The Isolation and Properties of Cardiac Ribosomes and Polysomes

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1. A method is described by which good yields of ribosomes and polysomes free of contamination by submitochondrial fragments can be prepared from rat cardiac muscle. These preparations are capable of incorporation of amino acids into protein *in vitro*. 2. The ribosome preparation consists of 32% of monomeric ribosomes and 68% of ribosomal aggregates or polysomes. The polysome preparation has a decreased monomeric content. Dimers, trimers, tetramers, pentamers and larger components can be differentiated. 3. The polysome aggregate structure is degraded to monomeric ribosomes on incubation with small amounts of ribonuclease or by preparation in the absence of Mg^{2+} ions. The degradation in the absence of Mg^{2+} ions was not reversible and drastically decreased the incorporation of amino acids *in vitro*. 4. The cardiac ribosomes contained two major RNA species sedimenting at 19s and 28s in a 1:2.4 ratio. 5. The RNA/protein ratio of cardiac ribosomes and polysomes was consistently lower than that of similar preparations from liver. The concentrations of Na^+ and K^+ ions present during preparation had a great effect on the RNA/protein ratio. 6. Optimum conditions for the incorporation of amino acids into protein *in vitro* are reported. Cardiac ribosomes have a lower rate of incorporation of amino acids *in vitro* than liver ribosomes. 7. Heart cell sap is less active than liver cell sap: evidence is presented that a factor, present in liver cell sap and concerned with stimulating the synthesis of the peptide chain, is lacking in heart cell sap. 8. Pulse-labelling of perfused hearts followed by examination of the subcellular structures showed that the ribosomal fraction was the most active in the incorporation of amino acids *in vitro*.

Knowledge of the mechanism of protein biosynthesis and its control in mammalian tissues has advanced considerably with the development of methods for the isolation of well-defined ribosome and polysome preparations (see, e.g., Korner, 1961, 1963; Wettstein, Staehelin & Noll, 1963; Noll, Staehelin & Wettstein, 1963; Gierer, 1963; Marks, Rifkind & Danon, 1963). Muscle, the major tissue of the body, has not often been used as a source of ribonucleoprotein particles because muscle is particularly difficult to homogenize. The rigorous procedures normally adopted to break up the tough cytostructure to release the ribosomes result in excessive damage to subcellular structures and lead to poor yields of ribosomes heavily contaminated by other cellular structures.

The present paper describes the isolation, in good yield, of ribosomes and polysomes free from contamination with submitochondrial particles, from rat cardiac muscle. Some of the properties of the preparations are given and the conditions for the incorporation of amino acids into protein *in vitro*

by them is examined and compared with those of cell-free systems from rat liver.

A preliminary report of this work has been published (Earl & Korner, 1963).

METHODS

Animals. Female albino rats weighing 150–200g. were used. They were bred in the laboratory or supplied by Tuck's Ltd., Rayleigh, Essex.

Radioactive compounds. DL-[1- ^{14}C]Leucine (7.2mc/m-mole), L-[G- ^{14}C]phenylalanine (304mc/m-mole) and ^{14}C -labelled algal-protein hydrolysate (145 μ c/mg.) were obtained from The Radiochemical Centre, Amersham, Bucks.

Other materials. Ribonuclease and the disodium salts of ATP, GTP and CTP were obtained from the Sigma Chemical Co., St Louis, Mo., U.S.A. The sodium salt of creatine phosphate was obtained from British Drug Houses Ltd., Poole, Dorset. Creatine phosphokinase was purchased from Boehringer und Soehne G.m.b.H., Mannheim, Germany, and the potassium salt of polyuridylic acid was obtained from the Miles Chemical Co., Clifton,

N.J., U.S.A. Amino acids were obtained from Roche Products Ltd., Welwyn Garden City, Herts.

The proteinase was purchased from the Teikoku Chemical Co., 3-Chome Higashi-ku, Osaka, Japan, under the trade name Nagarse.

All reagents were of AnalaR grade where possible. Solutions were made up in glass-distilled water. Tris buffers were adjusted to the required pH with HCl.

All apparatus was acid-washed, rinsed with tap water and glass-distilled water and baked in an oven overnight before use.

Preparation of heart-subcellular fractions. Rats were decapitated, the hearts were excised carefully so as to exclude as much of the aorta and fibrous tissue as possible and dropped into ice-cold 0.2M-sucrose in 50mM-tris buffer, pH7.6, containing magnesium acetate (5mM). All subsequent operations during the preparative procedure were carried out at 0–4°.

The heart muscle was cut up finely with scissors, washed free of blood, blotted on filter paper and weighed by displacement in 0.2M-sucrose in 50mM-tris buffer, pH7.6, containing magnesium acetate (5mM). Univalent cations were omitted until after myofibrils were removed, to avoid solubilization of myosin.

Two methods of homogenization were used: a method developed with proteinase digestion as an aid to breaking the cellular structure and, for comparison, a traditional method of homogenization with a Potter-Elvehjem homogenizer (Potter & Elvehjem, 1936).

(a) Proteinase method. To each 2g. of muscle, 5ml. of 1% (w/v) proteinase solution (Nagarse) in the same medium was added and the mixture incubated for 15min. at 0° (Chance & Hagihara, 1961). The supernatant from this incubation was poured off and the chopped muscle washed three times, by decantation, with 0.2M-sucrose in 50mM-tris buffer, pH7.6, containing magnesium acetate (5mM).

The tissue was homogenized in 5vol. of the same medium by five strokes of a loose-fitting hand-operated homogenizer similar to that described by Dounce, Witter, Monty, Pate & Cottone (1955). The homogenate was then centrifuged at 11000g for 10min. in an MSE refrigerated centrifuge and the supernatant was made 0.1M with respect to KCl and 40mM with respect to NaCl. This was then used to prepare microsomes, ribosomes and polysomes.

Microsomes were prepared by layering 7ml. of this 11000g supernatant over 5ml. of 0.5M-sucrose in 20mM-tris buffer, pH7.6, containing KCl (0.1M), NaCl (40mM) and magnesium acetate (5mM), and centrifuging at 105000g for 2hr. at 0° in the no. 40 rotor of a Spinco model L ultracentrifuge. The pellet was resuspended in the tris-salts buffer by gentle homogenization in an all-glass Potter homogenizer.

The lipoprotein membranes of the sarcoplasmic reticulum were solubilized (Palade & Siekevitz, 1956; Strittmater & Ball, 1952) by the addition to the 11000g supernatant of a fresh solution of 10% (w/v) sodium deoxycholate in 50mM-tris buffer, pH8.2, to give a final deoxycholate concentration of 0.5%.

Preparations of the total ribonucleoprotein particles (ribosomes) were made by layering 7ml. of this deoxycholate-treated 11000g supernatant on to 5ml. of M-sucrose in 20mM-tris buffer, pH7.6, containing KCl (0.1M), NaCl (40mM) and magnesium acetate (5mM), and centrifuging at

105000g for 4hr. in the no. 40 rotor of a Spinco model L ultracentrifuge.

Polysomes were prepared essentially as described by Wettstein *et al.* (1963): 6ml. of the deoxycholate-treated 11000g supernatant was layered over 3ml. of M-sucrose in turn layered over 3ml. of 2M-sucrose, both in 20mM-tris buffer, pH7.6, containing KCl (0.1M), NaCl (40mM) and magnesium acetate (5mM), and centrifuged at 105000g for 4hr. After centrifugation the supernatant, containing deoxycholate and traces of proteinase, was carefully sucked off to the interface of the 2M-sucrose layer, disturbance of the sucrose layer and contamination of the clear pellet being avoided. The sides of the tube were washed carefully with resuspension medium (see below) and this was also sucked off to the sucrose interface. The sucrose was then poured off, and the inside of the tube and the surface of the pellet were quickly rinsed with resuspension medium. Both ribosomes and polysomes were resuspended easily by agitation with a Pasteur pipette in 20mM-tris buffer, pH7.6, containing KCl (0.1M), NaCl (40mM) and magnesium acetate (5mM), for sucrose-gradient studies, or in 20mM-tris buffer, pH7.6, containing KCl (0.1M), NaCl (40mM), magnesium acetate (10mM) and mercaptoethanol (6mM), for studies on the incorporation of amino acids *in vitro*.

(b) Standard method. The chopped heart muscle, after being weighed by displacement, was homogenized in 3vol. of ice-cold 0.2M-sucrose in 50mM-tris buffer, pH7.6, containing magnesium acetate (5mM), by using a hand-operated loose-fitting ground-glass Potter-Elvehjem-type homogenizer kept in an ice bath. Mitochondria, nuclei and cell debris were removed by centrifugation at 11000g for 10min. in an MSE refrigerated centrifuge. The supernatant was carefully decanted and made 0.1M with respect to KCl and 40mM with respect to NaCl. Then 7ml. of this was layered on to 5ml. of 0.5M-sucrose in 20mM-tris buffer, pH7.6, containing KCl (0.1M), NaCl (40mM) and magnesium acetate (5mM), and recentrifuged at 105000g for 2hr. in a Spinco model L ultracentrifuge. The supernatant was sucked off and the surface of the microsome pellet rinsed as described above. Resuspension of the brown microsome pellet in 20mM-tris buffer, pH7.6, containing KCl (0.1M), NaCl (40mM) and magnesium acetate (5mM), required relatively vigorous homogenization.

The addition of $\frac{1}{5}$ vol. of 10% sodium deoxycholate in 50mM-tris buffer, pH8.2, followed by the centrifugation procedure described above for the proteinase method, yielded polysomes and ribosomes. The brown hard ribosomal pellet was resuspended in 20mM-tris buffer, pH7.6, containing KCl (0.1M), NaCl (40mM) and magnesium acetate (5mM), by homogenization with a small all-glass homogenizer. The very small polysomal pellet was more easily resuspended.

Preparation of heart cell sap. The 11000g supernatant after Potter-Elvehjem homogenization (i.e. no proteinase treatment) was centrifuged at 105000g for 2hr. and the cell sap passed through a column of at least ten times its own volume of coarse-grain Sephadex G-25 equilibrated at 0° with 20mM-tris buffer, pH7.6, containing KCl (0.1M), NaCl (40mM), magnesium acetate (10mM) and mercaptoethanol (6mM) to remove inhibitors (J. N. Mansbridge, personal communication) and to equilibrate the cell sap with the incorporation medium.

Preparation of rat-liver ribosomes, polysomes and cell sap.

These were prepared by the methods described by Munro, Jackson & Korner (1964).

Preparation of RNA from cardiac ribosomes. Ribosomes were resuspended in ice-cold 50 mM-tris buffer, pH 7.6, and RNA was extracted by a method similar to that described by Hall & Doty (1959). The suspension was made 0.1% with respect to sodium dodecyl sulphate by the addition of a fresh 10% (w/v) solution in 50 mM-tris buffer, pH 7.6. The mixture was shaken and allowed to stand in an ice bath for 10 min. An equal volume of 10% (v/v) water-saturated freshly-redistilled phenol was added and the flask shaken for a further 10 min. After centrifugation at 3000 g in an MSE refrigerated centrifuge for 10 min. the aqueous layer was separated and re-extracted with phenol. To the final aqueous fraction $\frac{1}{5}$ vol. of 20% (w/v) sodium acetate (adjusted to pH 5.2) and 3 vol. of ice-cold 96% (v/v) ethanol were added. The RNA was precipitated in a deep-freeze overnight, collected by centrifugation and dissolved in 10 mM-acetic acid (adjusted to pH 5.2 with dilute NaOH) containing NaCl (0.1 M) and reprecipitated as described above. The RNA was finally washed with ethanol and ether and dissolved in the acetate-salt buffer.

Determination of RNA. RNA was determined by the modification proposed by Fleek & Munro (1962) of the method of Scott, Fracastoro & Taft (1956) (see Munro *et al.* 1964).

Determination of protein. Triplicate samples of sub-cellular fractions were used for protein estimation by the method of Lowry, Rosebrough, Farr & Randall (1951). Bovine serum albumin was used as a standard.

Assay of succinate dehydrogenase. Succinate-dehydrogenase activity was taken as indicative of the presence of mitochondria or submitochondrial particles. The method used was essentially that of Green, Mii & Kohout (1955).

A cuvette containing 3 ml. of 50 mM-KH₂PO₄ (adjusted to pH 7.6 with dilute NaOH), containing KCN (1 mM), 2,4-dichlorophenol-indophenol (0.04 mM) and sodium succinate (20 mM), was placed in a Beckman model DK2 recording spectrophotometer at 37° for 5 min. to equilibrate and the extinction at 600 m μ recorded until it was constant. A known volume of particle preparation was added, with mixing, and the extinction at 600 m μ followed for 10 min. From the rate of change of extinction the amount of succinate oxidized was determined and the specific activity calculated as the amount of succinate oxidized/hr./mg. of protein. Succinate was always in excess. With limiting succinate the fall of extinction was found to be that predicted from the known amount of succinate added, demonstrating that succinate-dehydrogenase activity was in fact being assayed.

Assay of esterase. A cuvette containing 3 ml. of 50 mM-KH₂PO₄ (adjusted to pH 7.6 with dilute NaOH) and *p*-nitrophenyl acetate (0.02 mg./ml.) was placed in a Beckman model DK2 recording spectrophotometer at 37° for 5 min. to equilibrate and the extinction at 400 m μ recorded until it was constant. A known volume of the particle preparation was added, with mixing, and the extinction at 400 m μ followed for 10 min. From the rate of change of extinction the amount of *p*-nitrophenyl acetate hydrolysed was determined and hence the specific esterase activity. *p*-Nitrophenyl acetate was always in excess. When the substrate was limiting the change in extinction was found to be that predicted from the known amount of

p-nitrophenyl acetate added. This assay was based on the manometric method of Hulsmans (1961).

Incubation medium for the incorporation of amino acids into protein in vitro. The exact composition of the incubation medium varied from experiment to experiment and details are given in the text. The standard incubation mixture was 20 mM-tris buffer, pH 7.6, containing KCl (0.1 M), NaCl (40 mM), magnesium acetate (10 mM), mercaptoethanol (6 mM) (Sachs, 1957), ATP (5 mM), GTP (0.05 mM), creatine phosphate (1 mg./ml.) and creatine phosphokinase (10 μ g./ml.). The radioactive amino acid was at a concentration of 0.5 μ Ci/ml., and a mixture of all the natural amino acids, other than the radioactive one, was added to give a concentration of 0.1 μ mole of each/ml. Ribosomes were added together with cell sap at a ratio of 1 mg. of ribosomal protein to 15 mg. of heart-cell-sap protein and 25 mg. of liver-cell-sap protein. The incubation volumes were 0.5 ml. for the heart system and 1 ml. for the liver system in 15 ml. glass centrifuge tubes. Unless otherwise stated in the text, heart ribosomes were incubated with heart cell sap, and liver ribosomes with liver cell sap.

The incubation mixture was prepared in an ice bath and incubated at 37° in a Dubnoff water bath, with shaking.

The incorporation was stopped by the addition of 0.5 N-HClO₄ containing non-radioactive DL-leucine or DL-phenylalanine (1 g./l.), as appropriate. The proteins were washed and prepared for counting as described by Munro *et al.* (1964). The protein was dissolved in 0.4 ml. of 99% formic acid and the solution of protein poured into the centre of a weighed aluminium planchet (diam. 3 cm.). The solution was prevented from flowing from the centre by a ring of silicone high-vacuum grease applied round the edge. The formic acid was evaporated under an infrared lamp. The incubation tube was washed with a further 0.4 ml. of 99% formic acid, and this washing was also poured on to the planchet and dried. The planchet was reweighed and the radioactivity estimated in a Nuclear-Chicago gas-flow counter. The counting efficiency was about 20% and sufficient counts were recorded to ensure a standard error of less than 3%. Corrections were made for background and for self-absorption. All specific activities (counts/min./mg. of protein) were corrected to infinite thinness. The results were expressed as counts/min./mg. of ribosomal RNA incubated.

Where the amount of protein incubated was low, a known quantity of carrier protein was added when the incorporation was stopped and suitable corrections were applied to the result.

Analysis of ribosomes and polysomes and RNA by sucrose-gradient centrifugation. Particle preparations and RNA were analysed by sucrose-density-gradient centrifugation by the methods described by Munro *et al.* (1964). In some experiments the sedimentation properties were obtained automatically by a method essentially similar to that described by Edwards & Matthias (1963). 2 M-Sucrose was pumped by a Palmer injection pump (C. F. Palmer Ltd., London) at a constant rate through a hypodermic needle puncturing the bottom of the Lusteroid tube. The gradient was pushed from the top of the tube through polythene cannula tubing to a micro flow cell in a Beckman recording spectrophotometer.

Sedimentation analysis with the analytical ultracentrifuge. A Spinco model E analytical ultracentrifuge with schlieren and ultraviolet optics was used. The rotor was at room

temperature. Analyses on ribosomes and polysome preparations were carried out by centrifuging at 37000 rev./min. with variable photographic intervals. The sedimentation coefficients were calculated by the graphical method of Markham (1960) and corrected to $S_{20,w}$.

Pulse-labelling of cardiac muscle by perfusion with ^{14}C -labelled algal-protein hydrolysate. Rats weighing 150 g. were anaesthetized and given heparin by an intraperitoneal injection of a mixture of veterinary Nembutal (Abbott Laboratories, London) and 500 units of heparin (Evans Medical Ltd.) (Newsholme & Randle, 1961). The hearts were quickly excised to minimize anoxia and dropped into ice-cold Krebs-Henseleit Ringer solution (Krebs & Henseleit, 1932), containing sodium β -hydroxybutyrate (60 mg./100 ml.), which had been equilibrated for 1 hr. with $\text{O}_2 + \text{CO}_2$ (95:5) (called perfusion medium).

The hearts were perfused at 37° in an apparatus similar to that described by Morgan, Henderson, Regen & Park (1961) at a pressure of 50 mm. Hg., a perfusion rate of 10 ml./min. and continual aeration with $\text{O}_2 + \text{CO}_2$ (95:5). This initial perfusion was continued until the ventricular beat was regular (usually about 5 min.). The perfusion fluid was discarded.

By a three-way tap in the apparatus the perfusion medium was then changed to one containing ^{14}C -labelled algal-protein hydrolysate ($0.25 \mu\text{C}/\text{ml}$). The perfusion volume was 20 ml. and was recycled by a Brunswick PA-56 peristaltic pump (Brunswick Scientific Co., N.J., U.S.A.).

After 3 min. of perfusion with radioactive medium, the heart was reconnected to the non-radioactive perfusion medium, the perfusion fluid now being discarded and not recycled. This perfusion with non-radioactive medium was continued for various times from 3 to 15 min.

The hearts were taken from the perfusion apparatus and dropped into ice-cold 0.2M-sucrose in 50mM-tris buffer, pH 7.6, containing magnesium acetate (5mM). Homogenization was by the proteinase method as described above. Microsomes and cell sap were prepared from the 11000 g supernatant essentially as described above. The microsomes were resuspended in 20mM-tris buffer, pH 7.6, containing KCl (0.1M), NaCl (40mM) and magnesium acetate (5mM), and samples taken for assay of radioactivity. The remainder was made 0.2% with respect to sodium deoxycholate, higher concentrations giving a precipitate of magnesium deoxycholate under these conditions where little protein was present. Ribosomes and deoxycholate-soluble material were separated essentially as described above. The particles were suspended in tris-salts buffer, and samples of them and of cell sap were precipitated with 0.5N- HClO_4 , containing casein hydrolysate. A known amount of carrier protein was added to the microsomes, ribosomes and deoxycholate-solubilized material.

Fractions of labelled cardiac muscle were also separated from the pellet sedimented by centrifugation at 11000 g. It was resuspended in 0.2M-sucrose in 50mM-tris buffer, pH 7.6, containing magnesium acetate (5mM), by homogenization and recentrifuged at 11000 g for 10 min. This procedure was repeated twice and the material resuspended once again and filtered through Terylene gauze at 0° . The suspension was then centrifuged at 2000 g for 10 min. to sediment a heterogeneous pellet consisting mainly of myofibrils but containing some mitochondria and nuclei. Centrifugation of the 2000 g supernatant at 11000 g for 10 min. gave a pellet that consisted almost entirely of

mitochondria, as ascertained by examination in an electron microscope. This fraction had a very high succinate-dehydrogenase activity. The 2000 g pellet and the mitochondrial fraction were resuspended and precipitated by 0.5N- HClO_4 , containing casein hydrolysate.

All samples were prepared for assay of radioactivity in the Nuclear-Chicago gas-flow counter as described above.

RESULTS

The incubation with proteinase makes subsequent homogenization easier, with a considerable decrease in the damage to subcellular components. The ribonucleoprotein-particle preparations are less contaminated by other subcellular components (see Table 1). Esterase activity is taken as indicative of endoplasmic reticular material (Hulsmans, 1961), and succinate-dehydrogenase activity as an indication of the presence of submitochondrial particles. The RNA/protein ratios for microsomes and ribosomes prepared with the proteinase method are of a similar order to those obtained from liver. A greater yield of ribosomal particles was obtained by the proteinase method than by the standard method with a Potter-Elvehjem homogenizer.

The microsomal and ribosomal pellets isolated after homogenization of the heart muscle by the two techniques described were examined by electron microscopy. The pellets were fixed by the glutaraldehyde-osmic acid technique of Sabatini, Bensch & Barnett (1963), dehydrated and stained by 1% (w/v) potassium permanganate in acetone. After being embedded in Araldite, sections were cut, mounted on copper grids and stained with uranyl acetate and a basic lead stain (Millionig, 1961). The microsomal material, after homogenization by the proteinase method, sedimented as two layers. An upper one consisted of double-walled vesicles varying considerably in size, whereas the lower one was a mass of dense particles about 200 Å in diameter. Few particles were attached to the vesicles of the upper part of the pellet. Treatment with deoxycholate removed the vesicular material, leaving the dense particles.

Examination of the microsomal preparation after use of a Potter-Elvehjem homogenizer in the standard method gave a more complex picture. An upper layer consisted of a mass of small badly-differentiated vesicles, whereas below this was a layer of clearly defined mitochondrial elements, small vesicles and dense particles. Treatment of this preparation with deoxycholate gave an amorphous badly-differentiated mass of material.

These observations were taken as further evidence for the lack of contamination of microsomes isolated after homogenization by the proteinase method.

Table 1. *Yield and purity of microsomes and ribosomes prepared by the two methods*

Experimental details are given in the Methods section. Succinate-dehydrogenase activity is expressed as μ moles of succinate oxidized/hr./mg. of protein, and esterase activity as μ moles of *p*-nitrophenol released/hr./mg. of protein. Enzymic activities are presented merely as an indication of contamination. Values for a preparation of mitochondria (identified by electron microscopy) are given for comparison.

	Standard method		Mitochondria	Proteinase method		Mitochondria
	Microsomes	Ribosomes		Microsomes	Ribosomes	
Appearance	Brown and large	Brown, large and hard	—	White fluffy	Translucent	—
Resuspension	Homogenization required	Vigorous homogenization required	—	Gentle homogenization	Agitation with Pasteur pipette	—
Succinate-dehydrogenase activity	8	0	7.6	0	0	6
Esterase activity	18	0	7	58	0	1.4
RNA (mg./100 mg. of protein)	3	6	—	10	49 (60*)	—
Yield (mg. of RNA/100 g. wet wt. of muscle)	—	18	—	—	23	—

* mg. of RNA/100 mg. of protein for liver ribosomes.

Table 2. *Effects of variations in the proteinase concentration on the yield and some properties of ribosomes*

Incorporation conditions were as described in the Methods section. Incubation was for 1 hr. The radioactive amino acid used was DL-[1-¹⁴C]leucine.

Concn. of proteinase in homogenate	Yield (mg./100 g. wet wt. of muscle)	RNA (mg./100 mg. of protein)	Incorporation of amino acids <i>in vitro</i> by cardiac ribosomes (counts/min./mg. of ribosomal RNA)	Incorporation of amino acids <i>in vitro</i> by liver ribosomes prepared normally and in the presence of various concentrations of proteinase (counts/min./mg. of ribosomal RNA)
No proteinase added	—	—	—	7000
0.5 mg./ml.	23	49	540	—
0.2 mg./ml.	24	50	480	—
0.1 mg./ml.	26	47	646	1400
Washed out, as described in the Methods section	20	49	912	4550

Table 2 shows the effect of variations in the concentration of proteinase in the homogenization medium. As the proteinase concentration was decreased the yield of ribosomes fell slightly, whereas the ability of the preparation to incorporate DL-[1-¹⁴C]leucine into protein *in vitro* (expressed as counts/min./mg. of RNA) was enhanced. The technique described in the Methods section, where proteinase is washed out before homogenization, was adopted as a compromise between yield and incorporating activity *in vitro*.

Table 2 shows the inhibitory effect on the incorporation of amino acids into protein *in vitro* by liver ribosomes when prepared by the proteinase method.

The inhibitory action of the proteinase cannot be

ascribed to a ribonuclease contaminant, for no trace of hydrolysis of RNA could be detected when RNA was incubated with 1 mg. of proteinase/ml. Ribonuclease activity was assayed by the method of Fellig & Wiley (1959). The fact that polysomes, which are degraded by very low concentrations of ribonuclease, can be prepared from homogenates in the presence of proteinase supports the contention. Further, incubation of liver polysomes with 10 μ g. of proteinase/ml. for 15 min. in an ice bath had no effect on the sedimentation pattern on subsequent analysis by sucrose-density-gradient centrifugation.

Two other possibilities are apparent. The proteinase may adhere to ribosomes during preparation and result in digestion of nascent protein during

incorporation *in vitro* or in damage to proteins in the cell sap essential for incorporation. The second possibility is that the proteinase has damaged the polysome structure without disaggregation.

These possibilities were examined. Liver ribosomes prepared normally and by the proteinase method were incubated at 37° for 45 min. in a tris-salts buffer with liver cell sap. The cell sap was subsequently reisolated and its ability to

catalyse the incorporation of DL-[1-¹⁴C]leucine into protein by fresh normal liver ribosomes was examined. No difference was detected between cell sap preincubated with normal ribosomes and that preincubated with ribosomes prepared by the proteinase method. In further experiments liver ribosomes were labelled by incubation in the complete incorporation medium containing DL-[1-¹⁴C]leucine. Incubation of these labelled ribosomes with 50 µg. of proteinase/ml. in an ice bath caused loss of radioactivity. However, when these ribosomes were reisolated from the proteinase by centrifugation through a M-sucrose-salts medium, no further loss of nascent radioactive protein could be demonstrated, even by reincubation at 37° for 30 min. The conclusion is drawn that the proteinase is therefore not adhering to the ribosomes, the inhibitory effect probably being due to damage.

Characterization of ribosomes and polysomes from heart muscle. Fig. 1 shows sucrose-density-gradient analyses of heart-muscle ribosomes and polysomes. The ribosomal preparation has a main component, sedimenting at 80s, of monomeric ribosomes. Material sedimenting at rates greater than this can be differentiated into dimers (110s), trimers (140s), tetramers (170s) and pentamers (200s), with larger aggregates undifferentiated from one another. The polysome preparation is similar except that the amount of 80s monomers is considerably less. The position of a ferritin marker (65s) is shown by the arrow (its position was estimated by previous analysis). Material sedimenting at about 60s is interpreted as ribonucleoprotein material and not ferritin, as it is degraded by ribonuclease. Similar particles have been observed in preparations from other tissues (see, e.g., Takanami, 1960; Hamilton & Peterman, 1959).

Preincubation of polysomes with 1 µg. of ribonuclease/ml. for 10 min. at 20° (in the presence of 5 mM-magnesium acetate) degrades aggregates to monomers, whereas 10 µg. of ribonuclease/ml. for 10 min. at 37° degrades all the material to acid-soluble nucleotides sedimenting at the top of the gradient (Fig. 2). Preincubation of polysomes without ribonuclease under identical conditions caused little alteration of the sedimentation properties.

The proportion of aggregates in the ribosome preparation from cardiac muscle has been calculated by measuring the areas under the peaks shown in Fig. 1 with a planimeter: 32% of the total material sediments at 80s, 17% sediments at 110s (dimers) and 51% sediments at rates greater than 110s.

Similar results were obtained when the particles were examined in the analytical ultracentrifuge (Fig. 3).

Fig. 1. Sedimentation properties of cardiac ribosomes (a) and polysomes (b) in a linear 15–30% sucrose gradient. Fractionation was by the automatic technique by using a micro flow cell and a Beckman recording spectrophotometer to measure the extinction at 260 mμ. Details are given in the Methods section. The vertical arrow represents the sedimentation position of ferritin (65s).

Fig. 2. Effect of ribonuclease on cardiac polysomes: ○, polysomes; ▲, polysomes preincubated with ribonuclease ($1\mu\text{g./ml.}$) for 10 min. at 20° ; ●, polysomes preincubated with ribonuclease ($10\mu\text{g./ml.}$) for 10 min. at 37° . Sucrose gradients were 5–20%, as described in the Methods section. Fractionation was by the drop method.

Fig. 3. Analytical ultracentrifuge sedimentation pattern of rat cardiac ribosomes. Sedimentation was from left to right. Details are given in the Methods section.

Fig. 4 demonstrates the instability of the polysome structure at low Mg^{2+} ion concentrations. Ribosomes were prepared in the absence of Mg^{2+} ions and analysed on a gradient also without Mg^{2+} ions. Degradation of polysomes to 80s monomers and some 60s and 110s material occurred. Preincubation before gradient analysis in the presence of 5mM-magnesium acetate for 10 min. at 37° and analysis on a gradient containing Mg^{2+} ions failed to reaggregate the polysomes, except for some

Fig. 4. Instability of the cardiac polysome structure in the absence of Mg^{2+} ions. Cardiac ribosomes were prepared: ●, in the presence of 5mM-magnesium acetate; ○, in the absence of Mg^{2+} ions; ▲, in the absence of Mg^{2+} ions but preincubated with Mg^{2+} ions. Gradients were as described in Fig. 1, except that for the preparation prepared in the absence of Mg^{2+} ions the gradient was run without Mg^{2+} ions. The preincubated preparation, like the normal, was run in the presence of 5mM-magnesium acetate.

Fig. 5. Sedimentation properties of cardiac ribosomal RNA. The gradients were run as described in the Methods section.

formation of dimers, possibly by non-specific aggregation.

The sedimentation profile of cardiac ribosomal RNA after analysis by sucrose-density-gradient centrifugation is shown in Fig. 5, and is similar to RNA extracted from rat-liver ribosomes (Gierer, 1958; Hall & Doty, 1959). Major peaks at about 28s and 19s were found with a trace of material sedimenting at 4s, which is assumed to be transfer RNA (s-RNA) bound to ribosomes during preparation (Gilbert, 1963a,b). The 19s RNA/28s RNA ratio was about 1:2.4.

Although some variation was encountered in the RNA/protein ratio of cardiac ribosomes and polysomes from experiment to experiment, the RNA content was found to be somewhat lower than that in analogous preparations from liver. The protein content of polysomes and ribosomes is markedly increased by the absence of Na^+ and K^+ ions from the preparation media. The K^+ ions seem to displace protein from polysomes but not from ribosomes, possibly because the centrifugation of polysomes through 2 M-sucrose during their preparation removes protein from the RNA after its displacement by a cation.

Incorporation of amino acids into protein of heart ribonucleoprotein particles. Heart ribosomes and polysomes incubated with DL-[1- ^{14}C]leucine and heart cell sap incorporate radioactive amino acids into protein (Fig. 6). There is a progressive decrease in the rate of incorporation with time, but even after 1 hr. the rate is still considerable. It is evident that polysomes are more active/mg. of RNA incubated than are the ribosomes.

Fig. 6. Time-course of incorporation of DL-[1- ^{14}C]leucine into protein *in vitro* by cardiac polysomes (●) and cardiac ribosomes (○). Incorporation conditions were as described in the Methods section, with saturating concentrations of heart cell sap.

Heart ribosomes showed a marked requirement for Mg^{2+} ions: the concentration for optimum incorporation was 10 mM in the presence of 5 mM-ATP (Table 3). The very sharp rise to optimum incorporation is apparent with a more gradual fall off at higher concentrations.

Ribosomes prepared in the absence of Mg^{2+} ions were degraded to monomeric and dimeric ribosomes, and showed a marked decrease of the incorporation of radioactive amino acids into protein *in vitro* when compared with those prepared in the presence of Mg^{2+} ions.

The preparation of cardiac ribosomes and polysomes in the absence of K^+ or Na^+ ions or both has a considerable effect not only on the RNA/protein ratio but also on their ability to incorporate radioactive amino acids into protein. The absence of K^+ and Na^+ ions from the preparation media decreases the incorporation and increases (possibly by non-specific absorption) the protein content of both ribosomes and polysomes. The addition of K^+ and Na^+ ions separately or together to the preparation media results in the preparation of ribosomes and polysomes with a lower protein content but a higher incorporating ability; K^+ ions show a greater activity in decreasing adherent protein than Na^+ ions (see Table 4). The presence of Na^+ as well as K^+ ions increases the protein content of and the incorporation *in vitro* by polysomes when compared with those prepared in the presence of K^+ ions alone. This might suggest a specific action of Na^+ ions in stabilizing on the polysome some protein factor, essential to incorporation, that is displaced by K^+ ions.

Table 5 demonstrates the effect of varying the K^+ and Na^+ ion concentrations on the incorporation of DL-[1- ^{14}C]leucine into protein *in vitro* by cardiac ribosomes prepared in the presence of both cations.

Table 3. Effect of concentration of Mg^{2+} ions on the incorporation of DL-[1- ^{14}C]leucine into protein *in vitro* by cardiac ribosomes

Incorporation conditions were as described in the Methods section with ATP (5 mM) in the medium, except that the Mg^{2+} ion concentration was as shown. Incubation was for 1 hr.

Concn. of Mg^{2+} ions (mM)	Incorporation of amino acids <i>in vitro</i> (counts/min./mg. of ribosomal RNA)
0	0
2.5	0
5	60
7.5	350
10	625
12.5	420
15	410
20	320

Table 4. *Effect of preparation of cardiac ribosomes and polysomes in the presence and absence of K⁺ and Na⁺ ions or both on the incorporation of DL-[1-¹⁴C]leucine into protein in vitro and on the RNA/protein ratio*

Incorporation conditions were as described in the Methods section.					
Concn. of ions in preparation medium (mm)		Cardiac polysomes		Cardiac ribosomes	
K ⁺ ions	Na ⁺ ions	Incorporation of amino acids <i>in vitro</i> (counts/min./mg. of ribosomal RNA)		Incorporation of amino acids <i>in vitro</i> (counts/min./mg. of ribosomal RNA)	
		RNA (mg./100mg. of protein)		RNA (mg./100mg. of protein)	
0	0	309	69	157	30
100	40	500	82	276	47
100	0	326	89	277	58
0	40	408	80	284	36

Table 5. *Effect of the concentrations of K⁺ and Na⁺ ions on the incorporation of DL-[1-¹⁴C]leucine into protein in vitro by cardiac ribosomes*

Incorporation conditions were as described in the Methods section, except that the concentrations of K ⁺ and Na ⁺ ions were varied as indicated.							
		Incorporation of amino acids <i>in vitro</i> (counts/min./mg. of ribosomal RNA)					
Concn. of K ⁺ ions (mm) ...	...	0	20	50	100	150	200
Concn. of Na ⁺ ions (mm)							
0		290	—	305	459	406	288
20		288	373	322	509	340	—
40		372	358	490	510	374	—
80		374	509	424	338	322	—
100		—	—	440	322	338	—
200		338					

Table 6. *Effect of the addition and omission of some components of the incorporation medium on the incorporation of DL-[1-¹⁴C]leucine into protein in vitro by cardiac ribosomes*

Incorporation conditions were as described in the Methods section, except for the additions and omissions indicated.	
Incubation mixture	Incorporation of amino acids <i>in vitro</i> (counts/min./mg. of ribosomal RNA)
Complete system	479
ATP and ATP-generating system omitted	2
ATP-generating system omitted	250
GTP omitted	100
Amino acid mixture omitted	315
Ribosomes omitted	2
Cell sap omitted	0
Puromycin (0.2mm) added	0
Ribonuclease (5µg./ml.) added	70
Ribosomes preincubated for 5 min. at 37° with ribonuclease (1µg./ml.)	0
CTP (0.05mm) added	472

The optimum incorporation is obtained when a total cation concentration of about 90–140mm is present: the distribution between the two seems to be less important.

When ATP and an ATP-generating system (creatine phosphate and creatine phosphokinase) are omitted from the incubation medium, little incorporation of amino acid takes place (Table 6). Omission of the ATP-generating system alone diminished the incorporation by 50%, in direct contrast with optimum conditions for incorporation of amino acids *in vitro* by liver ribosomes, which is not stimulated by the addition of an ATP-generating system in the presence of 5mm-ATP (Korner, 1961). This could be due to the presence of solubilized myosin adenosine triphosphatase in the cell-sap fraction (Huys, 1961).

Omission of GTP caused a considerable inhibition of incorporation, again in contrast with the liver system. Increasing the concentration of GTP in the incubation mixture gives a sharp rise to a maximum rate at 25µM, further additions up to ten times this concentration giving no further increases. The addition of CTP had no effect.

Incubation without added amino acid mixture decreased the incorporation of DL-[1-¹⁴C]leucine to two-thirds of that obtained with the complete incubation mixture, but only when the cell sap used had been passed through a Sephadex-gel filtration column.

The addition of 5 μ g. of ribonuclease/ml. to the incubation mixture considerably decreased the incorporation of amino acids. Preincubation with 1 μ g. of ribonuclease/ml. abolished all incorporation.

Puromycin added to the incubation mixture caused a complete inhibition of incorporation of radioactive amino acid.

The effect of increasing the amount of heart cell sap on the incorporation of DL-[1-¹⁴C]leucine into heart ribosomes is shown in Fig. 7. The incorporation was maximal when the heart-cell-sap protein/ribosomal protein ratio was 15:1.

Table 7 summarizes the rate of incorporation of DL-[1-¹⁴C]leucine into protein by heart and liver

ribosomes obtained by using different cell saps. It is evident that both heart ribosomes and heart cell sap are much less active in catalysing incorporation into protein *in vitro* than are comparable liver preparations.

Maximal incorporation by both liver and heart ribosomes with increasing cell-sap concentration is reached when the s-RNA/ribosomal RNA ratio is 0.23 with heart cell sap and 0.53 with liver cell sap (equivalent to ratios 15:1 and 25:1 cell-sap protein/RNA protein respectively).

Heart cell sap has an RNA content similar to that found for liver sap (10–15 mg./100 mg. of protein). The amino acid-activating enzymes also show a similar activity (0.6–0.8 μ mole of hydroxamate formed/hr./100 mg. of cell-sap protein; Osawa, 1962).

The lower activity of heart cell sap might be explained by a higher ribonuclease activity than in liver cell sap. This possibility was eliminated in two ways. First incubation of liver polysomes with heart and liver cell sap followed by sucrose-density-gradient analysis of the polysomes showed little breakdown, yet 1 μ g. of ribonuclease/ml. for 10 min. at 20° is sufficient to degrade polysomes to 80s monomers. The second approach was to incubate heart and liver cell sap with ³H-labelled RNA and measure the radioactivity extracted by ice-cold 0.5N-perchloric acid: no release of radioactivity above blank levels was detected.

The presence of an inhibitor of protein biosynthesis in heart cell sap is unlikely because a mixture of heart and liver cell saps shows a similar time-course for incorporation as liver cell sap alone (see Fig. 8). Various other possible explanations, e.g. an unstable factor in heart cell sap or inactivation of ribosomes by heart cell sap were also eliminated (Table 8). Cell sap was still capable of catalysing incorporation even when it had been incubated with ribosomes for 15 min. Liver ribosomes, incubated with heart cell sap for 15 min. at 37°,

Fig. 7. Effect of concentration of heart cell sap on the incorporation of DL-[1-¹⁴C]leucine into protein *in vitro* by cardiac ribosomes. Incorporation conditions were as described in the Methods section, except that the concentration of heart cell sap was varied. Incubation was for 1 hr.

Table 7. Comparison of the rates of incorporation of DL-[1-¹⁴C]leucine into protein *in vitro* by heart or liver ribosomes with heart or liver cell sap

Incorporation conditions were as described in the Methods section.

Source of ribosomes	Source of cell sap	Incorporation of amino acids <i>in vitro</i> (counts/min./mg. of ribosomal RNA)	
		After 20 min. incubation	After 60 min. incubation
Liver	Liver	6600	7500
Liver	Heart	1800	2500
Liver	Liver + heart	6500	6800
Heart	Heart	150	275
Heart	Liver	640	900

were capable of carrying out the incorporation of amino acids on the addition of liver cell sap (Table 8).

It was concluded from these results that heart cell sap lacks a factor, present in liver cell sap, that stimulates the incorporation of amino acids into protein *in vitro* by ribosomes. It is present on the ribosomes in strictly limiting amounts.

Fig. 8. Time-course of the incorporation of DL-[1-¹⁴C]leucine into protein *in vitro* by liver ribosomes: ○, with liver cell sap; ●, with heart cell sap; ▲, with a mixture of liver cell sap and heart cell sap. All cell saps were at saturating concentrations. Incorporation conditions were as described in the Methods section.

The relative abilities of heart and liver cell sap to catalyse the stimulation of the incorporation of L-[G-¹⁴C]phenylalanine by polyuridylic acid has been studied with cardiac ribosomes prepared in the presence and absence of Mg²⁺ ions (Table 9). Ribosomes prepared in the presence of Mg²⁺ ions showed the expected higher incorporation of L-[G-¹⁴C]phenylalanine with liver cell sap than with heart cell sap. Stimulation of incorporation by polyuridylic acid was noted.

With ribosomes prepared in the absence of Mg²⁺ ions, less difference in incorporations with liver and heart cell sap was noted. Although the basal level of incorporation is much decreased by preparation in the absence of Mg²⁺ ions, the stimulation by polyuridylic acid is much greater, showing that the 80S ribosomes are not damaged by preparation in the absence of Mg²⁺ ions and are capable of reacting with a new messenger RNA and synthesizing protein.

Incorporation of ¹⁴C-labelled algal-protein hydrolysate into subcellular fractions of cardiac muscle by pulse-labelling. Experiments reported in this section were carried out with the perfused heart preparation, as described in the Methods section.

Fig. 9 shows the effect of perfusion for 30 min. with a medium containing ¹⁴C-labelled algal-protein hydrolysate followed by various periods (from 0 to 15 min.) with a non-radioactive medium.

The ribosome fraction is the most active site of protein biosynthesis. The specific activity of this fraction rose sharply during the perfusion with radioactive amino acids. The rate decreases slightly when the perfusion with non-radioactive medium is started, and then reaches a peak and declines. The microsomes behave similarly but with a much lower specific activity.

The microsomal material solubilized by 0.2% sodium deoxycholate has a relatively slow initial rise of specific activity compared with the ribosomes. The subsequent very sharp rise to a peak

Table 8. Effect of a further incubation, with added liver ribosomes or liver or heart cell sap, on the incorporation of DL-[1-¹⁴C]leucine into protein *in vitro* by liver ribosomes and heart cell sap

Liver ribosomes and heart cell sap at optimum concentrations were incubated in a complete incorporation medium for 15 min. at 37°, as described in the Methods section. Additions were then made as indicated and the incubation was continued for a further 45 min. The total radioactivity incorporated into protein after the initial 15 min. incubation was 250 counts/min.

Additions after first incubation	Total radioactivity incorporated during second incubation (counts/min.)	Total radioactivity incorporated during first and second incubations (counts/min.)	Incorporation of amino acids <i>in vitro</i> (counts/min./mg. of ribosomal RNA)
Tris-salts medium (0.1 ml.)	215	465	1410
Liver ribosomes (0.1 ml.)	687	937	1400
Liver cell sap (0.1 ml.)	410	660	2015
Heart cell sap (0.1 ml.)	275	525	1590

Table 9. *Effect of polyuridylic acid on the incorporation of L-[G-¹⁴C]phenylalanine by cardiac ribosomes prepared in the presence and absence of Mg²⁺ ions with heart or liver cell sap*

Incorporation conditions were as described in the Methods section, except that polyuridylic acid was added where indicated.

	Source of cell sap	Polyuridylic acid (100 µg./ml.)	Incorporation of amino acids <i>in vitro</i> (counts/min./mg. of ribosomal RNA)	Percentage stimulation
Ribosomes prepared in the presence of Mg ²⁺ ions	Heart	—	4350	—
	Liver	—	9470	—
	Heart	+	8550	97
	Liver	+	15300	60
Ribosomes prepared in the absence of Mg ²⁺ ions	Heart	—	1250	—
	Liver	—	1450	—
	Heart	+	11850	950
	Liver	+	6260	430

Fig. 9. Specific activities (counts/min./mg.) of protein of subcellular cardiac fractions after 3 min. pulse-labelling by perfusion with ¹⁴C-labelled algal-protein hydrolysate and then by cold perfusion for various times. Details are given in the Methods section. The vertical line shows the point of transfer from radioactive to non-radioactive perfusion medium. (a) ●, Ribosomes (deoxycholate-insoluble); △, soluble protein of cell sap; ▲, deoxycholate-soluble microsomal material; ○, a myofibrillar fraction. (b) □, Microsomes; ■, mitochondria.

followed by a loss of radioactivity might imply that this material is receiving radioactivity from ribosomes and then passing it on.

The mitochondria show a continued steady rise in specific activity, but were much less active than ribosomes or microsomes.

DISCUSSION

The use of proteinase to assist homogenization is open to the criticism that it damages the ribosomes and inhibits the incorporation observed *in vitro*.

However, by standardizing the procedure the inhibition can be kept constant in experiment to experiment. The yield of ribonucleoprotein particles is increased by the use of proteinase, and the preparations are free of contamination by submitochondrial fragments. The cardiac-ribosome preparations obtained contain aggregates analogous to the polysomes of liver and other tissues (see Warner, Rich & Hall, 1962; Marks, Burka & Schlessinger, 1962; Wettstein *et al.* 1963; Noll *et al.* 1963; Goodman & Rich, 1963; Gierer, 1963; Marks *et al.* 1963; Burka, Danon & Marks, 1963;

Warner, Knopf & Rich, 1963; Williamson, Mathias, Huxley & Page, 1963; Munro *et al.* 1964), and exhibit many similar properties.

The electron-dense particles (200 Å in diameter) observed in the electron-microscopic studies of microsomes and ribosomes are interpreted as ribosomes (see Warner *et al.* 1962). From the examination of the microsomal fraction it is apparent that these particles are not attached to the vesicular elements in this preparation (see also Porter & Palade, 1957). This contention is supported by the observation that polysomes can be prepared from the 11000 g supernatant fraction from cardiac muscle without the use of deoxycholate in as great a yield as in the presence of the detergent. When deoxycholate is omitted in the preparation of polysomes the vesicular material sediments on to the surface of the 2M-sucrose layer (see the Methods section). In view of the findings of Henshaw, Bojarski & Hiatt (1963) that in liver only ribosomes attached to the endoplasmic reticulum are active in protein biosynthesis, the significance of these observations is not clear. The degradation of polysomes to 80s particles by small amounts of ribonuclease gives weight to the identification of 110s and larger aggregates as monomeric ribosomes held together by messenger RNA (Wettstein *et al.* 1963).

Cardiac polysomes are completely degraded, by preparation in the absence of Mg^{2+} ions, to monomeric ribosomes, with considerable loss of ability to incorporate amino acids into protein *in vitro*. Munro *et al.* (1964) report some degradation of liver-polysome structure during preparation in the absence of Mg^{2+} ions, but not much loss in incorporation of amino acids *in vitro*. Cardiac polysomes clearly show sensitivity to Mg^{2+} ions, and the messenger RNA is easily dissociated from ribosomes by their absence. This feature may be a result of damage due to the use of proteinase. The addition of polyuridylic acid to ribosomes prepared in the absence of Mg^{2+} ions gives a ninefold stimulation of incorporation of amino acids *in vitro* showing that the structure of the ribosome is not damaged and is capable of forming an active particle with new messenger RNA.

The RNA isolated from cardiac ribosomes is of two species, similar in size to those of the RNA isolated from liver ribosomes (Gierer, 1958; Hall & Doty, 1959), and they occur in similar proportions.

The RNA/protein ratio of cardiac ribosomes and polysomes was consistently lower than that of similar preparations from liver. The protein content was considerably increased by preparation in the absence of K^+ and Na^+ ions with a concomitant fall in the incorporation of amino acids *in vitro*, probably due to binding of protein (Peterman & Hamilton, 1961). Increasing the K^+ ion

concentration and to a smaller extent the Na^+ ion concentration markedly decreased the protein content, increasing the incorporation of amino acids *in vitro*.

The conditions for optimum incorporation of amino acids into protein *in vitro* by cardiac ribosomes and polysomes in the presence of heart cell sap are essentially similar to those reported for liver ribosomes and polysomes by Korner (1961) and Munro *et al.* (1964). The major discrepancy is the requirement by cardiac ribosomes for an ATP-generating system.

Cardiac ribosomes are less active than liver ribosomes, and the optimum incorporation attained with heart cell sap is one-third of that attained with liver cell sap. The possibility of the presence of an inhibitor or an unstable factor in heart cell sap was eliminated (see also Florini, 1964). The heart cell sap does not appear to damage ribosomes more than liver cell sap. It is concluded that a factor present in liver cell sap and stimulating the incorporation of amino acids *in vitro* is absent from heart cell sap. This factor is not amino acid-activating enzymes, for Osawa (1962) has demonstrated the presence of activating enzymes for 18 natural amino acids in dog cardiac cell sap. He presents evidence that heart muscle is less than half as active as liver on a wet weight basis in the activation of amino acids. The amount of cell-sap protein/g. wt. wet of tissue is considerably lower for heart muscle than for liver.

It is unlikely that heart cell sap lacks an enzyme concerned in the interaction of messenger RNA with ribosomes, since good stimulation of the incorporation of phenylalanine by the addition of polyuridylic acid was obtained.

When hearts are pulse-labelled with radioactive amino acids and fractionated, the ribosomes are the most rapidly labelled subcellular fraction: the radioactivity reaches a peak and declines. This is the situation found in liver by Littlefield, Keller, Gros & Zamecnik (1955) and Simkin & Work (1957). However, these authors find maximum labelling 3 min. after introduction of the pulse, whereas in heart muscle the peak of radioactivity is not reached until 10 min. after the pulse.

Simpson & McLean (1955), McLean, Cohn, Brandt & Simpson (1958), Winnick & Winnick (1960) and Wool (1961) find that microsomes and mitochondria in skeletal muscle and diaphragm, after a pulse of radioactive amino acids, attain a similar specific activity, both reaching a peak and then falling off at the same time. In the experiments reported in the present paper the microsomes reach a peak of labelling at a similar time to the ribosome fraction and then decline. The mitochondria, on the other hand, are considerably less radioactive and their radioactivity continues to rise. The differences

between the present results and those of McLean *et al.* (1958) and Winnick & Winnick (1960) might be caused by contamination of their mitochondrial preparations.

Florini (1962) reported incorporation of amino acids into protein *in vitro* by a cardiac preparation, sedimenting between 10000g and 105000g, that he tentatively called microsomes. The incorporation of amino acids *in vitro* was found to be insensitive to ribonuclease, a characteristic of mitochondrial and not microsomal protein synthesis (Truman & Korner, 1962; Kroon, 1963). Hulsmans (1961) points out the heavy contamination of cardiac microsomes by mitochondrial fragments when heart muscle is homogenized with a Potter-Elvehjem homogenizer. Later, Florini (1964) reported studies on the optimum conditions for the incorporation of amino acids into protein *in vitro* by a ribonuclease-sensitive microsomal system from skeletal muscle. His results and those of Rampersad & Wool (1964), Florini & Breuer (1964) and Zak, Rabinowitz, Beller & De Salles (1964) are in essential agreement with our own save in those points where differences in yield and properties of ribonucleoprotein particles are to be expected because of our use of proteinase.

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