# Ribonucleic Acids Associated with Myofibrils*

Radovan Zak, Murray Rabinowitz,† and Constance Platt

ABSTRACT: In hearts of 16-day-old chick embryos, 85% of the ribonucleic acid (RNA) is of ribosomal type, *i.e.*, 28 and 18 S; the remaining 15% is 4S RNA. The ribosomal ribonucleic acid (rRNA) is composed of 64% 28 S and 36% 18 S. The RNA isolated from myofibrils has a sedimentation profile similar to RNA from whole heart but only 5% of it is 4S RNA. This 4S RNA can be greatly reduced in quantity by washing the myofibrils. Myofibrillar RNA and rRNA have similar base compositions and identical ratios of 28S:18S components. The RNA isolated from nucleotropomyosin is composed of 28S and 18S components plus a small 4S peak. Ribonucleoprotein separated from myosin by high-speed centrifugation contains RNA which has sedimentation properties similar to rRNA. Ribosomes were isolated from highly purified myofibrils. Their sedimentation profiles and *in vitro* incorporation of [$^{14}$C]phenylalanine into protein are the same as for microsomal ribosomes. Of the total RNA associated with myofibrils, 85% may be extracted as ribosomes.

Ribonucleic acid has been detected in isolated myofibrils (Perry, 1952; Perry and Zydowo, 1959b) and in purified myosin (Perry, 1960; Mihalyi *et al.*, 1957; Baril *et al.*, 1964). Other myofibrillar proteins rich in RNA have also been isolated; Hamoir (1965) prepared nucleotropomyosin containing 15% RNA from whole muscle of fish, and Perry and Zydowo (1959a,b) prepared a nucleoprotein containing almost 50% RNA from rabbit myofibrils.

It is not yet established whether the association of RNA with muscle proteins has significance relative to myofibrillar structure and function, or whether RNA is present as an impurity. The first possibility is suggested by the demonstration of basophilic material in the A band of the myofibril that is removed by RNase treatment (Clavert *et al.*, 1949). Moreover, Baril *et al.* (1966) have recently demonstrated that the association of RNA with myosin may influence ATPase[1] activity. On the other hand, fortuitous combination of RNA and muscle proteins is suggested by observation in the electron microscope of remnants of sarcoplasmic reticulum in preparations of isolated myofibrils (Muscattello *et al.*, 1962). As in other tissues the endoplasmic reticulum of muscle contains a high concentration of RNA (Hulsmans, 1961; Margreth and Novelli, 1964).

To help decide between these possibilities the distribution of ribosomes and RNA in heart muscle and the properties of RNA associated with myofibrils and isolated muscle proteins were analyzed. It is concluded that at least a large fraction of myofibrillar RNA is of ribosomal type. The possibility of the presence of a small amount of a different species of RNA which could not be detected by the methods used is not eliminated. Some of these results have been previously reported in preliminary form (Zak *et al.*, 1964).

## Experimental Procedure

*Materials.* Hearts were excised from 16- to 18-day-old chick embryos, opened, washed, and blotted to remove any adherent clotted blood.

*Myofibrils* were isolated according to Perry and Grey (1956). Hearts were homogenized with 20 volumes (milliliter per gram wet weight) of 0.025 M KCl and 0.035 M borate buffer (pH 7.1) in a Waring Blendor. The disintegration of the tissue to myofibrils was followed by examining the homogenate with a phase-contrast microscope. The liberated myofibrils were separated from smaller particles and sarcoplasm by centrifugation at 900*g* for 15 min and resuspension in 20 volumes of KCl-borate buffer. This step was repeated five times. Unbroken muscle and nuclei were removed by centrifugation at 600*g* for 2 min. This step was repeated three times. In some experiments the myofibrils were further purified as follows. The pellet containing myofibrils was suspended in a final concentration of 2.2 M sucrose containing KCl-borate buffer. The suspension (4 ml) was overlayered by 1 ml of 1.6 M sucrose and centrifuged at 20,000 rpm for 80 min in the 25.2 rotor of a Beckman L-2 ultracentrifuge. The myofibrils banded at the interphase between 1.6 and 2.2 M sucrose, the mitochondria floated on the top of 1.6 M sucrose, and the nuclei sedimented to the bottom of the tube.

*Ribosomes* were isolated as follows. The excised hearts were washed with ice-cold solution I (0.2 M sucrose, 0.1 M KCl, 6 mM $MgCl_2$, 1 mM EDTA, and 20 mM Tris buffer (pH 8.0)), finely minced with scissors,

* From the Departments of Medicine and Biochemistry and the Argonne Cancer Research Hospital, University of Chicago, Chicago, Illinois. *Received October 7, 1966.* the Argonne Cancer Research Hospital is operated by the University of Chicago for the U. S. Atomic Energy Commission.

† Recipient of a U. S. Public Health Service career development award.

[1] Abbreviations used: ATPase, adenosine triphosphatase; PEP phosphoenolpyruvate; GTP, guanosine triphosphate.

suspended in five volumes of solution I, and disintegrated in a MSE homogenizer for 30 sec at 8000 rpm and for 10 sec at 14,000 rpm. The homogenate, or in other experiments, the purified myofibrillar fraction, was further processed with three strokes in a Dounce-type all-glass homogenizer (Kontes Glass Co., piston size A). Cell debris and mitochondria were sedimented by centrifugation for 10 min at 10,000 rpm. The supernatant was filtered through glass wool, and one-tenth volume of 2.0 M $NH_4Cl$–2.5% sodium deoxycholate (pH 7.5) was added. After centrifugation for 10 min at 15,000*g* the supernatant was layered on 1 ml of 2 M sucrose and centrifuged 4 hr at 150,000*g*. The pellet was rinsed with solution I. The ribosomal profile was studied by sucrose density gradient centrifugation. Ribosomes (4–5 $OD_{260\,m\mu}$) were layered on a 15–30% linear sucrose gradient in solution I and centrifuged for 90 min at 39,000 rpm in the Spinco SW 39 head. The optical density profile at 260 m$\mu$ was recorded continually using a flow cell and a Gilford spectrophotometer Model 2000.

*Nuclei* were isolated by modification (K. G. Nair, M. Rabinowitz, and M. Tu, submitted for publication) of a method developed by Widnell and Tata (1964).

*Myosin.* Crude myosin preparations were obtained by extraction of isolated myofibrils with 0.3 M KCl containing 20 mM sodium pyrophosphate and 5 mM $MgCl_2$ at pH 7.8 for 2 hr. Myosin was precipitated from the extract by adding ammonium sulfate to 45% saturation. The precipitated myosin was dissolved by dialysis against 0.5 M KCl. The preparation contained a small amount of actin, since the relative viscosity of solutions containing 2.5 mg of protein/ml fell from 1.670 to 1.545 after the addition of 5 mM $MgCl_2$ and 5 mM ATP.

Purified myosin was obtained by centrifugation of the KCl–pyrophosphate–$MgCl_2$ myofibrillar extract at 50,000 rpm for 2 hr. The upper two-thirds of the contents of the tube was removed and the myosin was precipitated with ammonium sulfate. ATP decreased the relative viscosity only from 1.575 to 1.505, showing that much of the actin was eliminated.

*Nucleotropomyosin* was prepared from whole heart by the method Hamoir (1951) used to prepare nucleotropomyosin from carp muscle. All solutions and glassware used for isolation of myofibrils and proteins were sterilized, and sterile conditions were followed as closely as possible to reduce the RNA degradation.

*Isolation and Analysis of Nucleic Acids.* Total DNA and RNA was isolated by the method of Kirby (1965). The nucleic acids were extracted in a phenol–cresol mixture containing 4-aminosalicyclic acid and were precipitated with two volumes of ethanol. The precipitate was extracted twice with 10 ml of 3 M sodium acetate (pH 5.5). The extract contained DNA and RNA and the insoluble residue contained rRNA. The extract was dialyzed for 0.5 hr against distilled water and precipitated with two volumes of ethanol. Both fractions were washed twice with 75% ethanol and once with absolute ethanol. Finally, the nucleic acids were dissolved in 0.1 M sodium acetate (pH 6.5) and layered on top of a 5–20% sucrose density gradient containing 0.01 M sodium acetate (pH 6.5). The gradients were centrifuged in a Beckman L-2 ultracentrifuge in the SW 39 rotor for 16 hr at 22,000 rpm, or in the SW 25.3 rotor for 18 hr at 25,000 rpm. The bottom of the tubes was punctured and optical density profiles were analyzed at 260 m$\mu$ using a constant-flow system and the Gilford spectrophotometer Model 2000, or after drop collection, in the Zeiss spectrophotometer.

The base composition of the RNA fractions was determined by the method of Katz and Comb (1963) after hydrolysis for 18 hr in 0.3 M NaOH at 37°. RNA was determined by the orcinol method (Mejbaum, 1939), DNA by the diphenylamine method (Burton, 1956), and protein by the biuret reaction (Gornall *et al.*, 1949).

*Isotope Incorporation Experiments.* Chick embryos (15-days old) were injected intramuscularly with 5 $\mu$c of $^{32}P$ (carrier-free phosphate solution, Nuclear Consultants, Mallinckrodt Corp.) and sacrificed 20 hr later. Female, 200-g Sprague–Dawley rats were given 150 $\mu$c of $^{32}P$ intraperitoneally and sacrificed 24 hr later. The [$^{32}P$]RNA was isolated and analyzed by sucrose density gradient centrifugation as described above. Samples were counted in 15 ml of Bray's (1960) solution in a Tri-Carb liquid scintillation spectrometer. [$^{14}C$]Amino acid incorporation into proteins by ribosomes were measured by methods previously described (Rampersad *et al.*, 1965).

## Results

*Distribution of RNA in Cell Fractions.* When standard cell fractionation techniques are applied to muscle, much of the RNA sediments with myofibrils (Perry, 1952; Hulsmans, 1961; Margreth and Novelli, 1964).

TABLE I: Percentage Recovery of RNA in Purified Fractions of Chick Embryonic Heart.

| | Method I[a] | Method II[b] |
|---|---|---|
| Microsomes | 19 | 53 |
| Myofibrils | 45 | 24 |
| 150,000*g* supernatant | 20 | 15 |

[a] In method I hearts were homogenized in 0.25 M sucrose–0.15 M Tris (pH 7.2). Myofibrils, nuclei, and cell debris were sedimented by centrifugation at 900*g* for 15 min. Mitochondria were sedimented at 8000*g* for 10 min. The supernatant was centrifuged at 105,000*g* for 60 min and the microsomal pellet was suspended in the same buffer and pelleted in the same way. The fraction sedimenting at 900*g* was suspended in borate buffer and the myofibrils were purified as described in the Methods. [b] In method II the hearts were homogenized in 0.2 M sucrose, 0.1 M KCl, 1 mM $MgCl_2$, 1 mM EDTA, and 20 mM Tris (pH 8.1). Subsequent purification steps were identical with those described for method I.

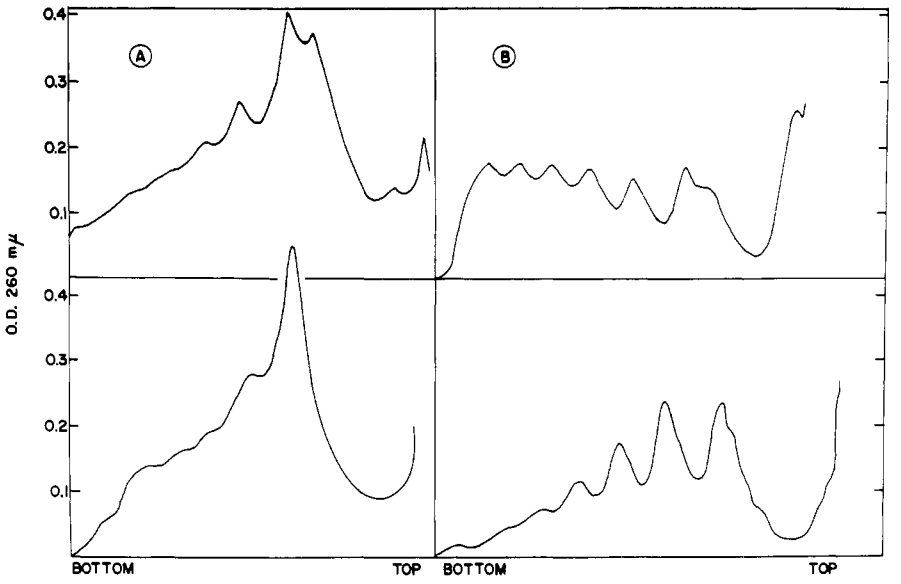


FIGURE 1: Sedimentation profiles of ribosomes. (A) Extracted from hearts of 16-day-old chick embryos. Upper curve: Ribosomes liberated by deoxycholate treatment of microsomes. Lower curve: Ribosomes liberated by deoxycholate treatment of isolated myofibrils. (B) Extracted from hearts of 3-month-old rats. Upper curve: Ribosomes pelleted from postmitochondrial supernatant treated with deoxycholate. Lower curve: Ribosomes liberated by deoxycholate treatment of myofibrils. Approximately 4 $OD_{260\ m\mu}$ of ribosomes was applied to a 15–30% sucrose density gradient and centrifuged 90 min at 39,000 rpm in Spinco SW 39 rotor.

The RNA distribution in chick embryo heart was analyzed in cell fractions obtained by differential centrifugation. The distribution of RNA was compared in preparations which used as suspending medium 0.25 M sucrose and 0.15 M Tris (pH 7.2) with fractionations using solution I which contains 0.2 M sucrose, 0.1 M KCl, 4 mM $Mg^{2+}$, 1 mM EDTA, and 20 mM Tris (pH 8.1) (Table I). In the first case 45% of the RNA is associated with the myofibrillar fraction; when solution I is used to homogenize the tissue, only 24% of the RNA sediments with the myofibrillar fraction and greater amounts appear in the microsomal fraction. This indicates that clean separation of subcellular elements is difficult to obtain in muscle and that the RNA distribution is greatly dependent on the composition of the suspending medium.

*Isolation of Ribosomes from Myofibrils.* Ribosomes were isolated from purified myofibrils as described in the methods and the properties compared with ribosomes isolated from the microsomal fractions. Ribosomes obtained from myofibrillar and from microsomal fractions of hearts from chick embryos and rat have similar sucrose density gradient profiles which display prominant multiple polysome peaks (Figure 1A,B). There are also no differences between the rates of [$^{14}$C]phenylalanine incorporation into protein by ribosomes isolated from myofibrils and from microsomes, in the presence or in the absence of poly U (Table II). The yield of myofibrillar ribosomes is approximately 15% of the total ribosomes extracted from the heart. Approximately 85% of myofibrillar RNA could be extracted as ribosomes.

*Nucleic Acids of the Whole Heart.* Four species of nucleic acids are distinguished by their sedimentation properties. The sedimentation profiles of nucleic acids not extractable in 3 M sodium acetate are seen in Figure 2 (upper curves). The sedimentation values were established to be 28 and 18 S from simultaneous density

TABLE II: [$^{14}$C]Phenylalanine Incorporation into Protein by Ribosomes Isolated from Chick Heart Microsomes and Myofibrils.[a]

| Source of Ribosomes | [$^{14}$C]Phenylalanine Incorp into Protein (cpm/mg of Ribonucleoprotein) −Poly U | +Poly U |
|---|---|---|
| Myofibrils | 5,190 | 45,100 |
| Microsomes | 7,510 | 46,130 |

[a] The reaction mixture contained per 1 ml: 55 μmoles of Tris-HCl buffer (pH 7.6), 30 μmoles of KCl, 10 μmoles of $MgCl_2$, 10 μmoles of mercaptoethanol, 1 μmole of ATP, 10 μmoles of PEP, 0.25 μmole of GTP, 10 μg of PEP kinase, and 1 mg of liver 105,000*g* supernatant. The final volume of 0.4 ml contained 1 $OD_{260\ m\mu}$ units of ribosomes, 100 μg (11,000 cpm) of [$^{14}$C]phenylalanyl-tRNA, and 100 μg of poly U where indicated. Incubation was at 37° for 15 min. The samples were processed as described previously (Rampersad *et al.*, 1965). Determinations were done in duplicates.

TABLE III: Percentage Distribution of Nucleic Acids Extracted from Chick Embryo Heart.[a]

| | Insoluble in 3 M Sodium Acetate | | | Soluble in 3 M Sodium Acetate | | |
|---|---|---|---|---|---|---|
| | | % of this Fraction | | | % of this Fraction | |
| | % of Total | 28 S | 18 S | % of Total | DNA | 4 S |
| Whole heart | 60 | 64 | 36 | 40 | 75 | 25 |
| Myofibrils | 75 (3.5)[b] | 65 | 35 | 25 | 80–90 | 10–20 |

[a] The nucleic acids were extracted from 5 g of heart or from 10 g of myofibrils and separated into 3 M sodium acetate soluble and insoluble fractions as described in Methods. The soluble and insoluble fractions were analyzed on sucrose density gradient. The relative amounts of nucleic acids in each type were obtained by integrating the areas under optical density curves of sucrose density gradient. [b] Percentage contributed by nuclear RNA.

TABLE IV: Base Composition of RNA Extracted from Heart Muscle Fractions of 18-Day-Old Chick Embryos.[a]

| RNA | G | A | C | U | G + C:A + U |
|---|---|---|---|---|---|
| Myofibrils | 32.9 ± 0.85 | 19.2 ± 0.64 | 25.1 ± 0.42 | 22.5 ± 0.65 | 1.40 ± 0.13 |
| Ribosomes | 34.4 ± 1.12 | 21.1 ± 0.29 | 23.4 ± 0.75 | 21.1 ± 1.14 | 1.37 ± 0.10 |

[a] The results are averages of three determinations, and are expressed as moles of individual base (plus and minus average deviation). The RNA analyzed is the fraction insoluble in the 3 M sodium acetate (ribosomal type).

TABLE V: The RNA Content of Protein Fractions of Muscle.[a]

| | Experimental Values | Values Reported in Literature | | |
|---|---|---|---|---|
| Muscle Component | Chick Embryo Heart | Type of Muscle | RNA Content | Reference |
| Whole muscle | 6.4 | Rat heart | 1.82 | Hulsmans (1961) |
| | | Rabbit skeletal | 0.69 | Perry and Zydowo (1959b) |
| Myofibrils | 1.6 | Rabbit skeletal | 0.30 | Perry and Zydowo (1959b) |
| Myosin | 0.3 | Rabbit skeletal | 0.2–0.8 | Perry (1960) |
| | | | | Mihalyi *et al.* (1957) |
| Nucleotropomyosin | 8.5 | Carp | 9.9–15.0 | Hamoir (1951) |
| Nucleoprotein of extraprotein | — | Rabbit skeletal | 50 | Perry and Zydowo (1959b) |
| Ribosomes | 182.0 | | | |

[a] The RNA content is expressed as mg of RNA/100 mg of protein. (To recalculate the data in the literature, the following factors were used: phosphate content of RNA = 8.2%; mg of protein nitrogen × 6.25 = mg of protein, and total protein content of muscle in percentage of wet weight = 17.8%.)

gradient runs, using chick embryo liver rRNA as standard. The two peaks were not affected by digestion with DNase.

The nucleic acid fraction soluble in 3 M sodium acetate also shows two peaks when analyzed by sucrose density gradient centrifugation (Figure 2, lower curves). The larger, heavier peak was quantitatively removed following digestion for 30 min with 10 μg/ml of DNase at 37° after adjusting the $MgCl_2$ concentration to $10^{-2}$ M. The lighter peak had a sedimentation value comparable to RNA of *Escherichia coli*.

The relative proportions of these four nucleic acids

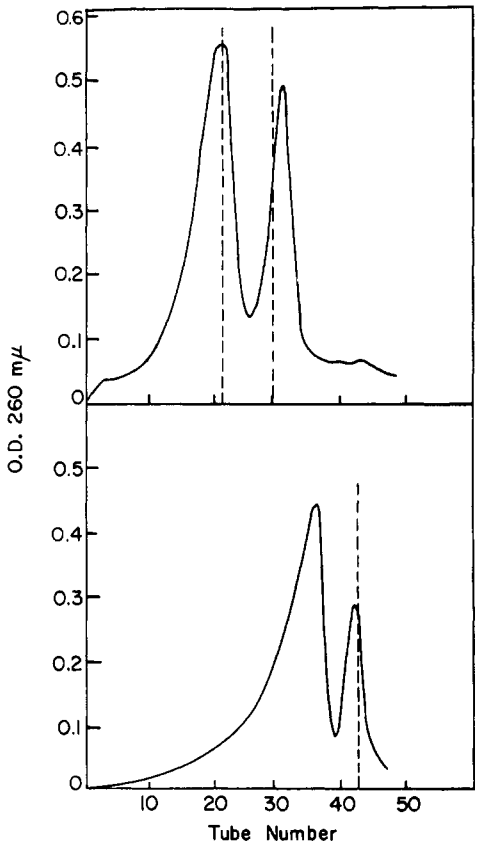


FIGURE 2: Sedimentation profiles of nucleic acids extracted from whole embryonic heart. Upper curve: Nucleic acid fraction insoluble in 3 M sodium acetate (the vertical lines indicate the position of RNA isolated from chick embryonic liver microsomes analyzed under the same conditions). Lower curve: Nucleic acids fraction soluble in 3 M sodium acetate (the vertical line indicates the sedimentation peak of *E. coli* RNA). A sample of approximately 10 $OD_{260\ m\mu}$ was layered on a 5–20% sucrose gradient and centrifuged for 18 hr at 25,000 rpm in Spinco SW 25.3 rotor. The optical density at 260 mμ of 20-drop aliquots, diluted with 1 ml of water, was determined in the Zeiss Model PMQ II spectrophotometer.

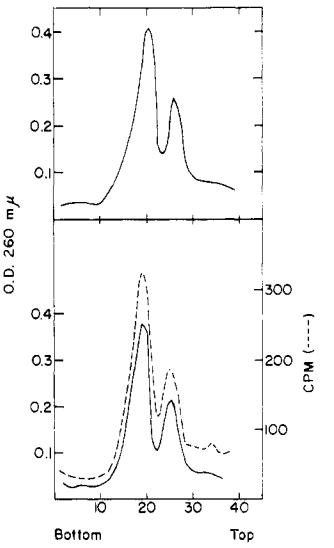


FIGURE 3: Comparison of the sedimentation profile of nucleic acids isolated from myofibrils and from heart ribosomes. Upper curve: RNA extracted from heart ribosomes. Lower curve: Nucleic acid fraction extracted from heart myofibrils insoluble in 3 M sodium acetate. Approximately 4 $OD_{260\ m\mu}$ myofibrillar RNA and 0.25 $OD_{260\ m\mu}$ containing 3600 cpm of $^{32}P$ chick liver rRNA were layered on a 5–20% sucrose density gradient and centrifuged 16 hr at 22,000 rpm in Spinco SW 39 rotor. Ten-drop aliquots were collected from the punctured tube, diluted with 1 ml of water, and read at 260 mμ. The radioactivity was counted after addition of 15 ml of Bray's (1960) solution in a Tri-Carb liquid scintillation spectrometer.

identified as 28S, 18S, and 4S RNA and DNA are given in Table III. In whole heart 4S RNA represents 15% of total muscle RNA. This value agrees with direct measurements of RNA in the 150,000*g* supernatant (Table I). RNA extracted from the 150,000*g* supernatant is almost all 4 S and contains only traces of 28S and 18S RNA.

*RNA Isolated from Myofibrils.* As was the case for whole muscle, four types of nucleic acids are detected in purified myofibrils. The sedimentation patterns of these nucleic acids are identical with those obtained from whole heart, although there are differences in the relative amounts of the four components (Table III). Approximately 75% of the myofibrillar nucleic acids is not extractable by 3 M sodium acetate and is composed of 28 and 18 S (Figure 3, lower curve). The sedimentation values were determined by mixing liver rRNA labeled with $^{32}P$ with myofibrillar RNA. The sedimentation profiles are identical with those of RNA isolated from purified chicken heart ribosomes (Figure 3, upper curve).

DNA and 4S RNA were identified in the fraction solublein 3 M sodium acetate (Figure 4). Treatment of this fraction with DNase led to the removal of the faster sedimenting peak. Since the amount of DNA was reduced by one-half when the myofibrils were purified by step-density gradient centrifugation, the DNA was assumed to represent contamination by nuclei.

Contamination by nuclei may account for a fraction of RNA found in myofibrils. This fraction may be estimated from the DNA content of the myofibrils and the ratio of DNA to RNA present in purified nuclei isolated from chick embryonic hearts. This ratio was determined to be 6.6:1. Approximately 3.5% of myofibrillar RNA was calculated to be derived from nuclei by this method.

The base composition of 28S and 18S RNA isolated from myofibrils is similar to that of RNA isolated from purified ribosomes (Table IV). The small dif-

TABLE VI: Incorporation of $^{32}P$ into RNA Associated with Myofibrils and Microsomes of Heart.[a]

| Source of RNA | Cpm/$OD_{260\,m\mu}$ | | | |
|---|---|---|---|---|
| | Chick Embryo | | Adult Rat | |
| | 28 S | 18 S | 28 S | 18 S |
| Microsomal ribosomes | 600 | 620 | 340 ± 24 | 344 ± 38 |
| Myofibrillar ribosomes | 595 | 605 | 341 ± 17 | 344 ± 13 |
| Myofibrils after DOC[b] extraction | 620 | 630 | 342 ± 51 | 355 ± 15 |

[a] Chick eggs (120 of 18-day old) or 20 rats were used in each experiment. The data for rats represents the average of four experiments (plus and minus average deviation). Twenty to twenty-four hours after injection of $^{32}P$, RNA was extracted from the various fractions and analyzed by density gradient centrifugation as described in the Methods.
[b] DOC, sodium deoxycholate.

ferences observed are within the error of the determination.

A small amount of 4S RNA is present in the myofibrillar preparations. The 4S RNA could be reduced in amount by repeated washing of the myofibrils, while DNA and 28S and 18S RNA remained constant. The DNA:4S RNA ratio increases from 1.56 in once-washed myofibrils, to 1.96 in two-times-washed, 3.10 in three-times-washed, and 5.30 in six-times-washed myofibrils.

*RNA Associated with Nucleotropomyosin.* Nucleic acids were extracted from nucleotropomyosin isolated from chick embryonic heart by the method of Hamoir (1951). RNA, but not DNA, was detected in phenol extracts, as indicated by a negative diphenylamine test for deoxyribose and the agreement between RNA content determined from absorption at 260 mμ and by orcinol test for ribose. Analysis by sucrose density gradient centrifugation showed that most of the RNA was 28 and 18 S, with small peaks in the 4S region (Figure 5).

*RNA Associated with Myosin.* The crude myosin contained 1.7 mg of RNA/100 mg of protein (Table V). Most of the RNA could be removed by centrifugation at 150,000*g* for 2 hr. Myosin purified in this way contained only 0.3 mg of RNA/100 mg of protein, while the pellet contained 38 mg of RNA/100 mg of protein. The RNA of purified myosin was obtained in small yield, and was polydisperse, with no distinct peak on sucrose density gradient. The RNA of the pellet separated from myosin by 2-hr centrifugation at 50,000 rpm was of ribosomal type, as judged from sucrose density gradient analysis (the results were identical with those shown in Figure 3).

*Incorporation of $^{32}P$ into RNA.* RNA labeled with $^{32}P$ was isolated from embryonic chicken or adult rat hearts 20–24 hr after injecting the radioactive $^{32}P$.

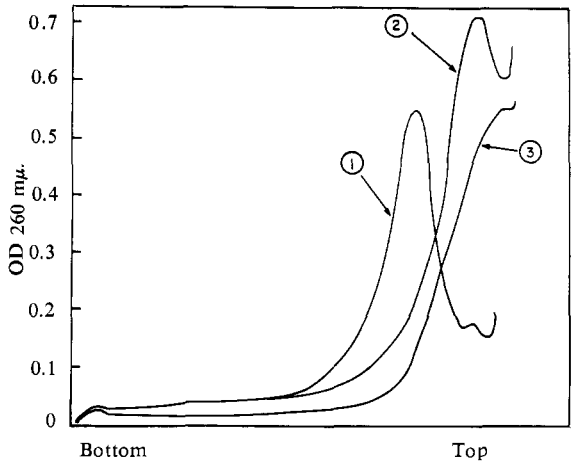


FIGURE 4: Sedimentation profiles of nucleic acids soluble in 3 M sodium acetate extracted from myofibrils. Curve 1: Nucleic acid fraction soluble in 3 M sodium acetate. Curve 2: *E. coli* RNA. Curve 3: Same as curve 1, reated with 10 μg/ml, of DNase for 60 min at 37°. The conditions were the same as those in Figure 2, except that a Gilford multiple sample absorbance recorder was used to trace the optical density distribution.

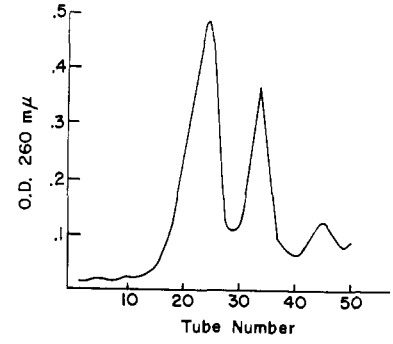


FIGURE 5: Sedimentation profile of nucleic acids extracted from nucleotropomyosin. The conditions were as given in Figure 2

The specific activities of the 28S and 18S RNA separated by sucrose density gradient centrifugation are given in Table VI. No difference in specific activity could be detected in: (1) RNA extracted from ribosomes prepared from 8000*g* supernatant, (2) RNA extracted from ribosomes obtained from myofibrils or, (3) RNA remaining in myofibrils after ribosomes had been extracted by deoxycholate. The RNA which remains associated with myofibrils after extraction of ribosomes represents 10–15% of the total RNA and shows 28S and 18S peaks having the same specific activity of $^{32}P$ as microsomal RNA. This rRNA, however, is much more susceptible to degradation into smaller fragments. In five preparations, degradation occurred twice. Activation of contaminating lysosomal RNase by deoxycholate may be responsible.

## Discussion

The nucleic acids extracted from heart muscle show sedimentation patterns and quantitative distributions similar to those of nucleic acids from other cells. The RNA is composed of 28S and 18S ribosomal fractions, and a low molecular weight 4S fraction. DNA extracted by the method used represents one-third of the total nucleic acids; it displayed a polydispersed sedimentation behavior.

The nucleic acids extracted from purified myofibrils have sedimentation properties similar to those extracted from the whole cell, although the amounts of DNA and 4S RNA are less in the myofibril. Approximately 75% of the nucleic acid, and over 95% of the RNA associated with myofibrils, have properties which are similar to those of rRNA. This RNA is insoluble in 3 M sodium acetate, and it sediments at 28 and 18 S just as purified chick heart microsomal RNA does. The ratio of 28S to 18S material is identical with that of authentic rRNA. Furthermore, its base composition is similar to that of chick microsomal RNA. The content of myofibrillar 28S and 18S RNA is not diminished by repeated washing of the myofibrils, and therefore must be firmly bound to the myofibrillar structure.

Isolation of ribosomes from myofibrils in high yield (85–90% of the total myofibrillar RNA) further indicates a ribosomal orgin for most "myofibrillar" RNA. "Myofibrillar" ribosomes are similar to microsomal ribosomes in their sedimentation profiles and in their ability to support incorporation of [$^{14}C$]-phenylalanine into proteins *in vitro*. Furthermore no difference in the incorporation of $^{32}P$ *in vivo* into myofibrillar and microsomal RNA could be detected.

The slowly sedimenting RNA fractions represent 2.5–5% of myofibrillar nucleic acid and have sedimentation values similar to those of *E. coli* RNA. Like RNA, they are soluble in 3 M sodium acetate. Repeated washing of the myofibrils results in significant reduction, but incomplete elimination, of the 4S RNA content. Because of the small amount present, direct demonstration of amino acid acceptor activity of this RNA fraction was not attempted.

The DNA present in myofibrils almost certainly represents contamination with nuclei or nuclear fragments. DNA constituted about 20% of the muscle nucleic acid in the myofibrillar preparation, a figure similar to that reported by Perry (1952). After purification of the myofibrils by sucrose density gradient centrifugation, the DNA content was reduced by 50%, but remained appreciable.

Several proteins isolated from myofibrils have been reported to contain RNA (Table V): myosin (Mihalyi *et al.*, 1957; Perry, 1960; Baril *et al.*, 1964, 1966), nucleotropomyosin (Hamoir, 1951), and nucleoprotein isolated from extraprotein (Perry and Zydowo, 1959a,b). In the few studies which examined the properties of this RNA, it was shown to be of low molecular weight. Recently, it has been emphasized that RNA is extremely susceptible to degradation by nucleases during isolation procedure, which might account for these results. In this study, it has been possible to isolate from nucleotropomyosin and impure myosin 28S and 18S RNA, representing 90% of total RNA. It would appear that a large part of the RNA associated with these proteins is of ribosomal type.

It is concluded that most RNA present in myofibrillar proteins is of ribosomal type. The presence of small amounts of other RNA species can not be eliminated.

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# Reaction of Formaldehyde with Heterocyclic Imino Nitrogen of Purine and Pyrimidine Nucleosides*

Edward J. Eyring† and James Ofengand

ABSTRACT: Hydroxymethylation of pseudouridines B and C, uridine, thymidine, inosinic acid, and polyuridylic acid by reaction with formaldehyde has been shown to take place by the use of spectrophotometric techniques. Reaction is almost instantaneous at room temperature and neutral or alkaline pH, resulting in addition to the $N_1$ and $N_3$ atoms of the pyrimidine ring, where available, and to the $N_1$ atom of the purine ring. Reactants and products are in rapid equilibrium such that after excess formaldehyde is removed by chromatography, no hydroxymethyl adduct remains. The equilibrium constant for reaction with uridine and inosinic acid was determined to be 2.5 and 1.7 l./mole, respectively. This reaction becomes significant when nucleic acids are studied in the presence of formaldehyde, since under these conditions an appreciable fraction of the uridylic or thymidylic acid will be derivatized.

Hydroxymethylation with formaldehyde has been widely used as a mild means of alteration of the secondary structure of nucleic acids as a result of the initial observations of Fraenkel-Conrat (1954) and Staehelin (1958), confirmed by others (Grossman *et al.*, 1961; Haselkorn and Doty, 1961), that reaction occurs wi h amino group containing nucleotides in both RNA and DNA. Several studies have shown that nucleic acids possessing large amounts of secondary structure such as native DNA, synthetic copolymers, or RNA in the presence of magnesium do not react with formaldehyde, while nucleic acids in which the secondary structure has been disrupted do so readily (Staehelin, 1958; Grossman *et al.*, 1961; Haselkorn and Doty, 1961; Staehelin, 1959; Sinsheimer, 1959; Sarkar and Dounce, 1961; Penniston and Doty, 1963; Marciello and Zubay, 1964; Fasman *et al.*, 1965). Attempts have been made to use this reaction as a means of distinguishing structured from nonstructured regions and to quantitate the degree of structure (Haselkorn and Doty, 1961; Penniston and Doty, 1963). In all of these studies, it has been assumed that formaldehyde does not react with the heterocyclic imino nitrogen atoms of purine or pyrimidine nucleotides such as uridylic acid because of the spectral study reported by Fraenkel-Conrat (1954). He observed that while the reaction with adenylic, cytidylic, and guanylic acids was relatively slow and led to marked spectral changes, only minor changes were noted in the absorption of uridine upon addition of formaldehyde, and there was no progressive alteration of the spectrum with time. These results were supported by the failure of [$^{14}$C]formaldehyde to become bound in a stable form to poly U or to poly I (Staehelin, 1958).

In this paper we report experiments showing that formaldehyde does react with the imino nitrogen atoms of pseudouridine, uridine, thymidine, and inosinic acid as well as with polyuridylic acid. The reaction is very rapid. However, the hydroxymethyl adducts are unstable and decompose when formaldehyde is removed. The reaction is of significance, therefore, only in those studies in which the nucleic acid is studied in the presence of formaldehyde.

## Experimental Section

### *Materials*

Uridine (pseudouridine free),[1] thymidine, and inosinic acid, all A grade, were obtained from CalBiochem and used without further purification. Pseudouridine, purchased from CalBiochem, was separated into its B and C isomers ($\alpha$ and $\beta$ anomers, respectively) by thin layer chromatography (Ofengand and Schaefer, 1965). [$^{14}$C]Formaldehyde was obtained from New England Nuclear Corp. Polyuridylic acid was synthesized

* From the Department of Biochemistry, University of California Medical Center, San Francisco, California. *Received March 27, 1967.* Supported by Grant GM 11506 from the National Institutes of Health.

† Special Fellow of the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health. Present address: Children's Hospital, Department of Orthopaedics, Columbus, Ohio.

[1] Actual chromatographic analysis showed the presence of 1.0% pseudouridine in this sample (lot 40046).