference could be detected between 30S and 18S rRNAs of the rat tumor, Jensen sarcoma. Neither precipitated and both gave band widths commensurate with their molecular sizes. The buoyant density of the unfixed protein was 1.256 ± 0.017 g per ml (three measurements). Single measurements on fixed subunits are denoted by X.

close to a line drawn between the values for anhydrous protein and RNA. Figure 3 also shows that the points do not fall on the line drawn between the values for CsRNA and the value for the protein in CsCl. In Cs_2SO_4 the buoyant densities of the subunits are lower than in CsCl, and their reciprocals do fall on the line drawn between the values of CsRNA and protein. It appears that the reciprocal of the buoyant density may be used empirically as a measure of the protein content, provided that the appropriate values of RNA and protein are used. It also seems that the reciprocal of the buoyant density in CsCl may be used directly as a measure of $\bar{v}$, since it is numerically the same as the value calculated from the chemical composition.

The Molecular Weights of the Ribosomal Subunits. Peak fractions from the zonal separation (see Figure 1) were used for the molecular weight measurements. Figure 1 (inset) illustrates the monodispersity of the subunits in sedimentation velocity experiments. When the patterns of the large subunit were subjected to boundary-spreading analysis (Trautman, 1968), no significant heterogeneity was detected. For the equilibrium runs two samples of the large subunit were analyzed: one in two separate runs and the other in one triplicate run; five values were averaged. For the small subunit, two samples were analyzed, one in duplicate in two runs, the other in two separate runs; data from both speeds were used, and nine values were averaged. For all of the samples, the plots of log absorbance against square of the radius were linear. The results of the measurements are given in Table I. The large subunit, 3.1 million daltons, is about 2.6 times the size of the small subunit, which has a molecular weight of 1.2 million daltons.

Discussion

Although ribosomes are made up of only two subunits, we found three peaks in the zonal centrifugation of EDTA-treated ribosomes. The slowest peak contained a mixture of tRNA, 5S RNA, and larger RNAs, 6–9 S, which may represent remnants of mRNA, as well as some "protein" material, probably nascent peptides; it did not have the solubility properties of ribosomal protein. As expected, the sedimentation coefficients of the subunits were highly dependent upon the ionic conditions (Petermann and Pavlovec, 1969). In the absence of $MgCl_2$ the sedimentation coefficient of the large subunit was 44 S. In the presence of $MgCl_2$ it increased to 50 S. Only at high concentrations of $MgCl_2$ did it have a value of 56 S, close to the value of 58 S observed by Petermann and Pavlovec (1969) for the compact form of a large subunit lacking 5S RNA; then, however, it tended to self-associate into dimers, trimers, etc. For the small subunit the sedimentation coefficient was about 23 S in the absence of $MgCl_2$ and 29 S in its presence. With a higher concentration of $MgCl_2$, it associated into 48S and 63S particles, but conversion into a 40S form did not occur. Thus the subunits, obtained by EDTA dissociation and studied with sufficient $MgCl_2$ to exhibit their maximum sedimentation rates without aggregation, were not 60S and 40S particles but 50S and 30S particles. They should not, of course, be confused with the 50S and 30S subunits of bacterial ribosomes (Tissières *et al.*, 1959; Hess *et al.*, 1967).

The molecular weights of the liver subunits were 3.1 and 1.2 million daltons. The first value agrees with the value of 3.05 to 3.2 million daltons which Tashiro and Yphantis (1965) obtained by equilibrium centrifugation for the large subunit of guinea pig liver ribosomes; their small subunit was unstable.

Calculation of the sizes of the rRNAs from the molecular weights and RNA contents of the subunits results in values of 1.7 and 0.6 million daltons, in agreement with accepted values for mammalian rRNAs (Petermann, 1964), and with the value obtained by the equilibrium method for the isolated 30S RNA of rat tumor ribosomes (Hamilton, 1967); a single measurement by that technique on the isolated 18S RNA of rat liver gave a value of 0.6 million daltons (M. G. Hamilton, 1967, unpublished data). The molecular weight of a ribosome formed by the two subunits would be 4.3 million daltons. It would contain 53% RNA, much more than the 45% we reported some years ago (Hamilton *et al.*, 1962). An improved method of isolation, chiefly the use of a pH 8 wash, gives ribosomes that contain less extraneous protein than those obtained by the older procedure.

A molecular weight of 4.3 million daltons for the rat liver 80S ribosomes agrees with the value of 4.3 million daltons obtained by sedimentation velocity and diffusion by Petermann and Pavlovec (1966) for the 80S ribosomes of a rat tumor, the Jensen sarcoma. If the value of 5 million daltons obtained by Tashiro and Yphantis (1965) by the sedimentation equilibrium method for the ribosomes of guinea pig liver is recalculated with the new $\bar{v}$, 4.6 million daltons is obtained. These molecular weights are higher than the value which we had estimated by extrapolation of light-scattering measurements on mixture of monomers with dimers, etc. (Hamilton *et al.*, 1962). The present measurements are undoubtedly more accurate, since they were made on monodisperse solutions and at very low concentrations.

While the physical and chemical data for the 50S and 30S subunits are consistent, we are uncertain as to the relation of these subunits to the active ribosome since they did not reassociate completely to 80S particles. We are sure that they would have no biological activity since similar preparations were inactive in *in vitro* amino acid incorporation even in the presence of polyuridylic acid (M. L. Petermann, A. Pavlovec, and I. B. Weinstein, 1968, unpublished data).

Recently Petermann and Pavlovec (1969) have found that, when the liver ribosomal subunits are dissociated under conditions where magnesium is not completely removed and the 5S RNA remains attached, the subunits have sedimentation coefficients of 58 and 38 S, and more importantly, can be reassembled into active ribosomes. In preliminary experiments with Petermann, Pavlovec, and Weinstein, active subunits were characterized. In two measurements one sample of the 58S particles had a molecular weight of 3.1 million daltons.

As expected, detection of the 1% difference (*i.e.*, the weight of one 5S RNA) between the weights of the inactive 50S and the active 58S forms was beyond the precision of the equilibrium method. The active small subunit, however, seemed to have a higher molecular weight than the inactive one. Whereas the inactive subunits gave single bands in isodensity experiments, the active small subunit showed a second band that suggested the presence of cosedimenting particles of a different composition. Whether the latter are similar to the mRNA-containing particles observed by Infante and Nemer (1968), Perry and Kelley (1968), and Henshaw (1968) remains to be established.

We conclude that the ribosomal subunits obtained by removal of Mg ions are "bare" subunits. The large one has lost 5S RNA; the exact nature of the differences between the active and inactive small subunits is under investigation.

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References

Anderson, N. G. (1966), *Science 154*, 103.

Bruner, R., and Vinograd, J. (1965), *Biochim. Biophys. Acta 108*, 18.

Crampton, C. F., and Petermann, M. L. (1959), *J. Biol. Chem. 234*, 2642.

Galibert, F., Larsen, C. J., Lelong, J. C., and Boiron, M. (1965), *Nature 207*, 1039.

Hamilton, M. G. (1967), *Biochim. Biophys. Acta 134*, 473.

Hamilton, M. G., Cavalieri, L. F., and Petermann, M. L. (1962), *J. Biol. Chem. 237*, 1155.

Hamilton, M. G., and Ruth, M. E. (1967), *Biochemistry 6*, 2585.

Henshaw, E. C. (1968), *J. Mol. Biol. 36*, 401.

Hess, E. L., Chun, P. W. L., Utsunomiya, T., and Horn, R. (1967), *Biochemistry 6*, 861.

Hirsch, C. A. (1966), *Biochim. Biophys. Acta 123*, 246.

Infante, A. A., and Nemer, M. (1968), *J. Mol. Biol. 32*, 543.

Lozeron, H. A., and Szybalski, W. (1966), *Biochem. Biophys. Res. Commun. 23*, 612.

McPhie, P., Hounsell, J., and Gratzer, W. B. (1966), *Biochemistry 5*, 988.

Peacock, A. C., and Dingman, I. W. (1967), *Biochemistry 6*, 1818.

Perry, R. P., and Kelley, D. E. (1966), *J. Mol. Biol. 16*, 255.

Perry, R. P., and Kelley, D. E. (1968), *J. Mol. Biol. 35*, 37.

Petermann, M. L. (1964), The Physical and Chemical Properties of Ribosomes, Amsterdam, Elsevier.

Petermann, M. L., and Pavlovec, A. (1963), *J. Biol. Chem. 238*, 3717.

Petermann, M. L., and Pavlovec, A. (1966), *Biochim. Biophys. Acta 114*, 264.

Petermann, M. L., and Pavlovec, A. (1967), *Biochemistry 6*, 2950.

Petermann, M. L., and Pavlovec, A. (1969), *Biopolymers* (in press).

Schachman, H. K., and Edelstein, S. J. (1966), *Biochemistry 5*, 2681.

Spirin, A. S., Belitsina, N. V., and Lerman, M. I. (1965), *J. Mol. Biol. 14*, 611.

Tashiro, Y., and Yphantis, D. A. (1965), *J. Mol. Biol. 11*, 174.

Tissières, A., Watson, J. D., Schlessinger, D., and Hollingworth, B. R. (1959), *J. Mol. Biol. 1*, 221.

Trautman, R. (1968), *Ann. N. Y. Acad. Sci.* (in press).

Van Holde, K. (1967), Fractions No. 1. Spinco Division of Beckman Instruments, Inc., Palo Alto, Calif.

Vinograd, J., and Hearst, J. E. (1962), *Progr. Chem. Org. Natural Products 20*, 372.

Yphantis, D. A. (1964), *Biochemistry 3*, 297.