# Separation and Properties of 50S Ribosomes from *Streptococcus pyogenes**

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ABSTRACT: Ribosomes (50 S) have been isolated from *Streptococcus pyogenes* cells disrupted by sonic energy and by grinding with carborundum. Physical and chemical properties of purified 50S ribosomes are: sedimentation coefficient 50.0 S; diffusion coefficient $1.87 \times 10^{-7}$ cm$^2$ sec$^{-1}$; partial specific volume 0.65 dl g$^{-1}$; intrinsic viscosity 0.049 dl g$^{-1}$; extinction coefficient at 260 m$\mu$ 138 dl g$^{-1}$; refractive increment 0.001931 dl g$^{-1}$; electrophoretic mobility $-7.9$ and $-9.4 \times 10^{-5}$ cm$^2$ v$^{-1}$ sec$^{-1}$ in 0.10 ionic strength phosphate buffer, pH 7.6, and barbital buffer, pH 8.6, respectively; ribonucleic acid content 62–64%; and molecular weight $\times 10^{-6}$: by the Archibald method 1.8; by sedimentation diffusion 1.9; by sedimentation viscosity 1.8; and by light scattering 1.8.

Ribosomes, defined as ribonucleoprotein particles with discrete sedimetation properties in the size range 20–100 S (Roberts, 1958), have been isolated from many sources (Petermann, 1964; Taylor and Storck, 1964). These particles contain about 60% ribonucleic acid (Petermann, 1964), and in mammalian cells appear to be intimately associated with the membrane portion of ergastoplasm (Oberling, 1959; Hess and Lagg, 1963).

Ribosomes from bacteria and blue-green algae appear to be uniquely distinguished by the sedimentation coefficient (70 S) from those of other microorganisms and mammalian cells (Taylor and Storck, 1964). The 70S ribosome under appropriate concentrations of $Mg^{2+}$ dissociates into a 30S and 50S unit (Bolton *et al.*, 1958; Tissières *et al.*, 1959). With respect to physical properties, ribosomes from *Escherichia coli* have been more completely characterized (Tissières *et al.*, 1959) than those from other sources. Properties of ribosomes from group A streptococci have been considered previously (Utsunomiya and Hess, 1961; Hess and Horn, 1964). This report concerns the isolation and physical and chemical properties of 50S ribosomes from *Streptococcus pyogenes*.

## Methods and Materials

*S. pyogenes* (type 3) cells, harvested during exponential growth phase, were obtained in the form of a frozen paste (Difco Laboratories, Detroit) and stored at −15° until used. Freshly thawed cells were washed three times prior to extraction. Whether the cells were washed with water or buffers containing defined concentrations of magnesium ions appeared to make little difference in terms of qualitative and quantitative composition of subsequent extracts. Frozen cells (10 g) usually provided about 8 g of packed washed cells on a wet weight basis. The washed cells averaged 20% dry solids when lyophilized.

*Buffer Compositions.* The phosphate buffer at pH 7.5 contained 0.0105 M phosphate as a potassium salt and 0.05 M NaCl. Magnesium sulfate was added to the buffer to a final concentration 0.0007–0.007 M depending upon particular experiments. Tris buffer consisted of 0.05 M Tris (Sigma Chemical Co., St. Louis, Mo.) adjusted at 20° to pH 7.5 with HCl. The concentration of $Mg^{2+}$ was varied from 0.002 to 0.01 M as required for particular experiments. The composition of barbital buffer, 0.10 ionic strength, pH 8.6, was defined by Longsworth (1942).

*Extraction Procedures.* A. GRINDING WITH CARBORUNDUM. The cells were thawed and washed three times with a buffer which contained 0.002 M $Mg^{2+}$. Prior to use, 400 mesh carborundum was washed four times with 6 N HCl followed by six rinses with water and dried at 85°. Packed cells amounting to 4 g (wet weight) were ground 30 min at 4° with 35 g of carborundum in a cold mortar. After this grinding procedure, 25 ml of buffer containing 0.002 M $Mg^{2+}$ was added and the suspension was stirred 10 min and poured into centrifuge tubes. Additional buffer (15 ml) was used to rinse the mortar and pestle. The suspension was centrifuged 15 min at 20,000*g*. The sediment was suspended in 30 ml of buffer, stirred 10 min, and centrifuged as before. The two supernatant solutions were combined to give the initial extract.

B. SONIC RADIATION. Washed wet cells (6 g) were

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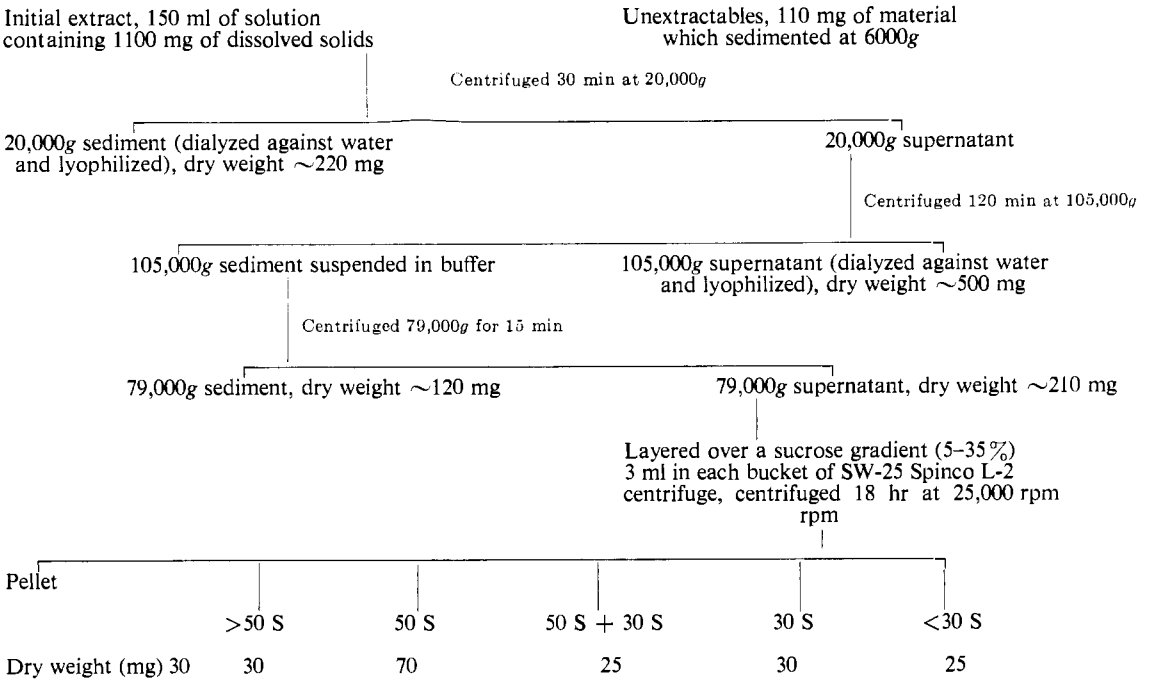


FIGURE 1: Schematic fractionation using extracts prepared by sonication procedures based on 6-g cells, wet weight. Either Tris or phosphate buffers were used in various experiments. A series of concentrations of $Mg^{2+}$ were employed in order to determine conditions optimum for obtaining 50S ribosomes.

suspended in 10 ml of buffer which contained 0.002 M $Mg^{2+}$ and placed directly in the steel cup of a 9-kcycle Raytheon magnetostriction sonic generator operated at 160 v. The cells were irradiated for 120 min. Ice water was circulated through the outer jacket of the cup during sonication. The power output of the sonic oscillator, Model S-120A, checked calorimetrically according to the procedure recommended by the manufacturer, was in accord with specifications.

The sonicated material was centrifuged 30 min at 6000*g* and the sediment was suspended in buffer, exposed for another 120 min to sonic energy, and centrifuged as indicated above. The above step was repeated three times. The five supernatant solutions obtained from the 6000*g* centrifugation step were combined and designated as the initial extract.

*Viscosity.* Viscosity measurements were carried out in phosphate buffer containing 0.002 M $Mg^{2+}$ at 20.0 ± 0.1° using a Fisher–Irany-type viscometer, as described previously (Hess and Cobure, 1950).

*Sedimentation Analyses.* Experiments were carried out in a Spinco Model E analytical ultracentrifuge at 20°. When the synthetic boundary cell was employed, the rotor was spun at 3000 rpm for a time sufficient to produce a well-defined symmetrical schlieren pattern. The speed of the rotor was then increased to 33,450 rpm where centrifugation was continued. Areas were measured planimetrically from enlargements of the schlieren patterns, with base lines established optically using buffer solutions in the antipodal cell. The synthetic boundary experiments were carried out in a 12-mm cell with a 2° sector modified according to the method of Kegeles (1952).

*Electrophoretic Analyses.* Moving boundary electrophoretic studies were carried out at 2.5° in a conventional Tiselius assembly using schlieren optics to include a phase plate. The experiments were performed in an all-glass cell assembly. Conductivities were measured at 0.0°; the mobility values obtain, therefore, at 0.0°. Mobility coefficients were calculated from tracings of enlargements of photographs of the descending limb of the cell in the customary manner.

*Partial Specific Volume.* The partial specific volume was determined pycnometrically in phosphate buffer containing 0.002 M $Mg^{2+}$ according to a method described by Kraemer (1940) from a plot of $w_1$ *vs.* $V$, where $w_1$ is the weight fraction of protein and $V$ the specific volume of the solution. Specific volumes were measured at 20° using a 5-ml pycnometer. Protein concentrations used to determine $w_1$ values were measured spectrophotometrically using an extinction coefficient, $E^{1\%}_{260}$, of 138 dl/g.

*Diffusion.* Diffusion studies were carried out at 20.0° in the electrophoresis assembly described above at a concentration of 0.4% nucleoprotein in phosphate buffer containing 0.002 M $Mg^{2+}$. Duplicate determinations resulted from using both limbs of the cell. Boundaries were sharpened according to the method of Kahn and Polson (1947). Diffusion coefficients were calculated from height and area, second moment, and inflection points of the gradient curves (Neurath, 1942). The zero-time correction suggested by Kahn

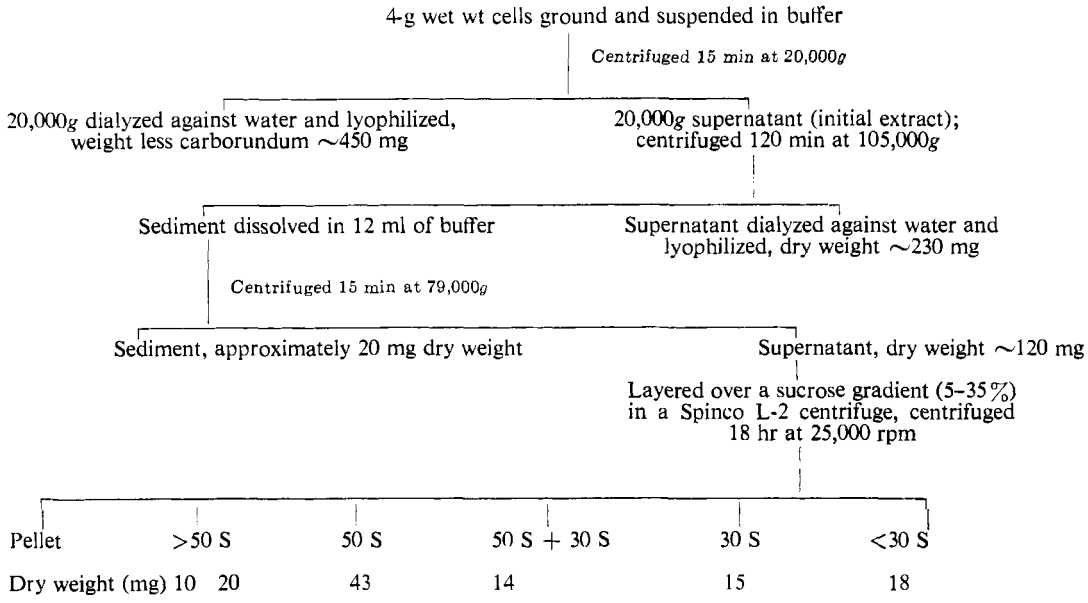


FIGURE 2: Schematic fractionation using extracts prepared by grinding cells with carborundum. Either Tris or phosphate buffers were employed. A series of concentrations of $Mg^{2+}$ were examined in order to determine the optimum conditions for obtaining 50S ribosomes.

and Polson (1947) and by Longsworth (1947) was applied.

*Light-Scattering Studies.* Light-scattering experiments were carried out with an Aminco microphotometer using procedures that have been considered previously (Hess *et al.*, 1956). In order to eliminate dust as a factor in light-scattering studies, a closed system was devised. Air and all solutions were passed through an MF-type Millipore filter (VC, pore size 1000 A). The light-scattering cell consisted of a cylinder 2.4 cm in diameter with two sides ground flat and plane parallel. The cell was oriented so that the incident light was normal to these planes. A fine glass capillary entered the cell at a point away from the light path. The capillary was used to fill the cell, dilute the contents, and remove samples. Both the dissymmetry method (Edsall, 1953) and the extrapolation method (Zimm, 1948) were used for calculating molecular weights.

*Extinction Coefficients.* Extinction coefficients measured in phosphate buffer containing 0.002 M $Mg^{2+}$ with a Beckman DU spectrophotometer are based on weights of samples dried *in vacuo* at 105°. Dry weight of the buffer salts was measured in the same way and subtracted from the weight of the ribosome samples. The observed extinction coefficient is attributable not only to the nucleic acid and protein moieties, but also to light scattering. A correction for light scattering (Bonhoeffer and Schachman, 1960) has not been applied to these measurements.

*Refractive Index.* Refractive indices, measured in phosphate buffer containing 0.002 M $Mg^{2+}$ with a Brice-Phoenix differential refractometer Model BP-2000V, are based upon concentrations measured spectrophotometrically using the extinction coefficient 138 dl/g.

*Chemical Analyses.* Chemical analyses are based upon samples dried to constant weight at 105°, *in vacuo*. In case of lipid analyses, lyophilized dry solids were extracted with an alcohol–ether (3:1) mixture using a Soxhlet extractor.

Two procedures were used for nucleic acid determinations. Total nucleic acid was determined with the Schneider (1945) method. The nucleic acid content of the trichloroacetic acid extract was measured spectrophotometrically using an extinction coefficient of 248 at wavelength 260 mμ. The absence of DNA was established with the diphenylamine test (Burton, 1956). RNA was also measured using the Webb (1956) method. The nucleic acid content from the Schneider (1945) procedure agrees well with the RNA determined according to the Webb (1956) method. Fractionation operations were carried out using cold equipment and solvents in a cold room held at approximately 2°.

## Results

*Fractionation.* The preparation of ribosomes is outlined schematically in Figures 1 and 2. A tabulation of yields of various fractions and analytical data are listed in Table I, and chemical and physical characterization data are found in Table II.

An earlier procedure for preparing 50S ribosomes (Utsunomiya and Hess, 1961) was abandoned in favor of the present procedure. The earlier procedure was based upon a shipment of cells (Difco) obtained in 1960. With a shipment of cells procured in February

TABLE I: Analytical Data Pertaining to Fractionation of 6-g Wet Weight Cells Ruptured with Sonic Energy.

| Fraction | Dry Wt (mg)[a] | Lipid (%)[b] | RNA (%)[b] Webb Procedure | RNA (%)[b] Schneider Procedure |
|---|---|---|---|---|
| Whole cells | 1200 | 2.9 | | 24.5 |
| Unextracted | 110 | 2.0 | | 13.6 |
| 20,000*g* sediment | 220 | 4.0 | 4.1 | 3.9 |
| 105,000*g* supernatant | 520 | 3.0 | 24.9 | 25.3 |
| 105,000*g* sediment | 330 | 4.0 | 41 | 39 |
| 79,000*g* sediment | 120 | 6.0 | | 24.2 |
| 79,000*g* supernatant | 210 | 0.9 | | 58 |

[a] Yield data based upon 30 fractionations. [b] Analyses based upon lyophilized samples without correction for moisture.

1962, we obtained smaller yields and a less pure product. Attempts to improve the yield and purity of the ribosomes by modifying steps in the earlier procedure did not lead to satisfactory results. We switched to density gradient procedures, therefore, as a final step in the preparation of purified material.

*Effect of Extraction Procedures.* As seen in Figure 3, there is little difference in the distribution of ribosomes in phosphate buffer from cells disrupted either by grinding with carborundum or by means of sonication.

*Effect of $Mg^{2+}$ Concentration.* The well-established effect of $Mg^{2+}$ concentration on the distribution of ribosomes was noted. According to Figure 4, the distribution of ribosomes in Tris buffer containing 0.005 M $Mg^{2+}$ shifted considerably when the corresponding solution was analyzed in buffer containing 0.01 M $Mg^{2+}$. The distribution in phosphate buffer containing 0.01 M $Mg^{2+}$ is seen in Figure 4c. In each buffer system the distribution of 50S and 70S ribosomes was essentially the same whether the initial extract or the 105,000*g* pellet was examined. Ribosomes prepared from *S. pyogenes* appear to require higher concentrations of $Mg^{2+}$ ions to maintain their integrity, as compared with those from *E. coli.* Tissières *et al.* (1959) found that in Tris buffer, pH 7.4, containing 0.001 M $Mg^{2+}$, the 70S peak predominated in ribosomes prepared from *E. coli.* As seen in Figure 4, at 0.005 M $Mg^{2+}$ in Tris buffer, ribosomes from *S. pyogenes* consisted of a mixture of 30 S, 50 S, and 70 S with the 50 S predominating. When the $Mg^{2+}$ concentration was increased to 0.01 M, the 70S particle predominated with lesser amounts of 30S and 50S material. The distribution of 30S, 50S, and 70S ribosomes was essentially equivalent in phosphate buffer containing 0.01 M $Mg^{2+}$ and Tris buffers containing 0.005 M $Mg^{2+}$. The 50S particle is stable in phosphate buffers within the $Mg^{2+}$ concentration range 0.001–0.005 M.

*Diffusion Coefficient.* Four methods using seven schlieren patterns obtained from two separate experiments were used to calculate the diffusion coefficient of 50S ribosomes. The four methods gave results as follows ($D_{20w} \times 10^7$ cm$^2$ sec$^{-1}$): inflection point method, 1.88; successive approximation, 1.86; height and area, 1.85; from the second moment of the gradient curve about the centroidal ordinate, 1.89; averaging 1.87 ± 0.02. The experiments were carried out at 20.0 ± 0.05° over a period of 60 hr. A sample removed from the diffusion cell after completion of the experiment and examined in the ultracentrifuge showed no evidence

TABLE II: Chemical and Physical Characterization Data for 50S Ribosomes from *S. pyogenes* (Type 3).

| | | |
|---|---|---|
| $s^0_{20,w}$ (S) | Sedimentation coefficient | 50.0 |
| $D_{20,w}$ (cm$^2$ sec$^{-1}$) | Diffusion coefficient | $1.87 \pm 0.02 \times 10^{-7}$ |
| $[\eta]$ (dl g$^{-1}$) | Intrinsic viscosity | 0.049 |
| $\bar{V}$ (ml g$^{-1}$) | Partial specific volume | 0.65 |
| $E^{1\%}_{260\ m\mu}$ (dl g$^{-1}$) | Extinction coefficient | 138 ± 3 |
| $\mu$ (cm$^2$ v$^{-1}$ sec$^{-1}$) | Electrophoretic mobility, $\mu$ = 0.1 pH 7.6 phosphate buffer containing 0.002 M $Mg^{2+}$ | $-7.9 \times 10^{-5}$ |
| | $\mu$ = 0.1 pH 8.6 barbital buffer | $-9.4 \times 10^{-5}$ |
| $M$ | Molecular weight, Archibald method | $1.8 \times 10^6$ |
| | Sedimentation diffusion | $1.9 \times 10^6$ |
| | Sedimentation viscosity | $1.8 \times 10^6$ |
| $M_w$ | Light scattering | $1.8 \pm 0.3 \times 10^6$ |
| $dn/dc$ (dl g$^{-1}$) | Refractive increment | 0.001931 ± 13 |
| RNA (%) | | 62–64 |

FIGURE 3: Ultracentrifugal evaluation of the effect of extraction procedures on ribosome fraction. The extracts were prepared using phosphate buffer containing 0.002 M $Mg^{2+}$. Photographs taken after 1920 sec at 33,450 rpm, phase plate angle 60°. The 105,000*g* pellets were dissolved in phosphate buffer, pH 7.6, containing 0.002 M $Mg^{2+}$ and 0.05 M NaCl, total ionic strength 0.10. Concentration of ribosomes approximately 0.8%. Three peaks represent 30S, 50S, and 70S units, respectively. Upper pattern obtained from extracts of cells ground with carborundum. Lower pattern obtained from extracts of cells ruptured by sonic energy.

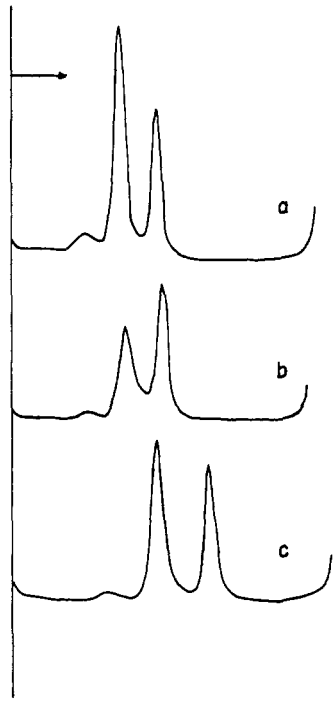


FIGURE 4: Ultracentrifugal evaluation of the effect of magnesium ions and buffer composition on the distribution of ribosomes. The extracts were prepared using the buffers specified in each instance. The 105,000*g* pellet was dissolved in (a) Tris buffer, pH 7.5, containing 0.005 M $Mg^{2+}$, photographed after 1300 sec, phase-plate angle 60°; (b) Tris buffer, pH 7.5, containing 0.01 M $Mg^{2+}$, after 1300 sec, phase-plate angle 60°; (c) phosphate buffer, pH 7.5, containing 0.01 M $Mg^{2+}$, after 1640 sec, phase-plate angle 50°. Centrifuged at 33,450 rpm and 20°. The three peaks represent 30S, 50S, and 70S ribosomes, respectively.

of degradation. The agreement between values of diffusion coefficients provides an additional criterion of homogeneity since the coefficients are calculated using different properties of the distribution curve (Neurath, 1942).

*Sedimentation Coefficient.* Sedimentation coefficients were measured over a concentration range of 0.1–0.8%. Results from fifteen separate determinations gave a linear plot and displayed a concentration dependency consistent with the observations of Tissières *et al.* (1959). The sedimentation coefficient extrapolated to zero concentration and corrected to the viscosity and density of water at 20° is 50.0 S.

*Extinction Coefficient.* The apparent extinction coefficient, 138 ± 3 dl $g^{-1}$, represents an average value[1] based on five separate preparations of purified 50S ribosomes.

*Refractive Increment.* The refractive increment, 0.001931 ± 13 dl $g^{-1}$, is an average from nine determinations using three separate preparations of 50S ribosomes.

*Electrophoresis.* Electrophoretic behavior of ribosomes is shown in Figures 5 and 6. Ultracentrifugal analyses with aliquots from samples used for electrophoretic studies indicated the presence of a small amount of 30S particles in the solution of 50S ribosomes. As seen in Figure 5, in barbital buffer at pH 8.6, the 50S ribosome has the mobility characteristics of component 9 as defined by Hess and Slade (1955). The pattern in Figure 5b contains a small amount of 30S material seen as component 5. A small amount of contaminating 30S ribosomes moving more slowly than the main component can be seen in Figure 6 also.

It is of interest that the 30S and 50S ribosomes also travel as separate components at pH 7.5 in 0.10 ionic strength sodium chloridephos-phate buffer

[1] Because of the size of ribosomes, light-scattering effects can introduce significant errors in measuring extinction coefficients.

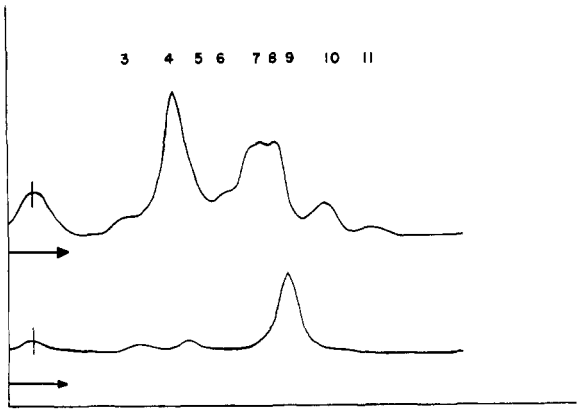


FIGURE 5: Electrophoretic characteristics of streptococcal fractions. Upper pattern is the 105,000*g* supernatant fraction, the so-called "cell sap" from streptococcal extracts, concentration about 1.5%. Lower pattern represents partially purified 50S ribosomes, concentration about 0.5%. Barbital buffer, pH 8.6, ionic strength 0.10. Photographed after 120 min under a potential gradient of 6.7 v $cm^{-1}$; photographs of descending limb of the cell. Initial boundaries are indicated by vertical lines through the $\epsilon$ stationary boundary.

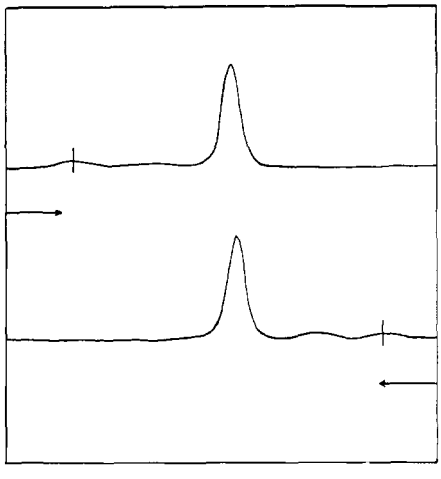

FIGURE 6: Electrophoretic properties of ribosomes. Partially purified 50S ribosomes in phosphate buffer containing 0.002 M $MgSO_4$ and 0.05 M NaCl, total ionic strength 0.10; pH 7.5; concentration about 1%, photographed after 120 min under a potential gradient 4.4 v $cm^{-1}$. Upper pattern from the ascending limb of cell. Lower pattern from the descending limb of cell.

containing 0.005 M $Mg^{2+}$ and at pH 7.5 in 0.10 ionic strength Tris buffer containing 0.001 M $Mg^{2+}$. In all three buffer systems, the 30S particle has the lower mobility, indicating the charge to mass ratio of the 30S particle is less than that of the 50S particle. The mobility difference is consistent with the observation of Spahr (1962) that 30S and 50S ribosomes differ in amino acid composition, and the more recent report of Waller (1964) that the proteins isolated from 30S and 50S particles have different electrophoretic properties.

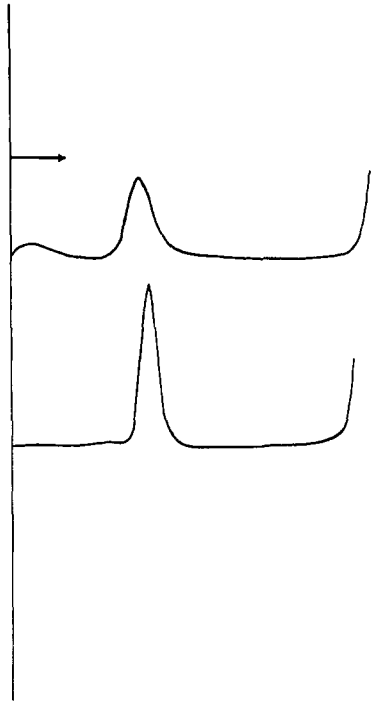

FIGURE 7: Ultracentrifugal evaluation of the effect of deoxycholate on 50S ribosomes. (upper pattern) The solution in the cell with a wedge window contained 0.5% deoxycholate. (lower pattern) The control solution in a standard cell. Concentration about 0.8% in phosphate buffer containing 0.002 M $Mg^{2+}$ and 0.05 M NaCl after 1920 sec at 33,450 rpm, phase-plate angle 60°.

*Effect of Deoxycholate.* We have observed that DOC[2] breaks down the 50S particle as shown in Figure 7. The area under the 50S boundary is reduced and material is observed at the meniscus. Whether this is a direct effect of DOC on the ribosome or an indirect effect of DOC reducing the effective concentration of $Mg^{2+}$ is not clear.

*Light-Scattering Studies.* We have experienced a great deal of difficulty in obtaining consistent and plausible results using light-scattering procedures. In earlier work, experimental results produced weight-average molecular weights between 5 and 10 $\times$ $10^6$, much higher than we obtained from sedimentation diffusion and sedimentation viscosity data. There are at least four reasons for high values using light-scattering procedures: (1) the presence of dust in the solution; (2) a bad light-scattering cell; (3) aggregation of ribosomes; (4) the presence of other high molecular

[2] Abbreviation used: DOC, sodium deoxycholate.

weight impurities in the preparation.

More recently we have used a light-scattering cell arranged to function in a closed system combined with a Millipore filter train. We believe that with this arrangement we have been able to prepare dust-free solutions. With solutions of 50S ribosomes centrifuged 10 min at 79,000*g* followed by filtration through a type MF Millipore filter, we obtained the molecular weight listed in Table II. We have observed an increase in scattering with time as well as increased dissymmetry readings as the solution stands in the cell. We attribute the change in scattering properties of the solution to aggregation of ribosomes, an interpretation in accord with synthetic boundary experiments with the analytical centrifuge.

Synthetic boundary studies with the analytical ultracentrifuge showed that the area of the 50S ribosome peak decreased with time to an extent greater than could be ascribed to radial dilution effects. We found a decrease of approximately 10% in the amount of optically recorded material at the end of such experiments. The results are consistent with the viewpoint that aggregation of ribosomes occurs during centrifugation.

*Molecular Weights.* Molecular weights determined by the four procedures listed in Table II are reasonably self-consistent and in good agreement with those reported by Tissières *et al.* (1959) for 50S ribosomes from *E. coli.* In calculating the molecular weight from sedimentation and viscosity data using the Scheraga–Mandelkern equation a value of $2.12 \times 10^6$ was used for $\beta$.

*A Heavier than 50S Fraction.* With ribosomes distributed through a sucrose gradient, we consistently observed a broad zone of material lying below the sharply defined band of 50S particles. We have designated the material "heavy 50 S." When examined in the ultracentrifuge, under essentially identical conditions, with the "heavy fraction" in a standard cell and the 50S fraction in a cell with a wedge window, the "heavy 50S" preparation sediments somewhat more rapidly than 50S particles as seen in Figure 8. An RNA content of 54% suggests that the "heavy 50S" material consists of 50S ribosomes containing attached nascent protein, an interpretation consistent with the report of Gilbert (1963).

When the "heavy 50S" material was examined in the analytical centrifuge in phosphate buffer containing 0.0007 M $Mg^{2+}$, a distinctive zone of slowly sedimenting material was noted. The observation is consistent with the report of Schlessinger and Gros (1963) that nascent protein dissociates from 50S particles at low concentrations of $Mg^{2+}$.

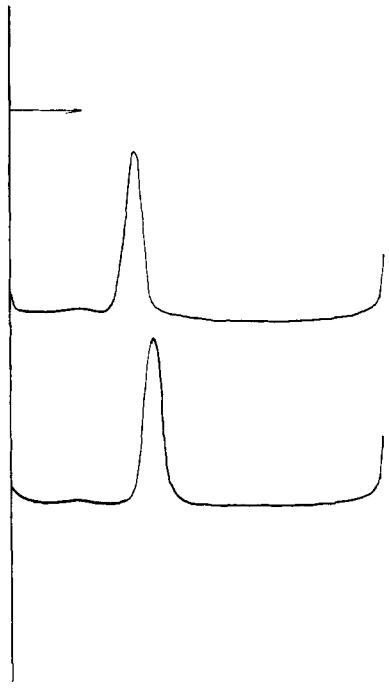

FIGURE 8: Sedimentation behavior of "heavy 50S" fraction. Upper pattern obtained from 50S ribosomes in a cell with a wedge window. Lower pattern obtained from "heavy 50S" fraction in a standard cell. Concentration 0.4% in phosphate buffer, pH 7.5, containing 0.002 M $Mg^{2+}$ and 0.05 M NaCl, total ionic strength 0.10, 30-mm cell with 4° sector. Photographed after 1920 sec at 33,450 rpm, phase-plate angle 60°.

## Discussion

We have observed limited mixing when the concentration of ribosomes layered on sucrose gradients exceeds 1%, a result in accord with observations of Brakke (1964). The extinction coefficient listed in Table II is considerably less than the values 149 and 182 reported by Tissieres *et al.* (1959) for 50S ribosomes from *E. coli*, whereas the RNA content of 50S particles from *S. pyogenes* and *E. coli* is the same. If the extinction coefficient of RNA in extracts prepared by hot trichloroacetic acid extraction of ribosomes is taken as 248 dl $g^{-1}$ (Hess *et al.*, 1964) and the weight fraction of RNA as 0.64, the contribution of RNA to the extinction coefficient of the ribosome is $0.64 \times 248 = 158.5$, ignoring hypochromicity. Schlessinger (1960) reported hypochromicity in *E. coli* ribosomes varying from 26 to 41% depending upon treatment. We have observed absorption at 260 mμ to be 26% greater in solutions heated to 85° than the absorption of unheated solutions of 50S ribosomes, indicating a hypochromicity consistent with the observations of Schlessinger (1960) and with Bonhoeffer and Schachman (1960). The contribution of the protein moiety to the extinction coefficient is no more than 3 dl $g^{-1}$ as judged by the amino acid composition of the 50S ribosome from *E. coli* reported by Spahr (1962). If $227 \pm 5$ is taken as the extinction coefficient for ribosomal RNA (Kurland, 1960), the contribution of RNA to the extinction coefficient of the ribosome is $(227 \pm 5) \times 0.64 = 145 \pm 3$. The value of the extinction coefficient

reported in Table II would appear, therefore, to be reasonable.

At least three reasons can be advanced to account for differences in the extinction coefficients: (1) errors[1] involved in measuring concentration and absorption, (2) differences in structural organization of RNA in the ribosomes, and (3) differences in the base composition of ribosomal RNA from the two organisms. Light-scattering effects will lead to an apparent extinction coefficient higher than the true value. The difference in extinction coefficients reported by Tissières *et al.* (1959) and that reported in this paper appears greater than what seems likely to result from errors of measurement. The second reason can be excluded from the similarity in hypochromicity. The third reason is consistent with the findings of Miura (1962) and Aronson (1963) who have reported differences in base composition of ribosomal RNA from several bacteria.

Except for the difference in the extinction coefficient, the chemical and physical properties of 50S ribosomes from *S. pyogenes* appear essentially identical with those from *E. coli*. One other feature distinguishing ribosomes from these two organisms appears to be the higher concentration of $Mg^{2+}$ required to keep 70S ribosomes from *S. pyogenes* intact.

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