Properties of Microsomes and Ribosomes from Thymus*

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The microsome fraction, ribosomes, and a fraction called M rich in lipids, isolated from cytoplasmic extracts of calf thymus were investigated. The electrophoretic and sedimentation properties of the microsome fraction are inconsistent with the expected behavior of a two-component noninteracting system. A nonlinear concentration dependence was observed in the sedimentation coefficient for the schlieren peak corresponding to the ribosome component present in the microsome fraction. The reduced sedimentation coefficient of the ribosomes, $s^0_{20,w}$, was found to be 74 S, and the RNA and lipid content 60% and 10%, respectively. Fraction M contained 1-4% RNA and 35% lipid. Results are interpreted in terms of reversible interaction between two molecular species M and R (ribosomes) in which a series of products M_iR_j are formed. Where i and j are of sufficient magnitude, the product sediments rapidly and is removed from the reaction. Additional large aggregates continue to form during centrifugation and leave the boundary region thereby reducing the area under the schlieren pattern. A major portion of the interaction product consists of a complex MR, permitting qualitative description in terms of the Gilbert and Jenkins treatment of a bimolecular reaction. The interpretation emphasizes dynamic aspects of the microsome fraction which would seem to prevail in the cytoplasm of the thymocyte.

The microsome fraction is described operationally as subcellular material which, after removal of mitochondria, sediments at 100,000 × g in 1 hour (Loftfield, 1957). In the case of secretory mammalian cells the fraction consists of membranes containing lipids, covered with dense ribonucleoprotein particles called ribosomes (Butler, 1961). Free ribosomes, that is, particles unassociated with membrane, are abundant in bacterial and nonsecretory mammalian cells (Butler, 1961; Birbeck and Mercer, 1957) Ribosomes from a variety of sources have similar chemical and physical properties (Petermann and Hamilton, 1961; Kuff and Zeigel, 1960; Utsunomiya and Hess, 1961; Hamilton *et al.*, 1962; Pogo *et al.*, 1962). The particles contain 40–65% RNA, and show a discrete spectrum of sedimentation coefficients dependent in quantitative disposition upon *p*H and magnesium ion concentration (Chao, 1957; Butler, 1961; Petermann and Hamilton, 1961).

In the process of isolating, purifying, and characterizing ribosomes from calf thymus lymphocytes (Hess *et al.*, 1962) it was evident that sedimentation properties of purified ribosomes differ from those in the microsome fraction.

In this report we present evidence indicating that, in the case of cytoplasmic extracts from thymus, ribosomes and a fraction rich in lipids exist in equilibrium with an interaction product. Results have been interpreted in terms of an interaction between two molecular species M and R, in which a series of products M_iR_j are formed. Where i and j are of sufficient magnitude, the product sediments rapidly even with moderate centrifugal force, and is removed from the reaction. Additional large aggregates continue to form during centrifugation and leave the boundary region, thereby reducing the area under the schlieren pattern. A major portion of the interaction product consists of a complex MR, permitting qualitative description in terms of the Gilbert and Jenkins (1959) treatment of the biomolecular reaction. The interpretation emphasizes dynamic aspects of the microsome fraction which would seem to prevail in the cytoplasm of the thymocyte.

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Experimental Procedure

Preparation of Microsome Fraction.—Freshly procured calf thymus, from which fat and other extraneous elements had been removed, was stored at −25° until used.[1] After thawing, the tissue was passed through an onion press, and mixed with 0.05 ionic strength potassium phosphate buffer, *p*H 7.6, containing 0.002 M $MgSO_4$ (standard buffer), 3 volumes/g of wet thymus, and stirred for 10 minutes at approximately 1000 rpm. Subsequent fractionation steps are indicated in a schematic diagram, Figure 1.

Preparation of Ribosomes and Fraction M.—The preparation of ribosomes and fraction M is outlined schematically in Figure 2. Fraction DOC-105_{sed}[2] contains small amounts of impurity, presumably consisting of the same material as present in fraction M, which was incompletely removed by deoxycholate treatment. Efforts designed to remove the contaminating materials will be discussed.

Electrophoretic Studies.—Moving boundary electrophoresis experiments were carried out as described previously (Hess *et al.*, 1961). Samples were removed from the electrophoresis cell by means of a 20-inch, 22-gauge stainless steel needle attached to a syringe mounted on a rack and pinion.

Ultracentrifuge Studies.—Sedimentation studies were carried out at 4–6° and 20° as described previously (Hess *et al.*, 1961). The only differences in sedimentation behavior observed were those which could be ascribed to viscosity and density factors resulting from temperature differences. All data reported, therefore, are based upon experiments carried out at 20°. Synthetic boundary experiments were carried out in a 12-mm cell with a 2° sector modified according to the method of Kegeles (1952). Planimetric measurements of enlargements of schlieren patterns were used to obtain area relationships, with baselines established optically using buffer solutions in the antipodal cell.

Chemical Analyses.—Lipid values are based upon Soxhlet extraction of lyophilized material with 3:1 alcohol-ether mixture. Nucleic acid (RNA) analyses

[1] In the work reported here we have found no differences in extracts prepared from fresh or frozen tissue.

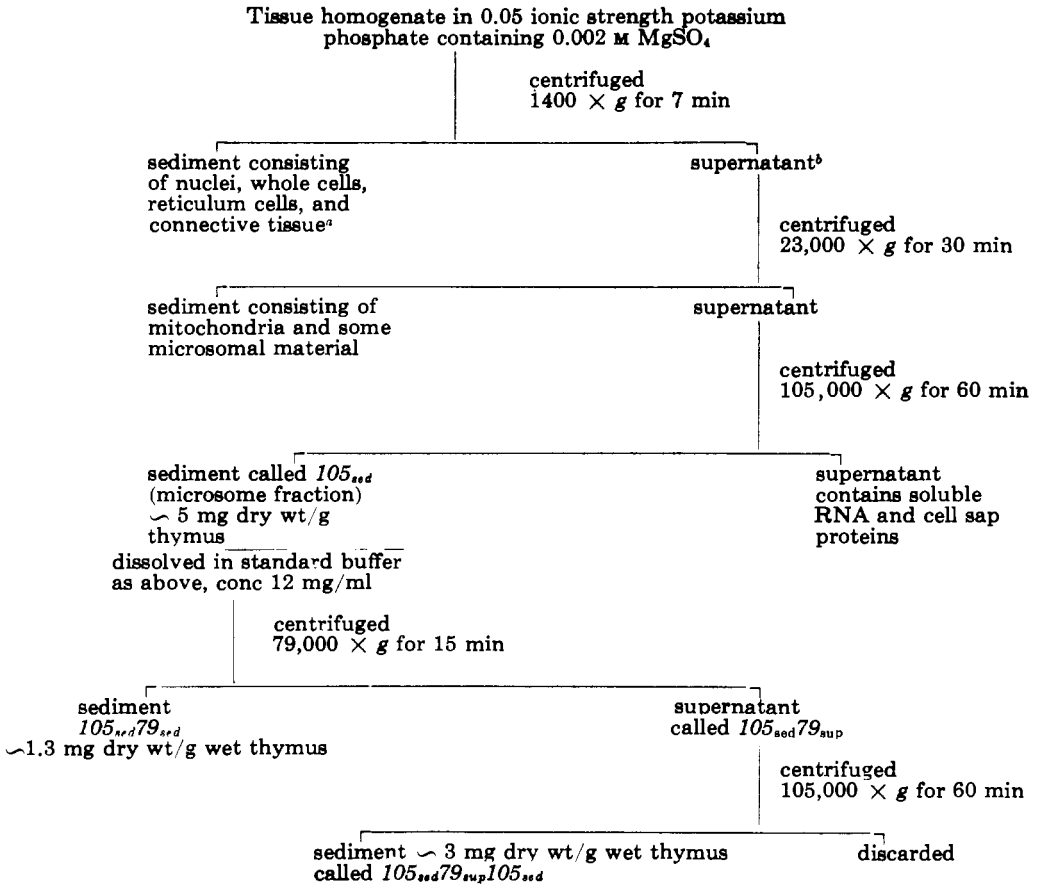

FIG. 1.—Schematic procedure for preparation of microsome fraction. [a] Additional material with characteristics of substances in the supernatant solution obtained at 1400× *g* was extracted when sediments were treated a second and third time in the manner described. Approximately 40% of the dry weight of materials in the first extract was obtained in the combined second and third extractions. [b] The materials in the supernatant represent the cytoplasm of the lymphocyte (Hess *et al.*, 1961; Herranen and Brunkhorst, 1962); connective tissue, lymphocyte nuclei, and most of the reticulum cells are found in the sediment (Allfrey *et al.*, 1957; Herranen and Brunkhorst, 1962).

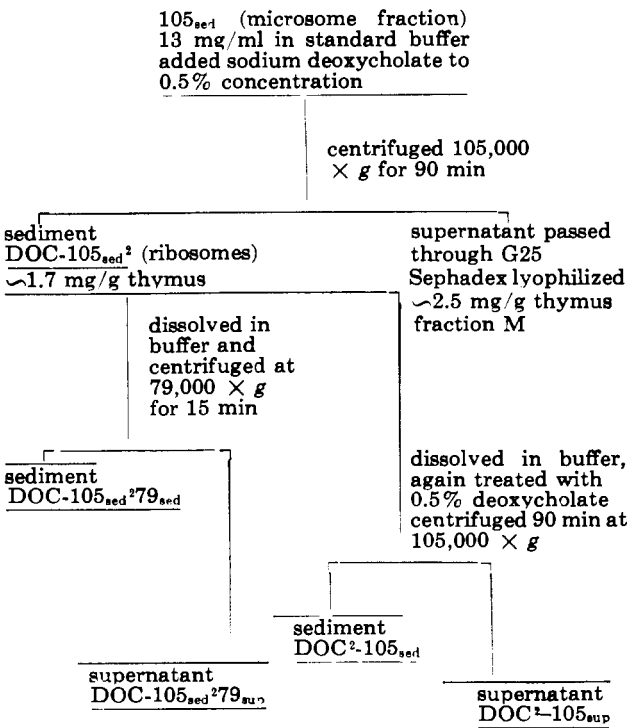

FIG. 2.—Schematic procedure for preparation of ribosomes and fraction M.

were carried out according to the procedure of Schneider (1945) using lyophilized material and an extinction coefficient $E_{260}^{1\%} = 248$. Analyses employing the orcinol procedure (Mejbaum, 1939; Davidson, 1947) were found to agree reasonably well with the Schneider procedure as can be seen in Table I. Absence of DNA in all preparations is based upon the failure to obtain color with the Dische (1930) diphenylamine reagent.

The RNA content of purified ribosomes refers to material dried *in vacuo* at 105°. All fractionation operations were carried out at 2–4°.

TABLE I

NUCLEIC ACID AND LIPID CONTENT OF SUBCELLULAR FRACTIONS

The data represent average values based upon a number of determinations and different preparations.

Fraction	RNA Content (%)		Lipid Content (%)
	Schneider	Orcinol	
105_{sed}	20–22		16
$105_{sed}79_{sed}$	10–12		
$105_{sed}79_{sup}$	27–33		16
DOC-105_{sed}[2]	56–58		
DOC-105_{sed}[2] 79_{sed}	30–36		9
DOC-105_{sed}[2] 79_{sup}	58–61	59–62	9–11
DOC[2]-105_{sed}	59–62	60–64	9–11
M	1–4		35

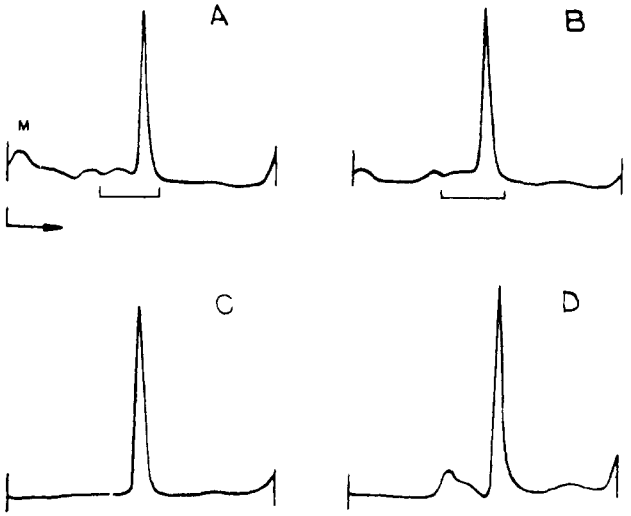

FIG. 3.—Ultracentrifuge patterns from calf thymus lymphocyte cytoplasm. Solvent system 0.05 ionic strength potassium phosphate buffer, *p*H 7.6, containing 0.002 M magnesium sulfate. Photographed approximately 1250 seconds after rotor attained speed of 33,450 rpm, phase plate angle 55° except plate C where the angle was 45°. (A) 105_{sed}; (B) $105_{sed}79_{sup}$; (C) DOC-105_{sed}?79_{sup}; (D) DOC?-105_{sed}.

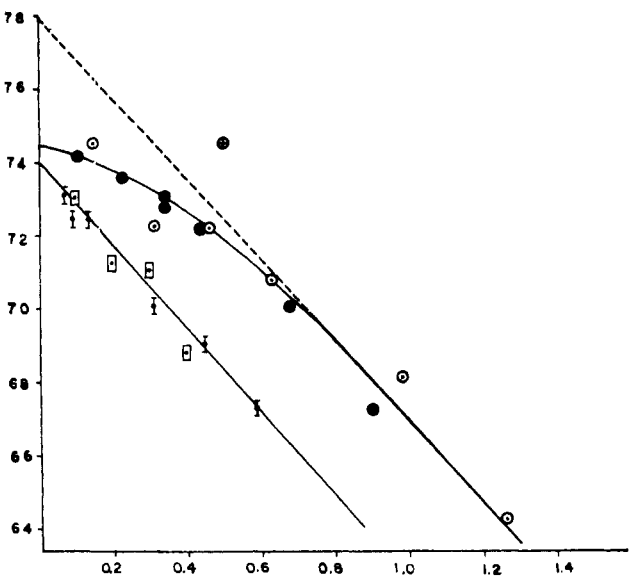

FIG. 4.—Concentration dependency of sedimentation coefficients. Buffer system as stated in the legend to Figure 3. ⊙, 105_{sed}; ●, $105_{sed}79_{sup}$; ‡, DOC-105_{sed}?79_{sup} preparation W80; ⊡, DOC-105_{sed}?79_{sup} preparation W70; ⊕, a synthetic mixture of ribosomes and fraction M; ordinate $s_{20,w} \times 10^{13}$; abscissa concentration in g/100 ml.

RESULTS

Microsome Fraction.—In the analytical centrifuge the 105_{sed} fraction gives the typical pattern seen in Figure 3A. The sedimentation coefficient of the major component, which appears to be free ribosomes, is substantially greater than that of purified ribosomes, and exhibits nonlinear concentration dependence as seen in Figure 4.

Solutions of the 105_{sed} fraction show a characteristic opalescence or turbidity suggestive of the presence of relatively large particles causing considerable scattering of light. A brightening of the viewing screen during the acceleration phase of the ultracentrifuge rotor indicated that the solution cleared during this period. In preparations of ribosomes from liver (Sherman and Petermann, 1961), rabbit appendix (Takata and Osawa, 1957), and streptococci (Utsunomiya and Hess, 1961) turbidity associated with a second component in electrophoretic patterns has been noted.

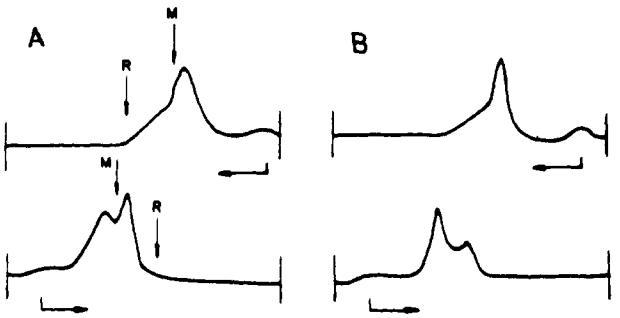

FIG. 5.—Electrophoretic patterns at *p*H 7.6 in 0.016 M potassium phosphate buffer containing 0.048M NaCl and 0.003 M $MgSO_4$. Photographs taken after 120 minutes under a potential gradient of 4.25 volt cm^{-1}. Upper patterns from ascending limb of the cell. Vertical arrows indicate corresponding positions of boundaries of ribosomes and fraction M run separately under equivalent conditions. (A) 105_{sed} 79_{sup}, conc 1.2%; (B) 105_{sed} 79_{sed}, conc 0.8%.

A comparison of relative areas of patterns at 3000 rpm and immediately after acceleration of 33,450 rpm using a synthetic boundary cell, allowing for radial dilution and stretching of the rotor, confirmed the presence of material removed from the boundary region during the acceleration step. A progressive decrease in the area of the faster component with time, greater than can be ascribed to radial dilution, indicated that interactions took place during the experiment. The pattern seen in Figure 3A represents, therefore, only the optically recorded portion of the 105_{sed} fraction.

The 105_{sed} fractions centrifuged at 79,000 × *g* for 15 minutes yield supernatants relatively free of material causing turbidity. The centrifugal effect during this period of centrifugation is approximately equivalent to that obtaining during experiments from which patterns such as those in Figure 3A were derived. The sediment, which amounted to about 30% of the 105_{sed} fraction, and which should include all material not optically recorded in Figure 3A, contained 10% RNA. The pattern obtained from fraction $105_{sed}79_{sed}$, when dissolved in buffer and examined in the analytical centrifuge, was qualitatively identical to that seen in Figure 3A. Quantitatively, the area fraction of the material labeled M was proportionately larger than that seen in Figure 3A. The ultracentrifuge pattern seen in Figure 3B was obtained from the supernatant solution, i.e., fraction $105_{sed}79_{sup}$ which contained 33% RNA.

The electrophoretic patterns characteristic of the sediment and supernatant materials are shown in Figure 5. Although the patterns differ quantitatively, the qualitative similarity is striking and provides no evidence of material in the sediment which is not present in the supernatant.

It is also significant that, as indicated in Table II, a component having a mobility corresponding to that of free ribosomes does not appear in the pattern seen in Figure 5A. In the case of both fractions, lack of enantiography between patterns from the ascending and descending limbs of the electrophoresis cell suggests an interacting system. In the case of the supernatant, samples removed from the forward edge of the boundary region in the ascending limb of the cell, according to ultraviolet absorption behavior, contained more than 50% RNA, whereas samples removed from the slowest

TABLE II

ELECTROPHORETIC MOBILITY DATA IN PHOSPHATE BUFFER

Buffer contained 0.02 M phosphate, 0.003 M Mg^{++}, and 0.048 M NaCl, at *p*H 7.6. Corresponding schlieren patterns are shown in Figures 5, 6, and 8.

	$u \times 10^5$ cm^2 volt^{-1} sec^{-1}			
	Descending Limb		Ascending Limb	
Microsomes	−5.3	−6.8	−5.9	−7.7
Fract. M + Ribosomes	−5.1	−7.9	−5.2	−8.4
Ribosomes		−8.6		−9.0
Fraction M		−6.7		−6.6

moving boundary region in the descending limb contained less than 10% RNA. The schlieren pattern seen in Figure 5B indicates that the interaction product on a gravimetric basis consists of several parts fraction M per part of ribosomes, although for interacting systems, as discussed below, such an interpretation may be misleading.

Synthetic boundary experiments using the 105_{sed}-$79_{sup}105_{sed}$ fraction gave results similar to those obtained with the original 105_{sed} fraction, i.e., area decreases indicative of the removal of heavier interaction products from the boundary regions during the experiment.

By means of alternate centrifugation at 79,000 × *g* for 15 minutes and 105,000 × *g* for 60 minutes, and recycling top and bottom fractions obtained from each step, a fairly pure ribosome fraction can be obtained. The procedure is tedious and from the point of view of yield, inefficient.

Ribosome Fraction.—The RNA content of purified ribosomes in a series of preparations varied from 58% to 62% and averaged 60%.

Examined in the analytical ultracentrifuge, ribosomes give the typical pattern seen in Figure 3C. As has been noted by previous workers (Chao, 1957; Petermann and Hamilton, 1961), the relative amounts of various components seen in schlieren patterns depend upon the concentration of Mg^{++}. The sedimentation coefficient of the major component seen in Figure 3C is 68 S. Extrapolated to infinite dilution a value $s^0_{20,w}$, approximately 74 S was found, as shown in Fig. 4.

Electrophoretic patterns typical of 74 S ribosomes are shown in Figure 6. Not only is the mobility of the ribosomes affected by Mg^{++} concentration as reported by Petermann (1960) but the enantiography of the patterns is altered. Under conditions where the patterns from the ascending and descending limbs are reasonably enantiographic, i.e., 0.005 M Mg^{++}, ultracentrifuge patterns indicate a smaller weight fraction of 74 S particles. At Mg^{++} concentration 0.005 M a considerable quantity of solute exists as aggregates of such size as to sediment prior to the rotor coming to a running speed of 33,450 rpm. As a consequence the area under the schlieren peaks is less than expected from the known concentration of solute. According to ultracentrifugal analysis the largest weight fraction of ribosomes in the form of 74 S particles is obtained at Mg^{++} concentrations 0.001–0.003 M. Conversely, at Mg^{++} ion concentration of 0.002 M, where ultracentrifuge analyses indicate 90% or more of the solute is in the form of 74 S particles, the pattern in the ascending limb of the electrophoresis cell has degenerated, as seen in Figure 6A.

Purity of Ribosomes.—The purity of ribosome preparations was judged by analytical ultracentrifugation, by moving boundary electrophoresis, by RNA content, and by the results of additional purification procedures.

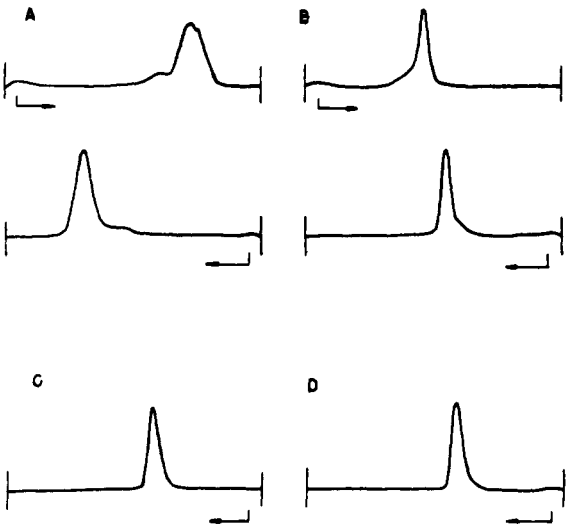

FIG. 6.—Electrophoretic patterns of ribosomes under various conditions. A and B upper patterns from ascending limb of cell; lower patterns from descending limb. C and D patterns from descending limb only. Potassium phosphate buffer *p*H 7.6 in all cases. (A) 0.020 M phosphate 0.002 M $MgSO_4$, photograph after 120 minutes, E = 5.9 volt cm^{-1}; (B) 0.013 M phosphate 0.005 M $MgSO_4$, photograph after 90 minutes, E = 4.5 volt cm^{-1}; (C) 0.016 M phosphate 0.003 M $MgSO_4$, photograph after 120 minutes, E = 4.1 volt cm^{-1}; (D) 0.016 M phosphate 0.003 M $MgSO_4$ 0.048 M NaCl, photograph after 130 minutes, E = 3.4 volt cm^{-1}.

In the case of ultracentrifuge patterns, as seen in Figures 3A, 7B, and 7C, there is very little movement of fraction M material. The schlieren patterns seen in Figures 3C and 7A may be deceptive in suggesting that fraction M is essentially absent in ribosome preparations. If fraction M interacts with ribosomes some complex is carried down during the acceleration phase. If the forward reaction rate is relatively rapid, equilibration continues during centrifugation and depletion of the slow component occurs (Schachman, 1959).

Ultracentrifugal analysis using a synthetic boundary cell provides a more sensitive criterion of purity. In the case of *DOC-105$_{sed}$*^{2}, an 8% decrease was found when the area under the pattern obtained at 33,450 rpm was compared with the area of the pattern at 3,000 rpm, whereas only 2% could be ascribed to radial dilution. A slight but significant decrease in area in excess of that attributable to radial dilution continued throughout the experiment. The area changes are consistent with the viewpoint of an interaction continuing during sedimentation.

The small pellet which forms when *DOC-105$_{sed}$*^{2} is centrifuged at 79,000 × *g* for 15 minutes contains 30–36% RNA and approximately 9% lipid, and consists of a mixture of ribosomes and fraction M as judged by ultracentrifugal and electrophoretic analysis. The material remaining in the supernatant contains 58–61% RNA and is nearly devoid of fraction M as judged by moving boundary electrophoretic analysis. When the supernatant was examined in the ultracentrifuge using a synthetic boundary cell the fraction M material was barely detectable, as seen in Figure 7A. More significant, however, was the area relationship. A decrease in area of only 5% was observed upon acceleration to 33,450 rpm of which 2% could be ascribed to radial dilution.

The difficulties encountered in removing small amounts of fraction M present in *DOC-105$_{sed}$*^{2} by sedimentation procedures led us to try a second treatment with deoxycholate. To DOC-105_{sed}^{2} dissolved in the

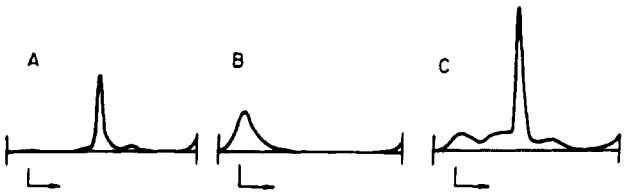

FIG. 7.—Ultracentrifugal patterns from preparations examined with the synthetic boundary cell. Buffer 0.020 M potassium phosphate, 0.002 M $MgSO_4$, *p*H 7.6, in all cases. Rotor speed 33,450 rpm, time approximately 1000 seconds. (A) DOC-$105_{sed}279_{sup}$ (ribosomes); (B) Fraction M; (C) $105_{sed}79_{sup}$ (microsomes).

same buffer as in the earlier step was added deoxycholate to 0.5% concentration and the mixture was centrifuged for 90 minutes at 105,000 × *g*. The pellet was not completely soluble in buffer. The insoluble portion was removed by centrifugation and the supernatant solution was analyzed in a synthetic boundary cell. The absence of a boundary representing free fraction M indicated that additional purification was achieved. Area analyses indicated that within the limits of error no changes greater than those attributable to radial dilution effects could be detected. An increase in material having sedimentation coefficients lower than the 74 S component was evident as shown in the ultracentrifuge pattern seen in Figure 3D. An RNA content of 62% provided further evidence of greater purity. According to electrophoretic analysis the soluble material did not appear to be as pure as the preparation seen in Figure 6D.

The insoluble material dissolved in water with the addition of alkali, according to ultraviolet absorption characteristics, contained less RNA than did the soluble portion. The possibility remains that the second deoxycholate treatment caused some destruction of ribosomes and the higher RNA content of the product is an artifact rather than a genuine measure of purity.

Fraction M.—Fraction M contains approximately 35% lipid and 1–4% RNA. The RNA may result from incomplete removal of ribosomal material during the separation step. Lyophilized material is soluble in water and in neutral buffers. Ultracentrifugal analyses, Figure 7B, indicate rather polydispersed material having sedimentation coefficients in the range 0–10 S. A small amount of material was removed from the boundary region throughout the centrifugation, as judged by area changes. The lack of enantiography between electrophoretic patterns from the ascending and descending limbs of the cell, Figure 8A, suggests interactions with buffer molecules. At *p*H 8.6 in 0.10 ionic strength barbital buffer a greater degree of enantiography obtains.

Recombination of Ribosomes and Fraction M.—Lyophilized fraction M and purified ribosomes were mixed in phosphate buffer and examined in the ultracentrifuge. The boundary comparable to the ribosome component sedimented at a faster rate than purified ribosomes as can be seen in Figure 4. In the case of electrophoretic analyses the mobilities of the two components seen in Figure 8B differ from those obtained when the ribosomes and fraction M were examined separately in the same buffer system, as indicated in Table II.

Addition of Ribosomes to Microsomes.—Changes in size or shape of the ribosome particles resulting from treatment with deoxycholate might have caused the change in sedimentation properties. In order to answer this question directly, purified ribosomes were added to an aliquot of microsome solution and the

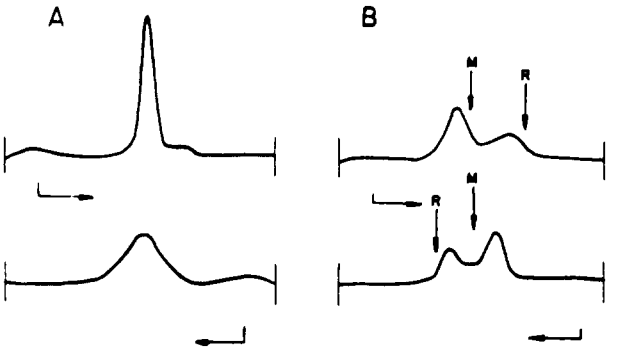

FIG. 8.—Electrophoretic patterns at *p*H 7.6 in 0.016 M potassium phosphate buffer, containing 0.048 M NaCl and 0.003 M $MgSO_4$. Photographs after 120 minutes, $E = 4.3$ volt cm^{-1}. Upper patterns from the ascending limb of cell. Vertical arrows indicate corresponding positions of ribosomes and fraction M examined separately under equivalent conditions. (A) Fraction M; (B) mixture of ribosomes and fraction M.

mixture was analyzed in the ultracentrifuge. Added ribosomes and ribonucleoprotein particles in the microsome fraction moved as a single peak indistinguishable in sedimentation behavior from the comparable boundary in the original microsome fraction.

Other Properties.—When solutions of purified 74 S ribosomes were frozen and thawed no changes in sedimentation properties were observed. In contrast, striking changes occurred when solutions of the microsome fraction were analyzed after freezing and thawing, and after results were compared with unfrozen material. The quantity of 74 S component decreased greatly and sizable amounts of 43 S and 55 S particles were observed in the schlieren patterns.

DISCUSSION

Ribosomes from lymphatic tissues appear to contain more RNA than do those from other mammalian cells. Takata and Osawa (1957) reported 66% RNA in a ribonucleoprotein obtained from rabbit appendix microsomes. Pogo *et al.* (1962) found 60–62% RNA in ribosomes from thymus nuclei. Our value of 60% RNA in ribosomes prepared from the cytoplasm of thymocytes is consistent with the above findings.

The value we obtained for the reduced sedimentation coefficient, $s^0_{20,w}$, = 74 S, for cytoplasmic ribosomes is somewhat lower than the 78 S value reported by Pogo *et al.* (1962) for nuclear ribosomes from thymus. The difference may be because of conditions employed in the ultracentrifuge studies. The value reported by Pogo *et al.* (1962) was based upon experiments carried out at 8° and corrected to 20°, whereas our value is based upon experiments carried out at 20°.

Rat liver ribosomes contain only 45% RNA and sediment as 83 S particles (Petermann and Hamilton, 1961). Ribosomes prepared from Novikoff hepatoma contain 49% RNA and the sedimentation coefficient according to Kuff and Zeigel (1960) is 79 S. In the case of rabbit reticulocytes, Dintzis *et al.* (1958) found that 82% of the particles centrifuged as a 78 S component containing 50% RNA.

Since small lymphocytes are morphologically unusual and biologically perplexing (Trowell, 1958) it is not surprising that the RNA content and sedimentation properties of ribosomes from these cells differ from those of other cells. Until ribosomes from a wider variety of cells have been characterized it would be premature to conclude that lymphocyte ribosomes are in this regard unique among mammalian cells.

The hypersharp trailing edge of the pattern seen in Figure 3C, a feature almost universally characteristic of purified ribosomes in all the buffer systems we have employed, appears to be consistent with the pronounced concentration dependence of sedimentation coefficient of the 74 S particle. Because of the self-sharpening action resulting from the concentration dependency these preparations may be less homogeneous than indicated by the schlieren patterns.

A number of observations support the viewpoint that the microsome fraction from thymus lymphocytes consists of a mixture of interacting components:

(1) Clearing of the viewing screen during acceleration indicated the presence, either suspended or dissolved, of "heavy material" which disappeared while the rotor was gaining speed. The sediment obtained when the microsome fraction was centrifuged for 15 minutes at 79,000 × *g* should have contained such material. Neither electrophoretic nor ultracentrifugal analyses of the redissolved sediment disclosed the presence of components other than those found in the 79,000 × *g* supernatant solution. Electrophoretic analysis provides a more critical test since all macromolecular solute appears in the schlieren pattern. As can be seen in Figure 5 there is no evidence for substances in the sediment which are not also in the supernatant.

(2) Chemical analyses are in accord with the interaction concept. As indicated in Table I, the $105_{sed}79_{sed}$ fraction contains less RNA than the 105_{sed}, and DOC-$105_{sed}{}^2$ 79_{sed} contains less RNA than DOC-$105_{sed}{}^2$.

(3) The relative proportions of the major boundaries, as judged by areas under the peaks, differ strikingly when schlieren patterns from ultracentrifuge and electrophoretic analyses are compared, e.g., Figure 3B and Figure 5A. The relative proportions also changed when the total solute concentration was altered by diluting stock solutions. In the case of ultracentrifuge patterns the relative area of the slower peak increased with dilution.

(4) There are progressive decreases in the areas under the peaks in the schlieren patterns during synthetic boundary experiments.

(5) The lack of enantiography between patterns from ascending and descending limbs of the electrophoresis cell is seen in Figure 5. These patterns are inconsistent with the expected behavior of a mixture of two non-interacting components.

(6) The relations seen in the concentration dependence of the sedimentation coefficients plotted in the upper curve in Figure 4 provide additional and convincing evidence for considering the microsome fraction an interacting system. The nonlinearity of the curves obtained with the microsome fraction contrasts with the linear relationship observed with purified ribosomes.

(7) When solutions of fraction M and purified ribosomes were mixed and centrifuged a value of 75 S was obtained for the sedimentation coefficient of the faster peak. This value is inconsistent with the sedimentation behavior of purified ribosomes.

(8) A pattern similar to Figure 3C was observed when purified ribosomes were added to $105_{sed}79_{sup}$. The result indicates not only that the sedimentation behavior of ribosomes is unaltered by the deoxycholate treatment, but that fraction M material was depleted in the presence of added ribosomes.

The first two observations can be explained by the presence in the microsome fraction of polydisperse material containing no, or only small amounts of, RNA, which is not recorded optically and is removed from solution during the acceleration of the ultracentrifuge rotor.

The third observation can be explained by postulating the presence of "heavy material" in the microsome fraction which is optically recorded and removed from solution during the acceleration of the ultracentrifuge rotor.

The above interpretations do not provide an explanation, however, for the last five observations. All eight observations are explicable qualitatively in terms of an interaction

$$iM + jR \rightleftharpoons M_iR_j$$

where M represents the protein fraction rich in lipid and R represents ribosome particles. According to this interpretation the larger aggregates M_iR_j are continually removed from solution during centrifugation, which accounts for the progressive decrease in area with time when the microsome fraction is examined in the synthetic boundary cell. Depletion of M occurs in the supernatant, which is consistent with what we have found. Since only a small portion of the solute disappears from the boundary region during centrifugation, the rate of accumulation of the larger aggregates must be relatively slow. As depletion of M occurs the equilibrium is shifted and smaller complexes, perhaps the bimolecular species MR, predominate. It is these lower complexes which are present presumably in the optically recorded boundaries.

The sedimentation properties of two reactants in equilibrium with a product has been discussed by Svedberg and Pedersen (1940), Gilbert and Jenkins (1956, 1959), and Schachman (1959). If, as assumed above, an interaction product of ribosome and protein sediments more rapidly than free ribosomes (R), which in turn sediment faster than (M), a number of features obtain which are consistent with results we have observed. Following the reasoning of Gilbert and Jenkins (1956, 1959), in place of two separate boundaries corresponding to the product and to the ribosomes there will occur one boundary with an intermediate sedimentation rate. The sedimentation coefficient and the shape of this boundary will depend upon the concentration of the reactants and upon the rate constants. The data plotted in Figure 4, as well as the results mentioned under (7) and (8) above, are in accord with this interpretation. Shape and hydration factors will of course also contribute to the concentration dependency relationship.

The conditions we have assumed are discussed as case (i) by Gilbert and Jenkins (1959). The theory predicts a broad generated boundary having a shape dependent upon the rate constants and the equilibrium concentrations of the reactants. It will be noted that in Figures 3A and 3B the gradient does not become zero at any point within the bracketed boundaries. Pattern 3D stands in contrast to A and B in this respect. We interpret the bracketed region as a single broad boundary throughout which a homogeneous phase cannot be generated.

The lack of enantiographic relationships seen in the patterns from the two limbs of the electrophoresis cell as seen in Figure 5 are difficult to explain except in terms of an interacting system. Case (iii) discussed by Gilbert and Jenkins (1959) appears to apply. The mobilities of the two boundaries in the pattern seen in Figure 5A, as shown in Table II, do not correspond to the mobilities of either M or R. Gilbert and Jenkins (1959) pointed out this would obtain if $v_c < v_M < v_R$, where v_c is the velocity of the complex, v_M that of the protein and v_R that of the ribosomes. We have not observed, however, hypersharp fronts predicted by Gilbert and Jenkins (1959) to occur with case (iii) under some circumstances.

Although circumstantial, available evidence indicates that fraction M represents the membrane portion of ergastoplasm. Both Haguenau (1959) and Oberling (1958) point out that "the so-called microsome fraction of cellular ultracentrifugates is composed almost exclusively of ergastoplastic components." In the process of isolating ribosomes from the microsome fraction, deoxycholate is commonly used to solubilize membrane materials. The large weight fraction of lipids characteristic of the microsome fraction is retained in the deoxycholate supernatant (Littlefield, *et al.*, 1955; Palade and Siekevitz, 1956; Moule *et al.*, 1960). In this respect our findings with thymus are consistent with the results of the above workers who used liver. In an investigation of thymus, using electron microscopy, Tanaka (1957) detected a small number of rough- and smooth-surfaced endoplasmic reticular membranes in lymphocytes.

It is of interest that Slautterback (1953) commented that small electron dense particles, approximately 25 mμ diameter, were concentrated in mouse liver microsome preparations under conditions where these particles were not expected to sediment to any extent. He concluded that these particles were carried down by membrane materials. We mention Slautterback's (1953) interpretation since his results would obtain for either a static or a dynamic association.

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