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QUANTITATIVE ISOLATION OF MESSENGER RNA FROM FREE AND BOUND POL--ETC(U)
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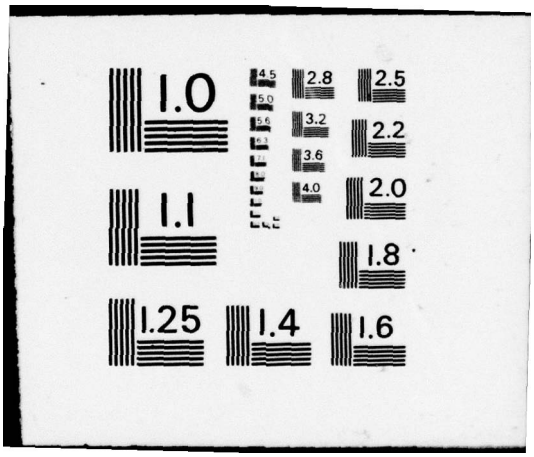
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A procedure is described for the quantitative separation of poly A-rich mRNA from free and bound ribosomes. A modification of the technique developed by Venkatesan et al. was employed for the quantitative subfractionation of rat hepatic cells. Detergent, rather than phenol-chloroform, were utilized for RNA extraction and the mRNA from free and bound ribosomes was isolated on oligo(dT)-cellulose columns. Evidence of the effectiveness of this procedure was demonstrated through electron microscopy, sucrose density gradient polyribosomal patterns, and RNA distribution studies. These techniques were utilized to study

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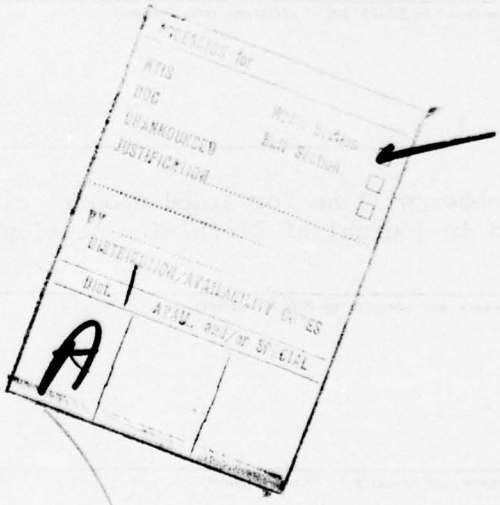
the effects of Streptococcus pneumoniae infection on hepatic transcription and translation rates. Increases in transcription rates directed toward the bound ribosomes were observed, which resulted in an increase in the rate of translation due to the availability of more ribosomal and messenger RNA. However, the efficiency of the translation process did not change.



# QUANTITATIVE ISOLATION OF MESSENGER RNA FROM FREE AND BOUND POLYSOMES OF CONTROL AND INFECTED RAT LIVER

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

24 November 1976

## Summary

A procedure is described for the quantitative separation of poly A-rich mRNA from free and bound ribosomes. A modification of the technique developed by Venkatesan et al. was employed for the quantitative subfractionation of rat hepatic cells. Detergents, rather than phenol-chloroform, were utilized for RNA extraction and the mRNA from free and bound ribosomes was isolated on oligo(dT)-cellulose columns. Evidence of the effectiveness of this procedure was demonstrated through electron microscopy, sucrose density gradient polyribosomal patterns, and RNA distribution studies. These techniques were utilized to study the effects of Streptococcus pneumoniae infection on hepatic transcription and translation rates. Increases in transcription rates directed toward the bound ribosomes were observed, which resulted in an increase in the rate of translation due to the availability of more ribosomal and messenger RNA. However, the efficiency of the translation process did not change.

## Introduction

Many properties of free and bound ribosomes have been discovered since the development of techniques for their separation. An increase in their rate of production has been observed in response to steroids [1], infection [2], a mediator released from leukocytic cells [3-5], and in certain types of stress [6]. They appear to have different turnover rates [7,8] and are dependent on certain soluble factors for the translation of proteins [9,10]. Also, it is generally believed that the free ribosomes are involved in the production of intracellular proteins and bound ribosomes in the production of extracellular proteins [11-14].

However, the procedures currently in use for subfractionation, isolation, and characterization of RNA-containing fractions are diverse and often lack good quantitative and/or qualitative steps. For example, the technique most commonly used for the separation of ribosomes involves an initial centrifugation at 10 000 to 15 000 X g for about 10 min [15-19]. The ribosomes are then isolated from the supernatant fraction. It has been shown that more than 60% of the ribosomal RNA (rRNA), most of which is associated with bound ribosomes, is lost in the pellet during this centrifugation [21]. Subsequent steps for further purification of ribosomes and isolation of other subfractions must be expedient and contain appropriate inhibitors to prevent the breakdown of RNA [21]. This involves the proper use of endogenous ribonuclease inhibitors along with certain cations and polyanions in addition to careful regulation of pH and temperature [21,22].

By extracting the various RNA species from the ribosomes, additional information on the processes taking place within the hepatic cell can be obtained. Most techniques for the extraction of RNA involve the use of phenol alone [23] or in combination with chloroform [24]. The problem with these methods is the use of toxic materials, time-consuming steps, and the loss of a portion of the starting RNA material.

By considering all the problems and limitation of these various steps and the alternatives available, a procedure was devised for the quantitative isolation of liver cell subfractions and subsequently, free and bound messenger RNA (mRNA). The initial step involved ultracentrifugation of the liver homogenate at the optimum time and g force for the separation of free and bound ribosomes, as described by Venkatesan et al. [21]. Modifications of this and other procedures were made for the further subfractionation of hepatic cells. Techniques for the extraction and separation of RNA species in each of the ribosome fractions that yielded the best possible products under well-controlled conditions were developed from various sources in the literature.

Since infection with Streptococcus pneumoniae has been previously shown to stimulate hepatic RNA production [2], it was utilized as a model to demonstrate the application of this procedure to the study of cellular RNA regulation and its relationship to the transcription and translation processes.

## Materials and Methods

### Subfractionation of hepatic cells

Male rats from Charles River Laboratories (Wilmington, Mass., U.S.A.) weighing 150-200 g were fasted overnight. For studies of infection, virulent S. pneumoniae, type Ia5, was given subcutaneously at a dose of $10^7$ organisms 16 h prior to sacrifice. Control rats were injected with an equal quantity of heat-killed S. pneumoniae (56°C, 20 min). In experiments where radioactive labeling of the RNA was desired, the rats were injected intraperitoneally with 20 μCi/100 g body weight of [6-$^{14}$C]orotate (New England Nuclear, Boston, Mass., U.S.A.) and killed 4 h later. The livers were perfused in situ with cold saline and subfractionated into nuclear, free and bound ribosomal and soluble RNA fractions using a modification of the procedure of Venkatesan and Steele [21]. The livers were homogenized in 4 vols. of 0.25 M sucrose in buffer A, which contained 50 mM N-2-hydroxyethypiperazine,-N'-2-ethanesulfonic acid (HEPES) buffer (pH 7.6), 75 mM KCl, 5 mM magnesium acetate, and 3 mM glutathione. Aliquots of the homogenate were taken for determination of total liver RNA and specific activity. Sixteen-milliliter aliquots of the remainder were precipitated in an SW-27.1 rotor (Beckman Instruments) at 27 000 rev/min for 12 min at top speed ($162 \cdot 10^4$ g · min). The supernatant containing the free ribosomes was centrifuged either through a 2-step discontinuous gradient containing 6 ml of 1.38 M sucrose in HEPES buffer at the bottom, or a 3-step gradient containing 3 ml each of 2.0 and 1.38 M sucrose. Both type gradients were overlayed with 4 ml of the supernatant and centrifuged 20 h at 105 000 X g (50 Ti rotor in Beckman L265B). The resulting polysomal

pellets, supernatant fraction and interphase between 1.38 and 2.0 M sucrose were collected and stored at -20°C for future studies.

The pellet from the initial centrifugation was resuspended in cell sap (prepared from liver homogenate of normal rats, centrifuged 2 h at 105 000 X g), treated with 1/10 volume of 10% Triton X-100 and centrifuged at 1470 X g for 5 min to remove the nuclei. The suspended material from this spin was either treated with sodium deoxycholate to a final 1.3% or left untreated. Both aliquots were then centrifuged at 105 000 X g for 3 h (50 Ti rotor). The resulting membrane-associated and nonmembrane-associated (treated with sodium deoxycholate) bound ribosome pellets were stored at -20°C for future use.

Sucrose density gradient fractionation

Sucrose gradients (10-40%) in TKM buffer (50 mM Tris, pH 7.6, 65 mM KCl, 10 mM $MgCl_2$) were prepared in polyallomer tubes for a Beckman SW-56 head. Free and detergent-treated bound ribosome pellets were resuspended to a concentration of 100 µg RNA/0.1 ml in TKM buffe using a loose dounce homogenizer. An aliquot of 0.1 ml of the suspensions was layered on the gradients and centrifuged at 36 000 rev/min for 75 min. The resulting gradients were analyzed for absorption at 254 nm using an automatic density gradient fractionator (ISCO, Lincoln, Neb., U.S.A.).

Extraction of RNA from free and bound ribosomes

Six free and 6 non-detergent-treated bound ribosome pellets (approximately 10 and 25 mg RNA respectively) were resuspended in 10 ml 0.01 M Tris buffer (pH 7.6), 0.5 M NaCl using a loose dounce homogenizer. Aliquots were taken to determine RNA content and

specific activity (if labeled with [$^{14}$C]orotate) of the total ribosome fractions. Sodium dodecyl sulfate was added to a final concentration of 0.5%. The samples were then precipitated at 2500 rev/min and the resulting pellet washed twice with starting buffer plus 0.5% dodecyl sulfate. The supernatant fractions were pooled (approximately 20 ml total volume) and analyzed for mRNA and rRNA content.

Separation of mRNA and rRNA

One by 7 centimeter glass columns were prepared containing 0.6 g oligo(dT)-cellulose (Collaborative Research, Inc., Waltham, Mass., U.S.A.) and equilibrated with 0.01 Tris buffer (pH 7.6), 0.5 M NaCl, 0.5% dodecyl sulfate. The extracted RNA from free and bound ribosomes was washed through the columns to a total volume of 40 ml using the starting buffer. The column was then washed with 10 ml of a solution containing 0.01 M Tris, 0.1 M NaCl, 0.5% dodecyl sulfate to remove rRNA. Poly A-containing mRNA was then eluted with 10 ml of distilled water. Aliquots of all fractions were taken for RNA and radioactivity determinations and the remainder precipitated overnight at -20°C with the addition of LiCl to a concentration of 0.3 M plus 2 vols. of ethanol.

RNA, DNA, and specific activity determinations

The RNA content and radioactivity of the total homogenate and all subfractions were determined by previously described techniques 8]. Aliquots of each fraction were precipitated and washed twice in cold 0.2 N perchloric acid (PCA).[1] They were then hydrolyzed in 2 ml of 0.3 N KOH for 1 h in a 37°C water bath. The samples were reprecipitated with 0.1 ml of 60% PCA, centrifuged and the pellets

washed twice with 0.2 N PCA, collecting the supernatant from each spin to a final volume of 5 ml. Aliquots of this were taken to measure radioactivity; optical density at 260 nm was determined on the remainder using a Gilford spectrophotometer. DNA content of the total homogenate and nuclear fraction was determined by treating the pellets after washing out the hydrolyzed RNA with 1.5 ml of 0.5 N PCA for 45 min at 95°C. The pellets were centrifuged and washed twice with 0.5 N PCA, and the supernatant fractions collected to 5 ml volume. The absorbance of the samples were then read at 265 and 284 nm; the difference was used to determine the DNA content according to a standard curve.

Electron microscope studies

Various subfractions of rat liver from the above techniques were examined in the electron microscope (see figure legends). The pellets were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.2-7.4) with sucrose added to bring vehicular osmolality to 300 mOsm. The material was then rinsed and post-fixed in 1% $OsO_4$ in the same buffer, imbedded in Epon 812, cut with a diamond knife on a Reicheart ultramicrotome, and stained with 5% uranyl acetate in 50% methanol and lead citrate. Stained sections were then viewed with an Hitachi HU-12 electron microscope at 75 kV.

In vitro amino acid incorporation assay

Free ribosomes and bound ribosomes with and without their membranes were tested in the amino acid incorporation system described by Cooper et al. [29]. Each tube contained the following additions: (1) 0.4 ml of solution containing 1.25 mM ATP, 25 μM GTP, 0.5 μCi/ml [U-$^{14}$C]L-leucine, other 19 naturally occurring amino acids at

optimum concentration [30], 0.25 M sucrose, 65 mM KCl, 10 mM $MgCl_2$, 50 mM Tris (pH 7.6); (2) 0.2 ml cell sap prepared from 105 000 X g supernatant of rat liver homogenate. The cell sap proteins were separated out on a G-25 Sephadex column. The yellow band eluting off the column was collected, the protein content was adjusted to 4 mg/ml and aliquots were stored at -70°C until ready for use; (3) 0.4 ml of resuspended ribosomes (see legends for concentration). The assay was run at 37°C in a shaking water bath. Time and concentration curves were run on all samples. The reaction was stopped with cold (4 ml) 0.4 N PCA containing 1 mg/ml unlabeled L-leucine. The proteins were extracted and counted by previously described procedures [28].

## Results

### Separation and extraction techniques for free and bound ribosomes

Centrifugation of the homogenate at $162 \cdot 10^4$ g · min, resulted in optimum separation of free and bound ribosomes with no loss of RNA. The suspended material containing the free ribosomes was centrifuged through both a 3-step gradient, which pellets out the free ribosomes on the bottom and leaves the contaminating bound ribosomes at the interphase of the 1.38 and 2.0 M sucrose, and a 2-step gradient which pellets out all of the ribosomes in the suspension. Using this procedure we found only about 3% contamination of bound RNA in the supernatant fraction. The bound ribosomes contained in the pellet from the first spin were resuspended in cell sap from high speed centrifugation of normal rat liver which contains an endogenous ribonuclease inhibitor [21]. After removal

of the nuclei, the bound ribosomes could be isolated most efficiently and quantitatively by centrifugation at 105 000 X g for several hours.

The distribution of polysomes in free and bound ribosomal fractions is shown in Fig. 1. Typical of previously reported findings [21], the free ribosomes had a larger distribution of heavier polysomes than were normally found in the bound ribosomes. However, the presence of polysome aggregates in both fractions was evidence of the fact that the polysomes were intact at least through isolation of the ribosomal fractions.

Electron microscopy studies also provided visual evidence of good separation of free and bound ribosomes. The free ribosomes isolated through a 3-step gradient were evenly distributed in each microscopic field with little evidence of contamination by membranes or membrane-bound ribosomes (Fig. 2). Free ribosomes prepared through a 2-step gradient showed minimal contamination of membrane-bound ribosomes. The electron micrograph prepared from the pellet of the initial centrifugation ($162 \cdot 10^4$ g $\cdot$ min) which contained the bound ribosomes showed most of the ribosomes to be associated with membranes (Fig. 3).

The use of phenol-chloroform for the extraction of RNA from ribosomes is predominant in the literature [23,24]. However, each extraction step results in a small loss of RNA. By measuring the RNA content of the ribosomes before and after extraction, precipitating overnight and washing, the loss of RNA could be determined. After three to five extractions and ethanol precipitation, 25-35% of the starting RNA was lost, whereas essentially all of the RNA was recovered using the detergent extraction technique.

The distribution of RNA in the subfractions of rat liver using the above described procedure is shown in Table I. Although the bound ribosomes constitute almost 60% of the total RNA, the mRNA associated with them was less than 0.1%. However, the free ribosomes, which were less predominant than the bound, contained about four times as much mRNA. Determination of the DNA content of the total homogenate and nuclear fractions has consistently yielded a 90-95% recovery of DNA in the nuclei. This observation plus the low RNA content provides evidence of good quantitative recovery of nuclei with negligible contamination of bound ribosomes.

Effect of infection on RNA distribution and specific activity

Table II shows the results of several experiments in which rats were exposed 16 h earlier to $10^7$ virulent S. pneumoniae and were compared to control rats given heat-killed organisms. There was a tendency toward an increase in hepatic RNA in response to infection in the total homogenate and nuclear fractions, but the only significant increase was observed in the bound ribosomes. A significant increase in the rate of uptake of labeled orotic acid was observed in all fractions but the nuclear.

The mRNA content and specific activities in a number of experiments are shown in Table III. Numerous runs were required due to the low yield of mRNA, particularly in the bound ribosome fraction. Little change was noted in either RNA content or specific activity in hepatic free mRNA of infected rats as compared to controls. However, infection stimulated an increase in both the concentration and specific activity of the bound hepatic mRNA in each instance, which was significant when compared to controls using a paired-t test.

Effect of infection on translational activity

The rate of incorporation of labeled amino acids by free ribosomes and bound ribosomes in the presence and absence of their membranes is shown in Fig. 4. A time-course study was done on each fraction containing 100 µg of RNA. A linear regression analysis was performed on the values from each time period, and the least square line was plotted for each sample. The free and bound without-membrane fractions were linear through 15 min. When compared there was no difference in their amino acid incorporation ability. However, the curves for the bound ribosomes in the presence of their membranes showed a significantly reduced rate of incorporation as compared to the other fractions. In order to determine if this was an inhibitory effect due to the presence of membranes, varying amounts of the three fractions were tested for amino acid incorporation ability after 15 min incubation. Linear regression curves for each sample were plotted from the log of the values at each concentration. The results shown in Fig. 5 demonstrate that with an increase in concentration of the membrane-associated bound ribosomes, the rate of incorporation of amino acids was depressed. Smaller decreases were observed in the other fractions, while the free ribosomes showed the least effect with increased concentration.

Studies were also conducted on the effects of infection on translation rates in free and bound ribosomes. When the amino acid incorporation rate was expressed in terms of either the amount of total RNA in the system shown in the top portion of Table IV or the amount of mRNA added, shown on the bottom, there was no significant increase in activity in response to infection in either fraction.

However, if the data were converted to CPM incorporated per gram of liver, a significant increase was observed in the bound-infected ribosomes when compared to controls.

## Discussion

A method is described in which rat liver is separated into various subfractions, the RNA extracted from the ribosomal fractions and the poly A-rich mRNA separated out on oligo(dT)-cellulose columns. These procedures were carried out under well-controlled conditions and resulted in quantitative yield of the final product.

Certain precautions had to be taken in order to coordinate the various techniques into one which resulted in an active, natural product. Of initial concern was the prevention of RNA degradation during the isolation procedures, particularly in the bound ribosome fraction, since most of the ribonucleases sedimented into this fraction. The use of cell sap containing an endogenous RNAase inhibitor and glutathione, which has been shown to prevent polysome breakdown [21], was one of the measures taken to prevent breakdown of RNA associated with the bound ribosomes. It is desirable to limit the use of detergents for the isolation of bound ribosomes, since they may result in the release of RNAases and/or the disruption of the bound RNA species. The resuspended bound ribosomes were treated only with a mild detergent (Trition X-100) to release the nuclei from the endoplasmic reticulum so that they could be centrifuged down. Then the remaining ribosomes were quickly centrifuged and stored. This procedure required less time and risk of RNA degradation than procedures using stronger detergents, such as sodium deoxycholate,

with overnight precipitation through a discontinuous gradient. Also, since detergents are normally used for the extraction of RNA from ribosomes, either in conjunction with phenol-chloroform or alone, it is of little use to treat the bound ribosomes twice with detergents. The use of detergents for the extraction of RNA from ribosomes has been shown to be as effective as the more commonly used phenol-chloroform technique [25]. Its advantages are that it requires less working time, no overnight precipitation in ethanol, and results in quantitative recovery of RNA. In contrast, the use of phenol-chloroform requires numerous extractions, overnight precipitation in ethanol, and, in our hands, results in a significant loss of the starting RNA material. The RNA from free or bound ribosomes therefore can be extracted and the ribosomal and messenger RNA separated out on oligo(dT)-cellulose on the same day, and in sufficient yield to recover the small proportion of mRNA that exists, particularly in the bound ribosome fraction.

The effectiveness of the separation of free and bound ribosomes was demonstrated on electron micrographs in which very little cross-contamination of one species in the other was observed. The maintenance of integrity in both free and bound ribosome fractions was shown by the presence of the heavier polyribosomes when separated out on sucrose density gradients and the ability of the polyribosomes to function after isolation was demonstrated by their capacity to actively incorporate amino acids in an in vitro system.

Results from distribution studies using this technique showed by direct measurement that the mRNA content of the hepatic cell represents only about 0.33% of the total RNA. Although almost 60%

of the RNA was associated with the bound ribosomes and less than 20% with the free, there was four times more mRNA in the free ribosome fraction than in the bound. This implies that the bound mRNA is more efficient and/or has a longer half-life, as some investigators have theorized [7] by other less direct methods.

The capacity to measure mRNA levels directly has enabled us to determine the mRNA response to infection in hepatic cells. The significant increase in activity and quantity of mRNA in the bound fraction during S. pneumoniae sepsis along with a lack of apparent change in free mRNA suggests that there is transcriptional regulation of the types of mRNA produced. The infection thus appears to cause an overall stimulation of the nonspecific RNA associated with the translational process along with an increase in production of the mRNA specific for the production of exportable proteins to be used in the host's defense. This is in agreement with recent findings of increases in specific serum proteins in response to infection [31] and fits well into the signal hypothesis mechanism proposed by Blobel and Dobberstein [12] in which the mRNA triggers the attachment of the ribosomes to the membrane. Therefore, the specificity for binding and hence, the quantity of bound polyribosomes, appears to be dependent on the quantity of bound mRNA transcribed, and the products on the specific type of mRNA produced.

Since the capacity for free and bound ribosomes to incorporate amino acids on a per ribosome basis was not enhanced with infection, the translational process does not appear to be involved in the regulatory process. It merely acts as the machinery for protein production. When more is available, there are more proteins produced.

However, care must be taken in interpretation of the results from an in vitro amino acid incorporation system, since the bound ribosomes are less active in the presence of their membranes than when stripped from them with detergents. The inhibition may be due to the "microsomal" or cirular conformation which the endoplasmic reticulum takes on when hepatic cells are broken up.

However, by removing the bound ribosomes from their membranes, a condition is created which might be more difficult to interpret. Also, in the case of infection versus noninfected controls, we are not interested in absolute rates of incorporation, but only in differences between the two groups in their capacity to incorporate amino acids. Therefore, since the membrane-bound ribosomes incorporate amino acids at a reduced, but linear, rate the differences between control and infected bound ribosomes should be more acceptable in this more natural state.

Whatever the situation may be, it has become apparent that mRNAs from free and bound ribosomes are distinct in their requirements or modes of action in relation to the translational process. Until the mechanism of action of bound mRNA is fully understood and an effective way of measuring its translational activity is found, it will be difficult to fully evaluate its involvement in the translational process.

## Footnote

[1]Begin with KOH hydrolysis for samples that were RNA-extracted and separated on oligo(dT)-cellulose. For samples containing small quantities of RNA, such as the mRNA fractions, measure $O.D._{260\ nm}$ and CPM directly.

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TABLE I

NORMAL DISTRIBUTION OF RNA IN SUBFRACTIONS FROM RAT LIVER

Quantity of RNA in each fraction is expressed in terms of the mean of all experimental values ± S.E.

| Fraction | mg RNA/g liver | % of total RNA |
|---|---|---|
| Total homogenate | 6.627 ± 0.068 | |
| Nuclear fraction | 0.490 ± 0.026 | 6.93 |
| Soluble fraction | 1.080 ± 0.012 | 15.28 |
| Free ribosomal fraction | | |
| Ribosomal RNA | 1.249 ± 0.021 | 17.67 |
| Messenger RNA | 0.0193 ± 0.0017 | 0.27 |
| Bound ribosomal fraction | | |
| Ribosomal RNA | 4.225 ± 0.025 | 59.78 |
| Messenger RNA | 0.00476 ± 0.0006 | 0.07 |

TABLE II

RNA DISTRIBUTION AND SPECIFIC ACTIVITY FROM LIVER SUBFRACTIONS OF INFECTED AND CONTROL RATS

All values are expressed in terms of the mean of all experimental values ± S.E.

| Fraction | RNA content (mg RNA/g liver) | | | Specific activity (DPM/μg RNA) | | |
|---|---|---|---|---|---|---|
| | Control | Infected | t | Control | Infected | t |
| Total homogenate | 6.67 ± 0.24 | 6.98 ± 0.21 | -1.08 | 173 ± 9.1 | 227.2 ± 6.0 | -4.76* |
| Nuclear | 0.67 ± 0.07 | 0.77 ± 0.02 | -1.27 | 1099 ± 45.2 | 1012.6 ± 30.9 | 1.58 |
| Soluble | 0.97 ± 0.05 | 1.02 ± 0.04 | -0.69 | 108 ± 6.2 | 174.8 ± 5.4 | -8.25* |
| Free ribosomal | 1.61 ± 0.09 | 1.59 ± 0.10 | 0.10 | 121 ± 1.6 | 165.4 ± 0.8 | -24.2* |
| Bound ribosomal | 2.81 ± 0.12 | 3.34 ± 0.19 | -2.32* | 77 + 4.7 | 128.5 ± 0.8 | -19.4* |

*Significant difference ($P < 0.05$) between infected and control rats.

TABLE III

MESSENGER RNA FROM FREE AND BOUND RIBOSOMES OF INFECTED AND CONTROL RATS

| Experiment number | Free ribosomes | | | | Bound ribosomes | | | |
|---|---|---|---|---|---|---|---|---|
| | µg RNA/g liver | | DPM/µg RNA | | µg RNA/g liver | | DPM/µg RNA | |
| | Control | Infected | Control | Infected | Control | Infected | Control | Infected |
| 1 | 15.28 | 17.36 | 1206.2 | 1305.6 | 3.01 | 6.12 | 77.8 | 200.0 |
| 2 | 23.55 | 12.98 | 936.8 | 833.5 | 3.97 | 15.27 | 200.5 | 396.2 |
| 3 | 13.28 | 11.70 | -- | -- | 2.22 | 5.45 | -- | -- |
| 4 | 19.21 | 17.53 | -- | -- | 10.93 | 17.42 | -- | -- |
| 5 | 10.00 | 14.42 | -- | -- | 5.77 | 7.36 | -- | -- |
| 6 | 26.24 | 22.73 | 1500.0 | 1845.0 | 3.16 | 4.93 | 148.9 | 581.5 |
| 7 | 19.00 | 27.24 | -- | -- | 5.53 | 11.91 | -- | -- |
| 8 | 29.96 | 22.42 | 1390.0 | 1793.0 | 6.03 | 10.65 | 314.8 | 433.3 |
| 9 | -- | -- | -- | -- | 6.01 | 11.21 | 372.0 | 577.2 |
| 10 (control runs; no label) | 24.55<br>15.14<br>18.90<br>17.01 | | | | 4.32<br>4.29<br>3.81<br>2.81 | | | |
| Mean | 19.343 | 18.296 | 1270.7 | 1444.3 | 4.76 | 10.04 | 222.80 | 437.64 |
| ± S.E. | 1.672 | 1.912 | 131.62 | 237.08 | 0.624 | 1.475 | 53.71 | 70.13 |
| t | 0.406 | | -0.639 | | -3.685* | | -2.431* | |
| F | 18 | | 6 | | 20 | | 8 | |

*Significant difference ($P < 0.05$) between infected and controls.

TABLE IV

IN VITRO AMINO ACID INCORPORATION ACTIVITIES OF FREE AND BOUND RIBOSOMES FROM INFECTED AND CONTROL RATS

| Fraction | CPM/mg total RNA/min (mean ± S.E.) | mg total RNA/ g liver | CPM/g liver |
|---|---|---|---|
| Total RNA added | | | |
| Bound control | 94.8 ± 16.3 | 2.79 | 264 |
| Bound infected | 100.4 ± 17.0 | 3.56 | 357* |
| Free control | 713.0 ± 32.8 | 1.50 | 1072 |
| Free infected | 554.4 ± 17.5 | 1.84 | 1020 |
| | CPM/µg mRNA/min | µg mRNA/g liver | CPM/g liver |
| Messenger RNA added | | | |
| Bound control | 45.0 ± 7.7 | 5.77 | 260 |
| Bound infected | 39.7 ± 6.7 | 7.36 | 292* |
| Free control | 107.2 ± 4.9 | 10.00 | 1072 |
| Free infected | 70.7 ± 2.2 | 14.40 | 1018 |

*Significant increase ($P < 0.05$) over control values.

## Figure Legends

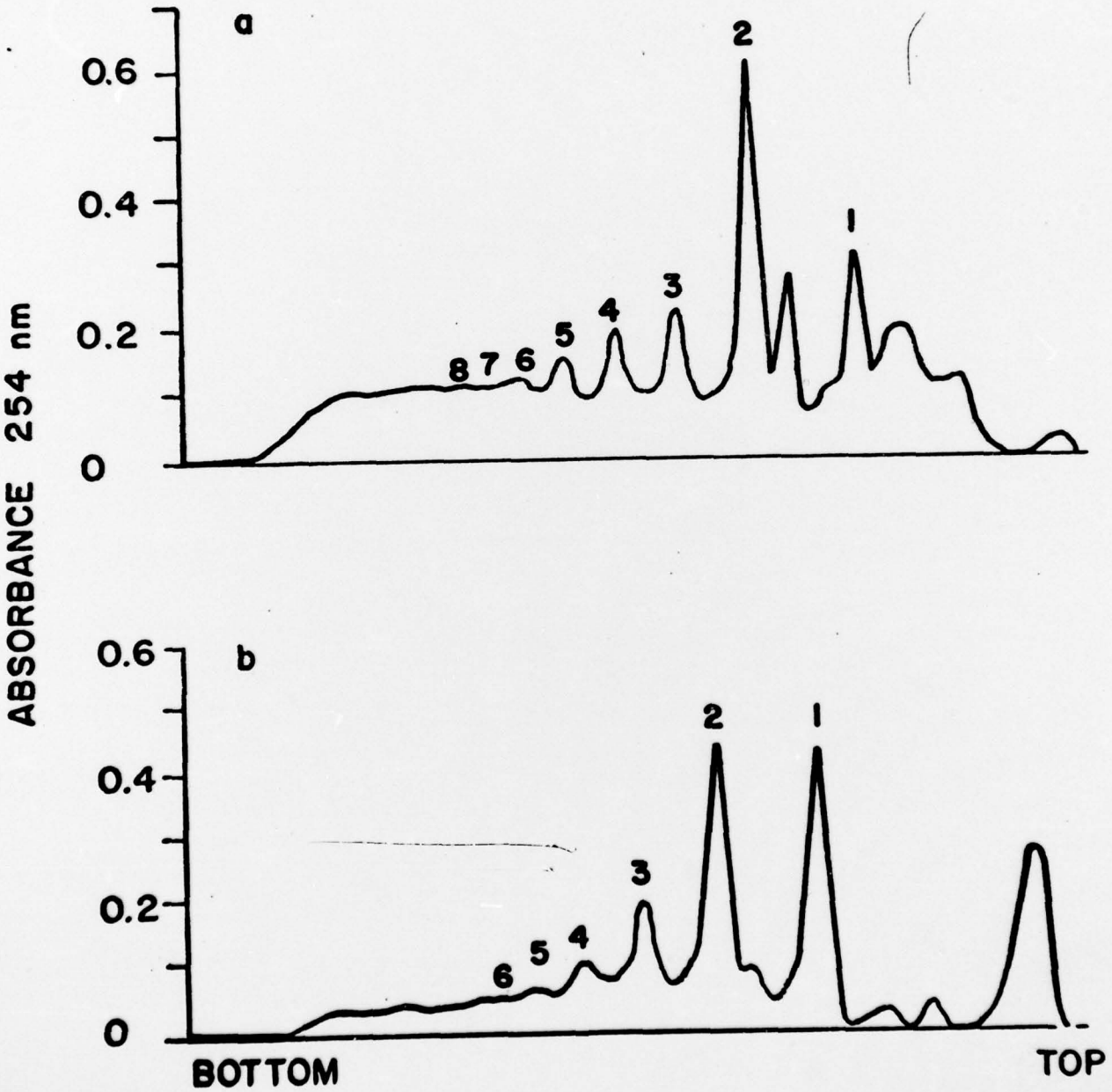
Fig. 1. Sucrose density gradient profiles of polyribosomes isolated from rat liver. a) From free polysomes; b) from bound polysomes which were treated with 1.3% sodium deoxycholate.

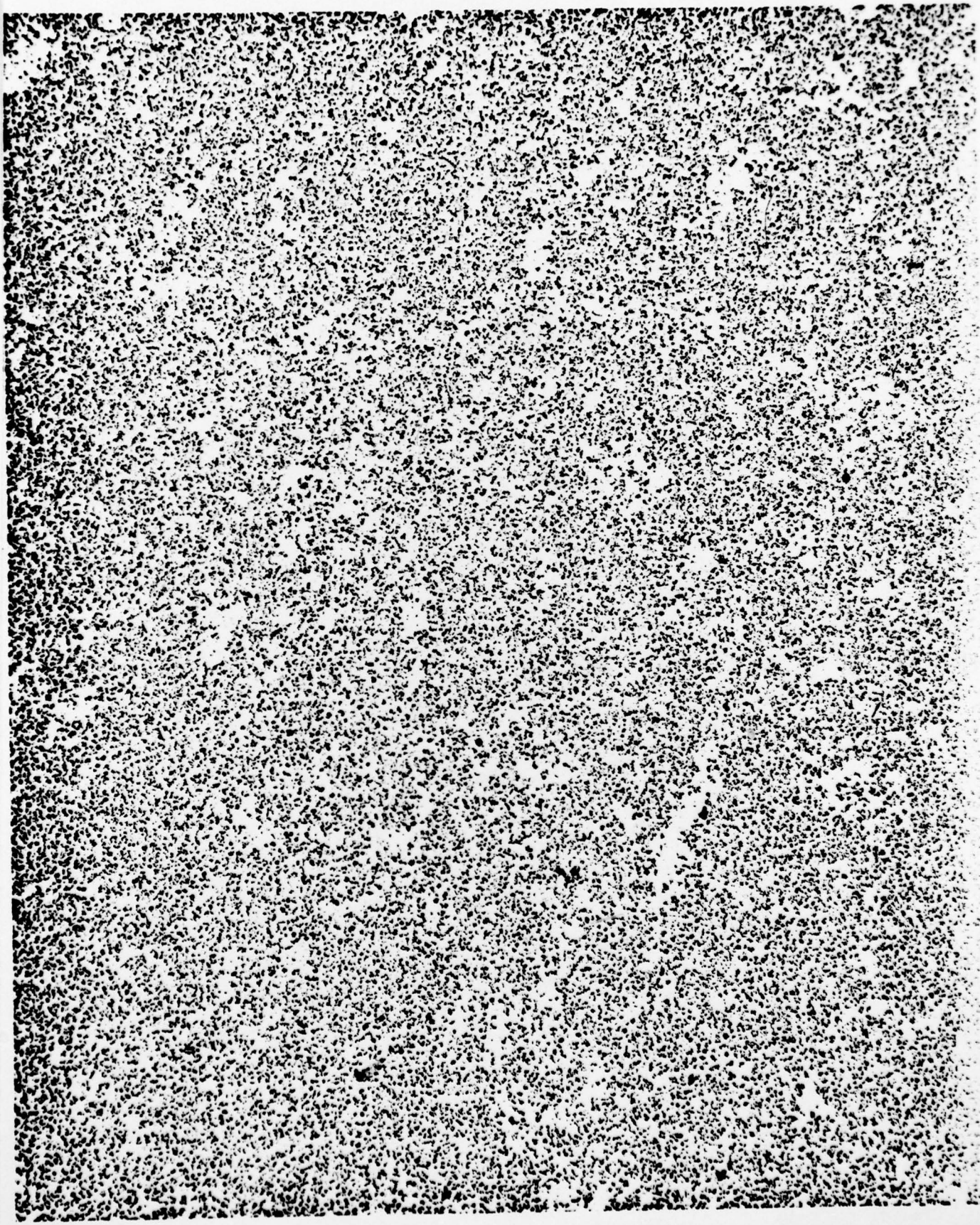
Fig. 2. Electron micrograph of free ribosomes isolated through a 3-step discontinuous sucrose gradient. Magnification, 14 400 X.

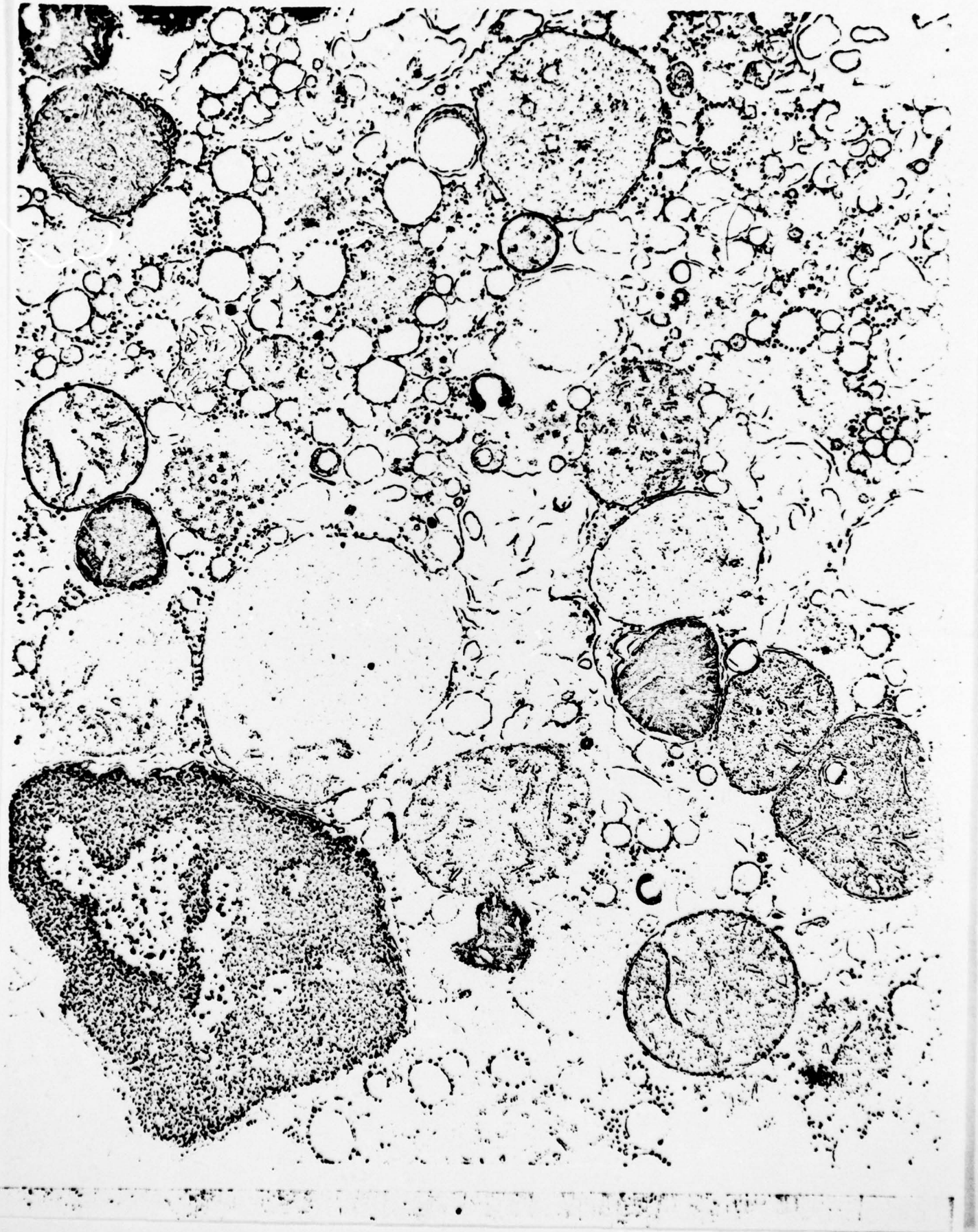
Fig. 3. Electron micrograph of sediment from initial $162 \cdot 10^4$ g · min spin illustrates that bound ribosomes attached to the endoplasmic reticulum are present as well as other cell organelles, such as nuclei and mitochondria which are shown here. Magnification, 30 600 X.

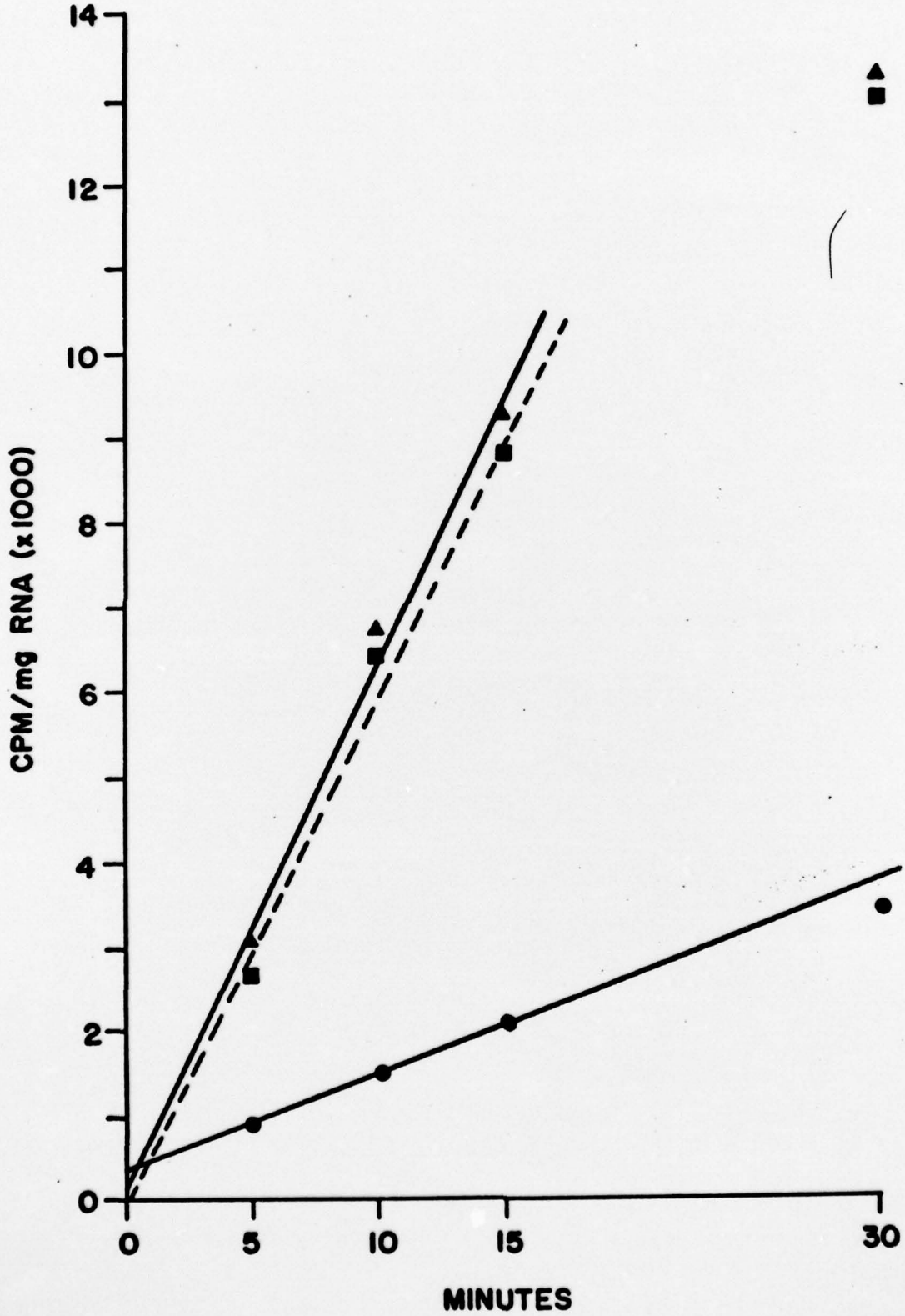
Fig. 4. In vitro amino acid incorporation rate of free and bound polysomes using 100 µg RNA per sample. A regression analysis was conducted on each sample through a 15-min incubation period and the appropriate regression curve plotted. (▲—▲) free ribosomes ($r = 0.994$); (■--■) bound ribosomes without membranes ($r = 0.989$); (●—●) bound ribosomes with membranes ($r = 0.999$).

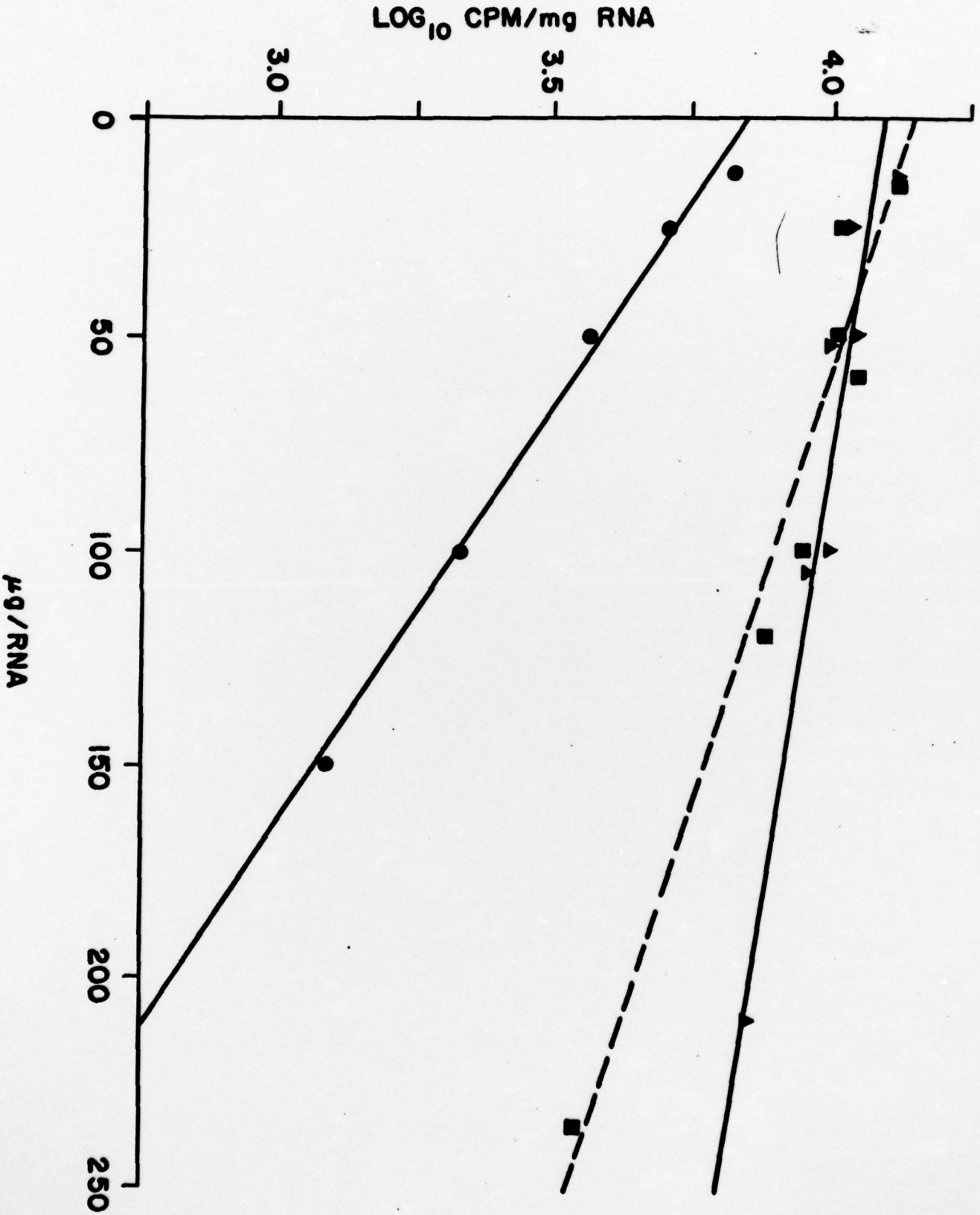
Fig. 5. In vitro amino acid incorporation of varying amounts of free and bound polysomes at 15 min incubation. The log of the value at each concentration was plotted and a regression curve determined for each fraction. (▲—▲) free ribosomes ($r = 0.925$); (■--■) bound ribosomes without membranes ($r = 0.980$); (●—●) bound ribosomes with membranes ($r = 0.990$).


a
b
0.6
0.4
0.2
0
ABSORBANCE 254 nm
1
2
3
4
5
6
7
8
BOTTOM
TOP






14
12
10
8
6
4
2
0
CPM/mg RNA (x1000)
0
5
10
15
30
MINUTES


LOG10 CPM/mg RNA
3.0
3.5
4.0
0
50
100
150
200
250
µg/RNA