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Preparation and some Properties of Rat Skeletal-Muscle Polyribosomes

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1. A method is described for the sucrose-gradient sedimentation analysis of ribosomes in a post-mitochondrial supernatant of rat skeletal muscle. 2. An essential feature of the method involves the use of buffer of ionic strength 0·3 for homogenization of the muscle tissue. 3. Polyribosomes can be prepared by precipitation from post-mitochondrial supernatant of skeletal muscle by adjustment of the potassium chloride content of the medium. These polyribosomes stimulate cell-free amino acid incorporation *in vitro* in an energy-dependent system. 4. Ribosome aggregates of uniform size distribution can be obtained by adjustment of the ionic strength of the post-mitochondrial supernatant, followed by differential sucrose-gradient centrifugation. 5. *In vivo*, rat skeletal-muscle polyribosomes became labelled by ^{14}C-labelled amino acid within 15min., and radioactivity was associated with the light ribosome species within 45min. 6. Electron microscopy of the polyribosomes revealed aggregations containing more than 40 single ribosomes.

In total body protein synthesis the contribution made by muscle tissue is important since this is the largest protein mass in the body. Although many studies have dealt with the mechanisms of muscular contraction and the composition and structure of the myofibrillar protein (Szent-Györgyi, 1960), few detailed investigations have been made of the amino acid-incorporating system of this tissue.

Studies with bacteria, plant and animal systems (Warner, Knopf & Rich, 1963; Risebrough, Tissières & Watson, 1962; Gierer, 1963; Wettstein, Staehelin & Noll, 1963) have demonstrated the central role played by polyribosomes in the synthesis of cytoplasmic proteins. Florini and coworkers (Breuer, Davies & Florini, 1964; Breuer & Florini, 1965; Florini & Breuer, 1965, 1966) have reported a series of investigations on the characteristics and factors associated with the control of the protein-synthetic apparatus of skeletal-muscle tissue.

As a preliminary to studies of the influence of diet, hormones and infectious stress on mammalian muscle-protein metabolism, we also examined the general mechanism of protein synthesis in this tissue. It appeared important to determine as accurately as possible the particle-size distribution of the ribosomes in the living muscle cell. Previous methods concerned with rat cardiac and skeletal muscle (Breuer *et al.* 1964; Rampersad, Zak, Rabinowitz, Wool & DeSalle, 1965; Earl & Korner, 1965; Florini & Breuer, 1966) indicated a low concentration of ribosomes in this tissue compared with liver. However, Heywood, Dowben & Rich (1967) used a high-ionic-strength homogenizing medium in their studies of chick-embryo muscle. The present study used the buffer system described by Heywood *et al.* (1967), and the present paper describes a method for the sedimentation analysis of rat skeletal-muscle ribosomes from a post-mitochondrial supernatant. Polyribosomes were prepared in good yield and their characteristics were studied.

MATERIALS

Male Sprague–Dawley rats (from Charles River Laboratories, Wilmington, Mass., U.S.A.), each weighing 60–100g., were used in these experiments. They were given an 18%-casein diet from the time of their arrival in the animal quarters until their use in the experiments.

Crystalline bovine pancreatic ribonuclease and deoxyribonuclease, trypsin, chymotrypsin, collagenase, ATP and tris hydrochloride were purchased from Sigma Chemical Co., St Louis, Mo., U.S.A. Creatine phosphokinase, creatine phosphate and GTP were obtained from Calbiochem, Los Angeles, Calif., U.S.A. Lubrol WX was given by I.C.I. Organics Inc., Providence, R.I., U.S.A. Sodium deoxycholate was purchased from Matheson, Coleman and Bell, New York, N.Y., U.S.A.; Hyamine hydroxide and 2,5-diphenyloxazole were obtained from Packard Instrument Co., La Grange, Ill., U.S.A. ^{14}C-labelled amino acid mixture (U.1) was obtained from New England Nuclear Corp., Boston, Mass., U.S.A. (specific activity 40mc/mg.atom of C). Other chemicals were reagent grade.

METHODS

Sucrose-gradient analysis of muscle ribosomes. Rats were decapitated with a guillotine, and muscle from the rear legs

was removed. After the muscle had been cleaned quickly of adhering fat and connective tissue, it was immersed in chilled medium *A* of the following composition: 0·25 M-KCl, 0·01 M-$MgCl_2$, 0·01 M-tris–HCl buffer, pH 7·6. All subsequent operations were performed in a cold-room at 4°. The muscle from each rat was blotted and a pooled sample prepared from three rats. Usually, a total of 4–6 g. of muscle was taken and transferred to a chilled 50 ml. beaker containing 5 ml. of medium *A*. The sample was minced finely with a pair of scissors and homogenized in 2 vol. of medium *A* for 20 sec. with the aid of a Polytron homogenizer (Kinematica G.m.b.H., Lucerne, Switzerland). The homogenate was centrifuged at 15000 rev./min. in a rotor 30 of a Spinco model L ultracentrifuge for 15 min. to sediment the cell debris, mitochondria and nuclei. The supernatant was filtered through two layers of nylon cloth (Nitex 230T; Lambert Silk Screen Co., Boston, Mass., U.S.A.) moistened with medium *A*. The filtrate was then treated with 10% (w/v) Lubrol WX dissolved in medium *A* and an aq. 10% (w/v) solution of sodium deoxycholate to achieve a final concentration of 0·5% (w/v) Lubrol WX and 1% (w/v) deoxycholate.

The resulting supernatant (0·7–0·8 ml., equivalent to approx. 0·4 g. of initial tissue), was then layered on a linear 15–40% (w/v) sucrose gradient (28 ml.) prepared in medium *A* according to the method of Britten & Roberts (1960). The gradient was centrifuged in the SW 25.1 rotor for 2 hr. at 25000 rev./min. Extinction profiles were measured at 260 mμ with a flow-cell with a 5 mm. light-path and a Gilford model 2000 Absorbance Recorder (Gilford Instrument Laboratories, Oberlin, Ohio, U.S.A.). The recorder was first set at zero against the 40% (w/v) sucrose medium. The gradients were removed at the rate of 3·4 ml./min. from the bottom of the tube.

Polyribosome preparation. Polyribosomes were prepared for experiments *in vitro* by the addition of 3 vol. of buffer medium *B* (0·01 M-$MgCl_2$, 0·01 M-tris–HCl, pH 7·6) to 1 vol. of the detergent-treated post-mitochondrial supernatant prepared as described above. The mixture was kept at 4° for 15 min. and then centrifuged for 10 min. at 10000 rev./min. in the rotor 30. The supernatant was discarded, and the white pellet was carefully resuspended in buffer medium *A* with four strokes of a loose pestle in an all-glass Dounce homogenizer. The suspension was centrifuged for 60 min. in a rotor 40 at 40000 rev./min. The pellet was again resuspended in medium *A*, and the suspension was used for the studies of amino acid incorporation in the cell-free system. The E_{260}/E_{280} ratio of the polyribosome preparation was 1·5. The yield was approx. 0·3–0·4 mg. of RNA/g. of muscle tissue. Recentrifugation of this suspension in a rotor 40 at 40000 rev./min. for 80 min. through a 5 ml. 1 M-sucrose cushion prepared in medium *A* resulted in an E_{260}/E_{280} ratio 1·7–1·8.

In some experiments (described in the Results section) the pellet of the precipitated polyribosomes was resuspended in buffer medium *A* and then layered on a linear 10–25% (w/v) sucrose gradient dissolved in buffer medium *A* and spun for 140 min. in a SW 25.1 rotor at 25000 rev./min. The pellet at the bottom of the gradient was again resuspended in medium *A* and the extinction profile determined in a 15–40% linear sucrose gradient as described above.

Radioactive labelling of nascent polypeptide chains in vivo. Intact rats were injected intrasmuscularly in each hind leg with 10 μc of the ^{14}C-labelled amino acid mixture. They were killed at 15 and 45 min. after injection and the muscle was prepared for sucrose-gradient analysis as described in the section on sucrose-gradient analysis of muscle ribosomes. Fractions of the gradients were collected in tubes kept at 4°. For the measurement of radioactivity, 0·25 mg. of bovine serum albumin was added to each tube and the protein was precipitated by the addition of ice-cold 5% (w/v) trichloroacetic acid. The tubes were kept overnight at 4°. The precipitates were collected on 0·45 μ Millipore filters and washed twice with ice-cold 5% (w/v) trichloroacetic acid. The filters were dried and counted on stainless-steel planchets in a Nuclear–Chicago gas-flow counter.

Cell-free amino acid incorporation. The system used for these studies was as follows: 5 μmoles of tris–HCl, pH 7·6; 10 μmoles of $MgCl_2$; 70 μmoles of KCl; 2 μmoles of ATP; 1 μmole of GTP; 15 μmoles of creatine phosphate; 20 μg. of creatine phosphokinase; 1·0 mg. of pH 5 enzyme fraction; 10 mμmoles of a complete amino acid mixture containing 1 μc of a U-^{14}C-labelled amino acid mixture. The pH 5 fraction was prepared from liver and muscle tissue according to the method of Keller & Zamecnik (1956). A final volume of 1 ml. was used for the assay system *in vitro*. The polyribosome fraction was added at zero time and the tubes were incubated under air at 37° in a Dubnoff metabolic shaker for the time-intervals indicated in the Results section. At the end of the incubation period the proteins were precipitated by adding ice-cold 5% (w/v) trichloroacetic acid, and the tubes were kept at 4° for 30 min. The tubes were heated at 95° for 30 min. and the precipitate was washed twice with ice-cold 5% (w/v) trichloroacetic acid and once with ethanol. The washed precipitate was then dissolved in 1 ml. of Hyamine hydroxide, 10 ml. of 0·6% (w/v) 2,5-diphenyloxazole in toluene was added and the radioactivity was assayed in a liquid-scintillation counter. Correction was made for zero-time controls. Protein determinations were made on polyribosomes diluted with 0·1 N-KOH (Lowry, Rosebrough, Farr & Randall, 1951).

Electron microscopy. Fractions were collected from a sucrose gradient and processed for electron microscopy. The positions of the gradient analysed are shown ('EM') in Fig. 1. Droplets of the gradient were diluted 1:1 with 0·06 M-uranyl acetate in 40% (w/v) formaldehyde and left to stand for 1 hr. A drop of the solution was then placed on a grid, dried by touching it with filter paper and rinsed twice with water, and a drop of 2·5% (w/v) uranyl acetate was added to the grid and dried. The grids were viewed with a Hitachi model HU-11A electron microscope.

RESULTS

Fig. 1 shows a sucrose-gradient analysis of the ribosomal particles from a post-mitochondrial supernatant of rat skeletal muscle. Also shown is the marked sensitivity to ribonuclease treatment. These results suggest that the heavy material sedimenting towards the bottom of the gradient consisted of ribosomes held together by RNA. Incubation of the post-mitochondrial supernatant with trypsin, chymotrypsin, collagenase and deoxyribonuclease had no apparent effect on the size-distribution of the ribosomal particles. Therefore these data are not presented here.

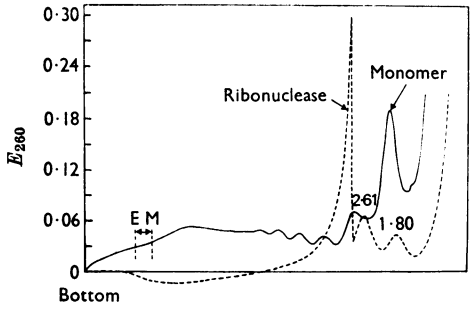

Fig. 1. Sucrose-density-gradient analysis of ribosomes from a post-mitochondrial supernatant of rat skeletal muscle. The supernatant was prepared from pooled muscles from three rats, and the amount layered on the linear 15–40% (w/v) sucrose gradient was equivalent to 0·4g. of muscle. Another portion of the post-mitochondrial supernatant was first incubated at 37° with 5μg. of bovine pancreatic ribonuclease for 15min. before being layered on the gradient. Details of the centrifugation are given in the text. E_{260} values of the gradient were recorded automatically by a flow-cell with a 5mm. light-path and a Gilford Absorbance Recorder. Control gradient (——); gradient of ribonuclease-treated supernatant (- - - -). EM, region studied by electron microscopy. Numbers indicate the E_{260} value of the monomer and dimer peaks.

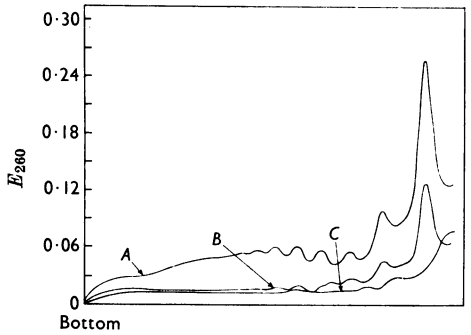

Fig. 2. Sucrose-density-gradient analysis of ribosomes from a post-mitochondrial supernatant of rat skeletal muscle. The muscle was homogenized in 0·01M-$MgCl_2$–0·01M-tris–HCl, pH7·6, buffer containing various concentrations of KCl. Supernatant equivalent to 0·4g. of muscle, prepared from a pooled sample from three rats, was applied to each gradient. Details of the preparation and the measurement of the gradient are given in Fig. 1 and in the text. Gradient *A*, 0·25M-KCl; *B*, 0·125M-KCl; *C*, 0·08M-KCl.

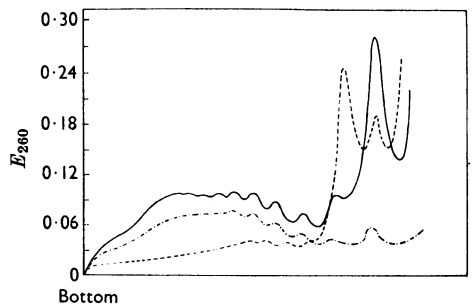

Fig. 3. Sucrose-density-gradient analysis of rat skeletal-muscle ribosomes. A control profile was obtained with the post-mitochondrial supernatant (in medium *A*) prepared as in Fig. 1 (——). A profile of resuspended polyribosomes was obtained after precipitation of ribosomes in the post-mitochondrial supernatant by the addition of 3vol. of 0·01M-$MgCl_2$, 0·01M-tris–HCl buffer, pH7·6, and resuspension of the pellet in medium *A*; the suspension was then applied to the 15–40% linear sucrose gradient prepared in medium *A* (-·-·-·-). A profile for resuspended polyribosomes was prepared as above except that the rats were starved for 52hr. before they were killed (- - - -). Material equivalent to 0·4g. of muscle, from a pooled sample from three rats, was layered on each gradient.

Myosin is precipitated at an ionic strength of below 0·1 (Szent-Györgyi, 1960) and, since Heywood *et al.* (1967) have demonstrated a loss of chick muscle ribosomes when a low-ionic-strength extraction buffer is used, the effect of potassium chloride concentration in the medium used for homogenization of rat muscle was studied. The results are shown in Fig. 2. The profile obtained with medium *A* (0·25M-potassium chloride) buffer indicates about 30% of monomers and 10–15% of dimers in the total ribosome population. The yield of polyribosomes was substantially lower when either a 0·08M or a 0·125M concentration of potassium chloride was used as compared with the 0·25M-potassium chloride buffer (medium *A*). Further, the heavy ribosomal aggregates could be precipitated from a post-mitochondrial supernatant prepared with 0·25M-potassium chloride by lowering the potassium chloride concentration of this supernatant to 0·06M-potassium chloride by the addition of 3vol. of 0·01M-magnesium chloride–0·01M-tris buffer, pH7·6 (medium *B*). Resuspension of the precipitate and subsequent sucrose-density-gradient analysis indicated a recovery of approx. 80% of the larger ribosomal aggregates present initially in the post-mitochondrial supernatant prepared with medium *A*. These results are shown in Fig. 3.

However, the recovery of lighter aggregates (dimers and monomers) was rather less than 30% of the original concentration in the 0·25M-potassium chloride post-mitochondrial supernatant. The profile obtained with medium *A* buffer from 50–60g. rats indicates approx. 30% of monomers and 10–15% of dimers in the total ribosome population (Fig. 3).

The proportion of lighter ribosome particles in the total ribosome population increases under a

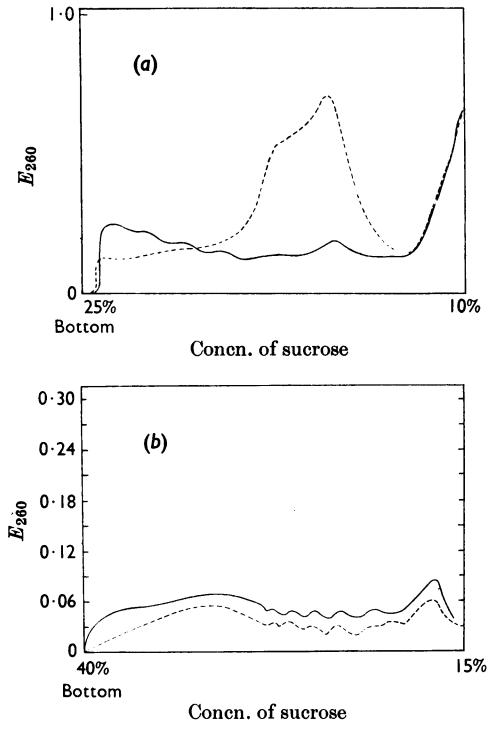

Fig. 4. Sucrose-gradient analysis of skeletal-muscle ribosomes from control rats and rats starved for 52 hr. before being killed. (*a*) Ribosomes were collected by precipitation from the post-mitochondrial supernatant prepared with medium *A* as described in the text. The pellet of precipitated ribosomes was resuspended in medium *A*, applied to a linear 10–25% sucrose gradient and spun for 140 min. at 25 000 rev./min. in an SW 25.1 rotor. Ribosomes equivalent to 5 g. of initial muscle were applied to each gradient. Well-nourished rats (——); starved rats (----). (*b*) The pellet obtained from the 10–25% sucrose gradient in (*a*) was resuspended in medium *A*, layered on a 15–40% linear sucrose gradient and spun for 2 hr. at 25 000 rev./min. Ribosomes equivalent to 1 g. of initial muscle were applied to each gradient. Well-nourished rats (——); starved rats (----).

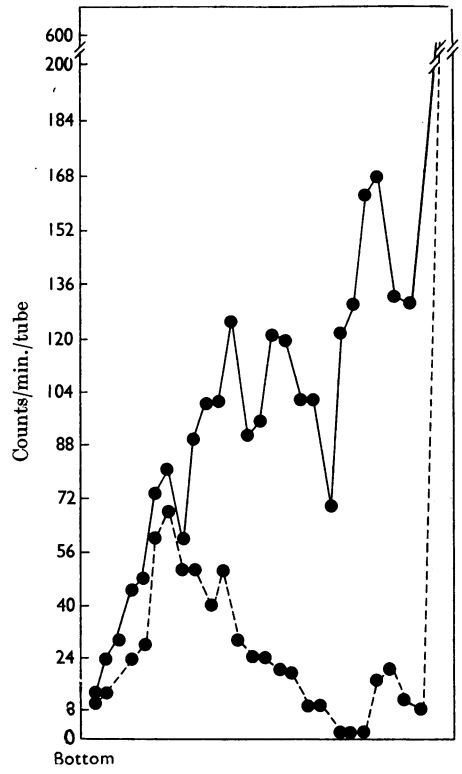

Fig. 5. Distribution of protein radioactivity in fractions obtained from a linear 15–40% sucrose density gradient of rat skeletal-muscle ribosomes. A total of eight rats were each injected with 20 μc of U-^{14}C-labelled amino acid mixture, and four were killed either 15 min. (----) or 45 min. (——) later. Ribosomes equivalent to 0·5 g. of initial muscle tissue were applied to each gradient. Each fraction is a pooled sample from three identical gradients.

number of experimental situations in rat liver (Webb, Blobel & Potter, 1966; Wunner, Bell & Munro, 1966). It is also known that protein synthesis is associated with the polyribosomes and not with individual ribosomes. Hence it appeared useful to obtain a ribosome preparation essentially free from the monomer species. As shown in Fig. 3, precipitation of the ribosomes in the post-mitochondrial supernatant prepared from starved rats resulted in the presence of a relatively high proportion of monomers and dimers in the precipitate, which was not the case for control rats. Therefore, after the precipitation of ribosomes from control and starved rats, the precipitate was first layered on a linear 10–25% sucrose gradient. The profiles obtained are shown in Fig. 4(*a*), and the results indicate a high content of light ribosome species in the precipitate obtained from starved rats compared with that from controls. The pellets obtained from the 10–25% gradient were then resuspended in medium *A* and layered on a 15–40% linear sucrose gradient. The profile is shown in Fig. 4(*b*). The particle-size distribution was similar for starved and control rats, and therefore the 10–25% sucrose gradient afforded the opportunity to remove the light ribosome species from the pellet of precipitated ribosomes prepared initially by decreasing the ionic strength of post-mitochondrial supernatant.

Amino acid incorporation by polyribosomes in vivo. Fig. 5 shows that ribosomal particles of muscle became labelled after 15 min. of exposure to the

Table 1. *Amino acid incorporation in a cell-free system by polyribosomes from rat skeletal muscle*

Incubation was carried out for 45 min. as described in the text. Each incubation mixture contained 0·5 mg. of polyribosomal protein in a total vol. of 1 ml. Polyribosomes were prepared from the post-mitochondrial supernatant by precipitation in a low-ionic-strength buffer (0·06 M-KCl, 0·01 M-$MgCl_2$, 0·01 M-tris, pH 7·6) as described in the Methods section.

Expt. no.	Incubation medium	Radioactivity incorporated (counts/min.)	Radioactivity incorporated (% of counts in complete system)
1	Complete, with liver pH 5 fraction	6672	100
	− ATP and GTP	662	10
	− Polyribosomes	688	10
	− pH 5 enzymes	1840	28
	Complete + 10 μg. of ribonuclease	986	15
2	Complete, with muscle pH 5 fraction	4624	100
	− ATP and GTP	488	11
	− Polyribosomes	599	13
	− pH 5 enzymes	2038	44
	Complete + 10 μg. of ribonuclease	504	11

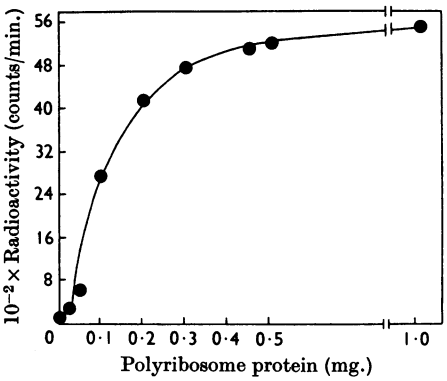

Fig. 6. Amino acid incorporation in a cell-free system by polyribosomes from rat skeletal muscle prepared as described in the Methods section by precipitation in a 0·06 M-KCl–0·01 M-$MgCl_2$–0·01 M-tris–HCl buffer, pH 7·6. The reaction was carried out in a final volume of 1 ml. at 37° for 45 min. The assay mixture contained 50 μmoles of tris–HCl buffer, pH 7·6; 10 μmoles of $MgCl_2$; 70 μmoles of KCl; 2 μmoles of ATP; 1 μmole of GTP; 20 μg. of creatine phosphokinase; 15 μmoles of creatine phosphate; 1 mg. of pH 5 enzyme from liver; 10 mμmoles of a complete amino acid mixture containing 1 μc of a U-^{14}C-labelled amino acid mixture. The amount of polyribosomal protein is given in the Figure.

^{14}C-labelled amino acid mixture after intramuscular injection. The labelling patterns obtained differed for two time-intervals studied. After 15 min. the radioactivity was associated with the heavy region of the gradient, and no radioactivity was associated with the lighter fraction. However, after 45 min., several peaks of radioactivity and a shift towards the lighter aggregates were observed. The radioactivity in the region of the light aggregates may have been associated with polypeptide chains formed initially on polyribosomes that were dissociated into dimers or single ribosomes at a later time. These results indicate that the heavy aggregates of ribosomes isolated by sucrose-gradient analysis are active sites of amino acid incorporation *in vivo*.

Cell-free amino acid incorporation. Rat muscle polyribosomes were found to be active in stimulating cell-free amino acid incorporation. The conditions for cell-free amino acid incorporation are shown in Table 1. In the absence of the pH 5 enzyme fraction, prepared from liver or muscle, ^{14}C-labelled amino acid incorporation was about one-third of that in the complete system. Ribonuclease inhibited the reaction. Fig. 6 shows that amino acid incorporation was proportional to the concentration of polyribosomes added to the cell-free system in the range 0·05–0·3 mg. of polyribosome protein. As indicated in Table 2, incorporation was linear for about 40–60 min. Similar results have been observed with ribosomes from chick-embryo muscle (Heywood *et al.* 1967).

Electron microscopy. The polyribosomes were examined under the electron microscope as described in the Methods section. Large polyribosomes, containing more than 40 ribosomes, were observed in the samples prepared from the heavy region (labelled 'EM' in Fig. 1) of the gradient. The appearance of the polyribosomes was very similar to that of those prepared from avian muscle by Heywood *et al.* (1967). Cedergren & Harary (1964) have reported the presence of a similar structure in intact heart-muscle cells. Single ribosomes were seen in the grids prepared

Table 2. *Time-kinetics of amino acid incorporation in a cell-free system by polyribosomes from rat skeletal muscle*

Incubations were carried out for the time-intervals shown. Each tube contained 0·5 mg. of polyribosome protein. The composition of the system is described in the Methods section.

Incubation time (min.)	Radioactivity incorporated (counts/min./tube)
5	927
10	1926
15	2937
20	3781
30	5766
40	6876
50	7461
60	7736

with a sample obtained from the region of the sucrose gradient labelled 'Monomer' in Fig. 1.

DISCUSSION

Methods for preparing muscle ribosomes published previously (Breuer *et al.* 1964; Earl & Korner, 1965; Rampersad *et al.* 1965) involved the use of a buffer of low ionic strength. Our results agree with the studies with avian muscle by Heywood *et al.* (1967), indicating that the use of a low-ionic-strength buffer substantially lowers the yield of muscle ribosomes. The precipitation of muscle ribosomes appears to involve co-precipitation with myosin (Heywood *et al.* 1967), and the present results as well as those of Heywood and co-workers suggest that polyribosomes are precipitated to a greater extent than the single ribosome species. It is of significance, therefore, that these workers found that myosin can precipitate up to 90% of the synthetic polyribonucleotides, forming a myosin–polyribonucleotide complex. Heywood *et al.* (1967) have also shown that ribosomes from chick liver and *Escherichia coli* can be precipitated in the presence of myosin by decreasing the ionic strength of the buffer medium. With the high-ionic-strength buffer of Heywood *et al.* (1967) for homogenization and subsequent precipitation of ribosomes from the post-mitochondrial supernatant, the present method yields about 0·3–0·4 mg. of polyribosomal RNA/g. of normal rat skeletal muscle. This yield is substantially greater than that obtained by previous workers (Breuer *et al.* 1964; Earl & Korner, 1965; Rampersad *et al.* 1965).

Polyribosomes prepared from rat skeletal muscle were active in incorporation of ^{14}C-labelled amino acids both *in vivo* and *in vitro*. Further, after a 15 min. exposure to ^{14}C-labelled amino acid mixture *in vivo*, the radioactivity was almost entirely confined to the polyribosome region. This also suggests that our method of preparation of the muscle tissue for sedimentation analysis of muscle ribosomes did not result in degradation of the polyribosomes and subsequent distortion of the size distribution of ribosomes as are present in the intact cell.

The present results demonstrate that a large proportion of the polyribosomes present in a post-mitochondrial supernatant may be easily collected by an adjustment of the ionic strength of the supernatant. This method yields polyribosomes with characteristics similar to those of ribosomes obtained from other mammalian tissues. Hence this simple procedure may allow the preparation of relatively large amounts of active polyribosomes for studies *in vitro* of mammalian muscle tissue. Further, it is a mild form of preparation with the reduced likelihood of degradation of the polyribosomes.

The size and concentration of polyribosomes from various tissues under different experimental conditions are well correlated with the general cellular protein-synthetic capacity and with amino acid incorporation *in vitro* (Murthy, 1966; Breuer & Florini, 1965; Webb *et al.* 1966). Several recent reports based on studies with animal and yeast cells suggest that polyribosome size and function and rate of protein synthesis may not necessarily be correlated to each other. These include investigations with yeast at different growth rates (Dietz, Reid & Simpson, 1965), hepatic polyribosomes of aflatoxin-treated rats (Clifford & Rees, 1967) and rat liver polyribosomes associated with starvation and re-feeding (Sox & Hoagland, 1966). According to Sox & Hoagland (1966), polyribosome activity in protein synthesis may be influenced by a regulatory mechanism residing in the polyribosome or neighbouring structures. To study this possibility in muscle, polyribosomes obtained under various physiological conditions and used for studies *in vitro* should ideally be of the same size. Since amino acid incorporation *in vitro* may depend both on the size of the ribosomal aggregates and on their concentration, the present method allows the use of a muscle ribosome preparation of uniform aggregation. This method should provide the opportunity for detailed studies of the characteristics and activity *in vitro* of polyribosomes obtained under different physiological conditions.

Fractions of the sucrose gradient examined under the electron microscope indicate that the two prominent peaks at the top of the gradient represent the monomer and dimer species. The diameter of the individual ribosomes of the polyribosomes is approx. 250 Å. This is in general agreement with the size reported for ribosomes from chick-embryo

muscle (Heywood *et al.* 1967) and mammalian cells (Sabatini, Tashiro & Palade, 1966) for negatively stained preparations. The appearance of polyribosomes obtained from the bottom of the gradient was similar to that of those obtained by Heywood *et al.* (1967) from chick-embryo muscle.

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