

A STRUCTURAL STUDY OF RAT LIVER RIBOSOMES

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ABSTRACT

Polyribosomes, ribosomes, and ribosomal subunits were prepared from rat liver using sodium deoxycholate and a variety of ionic media. They were examined in the electron microscope, mainly as negatively or positively stained preparations, and in the analytical ultracentrifuge. The polyribosomes consist of up to twelve or more ribosomes linked by a fine strand, 10 to 15 Å in diameter, probably of RNA. The ribosomes are approximately spherical with diameters of 250 to 300 Å, and are estimated to be about 50 per cent porous. Possibly because of their high protein content, whole ribosomes show no cleavage furrows. Ribosomes were dissociated in phosphate buffer and the subunits separated on sucrose density gradients containing 10 per cent formalin. Three classes of subunit were obtained with sedimentation coefficients of 71S, 50S, and 31S respectively. The smallest, 31S subunit is about 250 Å long by 100 Å wide. The largest subunits appear to be clusters of smaller particles. It is estimated from their linear dimensions in electron micrographs that the whole 83S ribosome could contain up to six 31S subunits, or their equivalent.

Sedimentation studies of rat liver ribosomes by Hamilton and Petermann (1, 2) have shown the existence of a stable 83S particle. The stability of this particle is dependent on the ionic environment and particularly on Mg^{++} concentration in the medium. A decrease in pH or an increase in Mg^{++} concentration causes non-specific aggregation of 83S particles, while an increase in pH above 8.0 and removal of Mg^{++} causes the 83S particle to dissociate into several subunits but principally into 46S and 30S components (1). The stability of the 83S particle suggested that it was the form of ribosome involved in the synthesis of proteins. Recent evidence (3; see also references 4 to 10) has indicated that the active unit in protein synthesis is an aggregate of 83S ribosomes. Such ribosomal aggregates or polyribosomes have also been termed polysomes (4) or ergosomes (3).

The present report describes observations in the electron microscope and ultracentrifuge on the

structure of rat liver polysomes, ribosomes, and ribosomal subunits.

MATERIALS AND METHODS

Sprague-Dawley rats, 3 to 5 months old, were starved for 24 hours and decapitated. After the blood had been drained, the livers were removed and chilled immediately in crushed ice. All subsequent steps were carried out in the cold. In most experiments, two rats were used giving about 22 gm fresh liver tissue.

Preparation of Ribosomes in Water

In general the procedure was based on the method of Palade and Siekevitz (11). The livers were minced and then homogenized in 0.88 M sucrose in water using a Potter-Elvehjem homogenizer. The homogenate was diluted with sucrose solution until the final volume in milliliters was 5 to 6 times the weight of liver in grams, and spun at 20,000 RPM for 20 minutes in a No. 40 rotor of a Spinco Model L Preparative Ultracentrifuge. The floating layer of lipid

was carefully removed from the tubes, the supernatants were pooled, and sufficient 2 per cent sodium deoxycholate (DOC), pH 7.4, was added to give a final concentration of detergent of 0.2 to 0.5 per cent. (Normally, the weight of DOC added was 8 to 10 times the weight of ribosomes in the final suspension. The latter was estimated from the optical density at 258 $m\mu$, assuming an extinction coefficient $E_{1\text{ cm}}^{1\text{ per cent}} = 120$, see reference 1). After standing for about 10 minutes, the preparation was spun at 40,000 RPM in a No. 40 rotor for 90 minutes. The resulting pellets were rinsed and resuspended in water with gentle stirring. The particle suspension was clarified at 25,000 RPM for 10 minutes to yield a supernatant representing a DC-1 preparation. This was spun at 40,000 RPM for 60 minutes, the resulting pellet was washed and resuspended in water, and the suspension clarified as before to give a final DC-2 preparation of ribosomes. Sections of a ribosomal pellet obtained from the final suspension and embedded in methacrylate showed the ribosomal preparation to be free of membranes.

Preparation of Polyribosomes

The procedure was that of Wettstein *et al.*, (3) except that the homogenization medium was made 0.44 M with respect to sucrose to prevent breakdown of mitochondria. All solutions contained 0.05 M Tris-HCl buffer, pH 7.8, with 0.025 M KCl, and 0.005 M Mg^{++} . The tissue was minced and homogenized briefly to minimize damage to polysomes. From this homogenate, 3 ml of a DC-1 suspension were prepared in Tris buffer with KCl and Mg^{++} by the procedure described above. Ribosomal aggregates were separated from individual ribosomes by centrifuging 1 ml of the sample through a layer of 2 ml 0.5 M sucrose in buffer and one of 1 ml 2 M sucrose in buffer in a tube of the SW 39 swinging bucket rotor for 3 hours at 39,000 RPM. The small transparent pellet was gently washed, resuspended with gentle shaking in glass-distilled water and then fixed in formalin for electron microscopy as described below.

Preparation of Ribosomes in Bicarbonate Buffer, 0.1 Ionic Strength, pH 8.0

Ribosomes were washed with a buffer of ionic strength 0.1 to remove extraneous protein after the method of Petermann and Pavlovec (12). A DC-1 suspension of ribosomes was prepared as before except that the resuspension was in 3 ml 0.02 M $KHCO_3$, 0.0015 M $MgCl_2$, and 0.0755 M KCl, pH 8. 1 ml of the suspension was then centrifuged through a layer of 2 ml 1.76 M sucrose in buffer and one of 1 ml 2.05 M sucrose in buffer in a tube of the SW 39 rotor for 3 hours at 39,000 RPM. The resulting transparent pellet was gently washed and resuspended in 1 ml glass-

distilled water and immediately fixed for electron microscopy.

Preparation of Ribosomes Free of Ferritin and Other Metallo-Proteins

The method employed was that of Hamilton and Petermann (1). A DC-1 suspension was prepared as before with the resuspension in 10 ml $5 \cdot 10^{-4}$ M phosphate buffer and $5 \cdot 10^{-4}$ M $MgCl_2$, pH 6.8. To this, 0.02 M barium acetate was added to give a final concentration of 0.005 M barium acetate to precipitate the ribosomes. After standing for 10 minutes, the mixture was centrifuged for 10 minutes at 11,000 RPM. The clear white pellet of ribosomes resuspended readily in phosphate buffer with Mg^{++} and the suspension was dialysed against excess phosphate buffer with Mg^{++} for 48 hours to remove traces of barium acetate. After a clarification spin at 20,000 RPM for 10 minutes, the ribosomes were spun down at 40,000 RPM for 60 minutes, resuspended in glass-distilled water and fixed for electron microscopy.

Gradient Separation of Ribosomal Subunits

Ribosomes were dissociated into subunits by suspending them in phosphate buffer, 0.05 or 0.1 ionic strength, pH 7.5. In some preparations, reasonably extensive dissociation was only obtained after the ribosomes had been centrifuged and resuspended a second time in phosphate buffer. The final concentration of the suspension was adjusted to about 0.5 per cent, as judged from the optical density at 258 $m\mu$ and assuming an extinction coefficient $E_{1\text{ cm}}^{1\text{ per cent}}$ of 120 (1).

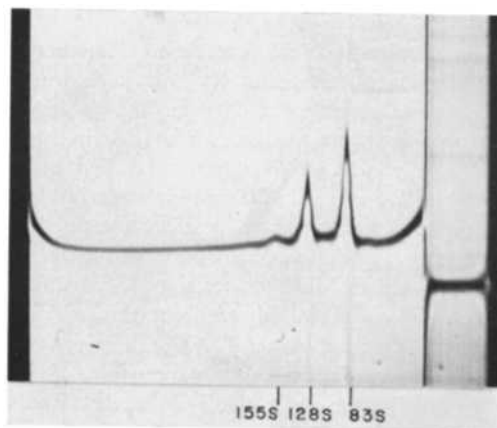
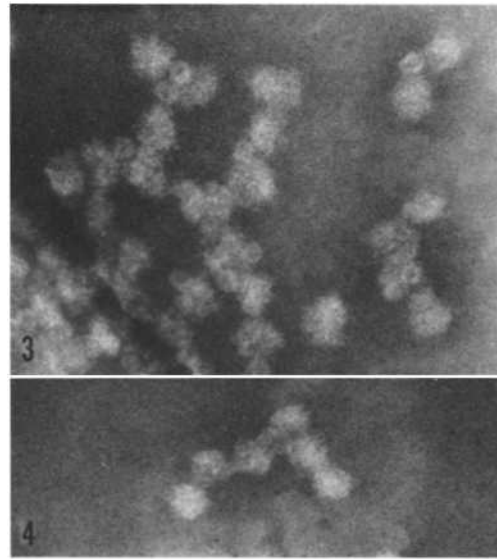
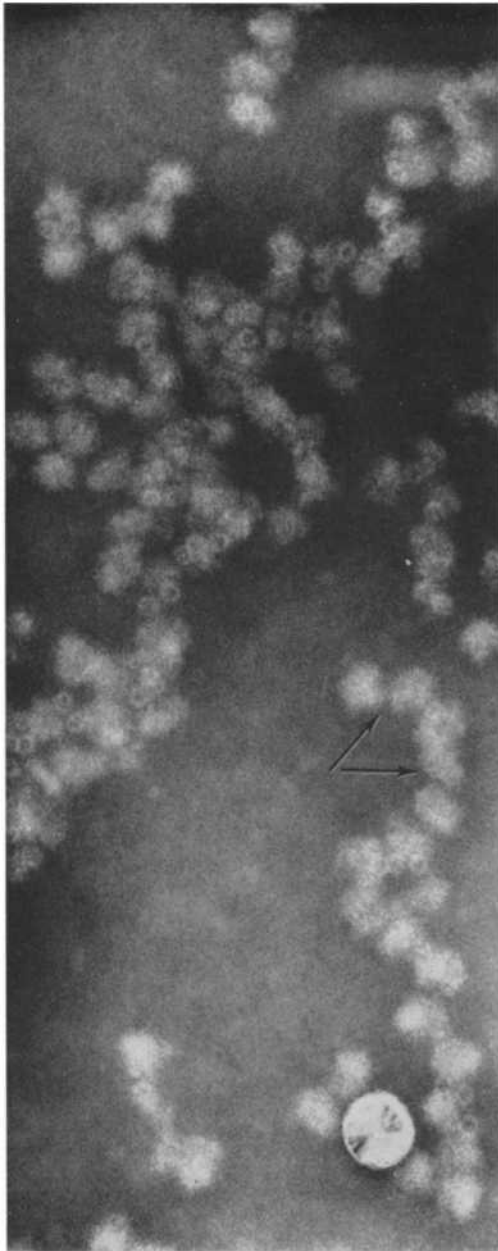


FIGURE 1 Sedimentation pattern of a DC-1 preparation of ribosomes in 0.05 M Tris-HCl buffer with $5 \cdot 10^{-3}$ M Mg^{++} , pH 7.8, after 4 minutes at 35,600 RPM. The main 83S peak represents single ribosomes while the two smaller, more rapidly sedimenting peaks represent dimers and trimers respectively (*cf.* reference 6).



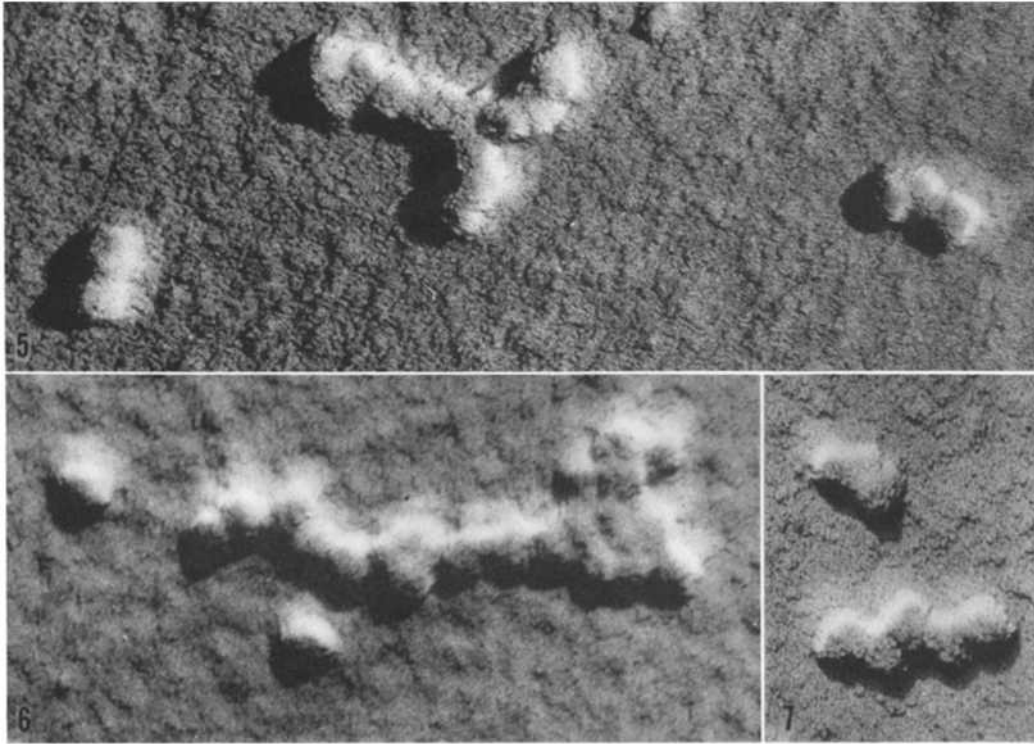
FIGURES 2, 3, and 4 Polyribosomes of different lengths negatively stained with PTA. The arrows in Fig. 2 indicate a gap of about 50 Å between adjacent ribosomes in a single polysome. Ferritin molecules can be seen as small doughnut-shaped particles. The larger, round object in Fig. 2 is an unknown contaminant. The polysome in Fig. 4 is in an area without a supporting carbon film and shows some compression. $\times 240,000$.

The subunits were separated on a linear, 5 to 20 per cent (w/v), sucrose gradient (13) containing 10 per cent formalin (4 per cent formaldehyde) to stabilize the subunits (14). The sucrose solutions contained phosphate buffer 0.05 ionic strength with 2.10^{-4} M Mg^{++} and 30 ml gradient was formed in a tube for the SW 25 head. 1 ml of the sample was added as an inverted gradient with 1 ml 4 per cent sucrose in the same buffer with Mg^{++} and formalin and spun at 16,000 or 17,000 RPM for 16 hours. The

base of the tube was then punctured, thirty or more 1-ml samples were collected and their optical densities at 258 $m\mu$ read. For electron microscopy, the pH of the selected tubes was adjusted to 6.0 and samples prepared as described below.

Chemical Analyses

Intact ribosomes were analysed for protein by the method of Lowry *et al.* (15), using bovine plasma



FIGURES 5, 6, and 7 Polysomes shadowed with Pt/C. The numbers of ribosomes in the chains can be judged from the shapes of the shadows. $\times 240,000$.

albumin as a standard, and for phosphorus by the method of Allen (16). The RNA content of ribosomes was taken as being eleven times their phosphorus content and the percentage of RNA was calculated by comparing the RNA and protein contents. These analytical procedures for protein and phosphorus did not work satisfactorily on components separated on linear sucrose gradients containing formalin.

Sedimentation Analyses

Preparations were analysed in a Spinco Model E analytical ultracentrifuge at 35,600 RPM and 20°C, using schlieren or ultraviolet optics. Traces of the ultraviolet absorption patterns were obtained on a Joyce, Loebel Double Beam Recording Microdensitometer. Relative viscosities were measured at $20 \pm 0.02^\circ\text{C}$ in an Ostwald Fenske viscometer holding 1 ml of sample.

Electron Microscopy

Samples of polysomes and whole ribosomes were prepared for electron microscopy according to the methods of Huxley and Zubay (17). Equal volumes of particle suspension and 20 per cent formalin, pH 6.0, were mixed and after 10 minutes or more, the

fixed suspension was diluted appropriately with 10 per cent formalin, pH 6.0. Particles fixed in this way appeared to be stable and to remain undamaged over long periods. For negatively stained preparations, a drop of particle suspension was placed on a carbon-coated grid with a Pasteur pipette, the excess was removed and immediately a drop of 1 per cent phosphotungstic acid (PTA) with $5.10^{-3} \text{ M Mg}^{++}$, pH 5.0 to 5.1, added. The excess PTA solution was removed and the grid air-dried. As prepared, the carbon-coated grids contained numbers of small holes. These served as focusing aids and, where they were spanned by the PTA film, enabled particles to be observed free of the supporting carbon film (17). The only significant difference in appearance of particles with and without a supporting film was that those over the carbon film were slightly larger in area than those over a hole. The former may have been flattened but the latter could have been contracted by the drying forces in the PTA film. Where the PTA film spanning a hole was torn, many of the particles were obviously distorted and these areas were avoided.

Preparations were positively stained with uranyl acetate by placing drops of the fixed suspension on

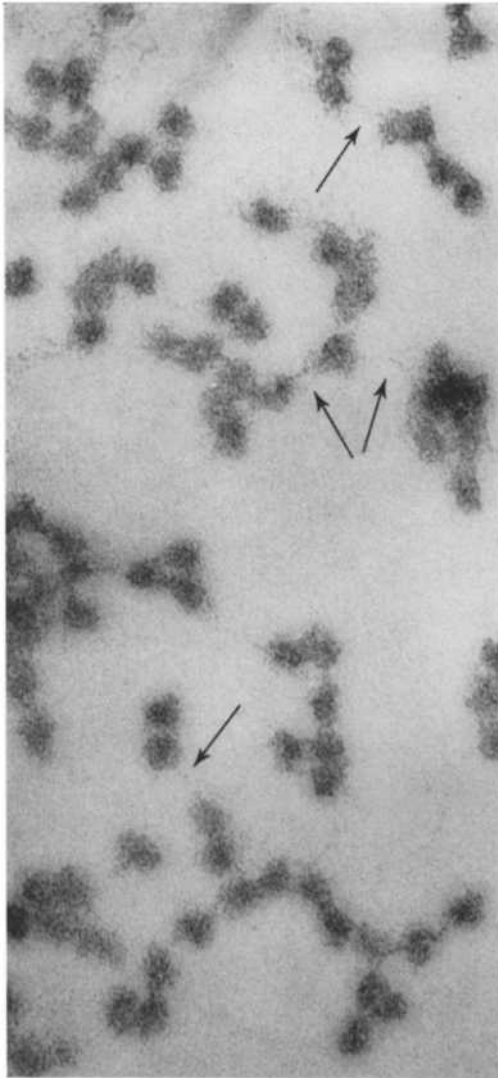


FIGURE 8 Polysomes positively stained with uranyl acetate. The interparticle spaces are clear and, where these are large, a fine connecting strand can be seen (see arrows). $\times 240,000$.

carbon-coated grids and removing the excess to leave a thin film of fluid over the grids. The grids were then immersed in 2 per cent uranyl acetate solution, pH 5.0, for 6 to 8 hours at room temperature, rinsed with distilled water and air-dried.

For shadowed preparations of polysomes, a drop of the fixed suspension was placed on a carbon-coated grid, which was air-dried and shadowed with Pt/C simultaneously in an oil-free, high vacuum evaporator (18).

With ribosomal subunits, drops of the fractions

from the gradient at pH 6.0 were placed directly on grids, the excess suspension was removed and the grids were rinsed with water to remove sucrose. The preparations were then negatively or positively stained as above.

All micrographs were taken on a Siemens Elmiskop I at 80 kv with double condenser and a 50μ objective aperture at a magnification of 80,000.

RESULTS

The sedimentation pattern of a typical DC-1 ribosomal preparation in Tris-HCl buffer, pH 7.8, showing ribosomes and ribosomal aggregates, is given in Fig. 1. The value of 83S is the estimated sedimentation coefficient at infinite dilution, $S_{20,w}^0$, for single ribosomes. This result, which agrees with that of Hamilton and Petermann (1), was obtained on a sample of ribosomes treated with 0.1 ionic strength bicarbonate buffer as described above, and resuspended in 0.05 M Tris buffer, 0.025 M KCl, and 0.005 M $MgCl_2$ at a range of concentrations down to $40 \mu\text{g/ml}$. The values of $S_{20,w}^0$ for the aggregates, 128S and 155S respectively, are less accurate extrapolations from higher concentrations.

Electron micrographs of aggregates purified by the polyribosome method are shown in Figs. 2 to 8. The numbers of ribosomes in these aggregates varies from two (Fig. 5) to more than twelve (Fig. 2 and 6). Single ribosomes can also be seen; some of these and some of the smaller aggregates may have resulted from disruption of larger aggregates during preparation. The aggregates are in the form of strings of ribosomes; adjacent ribosomes often appear in contact, but in some cases they are separated from one another by up to 50 Å (Fig. 2). Linear arrays of ribosomes, as distinct from random clumps (see *e.g.*, Fig. 13 below), were seen only in samples prepared by the polyribosomal method and are therefore unlikely to be artifacts of specimen preparation for the electron microscope. The way in which ribosomes are held together in these arrays cannot be seen in either the negatively stained or the shadowed preparations. However, positively stained preparations showed separation of particles clearly (Fig. 8), and, in instances where adjacent particles are further apart, perhaps as a result of the disruptive effects of surface tension forces on drying (see also Slayter *et al.*, reference 19), a fine connecting strand about 10 to 15 Å in diameter can be seen between the particles. Since uranyl acetate stains nucleic acids preferentially (20), these connecting strands may be RNA.

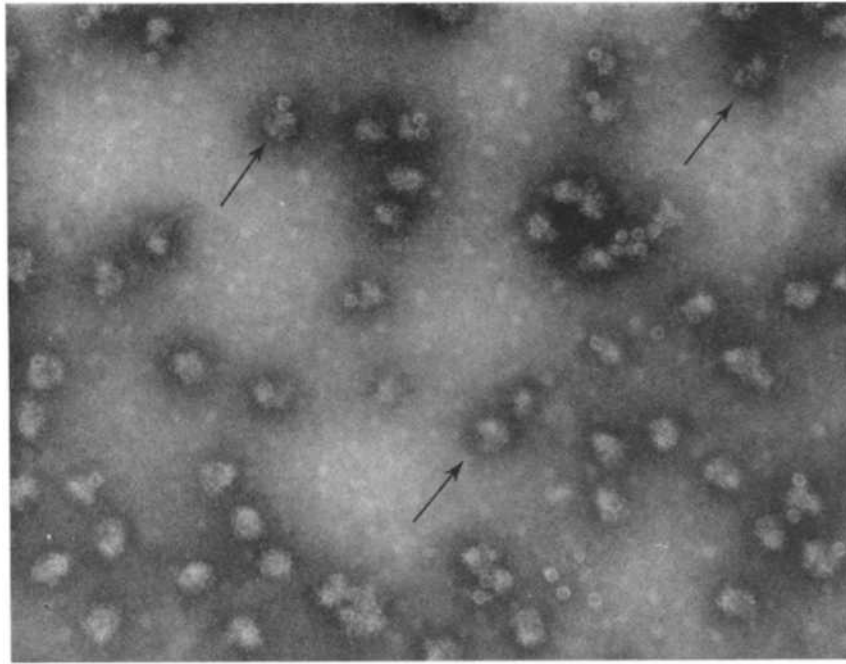


FIGURE 9 Preparation of polysomes treated with 1 $\mu\text{g}/\text{ml}$ RNase at 25°C for 1 minute, fixed and negatively stained with PTA. Only individual particles, some showing disruption (see arrows), can be seen. Contaminating ferritin molecules are also present. $\times 160,000$.

To determine whether the linkages between individual ribosomes in the aggregates were susceptible to ribonuclease, a suspension was treated with 1 $\mu\text{g}/\text{ml}$ RNase (Worthington) at 25°C for 1 minute (4, 5) and then fixed with formalin as usual. Negatively stained preparations of the sample showed no chains of ribosomes (Fig. 9), indicating the possible breakdown of aggregates to individual 83S particles by destruction of a connecting strand of RNA. Under similar conditions, in the absence of added RNase, the aggregates were stable.

83S Ribosomes

In positively stained preparations of aggregates (Fig. 8), the individual ribosomes appear as nearly spherical particles showing a finely granular internal structure.

After negative staining, the most compact ribosomes were seen in samples washed with bicarbonate buffer, 0.1 ionic strength (Fig. 10). These also appear as more or less spherical particles, 250 to 300 Å diameter. Where the contrast is particularly good, the ribosomes show a porous internal structure but no cleavage furrows.

Ribosomes prepared in water alone without

added Mg^{++} gave one predominant peak in the sedimentation pattern (Fig. 11), indicating that these particles are reasonably stable even in the absence of added Mg^{++} in the environment. In the electron microscope, these ribosomes (Fig. 12) showed a greater variety of forms and appeared more porous than those prepared in bicarbonate buffer (Fig. 10), suggesting that they are more easily disrupted during preparation for electron microscopy.

Ribosomes prepared in buffer or in water alone were contaminated with ferritin. This contamination was considerably reduced in a preparation treated with barium acetate as described above. Untreated samples contained 40 per cent RNA and had a ratio $\frac{\text{OD}_{258\text{m}\mu}}{\text{OD}_{235\text{m}\mu}}$ of 1.4; the corresponding

values for the treated sample were 48 per cent and 1.7, respectively. In negatively stained preparations, the treated ribosomes were irregular in shape (Fig. 13) and the presence of particles smaller than 200 Å in diameter showed that some disruption had occurred. Despite the evident disruption in Fig. 12 and 13, however, no cleavage furrows between subunits in the whole ribosomes, as reported in

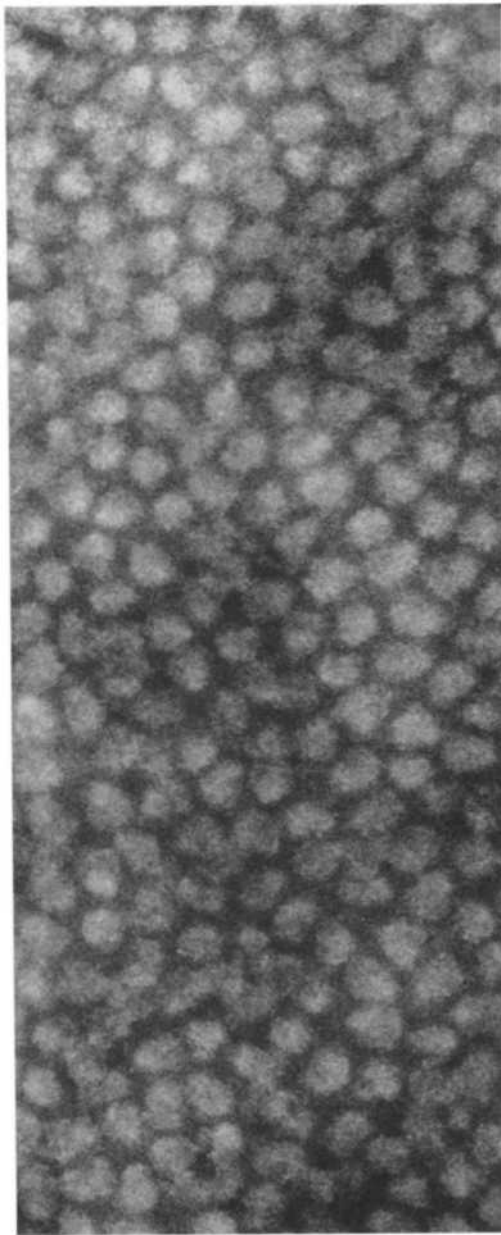


FIGURE 10 Ribosomes washed with bicarbonate buffer, 0.1 ionic strength and negatively stained with PTA. In some of the particles, internal structure can be faintly seen. $\times 240,000$.

those of *E. coli* (17) and rabbit reticulocytes (21), can be seen.

Ribosomal Subunits

To study the structure of 83S ribosomes further, ribosomal subunits were fractionated and exam-

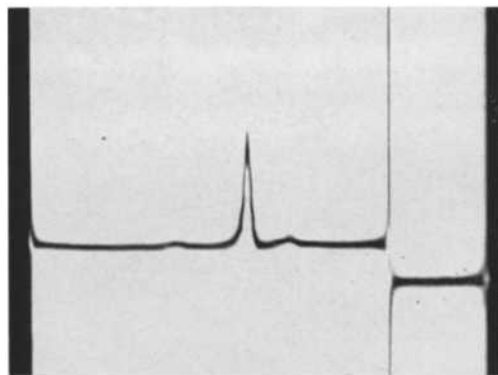


FIGURE 11 Sedimentation pattern of a DC-2 preparation of ribosomes in water, 11 minutes at 35,600 RPM, with a predominant peak due to 83S particles.

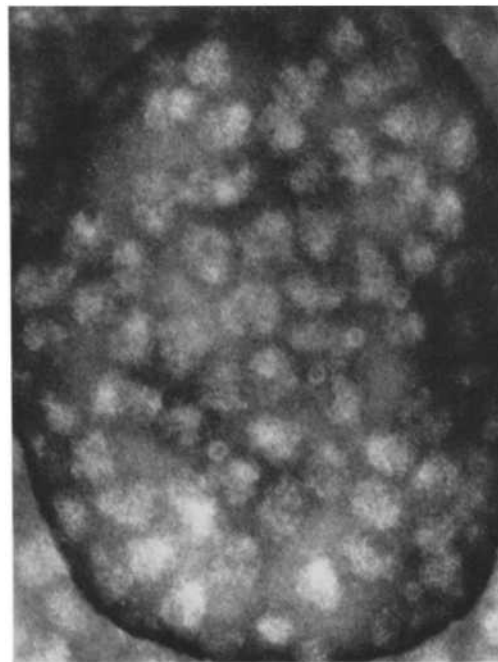


FIGURE 12 Ribosomes prepared in water and negatively stained with PTA; most of the field of view is across a hole in the supporting carbon film. Some ribosomes are compact but others have a spongy appearance or are partially disrupted. $\times 240,000$.

ined. The dissociating effect of phosphate buffer on 83S ribosomes is illustrated by the sedimentation pattern in Fig. 14. The fractionation of ribosomal subunits on a sucrose gradient containing phosphate buffer and 10 per cent formalin is shown in

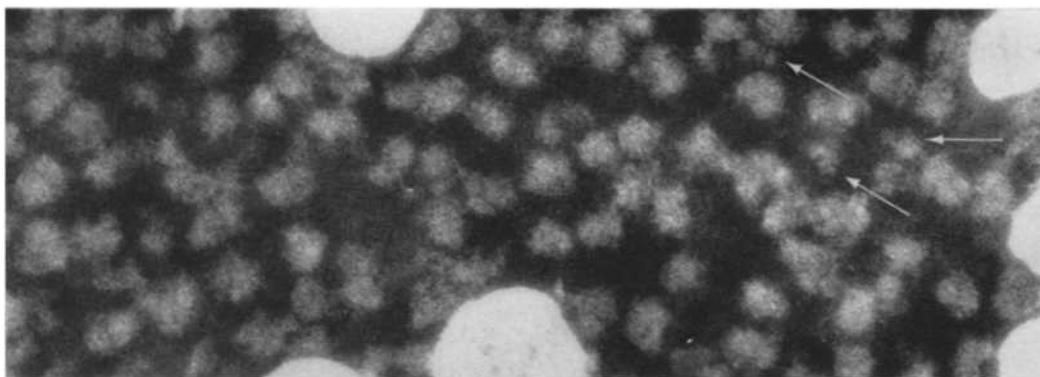


FIGURE 13 Negatively stained ribosomes which have been freed of ferritin. Particles smaller than whole ribosomes can be seen (see arrows). $\times 240,000$.

Fig. 15. From gradients similar to this, the fractions corresponding to each of the main components were pooled and centrifuged at 50,000 RPM in a No. 50 rotor for up to 2 hours. The pellets resulting from 2 hours centrifugation were rinsed and resuspended in phosphate buffer, 0.05 ionic strength, pH 7.5, plus $2 \cdot 10^{-4}$ M Mg^{++} and either with or without 10 per cent formalin. In some cases, shorter periods of high speed centrifugation were used to avoid packing the pellets too densely; after these, the upper portions of the supernatants were discarded and the remainders stirred and dialysed for about 20 hours against several changes of a small volume (50 ml) of the same buffer, again with or without formalin. The final suspensions, freed of sucrose, were analysed in the ultracentrifuge over a range of concentrations down to about $80 \mu\text{g/ml}$ (Figs. 16 to 18). The values of $S_{20,w}^0$ for the three separate components shown in Fig. 15 were $71 \pm 1S$, $50 \pm 1S$, and $31 \pm 1S$, respectively, and these values were consistent, regardless of the treatment the samples received after fractionation on the gradient. In many cases, there was some contamination of a component by its neighbours, and this contamination was somewhat greater when the final suspension contained formalin. Fractions at the base of the gradient contained heavier particles, whose sedimentation coefficients varied between experiments; these probably represented aggregates of the 71S, 50S, and 31S subunits.

Unfortunately, all the preparations of subunits examined were contaminated with ferritin, which had spread throughout the sucrose gradient. Similar spreading of ferritin was noticed when whole ribosomes were washed in bicarbonate

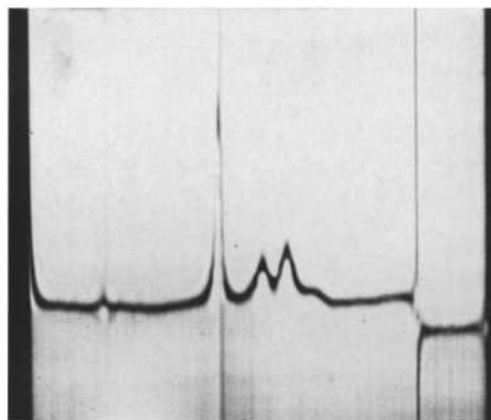
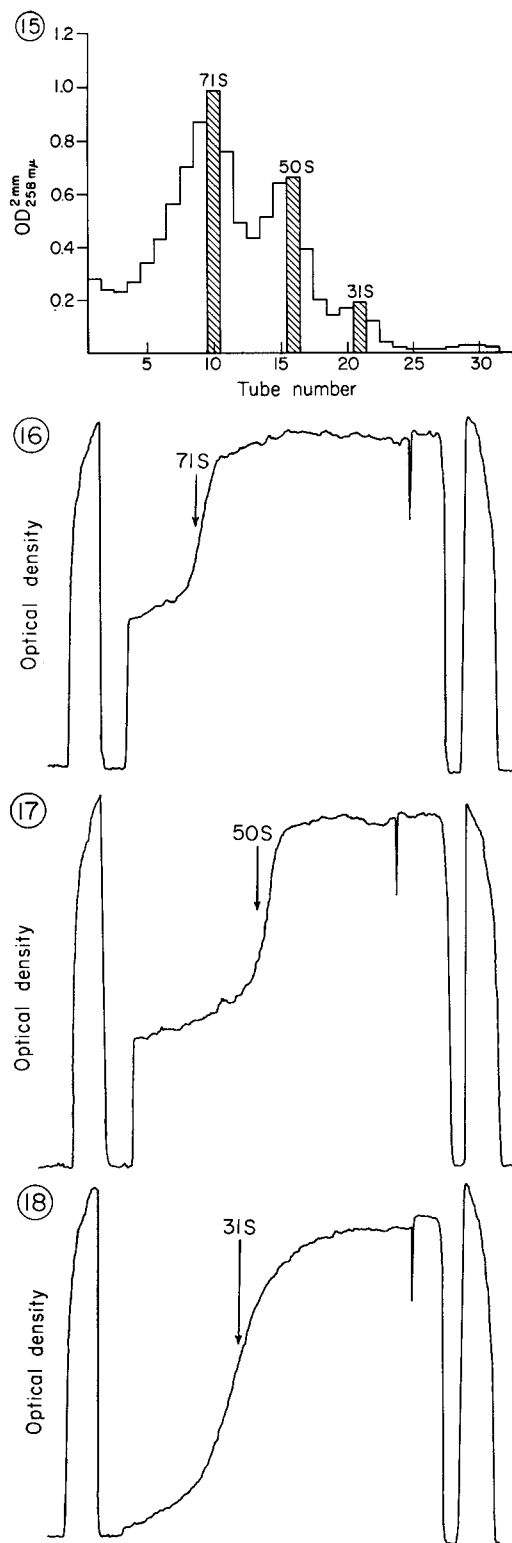


FIGURE 14 Sedimentation pattern of a DC-2 preparation of ribosomes in phosphate buffer, 0.05 ionic strength, pH 7.5, with $2 \cdot 10^{-4}$ M Mg^{++} , pH 7.5, for 12 minutes at 35,600 RPM, showing a main peak representing whole ribosomes and three more slowly sedimenting components representing subunits. Values of $S_{20,w}^0$ for the latter were not determined; the $S_{20,m}^0$ values were 54S, 45S and 36S respectively,

buffer to remove contaminating protein by passing them through a discontinuous sucrose gradient (see Materials and Methods); here the contaminating amber colour could be seen throughout the gradient, down to the upper surface of the densest, 2.05 M sucrose, layer. Washing by this method was not normally used to reduce ferritin contamination of ribosomes before preparing subunits as it reduced the yield of ribosomes too much. The alternative method of reducing contamination by precipitating ribosomes with barium acetate and dialysing against phosphate buffer clearly yielded



damaged ribosomes (Fig. 13). It is clear from Figs. 16 to 18, however, that ferritin contamination did not prevent the fractionation of distinct classes of subunit.

To compare the effect of formalin on whole 83S ribosomes, these were analysed in the ultracentrifuge in phosphate buffer, 0.01 ionic strength with 2.10^{-4} M Mg^{++} and 10 per cent formalin. This electrolyte system was used because, in the presence of other buffers, the ribosomes precipitated on the addition of formalin. Within experimental error, the sedimentation coefficient $S_{20,w}^0$ for ribosomes in the presence of formalin was indistinguishable from the value of 83S found previously.

Samples from each of the three components indicated in Fig. 15 were examined in the electron microscope. Of these, 31S subunits from tube 21 were the most clearly defined. In positively stained preparations (Fig. 19), where they are flattened onto the supporting film during drying, these particles appear elongated, with a length of 200 to 250 Å and a width of about 100 Å. Occasionally, pairs of particles can be seen in lateral association (Fig. 19), when in total cross-sectional area they resemble a whole ribosome (Fig. 8). In negatively stained preparations (Fig. 20), the rod shape is less apparent. This is probably because the particles are embedded in a film of PTA at different angles to the electron beam. It also seems likely that these particles are flexible, and that some of the distorted forms normally present in solution are preserved in the PTA film. The appearance of the 31S subunits in Figs. 19 and 20 is similar to that of the smallest subunit from pea ribosomes (14).

FIGURE 15 Fractionation of ribosomes and ribosomal subunits on a sucrose density gradient containing 10 per cent formalin, 0.05 ionic strength phosphate buffer and 2.10^{-4} M Mg^{++} , pH 7.2, after centrifuging in an SW 25 rotor at 17,000 RPM for 16 hours. The hatching indicates the samples used for electron microscopy.

FIGURES 16 to 18 Microdensitometer traces of sedimentation ultraviolet-absorption patterns in 0.05 ionic strength phosphate buffer, pH 7.5, with 2.10^{-4} M Mg^{++} and 10 per cent formalin, of the three components obtained by gradient fractionation as in Fig. 15. All at 35,600 RPM; sedimentation is from right to left. Fig. 16, 71S component at a concentration of about 80 μg/ml, after 26 minutes; Fig. 17, 50S component at a concentration of about 80 μg/ml after 24 minutes; Fig. 18, 31S component at a concentration of about 200 μg/ml after 54 minutes.

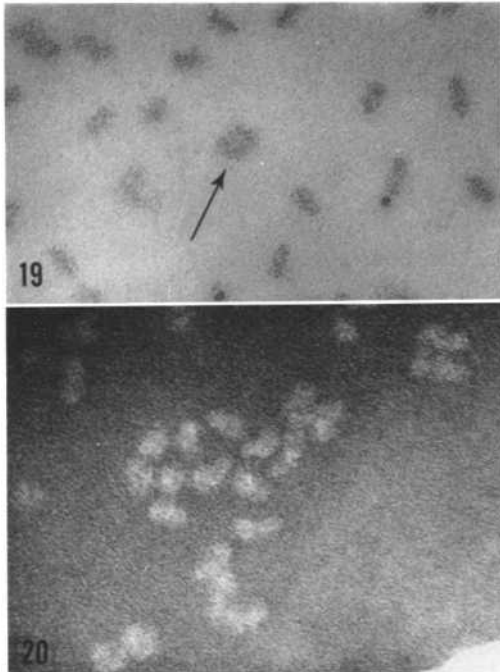


FIGURE 19 Positively stained 31S particles from tube 21, Fig. 15. The arrow indicates two particles in lateral association. $\times 240,000$.

FIGURE 20 Negatively stained 31S particles from tube 21, Fig. 15, in an area without a supporting carbon film. $\times 240,000$.

The shapes of particles from the other two components, *viz.* tubes 10 and 16, Fig. 15, with sedimentation coefficients of 71S and 50S, respectively, were less easily characterized in the electron microscope. In positively stained preparations of 71S subunits (Fig. 21, 22), where they are dried down onto their broadest faces, the particles show circular profiles with diameters similar to those of whole ribosomes. After negative staining, some particles similar to whole ribosomes can be seen, but many others appear as rather loose associations of smaller units (Fig. 23). Petermann and Hamilton (2) have suggested that the larger subunits are composed of multiples of 31S particles. The appearance of 71S subunits is consistent with this, although the exact arrangement of the smaller components cannot be distinguished.

Positively stained preparations of 50S subunits show particles that are of roughly the same diameter as 83S ribosomes and 71S subunits (Fig. 24), but are much less dense and are therefore presumably thinner. Occasionally, particles similar to 31S

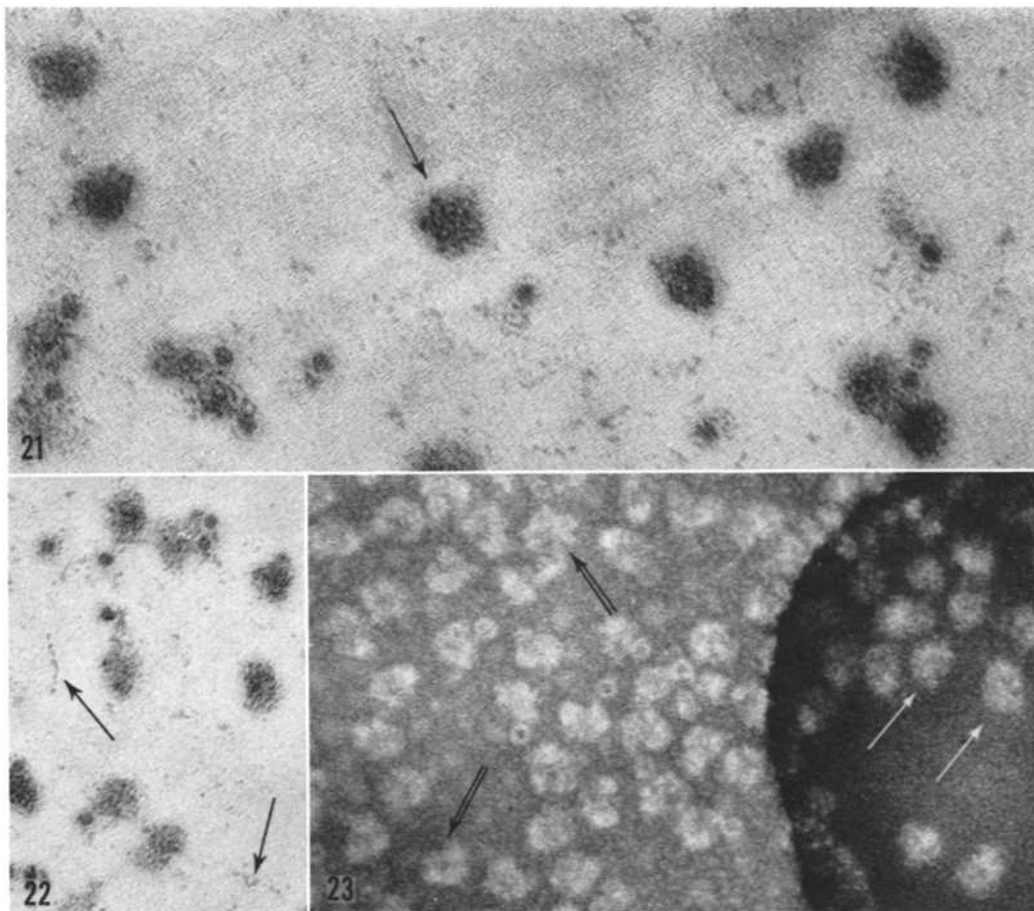
subunits can be seen. In negatively stained preparations, particles of a variety of sizes can be seen (Fig. 25), but on the average, they are smaller than those in 71S samples. In these preparations, the forms of the 50S particles are not clear enough to determine whether or not they are composed of 31S subunits.

A striking feature of positively stained preparations of the 71S and 50S subunits is the presence of fine strands about 10 to 15 A wide, either free (Fig. 22) or in association with subunits (Fig. 24). Such strands were not observed in preparations of 83S or 31S particles; this suggests that they are not artifacts due to the presence of excess stain (21). The diameter of these strands is similar to that of the interconnecting strands of the polysomes, while the length of the free pieces, about 300 A, is close to the centre-to-centre distance of adjacent ribosomes in negatively stained polysomes. These may therefore be fragments of the interconnecting strand formed by breakdown of polysomes into individual ribosomes.

DISCUSSION

The subunit composition of rat liver ribosomes and the manner in which they cleave into subunits are not known exactly. Hamilton and Petermann (1) reported subunits with sedimentation coefficients of 63S, about 50S, 46S, and about 30S, respectively, and suggested that the 63S and 50S are intermediates in the course of dissociating to 46S and 30S. Whether all 46S subunits can dissociate into 30S particles or whether some have identities of their own was not made clear. More recently, Tashiro *et al.* (22) and Henshaw (23) have suggested that the rat liver ribosome consists of one 47-50S and one 30-32S subunit, the former having twice the mass of the latter (22). Tashiro *et al.* (22) suggested further that a 60S particle, which they found when ribosomes dissociated, represents a compact configuration of the larger 47S subunit.

The present results confirm the existence of 50S and 31S subunits. The smaller of these is about 250 A long by about 100 A wide. If it is assumed that this is a deformable rod of this size, and that, to a first approximation, the whole 83S ribosome is a cylinder, 250 A long and 250 A in diameter, then clearly the 83S particle could contain as many as six of the smallest, 31S, subunits or their equivalent. In terms of relative mass, this estimate is twice that implied by the results of Tashiro *et al.* (22). It is, nevertheless, consistent with the relative elec-



FIGURES 21 and 22 Positively stained 71S particles from tube 10, Fig. 15. The small dense particles are molecules of ferritin. In Fig. 21, the arrow indicates a particle which shows a honeycomb structure. In Fig. 22, arrows indicate fine strands. Fig. 21, $\times 400,000$; Fig. 22, $\times 300,000$.

FIGURE 23 Negatively stained 71S subunits from tube 10, Fig. 15. Many particles appear compact (arrow, single tail), but others are disrupted (arrow, double tail) and appear to be clusters of smaller units. The darker area on the right is a hole in the carbon film spanned by PTA. $\times 240,000$.

tron densities of these particles after positive staining. Also it is identical with the equivalent ratio found in pea seedling ribosomes from measurements both of molecular weight (24) and of linear dimensions in the electron microscope (14). Indeed, the similarities between rat liver and pea seedling ribosomal particles in the sizes and shapes of the whole ribosomes and of the smallest subunits are close.

The sizes, shapes, and densities of the 71S and 50S particles after positive and negative staining suggest that these subunits are equivalent in mass to intermediate, but different, numbers of 31S

particles. The 50S subunit appears to be at least twice the size of the 31S, *cf.* Figs. 19 and 24. It seems unlikely that the heaviest, 71S, subunit is a compact form of the 50S (*cf.* reference 22), but the possibility that it represents an opened-up version of the whole 83S ribosome cannot be excluded.

The amounts of subunits separated on density gradients were insufficient for an accurate measure of intrinsic viscosities. This is unfortunate because the lack of precise estimates of molecular weight for the various subunits makes the subunit composition of 83S ribosomes difficult to determine. Measurements on ribosomal particles from pea

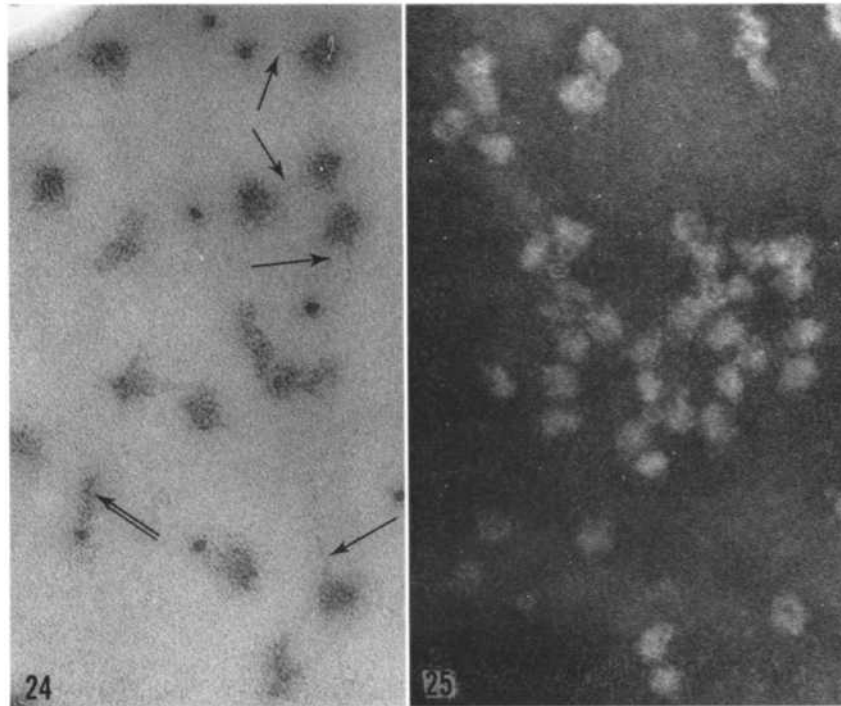


FIGURE 24 Positively stained 50S particles from tube 16, Fig. 15. Most particles are uniform in size and to some of them a fine strand can be seen attached (arrows, single tail). Occasionally a particle similar to the 31S subunits can be seen (arrow, double tail). $\times 300,000$.

FIGURE 25 Negatively stained 50S particles from Tube 16, Fig. 15. $\times 200,000$.

seedlings (24) and rat liver (25) suggest that the intrinsic viscosities of whole ribosomes and their subunits are not widely different. If, in the calculation of molecular weight, variations in viscosity are neglected, then molecular weight can be taken as roughly proportional to $(S_{20,w}^0)^{3/2}$ (see also Inouye *et al.*, reference 26). On this basis, the ratio of the molecular weights of the 83S and 31S particles is 4.5, a result which prevents a clear choice being made between the value of 3, proposed by Tashiro *et al.* (22), and of 6, based on electron microscope evidence. Either model could account for the trimeric form of ribosomal RNA from rat liver reported by Petermann and Pavlovec (27). However, the higher factor of 6 would appear to offer the more plausible explanation for the existence of the larger subunits, *viz.* the 63S reported by Hamilton and Petermann (1) and the 71S found here, if it is assumed that the masses of these are multiples of the smallest, 31S, particle.

In the whole ribosome, the subunits associate intimately since no cleavage furrows were seen be-

tween them. Furrows could exist but be obscured by adsorbed protein or RNA. However this seems unlikely, since the 0.1 ionic strength bicarbonate buffer used to wash ribosomes has been shown by Petermann and Pavlovec (12) to remove extraneous protein effectively from sarcoma ribosomes, while RNase treatment should have removed any remnants of RNA. Neither of these treatments revealed clefts in the ribosomes.

To account for the cleavage furrows in *E. coli* ribosomes, Huxley and Zubay (17) suggested that in the junction region between the two main subunits the concentration of protein is less than elsewhere in the ribosome so that the "negative" stain can penetrate. The RNA content of *E. coli* ribosomes is 60 per cent (28). On the other hand, ribosomes from pea seedlings (14) and rat liver, which show no cleavage furrows, contain 40 per cent (24, 29) and 45 to 48 per cent RNA (see above, and reference 25), respectively. In each case, the mass of RNA is about the same (30) and the balance of the ribosome is made up predominantly of protein.

Regions of low protein concentration between subunits may therefore not exist in rat liver and pea ribosomes because of the higher content of protein compared to ribosomes from *E. coli*. Ribosomes from rabbit reticulocytes appear to be intermediate; they contain 50 per cent RNA but after negative staining show clefts only faintly (21, 19).

Differences in the amounts of structural protein in the junction regions between subunits may also have a bearing on the dissociation properties of whole ribosomes from different sources, by affecting the loss of Mg^{++} . Magnesium ions appear to stabilize ribosomes by forming interchain bonds between the RNA molecules of the subunits (24). However, while *E. coli* ribosomes dissociate completely in water without added Mg^{++} (28), those from pea seedlings (29) and rat liver (see above) can be prepared in water in the absence of added Mg^{++} without serious dissociation. This difference in the ease with which Mg^{++} can be removed may reflect the difference in the degree of exposure of RNA in the junction region between subunits.

If the 83S rat liver ribosome is assumed to be a sphere with an average diameter of between 250 and 300 Å and a density of 1.51 (25), then the calculated molecular weight is about $7.3 \cdot 10^6$, which

is twice the value given by Hamilton *et al.* (25). This suggests that the ribosomes are 50 per cent porous.

The present observations confirm the existence of aggregates containing up to ten or more ribosomes. The sizes of the aggregates cannot be assessed accurately since some non-specific aggregation may have occurred when the preparation was sedimented into a pellet during purification (3). The aggregates are in the form of chains held together by a fine strand, probably of RNA. Similar observations have been made by Warner *et al.* (4), by Slayter *et al.* (19), and by Mathias *et al.* (21) on polysomes from rabbit reticulocytes. The evidence therefore suggests that the present aggregates are the polysomes or ergosomes first reported in rat liver by Wettstein *et al.* (3).

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