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Group Fractionation and Determination of the Number of Ribosomal Subunit Proteins from *Drosophila melanogaster* Embryos†

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ABSTRACT: Proteins were extracted from ribosomes and (for the first time) from ribosomal subunits of *Drosophila melanogaster* embryos. The ribosomal proteins were analyzed by two-dimensional polyacrylamide gel electrophoresis. The electrophoretograms displayed 78 spots for the 80S monomers, 35 spots for the 60S subunits, and 31 spots for the 40S subunits. On the basis of present information, we propose what we believe to be a reliable and convenient nomenclature for the proteins of the ribosomes and each of the subunits. A pair of acidic proteins from *D. melanogaster* appears to be very similar in electrophoretic mobility to the acidic proteins L7/L12 from *Escherichia coli* and L40/L41 from rat liver. The electrophoretogram of proteins from embryonic ribosomes shows both qualitative and quantitative differences from those of larvae, pupae, and adults previously reported by others. The proteins of the 40S subunit range in molecular weight from approximately 10000 to 50000, and those from the 60S subunit range from approximately 11000 to 50000.

The proteins of the 80S ribosomes were fractionated by stepwise elution from carboxymethylcellulose (CMC) with lithium chloride. The proteins were separated into seven groups (A–G) containing between 9 and 23 proteins each. Small samples were removed from every fifth fraction of the CMC column and analyzed by one-dimensional electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate ($NaDodSO_4$). Further fractionation of the proteins of the 80S ribosome was achieved by pooling adjacent fractions from the CMC column that shared similarities in their $NaDodSO_4$ profiles. Twenty-four pools were obtained. From the analysis of these pools by two-dimensional polyacrylamide gel electrophoresis, it was found that each pool contained between 1 and 12 major proteins. Most proteins occurred in only one pool each. This fractionation procedure has proved valuable as an initial step in the isolation and characterization in *Drosophila* proteins.

The purification and characterization of eucaryotic ribosomal proteins are important requisites for an understanding of the structure and function of ribosomes at the molecular level. Although much is known about the morphological, chemical, and immunological characteristics of procaryotic ribosomes, little is known of those from eucaryotes [see Wool & Stöffler (1974), Wittmann (1974), and Wool (1979) for reviews and references]. *Drosophila* nucleic acids have been extensively studied (Tartof, 1975; White & Hogness, 1977; Laird & Chooi, 1976), but far less information is available about their ribosomal proteins. Large differences have been detected between the protein patterns of *Drosophila* larval,

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pupal, and adult ribosomes (Lambertsson, 1975; Fekete & Lambertsson, 1978). A few preliminary studies have been directed toward the elucidation of the biogenesis of ribosomes in wild-type and some cold-sensitive mutant strains of *Drosophila* (Berger, 1977; Falke & Wright, 1975).

Ribosomes of *Drosophila melanogaster* embryos have not been previously studied. We sought to determine the number of proteins in the 80S and especially in the 40S and 60S particles from *Drosophila* embryos, since that information could prove helpful in the isolation and purification of the ribosomal proteins. Previous reports indicated either failure (Lambertsson, 1975) or only partial success (Vaslet & Berger, 1976) in the separation of clean 40S and 60S subunits from *Drosophila*.

Few of the eucaryotic ribosomal proteins have been isolated and purified in appreciable amounts, although a start has been made (Collatz et al., 1976a, 1977; Tsurugi et al., 1976, 1978; Möller et al., 1975). The purification of eucaryotic ribosomal proteins has been complicated by a number of problems. The principal difficulty lies in the greater number of proteins (78 compared with *Escherichia coli*, which has only 53). As most of the proteins are very basic and relatively similar in molecular weight, the increase in number greatly impedes their resolution.

We describe for the first time the fractionation of *Drosophila* ribosomal proteins by stepwise elution from carboxymethycellulose with LiCl. This procedure is efficient in resolving proteins into small groups that can be readily fractionated by other procedures like gel filtration.

Materials and Methods

Collection of Drosophila Embryos and Preparation of Ribosomes. Developing embryos of *Drosophila melanogaster* (Oregon R strain), 2–22 h old (25 °C), were collected and washed as described previously (Chooi & Laird, 1976).

The procedure for the isolation of ribosomes was based on a modification of the method described by Sherton & Wool (1974) for rat liver ribosomes. Dechorionated embryos were disrupted at 4 °C with a Thomas Teflon pestle tissue homogenizer in buffer A [10 mM Tris, pH 7.6, 80 mM KCl, 10 mM $MgCl_2$, 0.25 M sucrose, and 1 mM dithiothreitol (Bio-Rad)]. Unbroken nuclei and other cell debris were sedimented from the homogenate by two centrifugations in a Sorvall SS-34 rotor at 17000*g* for 20 min at 4 °C. The supernatant was then adjusted to 1% sodium deoxycholate and 1% Triton X-100 and centrifuged at 12000*g* for 20 min. This supernatant was layered over a 4-mL high salt cushion (10 mM Tris, pH 7.6, 500 mM KCl, 10 mM $MgCl_2$, and 1 M sucrose), and ribosomes were pelleted by centrifugation in a Beckman Type 60 Ti fixed-angle rotor at 360000*g* for 90 min at 4 °C. The salt-washed ribosomes were stored as pellets at −70 °C.

Preparation of Drosophila Ribosomal Subunits. Ribosomes were dissociated into 40S and 60S subunits by the method of Sherton & Wool (1974). Only the central fractions of the peaks containing the subunits were pooled. The subunits were recovered from the pooled fractions by centrifugation at 360000*g* for 20–24 h at 4 °C in a Beckman Type 60 Ti rotor.

Purity of the Drosophila Ribosomal Subunits. The purity of the isolated subunits was determined by their sucrose gradient profiles and by analysis of the amounts of the 18S and 28S ribosomal RNA (rRNA) present in each subunit fraction. After the first sucrose gradient centrifugation, the subunit fractions were pooled separately. The 40S and 60S subunits were recovered from solution by pelleting as described above. The ribosomal RNAs from each subunit fraction were extracted and separated by centrifugation according to the procedure outlined by Sherton & Wool (1974).

Extraction of Drosophila Ribosomal Proteins. Proteins were extracted from ribosomes and subunits with 67% acetic acid by using the procedure described by Sherton & Wool (1974). Ribosomal proteins were dialyzed (in Spectraphor membrane tubing, 3500-MV cutoff) against 1 N acetic acid for at least 2 days with three changes of acid. The dialyzed proteins were lyophilized and stored at −20 or −60 °C.

Two-Dimensional Polyacrylamide Gel Electrophoresis. Two-dimensional polyacrylamide gel electrophoresis was carried out in two sizes of gels, the large-scale ones initially described by Kaltschmidt & Wittmann (1970) but modified by Sherton & Wool (1974) and the microscale ones as described by Tsurugi et al. (1978). The "standard" conditions used for electrophoresis in the large gels were as follows: (1) first dimension, 8% acrylamide gel, pH 8.6, for 36 h at 90 V at room temperature; (2) second dimension, 18% acrylamide gel, pH 4.6, for 53 h at 70 V (initial current 90 mA/gel) at 4 °C. The concentration of acrylamide in the first-dimension separation gel was occasionally changed to 4% but maintaining a constant acrylamide/methylenebis(acrylamide) ratio. The amount of ribosomal proteins loaded into the first-dimension gel was also varied. Optimal amounts were 1–2 mg/gel.

The procedure for running the micro two-dimensional polyacrylamide gels was essentially that described by Tsurugi et al. (1978), which was based on the methods of Lastick & McConkey (1976) and Lin et al. (1976). However, the reducing agent for the second dimension was changed to 5% mercaptoacetic acid (Eastman Organic Chemicals), and gels were run for 5 h at 5 mA/gel. Optimal amounts of proteins were 10–30 μg for the resolution of basic proteins and about 10 times those amounts (or 220–280 μg) for acidic proteins.

Polyacrylamide Gel Electrophoresis in Sodium Dodecyl Sulfate. The molecular weights of subunit proteins were estimated by gel electrophoresis in 0.1% $NaDodSO_4$ by using the procedure of Laemmli (1970) except that the concentration of acrylamide was 15%. Fractions from the CMC column were analyzed by one-dimensional polyacrylamide gel electrophoresis using the same procedure. Samples of fractions from the CMC column were precipitated with an equal volume of 30% trichloroacetic acid (Fisher) for the analysis.

Column Chromatography. Fractionation of the 80S ribosomal proteins was performed by stepwise elution from a carboxymethylcellulose (CMC) column (Whatman CM-52) with increasing concentrations of LiCl, a procedure based on that described by Collatz et al. (1976b).

Results

Purity of the Drosophila Ribosomal Subunits. Typical profiles of ribosomal subunits recovered after centrifugation through a linear sucrose gradient are shown in Figure 1. The 60S subunits isolated after the first gradient were contaminated with a small amount of 40S subunits. Therefore, in all experiments involving the use of 60S subunits, they were purified by a second sucrose gradient (Figure 1a). However, there was no significant contamination of the 40S subunits after the first gradient (Figure 1b).

Since the 40S subunit contains only 18S RNA and the 60S subunit has 28S RNA but no 18S RNA, the purity of the isolated 40S and 60S subunits was determined by an analysis of their constituent rRNAs. Typical rRNA profiles are also shown in Figure 1. Figure 1c shows a profile of the 18S and 28S rRNAs extracted from the 80S monomers. The 28S rRNA extracted from the 60S subunits after purification in two gradients shows no significant contamination with 18S rRNA (Figure 1d). The 18S rRNA extracted from the 40S subunits after purification in one gradient shows no detectable

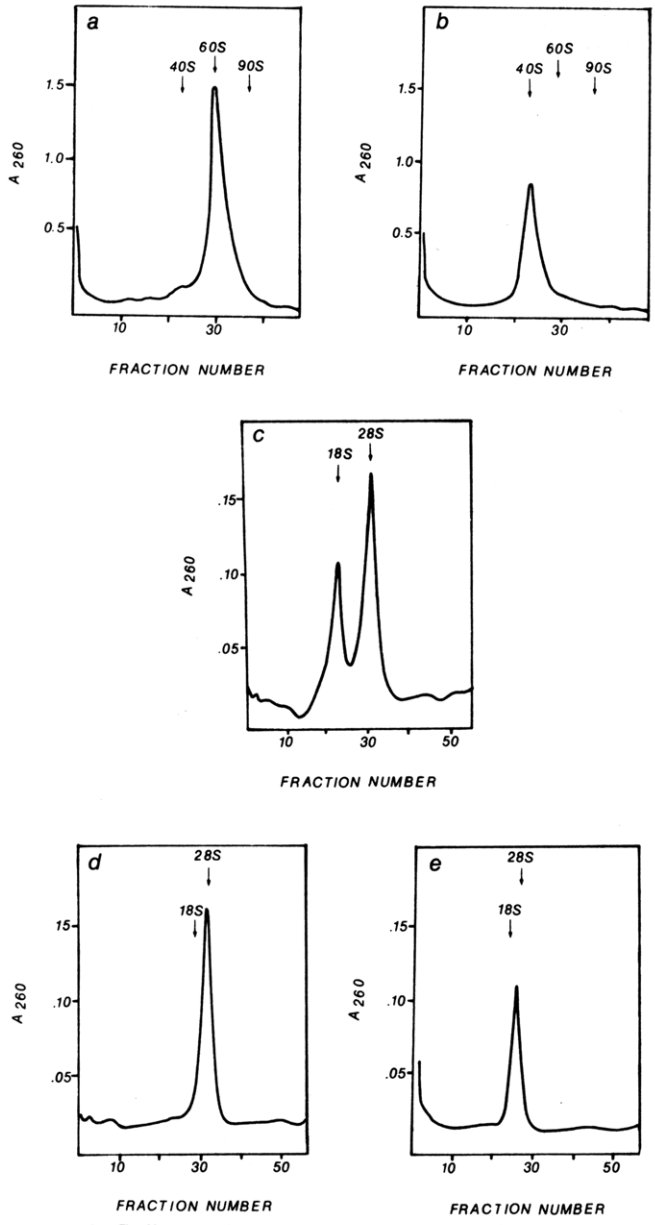

FIGURE 1: Sedimentation of subunit fractions in sucrose gradients. Subunits obtained from the first sucrose gradient centrifugation were pelleted from those fractions pooled as described under Materials and Methods. These subunits were reincubated with puromycin in buffer B, and the purity of the samples was analyzed on a second 10–30% linear sucrose gradient. (a) 60S subunits (6–8 mg). (b) 40S subunits (3–4 mg). (c) Sedimentation profile of 18S and 28S rRNAs from 80S monomers. (d) 28S rRNA from 60S subunits recovered after the second 10–30% sucrose gradient. (e) 18S rRNA from 40S subunits recovered after the first 10–30% sucrose gradient.

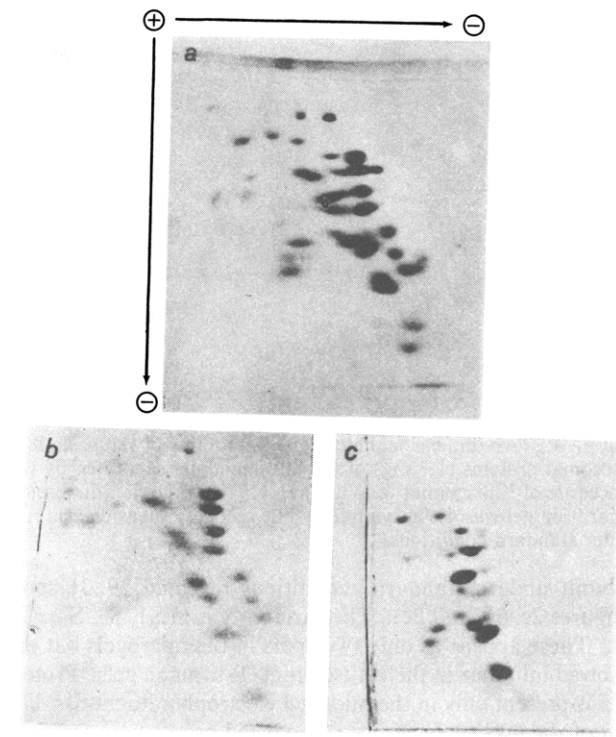

FIGURE 2: Two-dimensional microgel electrophoretograms of the basic ribosomal proteins. Standard conditions were used for electrophoresis. Origins are at the upper left corner of each of the electrophoretograms. Some of the spots on the gel were clearly visible to the eye but are barely discernible or cannot be seen in the photographs. Those proteins are included in the schematics in Figure 5. (a) 10 μg of 80S ribosomal proteins. (b) 10 μg of 60S subunit proteins. (c) 45 μg of 40S subunit proteins.

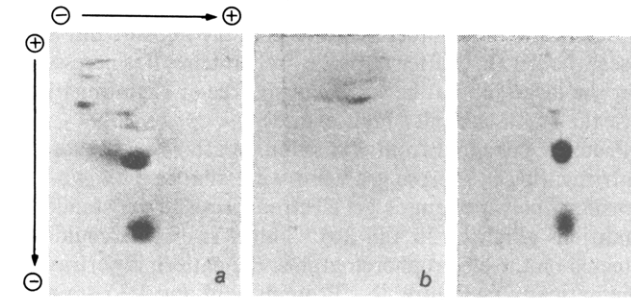

FIGURE 3: Two-dimensional polyacrylamide microgel electrophoresis of the acidic ribosomal proteins. The origins are at the upper left corner of each of the electrophoretograms. (a) 260 μg of 80S ribosomal proteins. (b) 150 μg of 60S subunit proteins. (c) 160 μg of 40S subunit proteins.

contamination with 28S rRNA (Figure 1e). Based on these results, *Drosophila* ribosomal subunits isolated in this manner were presumed to be clean.

Amounts of ribosomes are ordinarily measured by spectrophotometric absorption. Those extracted by the procedure described here yielded an estimate of 50 A_{260}/g of embryos (or 4.5 mg/g of embryos if the conversion of Sherton & Wool (1974) is adopted). The efficiency of recovery of the 40S subunit after one round of centrifugation and that of the 60S subunit after two rounds are 45 and 9%, respectively.

Protein Nomenclature. The proteins on electrophoretograms were located by staining with Coomassie Blue. The protein spots on electrophoretograms of ribosomal proteins from each subunit have been numbered separately along horizontal lines beginning at the upper left. In accordance with the conventions of Kaltschmidt & Wittmann (1970), the 60S subunit proteins are designated by an L (for the large subunit), and the 40S subunit proteins are designated by an S (for the small subunit). Proteins found in the ribosome monomers, but in neither subunit, have no letter prefix.

Some of the spots on the gel were clearly visible to the eye but are barely discernible or cannot be seen in the photographs. The schematics do not represent with fidelity the intensity of staining of the individual spots; moreover, they are composites from a large number of electrophoretograms and, as such, may differ in detail from individual gels. Material that remained at the origin in the first dimension and migrated directly toward the anode in the second dimension was not included in the schematics.

Proteins of the 40S Subunit. Two-dimensional polyacrylamide gel electrophoresis of the proteins of the 40S

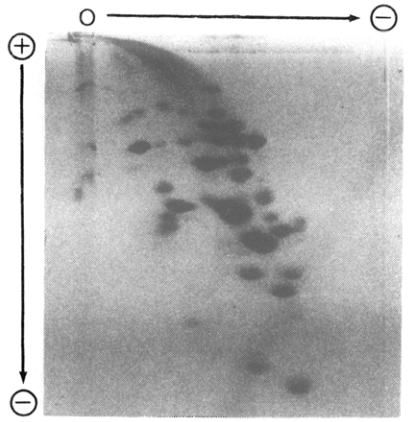

FIGURE 4: Two-dimensional electrophoretograms of the basic 80S ribosomal proteins (1.7 mg) of the 80S monomer separated by the procedure of Kaltschmidt & Wittmann (1970). The first-dimension separation gel was 4% acrylamide. The second dimension was run under standard conditions.

subunit under "standard" conditions revealed 29–31 spots (Figures 2c, 3c, and 5c). The variability is in S1, S3, S4, and S5. These appear as only two spots in the microgels but are resolved into four in the Kaltschmidt–Wittmann gels. Protein S15 is present only in the microgel electrophoretograms. The rest of the 40S spots can be reproduced precisely in terms of location and intensity.

Proteins of the 60S Subunit. When proteins extracted from the first sucrose gradient separation of 60S subunits were analyzed by two-dimensional gel electrophoresis, almost all of the proteins of the 80S monomer were present (results not shown). Thus, even though only a small number (three to five) of central fractions of the 60S subunit peak had been pooled, there was contamination with the 40S subunit (Figure 1a). To determine the number of proteins in the 60S subunit and to see whether they differ from the 40S proteins, it is necessary that the large subunit be scrupulously free of contamination with the small subunit (Figure 1a,d).

Proteins extracted from 60S subunits after *two* rounds of centrifugation in sucrose gradients were analyzed by two-dimensional polyacrylamide gel electrophoresis under standard conditions (Figures 2b and 3b). Thirty-five spots could be detected on the electrophoretograms; the pattern is portrayed schematically in Figure 5b. Proteins L34 and L35 stained lightly and were seen only when large amounts of proteins were used. Proteins L21 and L32 are found only in the microgel electrophoretograms. Acidic proteins L1, L2, L5, L6, L8, and L9 also stained lightly and were seen only when about 10 times the usual concentration of proteins was used. They tend to migrate in streaks to a region where one would expect to find proteins of the greatest molecular weight. The streaks may be caused by protein aggregates linked by disulfide bonds.

Proteins of the 80S Ribosome. Ribosome monomers were prepared by a method expected (Sherton & Wool, 1974) to yield particles free of contamination with extraneous proteins. Seventy-eight spots are resolvable during electrophoresis of the proteins extracted from the 80S ribosomes (Figures 2a, 3a, 4, and 5a). Sixty of the total migrated to unique positions apparently identical with that occupied by a protein of either the small or large subunit. Of the remaining 18 spots, six were present in electrophoretograms of both the 40S and 60S subunits. These are S20 and L24, S22 and L26, and S28 and L33. Twelve spots were not found in either of the subunit electrophoretograms. These proteins have been numbered 1–12, without an S or L prefix. Proteins L34, L35, and 9–12 are always present in the Kaltschmidt–Wittmann gel electrophoretograms. These proteins are lightly staining proteins of low molecular weight and are occasionally missing in the microgels.

Fractionation of the Proteins. Seven groups of proteins (A–G) were obtained when proteins of the 80S ribosome (TP80) of *D. melanogaster* were chromatographed on carboxymethylcellulose. Elution was at pH 6.5 with stepwise increase in the concentration of LiCl (Figure 6). The more acidic proteins did not bind to carboxymethylcellulose under conditions used here and were washed through the column with the starting buffer; these were referred to as group A proteins (A80). The six separate groups of relatively basic proteins eluted from the column stepwise with 0.135, 0.185, 0.23, 0.27, 0.3, 0.4, and 1 M LiCl were arbitrarily designated as B80, C80, D80, E80, F80, and G80, respectively. Identification of the proteins in each group was facilitated by analysis in two-dimensional polyacrylamide microgels. The sample was analyzed alone on half of the gel slab and in the presence of a small amount of TP80 on the other. The small amount of TP80 ($\simeq$10 μg) provided a background that assisted in identification of the proteins in the sample whereas examination of the sample without the TP80 permitted one to estimate the number of proteins in the pool. Except for the occasional presence of several very faintly staining spots in the vicinity of proteins 7/8 and S7, the proteins identified in A80 were similar to those seen in the electrophoretograms of the acidic proteins of TP80 (Figure 8).

Proteins in each basic group were further subdivided into pools as follows. Small samples were removed from every fifth fraction of the carboxymethylcellulose column, and the proteins were analyzed by using one-dimensional polyacrylamide gels containing $NaDodSO_4$. Neighboring fractions that shared similarities or overlaps in their $NaDodSO_4$ profiles were combined into pools. A total of 24 separate pools was obtained (Figures 6 and 7).

Table I shows the fractions from the CMC column that made up each pool of proteins and the proteins identified in each group. The proteins in each group are denoted in Table I as being present either in major amounts or in minor amounts. These designations refer to darkly staining spots that were always present vs. poorly staining spots. Some faintly staining spots were present only occasionally. Each pool contained between 1 and 12 major proteins. However, if the minor components of each pool were included, then each pool would have between 2 and 16 proteins. Pools 1, 2, 3, and 5 did not have significant amounts of any protein since they were derived from the flat parts of the graph (Figure 6). Although we obtained a few faint bands on deliberate overloading of $NaDodSO_4$ gels, we did not get any definitive spots on the two-dimensional polyacrylamide microgels.

Of the 63 basic ribosomal proteins that were identified with certainty after fractionation, 30 to 31 were eluted in only one pool each: 1, 2, 3, 7, 9, 11, 13, 14, S2, S11, S13, S17, S18, S21, S29, S30, S31, L1, L7, L13, L14, L15, L18, L23, L25, L27, L28, L30, L32, L33/28, and L34.

Seventeen to nineteen proteins were in one pool each in large amounts and in a second (and occasionally a third as in the case of L10, L11, and L19) in small quantities. These pools were adjacent to each other and their proteins were eluted in the same LiCl step. The proteins in this category were S8, S10, S12, S16, S23, S28/L23, L4, L10, L11, L16, L17, L19, L22, L23, L26/S22, L29, and L31. S24 was found in four pools in the same LiCl step.

Thus, at least 48–50 proteins out of the 63 are fractionated in one LiCl step each.

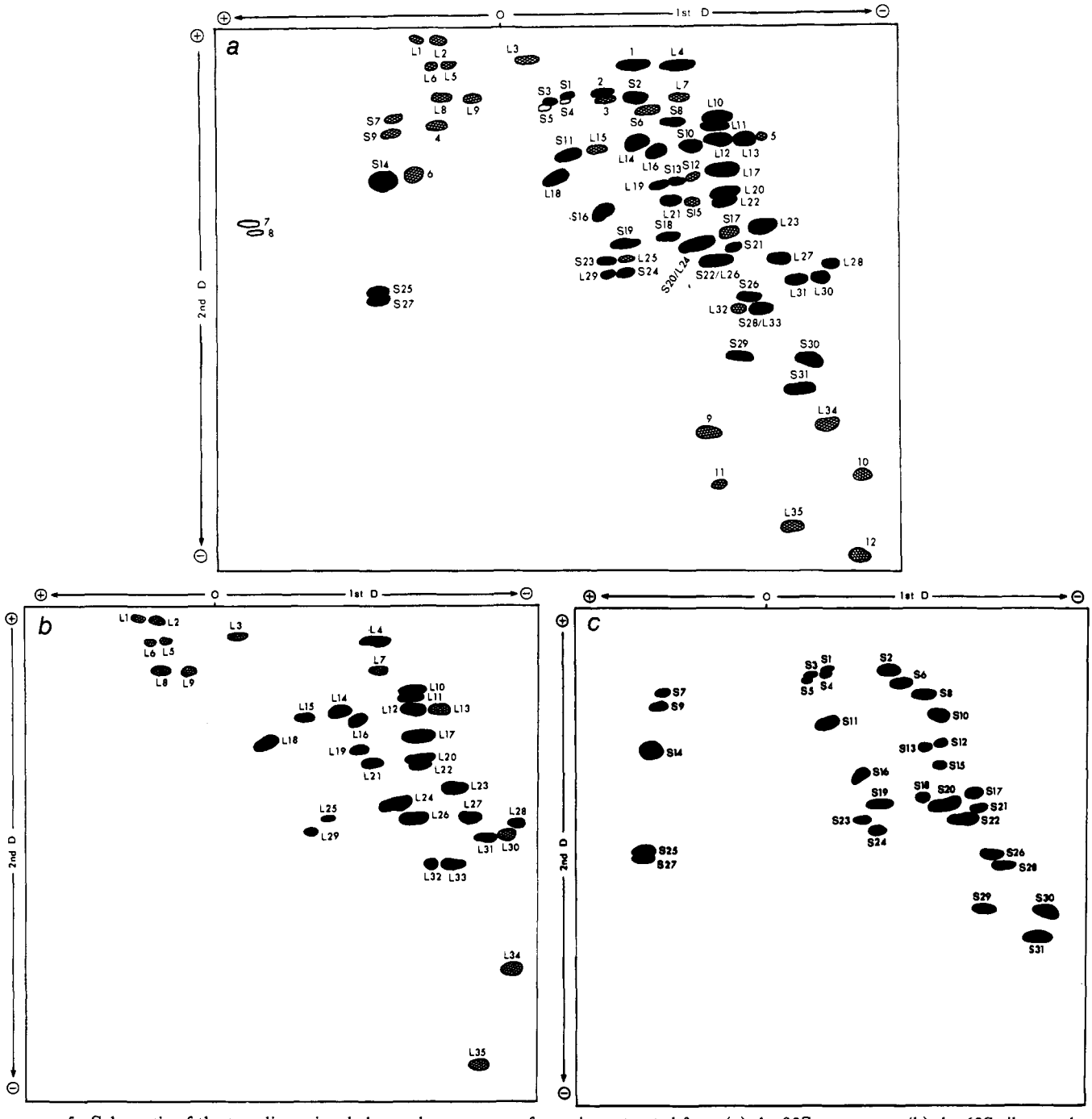

FIGURE 5: Schematic of the two-dimensional electrophoretograms of proteins extracted from (a) the 80S monomers, (b) the 60S ribosomal subunits, and (c) the 40S ribosomal subunits. This schematic represents a composite of both the Kaltschmidt–Wittmann gels and the microgels run under a variety of conditions and as such may differ in detail from the electrophoretograms presented in Figures 2–4. The solid spots were always seen; the crosshatched spots varied in intensity or were seen only when an excess of protein was used; the open spots were found only in the Kaltschmidt–Wittmann gels.

S1, S3, S4, S5, S28/L33, and L12 were found in two pools that were in adjacent LiCl steps. S20/L24, S22/L26, and L20 were found in at least three pools in adjacent LiCl steps. S26 was found in three pools that were not within the same LiCl step but rather in three consecutive LiCl steps.

L21 and L24/S20 were found in two and four pools, respectively, that were not in adjacent LiCl steps.

One major protein, 13 was the predominant protein in pool 24 (Figure 8). This protein had not been detected in our two-dimensional electrophoretograms.

Discussion

One difficulty encountered in the estimation of the number of ribosomal proteins is the variable amount of protein that remains at the origin during electrophoresis in the first dimension and causes the formation of bands by migration directly down from the origin during electrophoresis in the second dimension. The formation of bands is concentration dependent. The reason for this is uncertain although it has been suggested to be a solubility problem and has been previously encountered in mammalian ribosome studies (Sherton & Wool, 1974). The important question of whether a protein may be entirely lost in this fashion is unresolved. If these bands did represent unique proteins, it seems unlikely that they all would have mobilities in the second dimension identical with those of other proteins that migrated away from the origin in the first dimension. The question cannot be satisfactorily

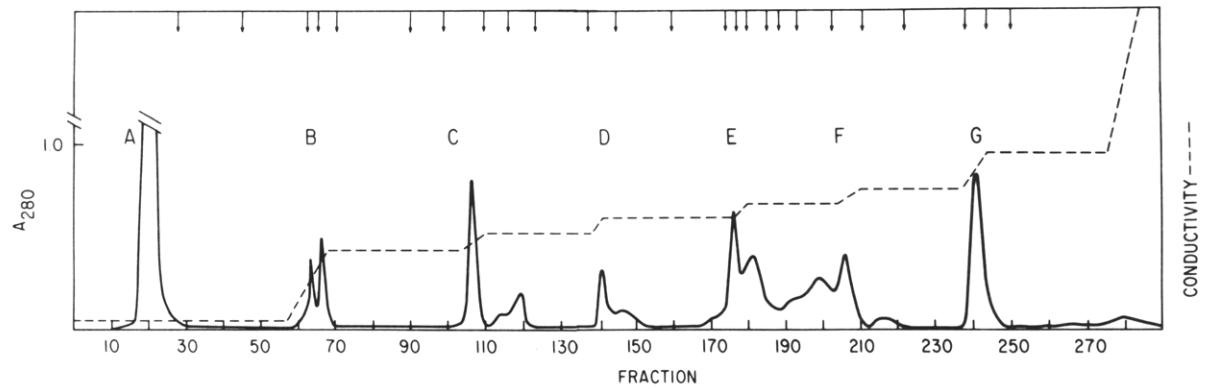

FIGURE 6: Group fractionation of 80S ribosomal proteins by chromatography on carboxymethylcellulose. The proteins (1.8 g) were separated into seven groups (A80–G80) by elution in steps with increasing concentrations of LiCl at pH 6.5 from a column (4 × 46 cm) of carboxymethylcellulose. The fractions were pooled as indicated by the arrows. For details, see Table I.

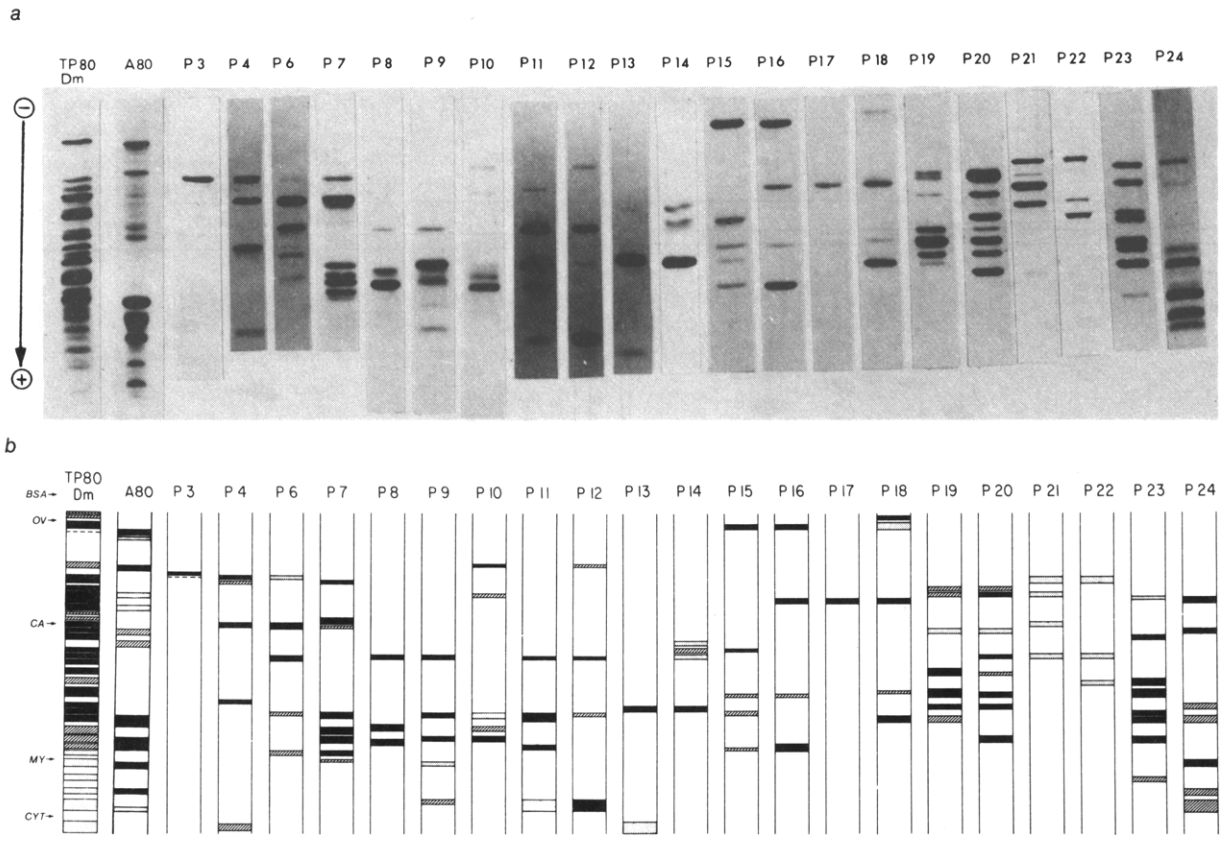

FIGURE 7: (a) Sodium dodecyl sulfate electrophoretograms of proteins from (left to right) TP80, A80, pool 3, pool 4, pool 6, pool 7, pool 8, pool 9, pool 10, pool 11, pool 12, pool 13, pool 14, pool 15, pool 16, pool 17, pool 18, pool 19, pool 20, pool 21, pool 22, pool 23, and pool 24. The conditions for electrophoresis were described under Materials and Methods. (b) Schematic representation of $NaDodSO_4$ electrophoretograms of proteins from TP80 and the pools listed in (a). The schematics were based on gels run with varying concentrations of each sample adjacent to a TP80 standard. The schematics therefore represent with fidelity the relative migration distances of the proteins, whereas the gel composite (a) does not. The lightly staining bands in the gels are represented by lightly stippled bands. The darker staining regions are represented by the enclosed hatched regions. The darkest staining bands are represented by solid bands. Dotted lines represent thin, faintly staining bands. Arrows represent the positions of the standard protein markers: BSA = bovine albumin (66 200), OV = chicken egg albumin (45 000), CA = bovine erythrocyte carbonic anhydrase (31 000), MY = horse skeletal muscle myoglobin (17 200), and CYT = horse heart cytochrome *c* (12 400). P = pool.

answered until individual ribosomal proteins have been isolated and purified.

Number of Proteins. It is generally accepted that different proteins with different sizes and charges would migrate separately during two-dimensional gel electrophoresis: thus, each spot on a gel is assumed to contain a single protein. On this assumption, the electrophoretograms of proteins from the 80S monomer of *D. melanogaster* (Figures 2a, 3a, 4, and 5a) show that there is a total of 78 proteins in that particle. The majority of the 80S ribosomal proteins (63) are basic, with isoelectric points above pH 8.6. Fifteen of the 80S ribosomal proteins are acidic, however, with isoelectric points between pH 4.6 and pH 8.6.

In the separation of ribosomal proteins from purified subunits, 31 spots are resolvable from the small subunit and 35 spots are resolvable from the large subunit. Of the 31 proteins in the small subunit, five are acidic (S7, S9, S14, S25, and S27). Six of the large subunit proteins are acidic (L1, L2, L5, L6, L8, and L9). Twelve of the 80S proteins have not been observed in any of the electrophoretograms of subunit proteins.

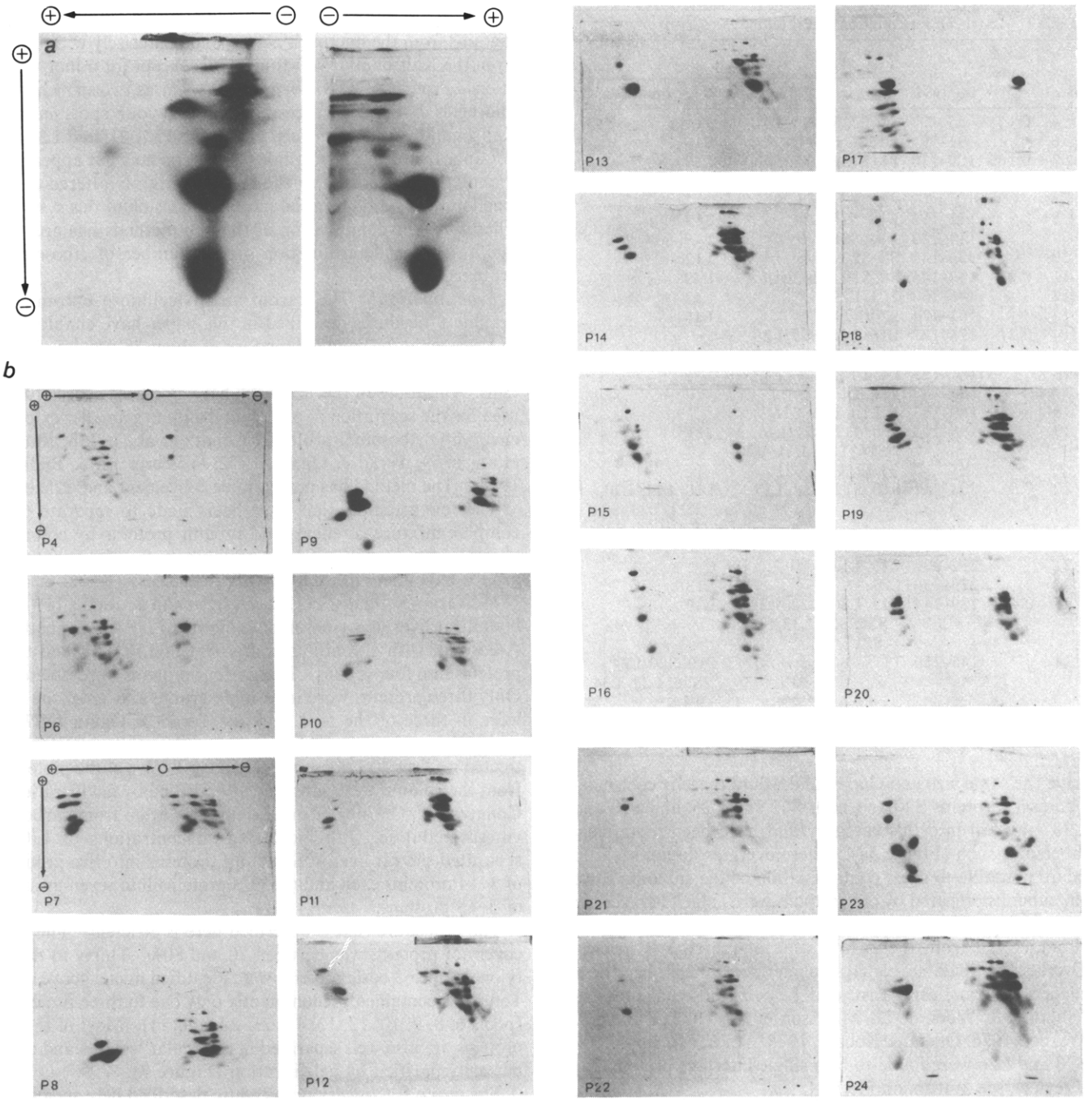

FIGURE 8: Two-dimensional polyacrylamide gel electrophoresis of proteins isolated from A80 and pools 4–24. (a) Proteins of A80 were analyzed on the left gel (origin at top right; electrophoresis was from right to left), and the acidic proteins of TP80 were analyzed on the right gel (origin at top left; electrophoresis was from left to right). Electrophoresis was from top to bottom in the second dimension. (b) Except for pools 4, 6, 15, and 17, proteins from each basic pool were analyzed alone on the left half of the second-dimension gel (origin at top left) and on the right side (origin at top center) with a small amount ($\simeq$10 μg) of TP80. For pools 4, 6, 15, and 17, proteins from each pool were analyzed alone on the right-hand side of the second-dimension gel (origin at top center) and on the left side (origin at top left) with a small amount of TP80. Electrophoresis was from left to right in the first dimension and from top to bottom in the second dimension. O = origin. P = pool.

The cause(s) of this discrepancy between the estimate from the 80S monomer and the estimate derived from the sum of the two subunits is uncertain. Some of the discrepancy may be the result of the following. (a) The discrepancy may be inherent in the use of the microgel system. The microgel system was adopted for use here because it requires only small quantities of protein and shorter running times. The low molecular weight proteins (L34, L35, and 9–12), normally present as lightly staining spots in the Kaltschmidt–Wittmann gels, are occasionally missing in the microgels. Their occasional absence may be due to the fact that they are normally very lightly staining in the Kaltschmidt–Wittmann gels and, as such, may be more difficult to see in the microgels unless they are excessively overloaded. Alternatively, the low molecular weight proteins could have run off the gel earlier during electrophoresis. This alternative seems unlikely because two proteins, L34 and L35, which are within a similar range in molecular weight are detected in the electrophoretograms of proteins from the large subunit run under standard conditions.

(b) The discrepancy may be attributable to coelectrophoresis of proteins. Three of the proteins found in the 40S subunit (S20, S22, and S28) coincide in electrophoretic mobility with three proteins found in the 60S subunit (L24, L26, and L32, respectively). It is possible that two proteins might fortuitously

Table I: Further Fractionation of Proteins

pool	LiCl step (M)	fractions	protein: major	protein: minor
4	0.135	65–70	S1, S3, S4, S5, L18	11
6		91–99	L15	S16
7	0.185	100–110	S1, S2, S3, S4, S5, S11, S16, S23, S24, L14, L25, L29	S19
8		111–116	L19, S20/L24	S19
9		117–123	L10, S18	L29, S24, 9, S13[a]
10		124–141	S8, S23, S24	S17
11	0.23	142–145	S8, S19, S24, L19	L29
12		146–160	2, 3, L19	
13		161–174	S20/L24	L16
14	0.27	175–177	L16, L21, S22/L26	
15		178–180	L4, S20/L24, S22/L26	L16
16		181–185	L4, L11, S20/L24, S29	
17		186–188	L10, L11	
18		189–193	1, L10, L11, S26	
19		194–203	S10, S12, S22/L26, S26, L10, L11, L20, L22, L23, L31	S21[a]
20	0.3	204–211	S10, S12, L12, L17	L10, L11, L20, L22, L23, S21, S22/L26, S26, L31, L32
21		212–222	L13, L17	
22		223–238	L27	L13
23	0.4	239–244	L12, L20, L22, L28, S26, S28/L33, S30, S31	L30
24		245–250	13	S10, S20/L24, S26, L23, L34, 14

[a] Occasionally present.

have the same size and charge and would thereby coelectrophorese. Proteins S20 and L24, S22 and L26, and S28 and L33 may fall into this category and have been tentatively labeled as such (Figure 5a). These overlaps do not seem to be attributable to cross contamination of the subunits since the subunits prepared by our methods were judged to be better than 95% pure both from the recentrifugation of the isolated subunits and from the examination of the rRNA species. Overlaps, attributable, perhaps, to interface proteins, have been reported in rat liver, yeast, and *E. coli* ribosomal proteins (Sherton & Wool, 1972; Morrison et al., 1973; Zinker & Warner, 1976; Otaka & Kobata, 1978). In *E. coli* ribosomes, S20 and L26 were found to be a single interface protein [for a review, see Wittmann (1974)].

Proteins 7 and 8. The most acidic proteins are 7 and 8. They migrate in a manner virtually identical with that of certain proteins of *E. coli* (L7/L12), rat liver (L40/L41), and two acidic proteins in yeast and in brine shrimp (Möller et al., 1975; Sherton & Wool, 1972; Zinker & Warner, 1976; Otaka & Kobata, 1978; Highland et al., 1973). In addition to similarities in the electrophoretic mobilities of these proteins, immunochemical and biochemical studies have shown these acidic proteins to be both structurally and/or functionally related (Richter & Möller, 1974; Stöffler et al., 1974). In the case of yeast and *E. coli* ribosomes, in vitro experiments on protein functions show these acidic proteins to be interchangeable. For instance, hybrid ribosomes have been shown to be active in GTP hydrolysis and in the EF-G dependent binding of GDP (Richter & Möller, 1974; Stöffler et al., 1974). Since these acidic proteins appear to have been conserved in the evolution from procaryotes to mammals, it is likely that they are also homologous to proteins 7 and 8 in *D. melanogaster*.

Comparison of the Two Gel Systems. The electrophoretograms from the microgels corresponded precisely with those from the Kaltschmidt–Wittmann gels except for minor variations. Proteins S1, S3, S4, and S5 appear as two spots in the microgels but are further resolved into four spots in the Kaltschmidt–Wittmann gels. Proteins S15, L21, and L32 do not appear in the Kaltschmidt–Wittmann gels but appear in the microgels, perhaps as the result of slightly altered electrophoretic conditions in the second dimension of this system. Therefore, the combination of the two methods has greatly facilitated the determination of the number of ribosomal proteins.

Fractionation. The carboxymethylcellulose chromatographic procedures described in this paper have enabled us to fractionate the complex mixture of about 78 proteins from the ribosome of *D. melanogaster* into smaller groups of proteins. Variations of this approach have also been successfully used for the separation of procaryotic and, to a smaller extent, eucaryotic ribosomal proteins (Collatz et al., 1976b; Kanai et al., 1969; Terao & Ogata, 1972; Westermann & Bielka, 1973). The method has proven to be convenient and efficient.

The few attempts that have been made to separate the complex mixture of eucaryotic subunit proteins by column chromatography without some preliminary fractionation into groups have met with only moderate success (Kanai et al., 1969; Terao & Ogata, 1972). Westermann & Bielka (1973) bound rat liver 40S ribosomal subunits to DEAE-Sephadex A-25 and, with a continuous gradient of LiCl, divided the proteins into four groups. Twenty-four proteins were isolated. Only three proteins were in a single group, and 13 of the 31 were in three of the four groups. Terao & Ogata (1972) resolved 19 fractions by CMC chromatography of EDTA-treated rat liver 40S ribosomal subunits. Twelve of the proteins from the column were subsequently purified by gel filtration. Collatz et al. (1976b), using a stepwise elution from carboxymethylcellulose, with increasing concentrations of LiCl, separated the rat liver 40S subunit proteins into five groups of 3–14 proteins each and the 60S proteins into seven groups of 3–15 proteins.

The procedure that is described here is efficient. The recovery of proteins was between 70 and 80%. Thirty to thirty-one of the 63 basic proteins were identified in one pool each. Ten pools contained predominantly only one to three proteins (pools 6, 8, 9, 10, 12, 13, 17, 21, 22, and 24). Most of these proteins are also well separated in molecular weights and can be easily purified by gel filtration (Figure 7).

The group fractionation procedure described here seems to be a valuable step in the purification of *Drosophila* ribosomal proteins. A number of important developments can follow from this initial step. For example, the groups consisting of mainly one or a small number of proteins offer promising leads to the purification of single proteins. Alternately, antisera raised against such a group of proteins could be used in the purification of proteins by the method of affinity chromatography. Finally, pure proteins could prove to be useful for in vivo studies of ribosomal RNA–protein interactions (Chooi, 1976). The latter is especially interesting since the genetics of the ribosomal RNA loci in *D. melanogaster* is well studied.

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Two Nonspecific Phospholipid Exchange Proteins from Beef Liver. 1. Purification and Characterization†

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ABSTRACT: Two proteins have been purified from the postmicrosomal fraction of beef liver homogenate. They accelerate the transfer of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, sphingomyelin, phosphatidylglycerol, phosphatidic acid, and cholesterol from unilamellar vesicles to either mitochondria or multilamellar vesicles and are therefore referred to as "nonspecific" exchange proteins. These are the first purified exchange proteins which have been found capable of accelerating the transfer of phosphatidic acid and phosphatidylglycerol. However, the transfer of diphosphatidylglycerol from unilamellar vesicles to multilamellar vesicles and cholesteryl esters from low-density lipoprotein to high-density lipoprotein is not accelerated by these proteins. Characterization of the two exchange proteins has revealed a striking similarity. Both accelerate the transfer of phosphatidylinositol, phosphatidylcholine, and phosphatidylethanolamine from unilamellar vesicles to multilamellar vesicles with the same relative specificity (2.2:1.7:1). Both have molecular weights of 13 600 as determined by molecular filtration through Sephadex G-50 and 14 500 as determined by electrophoresis on 12% polyacrylamide gels in the presence of sodium dodecyl sulfate. They have isoelectric points of 9.55 and 9.75 as determined on an isoelectric focusing column. Amino acid analyses reveal only two differences. One protein contains neither histidine nor arginine whereas the other contains one residue of each per protein molecule.

Proteins that accelerate the exchange of phospholipids between membranes have been isolated from a number of sources (Wirtz, 1974; Zilversmit & Hughes, 1976) and have been used to study the distribution and transbilayer movement of phosphatidylcholine in artificial and biological membranes (Johnson et al., 1975; Bloj & Zilversmit, 1976; Rothman & Dawidowicz, 1975). Recently, purified proteins from rat liver and rat liver hepatoma have been shown to enhance the exchange of phosphatidylethanolamine and sphingomyelin (Bloj & Zilversmit, 1977a; Dyatlovitskaya et al., 1978). These proteins should be useful in studying the asymmetry and transbilayer movement of phospholipids in mixed lipid vesicles and biological membranes. One of these has been used to

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