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The Nucleic Acids of *Drosophila melanogaster*

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1. Nucleic acids of whole *Drosophila* adults were prepared in good yield and substantially free from impurities by new modifications of the phenol method. 2. The average molar base compositions of the DNA (41% of guanine + cytosine) and transfer RNA (60% of guanine + cytosine) resemble those of mammalian nucleic acids; the ribosomal RNA has a DNA-like molar base composition (43% of guanine + cytosine), and it is considered that this is reflected in the lower stability of its secondary structure compared with mammalian ribosomal RNA. 3. The two main ribosomal forms were separated and average base compositions and sedimentation values determined.

RNA and DNA were isolated in good yield from mammalian sources by phenol extraction (Kirby, 1956, 1957). The RNA was relatively free from DNA and polysaccharides, but although ribonuclease is inhibited by phenol it is not inactivated and on purification some degradation of RNA occurred; the addition of 8-hydroxyquinoline to the phenol (Kirby, 1962*b*) resulted in an improved product but some ribonuclease activity was still apparent.

Application of these methods to the separation of nucleic acids from *Drosophila* flies showed that ribonuclease was equally resistant to complete removal. The isolation and fractionation were also made difficult by the presence of large amounts of glycogen, by the relatively small amount of DNA (25mg./100g. of flies) compared with the amount of RNA (650mg./100g. of flies) [RNA being isolated by the method of Kirby (1962*b*) and then DNA from the remaining phenolic DNA–protein gel by the method of Kirby (1957)] and by the relatively much greater tendency to loss of secondary structure of the ribosomal RNA (compared with mammalian ribosomal RNA).

These difficulties were overcome by extracting RNA first and then removing residual ribonuclease by a second extraction of the aqueous phase. DNA could be extracted from the insoluble residues in good yield and with only a small degree of protein contamination. Transfer RNA was separated by first precipitating ribosomal RNA and glycogen with *m*-cresol.

MATERIALS

Hydroxyquinoline–phenol mixture was made by dissolving 8-hydroxyquinoline (0·5g.) in a mixture of phenol (detached crystals, 500g.) and water (55ml.). Phenol–cresol mixture was made by adding redistilled *m*-cresol (70ml.) to the hydroxyquinoline–phenol (amount as above).

Sucrose gradients (5–20%, w/v) were normally made up in either 10mM-sodium acetate buffer, pH5·2, or 0·15M-NaCl–15mM-trisodium citrate buffer, pH7, and run on the SW25.1 head of the Spinco model L centrifuge at 4°. Unless otherwise stated 0·5–1mg. of purified RNA was dissolved in 1ml. of the corresponding buffer solution, and carefully layered on to the gradients; to test for freedom from ribonuclease, bentonite (1mg. prepared by the method of Fraenkel-Conrat, Singer & Tsugita, 1961) was in some cases added to the buffer solution before dissolution of the sample.

METHODS AND RESULTS

Initial extraction of total RNA. *Drosophila* adults (10–50g.) were anaesthetized by exposure to ether vapour for a few seconds and immediately homogenized in a cooled Waring Blendor for 1min. with ice-cold 0·5% (w/v) disodium naphthalene-1,5-disulphonate (5ml./g. of flies) together with cool hydroxyquinoline–phenol (5ml./g. of flies). The mixture was stirred for 30min. at room temperature (20°) and then centrifuged (20min. at 5000*g* at 5°). The supernatant fraction containing the bulk of the RNA together with much glycogen was carefully removed, and the gel-like interface containing DNA–protein complexes was collected and concentrated if necessary by further brief centrifugation. If repeated extractions of the fly residues at the base of the original phenol layer and of the interfacial DNA–protein gel were carried out with equal volumes of fresh 0·5% naphthalene-1,5-disulphonate together with hydroxyquinoline–phenol, a small additional yield of RNA was obtained (less than 10% of the yield from the initial extraction), but such re-extraction was not normally carried out because of the possibility of degradation resulting from excessive handling.

Purification of RNA. The supernatant fraction from the first naphthalene-1,5-disulphonate-and-hydroxyquinoline–phenol extraction was made 3% (w/v) with respect to sodium toluene-*p*-sulphonate and 4% (w/v) with respect to

NaCl, stirred for 20 min. at 20° with 0·5 vol. of phenol–cresol mixture and then centrifuged (15 min. at 5000*g* at 5°). The aqueous phase was carefully removed and the crude RNA precipitated with 2 vol. of cooled (5°) ethanol–M-sodium acetate (pH 6) (9:1, v/v). After recovery by low-speed centrifugation the precipitate was extracted three times with cold 3 M-sodium acetate, pH 6·0, which removed glycogen, traces of DNA and transfer RNA. The method of purification was shown to be effective by the absence of glucose on hydrolysis with N-HCl (method of Partridge, 1949), the absence of a colour in the Burton (1956) modification of the Dische reaction and the absence of RNA in the 4S region after centrifugation in a sucrose density gradient (cf. curve *A* in Fig. 1). The remaining ribosomal RNA was washed twice with cold aq. 75% (v/v) ethanol containing sodium acetate (1%, w/v), then with ethanol, and dried in a vacuum desiccator. It was preferable to carry out all operations from the final precipitation onwards at 0–5°. The yield was 500–550 mg./100 g. of flies.

Separation of the two ribosomal components. The two components were separated by centrifuging in sucrose gradients in 0·2 M-NaCl–10 mM-sodium acetate, pH 7·0, all operations being performed at 0–4° (cf. Kirby, 1965). From 15 mg. of RNA, 7·5 mg. of the faster component and 3·5 mg. of the slower component were obtained. Sedimentation-velocity measurements (Shooter & Butler, 1956) on the Spinco model E centrifuge gave $S_{20,w}$ 29·4 and 18·8S for the two components dissolved in 0·1 M-NaCl at 4° and run at 6° (see Fig. 2). Parallel sucrose-gradient profiles for the two components compared with the original material are shown in Fig. 1. No change took place after storage for 8 months at 2° or when bentonite was present in the gradient mixture.

Isolation of RNA from microsomal particles. Drosophila flies were cooled and homogenized in a blender with a cold solution (3 ml./g. of flies) of 0·44 M-sucrose–50 mM-tris–HCl (pH 7·6) – 60 mM-KCl – 5 mM-NaCl – 0·1 mM-magnesium acetate for 20 sec. at 2°. The mixture was centrifuged at 12000*g* for 10 min. at 5° and the supernatant fraction was carefully removed and centrifuged again in the same manner. The supernatant solution (8 ml. portions) was layered over a solution (2 ml.) of M-sucrose in the same salt mixture as above and the samples were centrifuged in the no. 40 angle head of the Spinco model L centrifuge at 105000*g* for 3 hr. at 5°. The supernatant fractions were discarded and the pellets used directly for the preparation of RNA. In this case the pellet was extracted directly with 3% (w/v) sodium toluene-*p*-sulphonate in aq. 4% (w/v) NaCl (5 ml./g. wet wt.) together with an equal volume of phenol–cresol mixture. After centrifuging as before and separation of the aqueous phase, it was re-extracted with 0·5 vol. of phenol–cresol mixture and the RNA isolated as before. The yield was equivalent to 60–120 mg./100 g. of flies.

The RNA/protein ratio in the microsomal particles was also measured and the distribution of particle sizes determined by sedimentation.

Isolation of transfer RNA. Three different methods were used: in the first the ribosomal RNA was precipitated by 4 M-potassium acetate; in the second transfer RNA was extracted directly with a butan-2-ol–phenol mixture; in the third (the most practical) ribosomal RNA, DNA and some glycogen were first removed by precipitation with *m*-cresol.

Method (*a*). To the crude precipitate from the first extract dissolved in water (20 mg./ml.) was added an equal volume of 8 M-potassium acetate, pH 6·0. Although most of the ribosomal RNA was precipitated final purification could only be achieved by countercurrent distribution (Kirby, Hastings & O'Sullivan, 1962) with solvent system 150/11. The transfer RNA travelled in the top organic phase faster than the DNA, which was left as a sharp peak in the first few tubes.

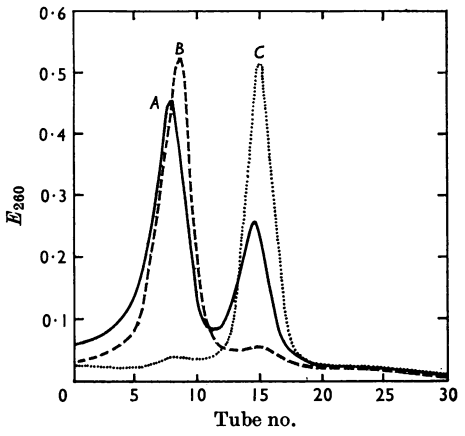

Fig. 1. Centrifugation patterns in parallel sucrose density gradients [5–20% (w/v) sucrose in 0·15 M-NaCl–15 mM-trisodium citrate buffer, pH 7; 24500 rev./min. for 14 hr. at 4° in the Spinco model L centrifuge (rotor SW 25)] of *Drosophila* ribosomal RNA. The fast and slow components were first separated on preparative gradients with recovery and redissolution at 2° in the above buffer; no ribonuclease inhibitors were used. Curve *A* (——), 0·6 mg. of whole RNA; curve *B* (– – – –), 0·4 mg. of fast component; curve *C* (• • • •), 0·3 mg. of slow component.

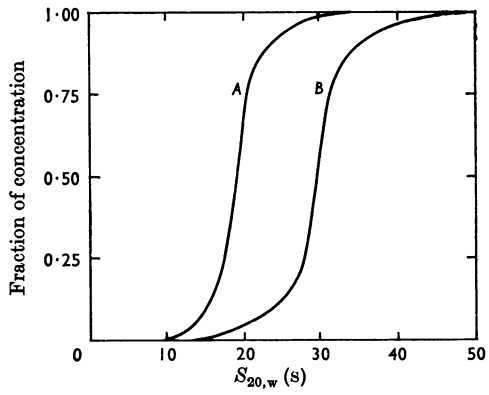

Fig. 2. Sedimentation-distribution diagrams (*A*, slow component, 47660 rev./min. at 6°; *B*, fast component, 39460 rev./min. at 6°; both in the Spinco model E centrifuge, with u.v. optics) of the separated components of *Drosophila* ribosomal RNA as shown in curves *B* and *C* in Fig. 1. Samples were dissolved at 2° in 0·1 M-NaCl ($E^{1\,cm.}_{260}$); no ribonuclease inhibitors were used.

Method (*b*). Flies were extracted for 5min. with 0·5% (w/v) naphthylamine-4,8-disulphonate in aq. 0·5% (w/v) EDTA, pH6·8, and an organic mixture of butan-2-ol–phenol–8-hydroxyquinoline (95:5:0.1, v/v/w). Transfer RNA was present in the aqueous phase, from which it was precipitated with ethanol, re-extracted with a two-phase phenol system and purified by the methoxyethanol–phosphate system (Kirby, 1956). The yield was 50mg./100g. of flies.

Method (*c*). To the supernatant solution containing toluene-*p*-sulphonate–NaCl from the second extraction of the material from the flies was added sodium benzoate (final concn. 20%, w/v) and then *m*-cresol (final concn. 20%, v/v). Ribosomal RNA, DNA and glycogen were precipitated and centrifuged off. An equal volume of ethanol was added to the supernatant solution and transfer RNA was collected by centrifugation. It was dissolved in 0·1M-NaCl, centrifuged for 1hr. at 59000*g* in the no. 40 angle head of the Spinco model L centrifuge and then purified by adsorption on DEAE-cellulose (Midgley, 1962), eluted with 0·7M-NaCl and recovered by precipitation with ethanol. The yield was 40mg./100g. of flies.

The 4s RNA prepared in this manner from rat liver was shown to be active in accepting amino acids with the pH5·0 enzyme system and an energy source (R. A. Cox, personal communication).

Sedimentation analysis of transfer RNA isolated from *Drosophila* by both methods (*b*) and (*c*) resulted in a single peak in the region of 4s.

Isolation of DNA. The DNA–protein gel, freed as far as possible from phenol and aqueous phase, was extracted with aq. 6% (w/v) 4-aminosalicylate and phenol–cresol mixture (each 25% of the original volumes used for the extraction). After centrifuging for 30min. at 5000*g* at 5° the aqueous phase was carefully removed, made 4% (w/v) with respect to NaCl, re-extracted with 0·5vol. of phenol–cresol mixture for 20min. at 20° and then centrifuged for 20min. at 5000*g* at 5°. The aqueous phase was again removed, the concentration of Na^+ brought up to approximately 3M by adding 12% (w/v) NaCl, and 0·5vol. of ethanol was then added. The largely granular precipitate containing DNA, glycogen and a small amount of RNA was collected by low-speed centrifugation (500*g*) and shaken (three times at 20°) with 3M-NaCl–ethanol (2:1, v/v), which removed lower-molecular-weight contaminants and also much of the glycogen. The remaining sediment was extracted with the minimum volume of concentrated salt to dissolve the DNA and leave contaminating high-molecular-weight RNA insoluble. The extraction was usually carried out with 2M-NaCl over several hours at 0° with occasional shaking; dissolution at 20° was more rapid, but it was then desirable to leave the solution for at least 24hr. at 0° to precipitate some dissolved RNA. The solution was centrifuged for 1hr. at 59000*g* (no. 40 angle head, Spinco model L centrifuge) to sediment RNA and glycogen. Removal of RNA was followed by carrying out thermal-denaturation measurements and the sample was considered satisfactory when negligible changes in extinction occurred between room temperature and the beginning of the main transition zone; differences in E_{260}/E_{280} ratios for purified RNA and DNA were also used to detect RNA contamination. If much RNA was present in the sample, the NaCl concentration was raised to 3M, the solution again kept for several hours at 0°, followed by recentrifugation at 59000*g*. The final sample was stored at 0° in M-NaCl. The yield (estimated from the extinction) was 15–20mg./100g. of flies.

Analyses of products. RNA base compositions were determined after hydrolysis with N-HCl at 100° (Smith & Markham, 1950) by ascending paper chromatography in methanol–ethanol–11·6N-HCl–water (50:25:6:19, by vol.) (Kirby, 1956); each hydrolysate was run in triplicate, and the results corrected for 5% estimated loss of pyrimidine nucleotides during hydrolysis.

DNA base compositions were determined after hydrolysis with 90% (v/v) formic acid at 175° by ascending paper chromatography in methanol–11·6N-HCl–water (7:2:1, by vol.) (Kirby, 1957), each hydrolysate being run in triplicate.

Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951), with chymotrypsin or bovine serum albumin as standard.

Contamination of nucleic acids by glycogen was determined by hydrolysis with N-HCl and reaction with aniline hydrogen phthalate (Partridge, 1949).

Contamination of RNA by DNA was determined by the Burton (1956) modification of the Dische reaction.

RNA content of microsomal particles was determined by the Dische & Schwartz (1937) orcinol reaction.

Phosphorus was determined colorimetrically with ammonium molybdate, after $HClO_4$ hydrolysis (Fiske & Subbarow, 1925).

(i) RNA analyses. Mean base compositions of RNA are summarized in Table 1; where five or more separate determinations were carried out in arriving at the mean, the standard error of the mean (S.E.M.) as expressed by $\Sigma d^2/\sqrt{n(n-1)}$ is also indicated.

Additional analyses of ribosomal RNA were as follows (average of determinations on several samples obtained by phenol extraction of whole flies): P, 7·5%; $E^{1\%}_{260}$ in water, 205; E_{260}/E_{280} in 0·1M-NaCl, 2·22; $\epsilon_{(P)}$ in 0·1M-NaCl, 7150; $\epsilon_{(P)}$ in 0·15M-NaCl–15mM-trisodium citrate buffer, pH7, 6800; $\epsilon_{(P)}$ in mM-magnesium acetate, 6850; protein, 0·2%.

Addition of 2-methoxyethanol to a solution of ribosomal RNA in aqueous sodium acetate or NaCl resulted in a maximum net increase in E_{260} of 27% obtained at final net concentrations of 10mM-salt and 70% (v/v) organic solvent. Thermal denaturation in 15mM-NaCl–1·5mM-trisodium citrate buffer, pH7, of both transfer RNA prepared by method (*c*) and ribosomal RNA prepared by phenol extraction of whole flies resulted in a 23–24% maximum increase in E_{260}, but the mid-point of denaturation was 15° higher with transfer RNA (53°) than with ribosomal RNA (38°).

(ii) DNA analyses. Mean analyses of DNA (dried) were as follows, the standard error of the mean being indicated for the base compositions (six separate samples): guanine, 20·8±0·52%; adenine, 29·7±0·22%; cytosine, 20·1±0·12%; thymine, 29·4±0·25%; P, 6·9%; $\epsilon_{(P)}$ in 0·1M-NaCl, 6500.

The guanine+cytosine content of DNA estimated from buoyant density (1·700–1·701) in CsCl on the Spinco model E ultracentrifuge, with various known DNA standards, was 41–42% when estimated from the data of Schildkraut, Marmur & Doty (1962) or 37–38% when estimated from the data of Sueoka, Marmur & Doty (1959), thus showing close agreement with the analytical values. Velocity sedimentation in 0·1M-NaCl gave $S_{20,w}$ in the range 16–18s.

Other properties were: E_{260}/E_{280}, 1·92; protein, 0·2%.

Table 1. *Base compositions of RNA isolated from* Drosophila

Experimental details are given in the text. Where appropriate, results are given as means ± S.E.M.

Type of RNA	Base composition (moles/100 moles of total bases) Guanine	Adenine	Cytosine	Uracil	DNA content (%)
Total ribosomal RNA	23·0±0·04	29·9±0·07	19·7±0·03	27·3±0·25	0·1
29·4s Ribosomal component	22·5±0·14	30·8±0·07	19·6±0·02	27·1±0·13	
18·8s Ribosomal component	23·5±0·02	28·8±0·11	20·3±0·08	27·4±0·07	
RNA from microsomes	23·3	30·5	19·9	26·3	
Transfer RNA					
Method (*a*): countercurrent-distribution purification of 4M-potassium acetate-soluble fraction	31·2	20·5	28·9	19·4	1·0
Method (*b*): direct extraction	30·5	21·5	30·0	18·0	0·5
Method (*c*): fractionation of RNA	30·6	22·1	29·3	18·0	0·7

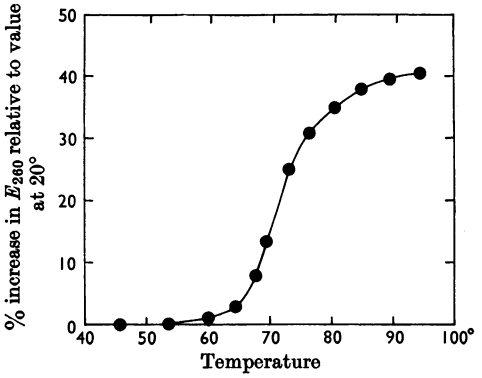

Fig. 3. Thermal-denaturation curve for *Drosophila* DNA in 15mM-NaCl–1·5mM-trisodium citrate buffer, pH7. The percentage increase in E_{260} was measured relative to that at 20°.

Addition of 2-methoxyethanol to a solution of DNA in aqueous NaCl resulted in a net maximum increase in E_{260} of 45% obtained at final net concentrations of 10mM-salt and 80% (v/v) organic solvent.

Thermal denaturation of DNA in 15mM-NaCl–1·5mM-trisodium citrate buffer, pH7, resulted in maximum increases in E_{260} of 38–40%, T_m about 70°, with negligible tailing in the lower range. A typical denaturation curve is shown in Fig. 3. Rat-liver DNA (molar guanine + cytosine content, 41%) obtained by the 4-aminosalicylate–phenol method had the same transition temperature as *Drosophila* DNA under comparable experimental conditions.

(iii) Analyses of microsomal particles. The protein/RNA ratio was 51:49 (w/w).

The 105000*g* microsome pellets dispersed in 50mM-tris-HCl (pH7·6)–60mM-KCl–5mM-NaCl–0·1mM-magnesium acetate buffer were centrifuged in sucrose gradients (5–20%, w/v) containing the same buffer for 3hr. at 20000 rev./min. at 4° (Spinco model L centrifuge, SW25 rotor). The bulk of the absorbing material (about 70%) sedimented as a single peak, the remainder appearing as decreasing shoulders of several minor components of increasing sedimentation coefficient together with small amounts (about 10% combined) of material near the top and bottom of the gradient. Almost identical profiles were obtained on parallel gradients with or without prior treatment of the dispersed microsomes for 15min. with 0·5% (w/v) sodium deoxycholate, indicating the virtual absence of polysomes bound to membrane. The E_{260}/E_{280} ratio of a suspension of the total microsome pellets was 1·83, in exact agreement with the corresponding ratio obtained throughout the region of high extinction in the sucrose gradients.

Sedimentation of the microsome pellets dispersed in the above buffer (without prior deoxycholate treatment) was also carried out on the Spinco model E centrifuge with schlieren optics (25980 rev./min. at 7·5° at a concentration of 16mg. of RNA plus protein/ml.). Photographs were taken at intervals of 2 or 4min. up to 56min. The major component sedimented at 69s, with decreasing amounts of other discrete peaks at 95s, 119s and 136s. Additional minor components were present at 56s, 87s and about 30s. Small amounts of material of very high and very low S values were also present.

Modification of the structure of ribosomal RNA shown by sucrose-density-gradient profiles. When vacuum-dried ribosomal RNA was dissolved initially at room temperature (20–25°), particularly in dilute salt (10–20mM with respect to Na^+), sucrose-gradient profiles (run at 4°) showed a variable tendency to become distorted and at the same time to spread to upper regions of lower apparent S value, when compared with the typical two-component pattern obtained after dissolution at 0° in more concentrated salt, such as for example in 0·15M-NaCl–15mM-trisodium citrate buffer, pH7. The faster-sedimenting component appeared to change its pattern more readily than the slower component.

Similarly, when sedimentation-velocity measurements were carried out on the separated components (with the same specimens as used in Fig. 2) on the Spinco model E centrifuge in the temperature range 20–25°, distortion and tailing of the patterns occurred in the regions of lower apparent S value.

The effect of deliberate heat treatment of the total ribosomal RNA before sedimentation on sucrose gradients is shown in Fig. 4.

When once dissolved at low temperature, ribosomal RNA remained stable for several hours in concentrated salt at room temperature in the absence of bentonite (shown in

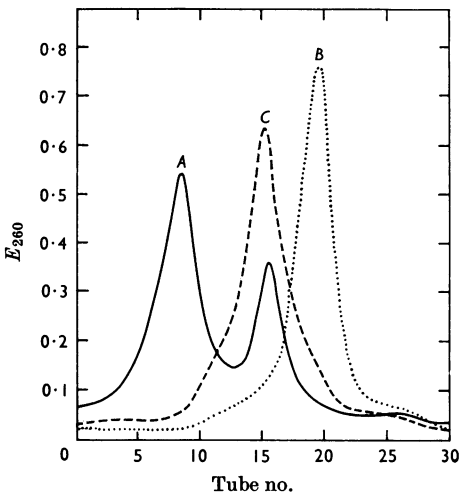

Fig. 4. Centrifugation patterns in parallel sucrose density gradients [5–20% (w/v) sucrose; 24000 rev./min. for 15hr. at 4° in the Spinco model L centrifuge (rotor SW25)] of *Drosophila* ribosomal RNA. Samples (0·8mg./gradient) were dissolved initially at 2° in 10mM-sodium acetate, pH6; no ribonuclease inhibitors were used. Curve *A* (——), sample unheated: gradient made up in 0·15M-NaCl–15mM-trisodium citrate buffer, pH7; curve *B* (····), sample heated at 70° for 10min., then cooled to 20° during 5min.: gradient made up in 10mM-sodium acetate, pH6; curve *C* (----), sample heated at 70° for 10min., then cooled to 20° during 5min.: gradient buffer as for curve *A*.

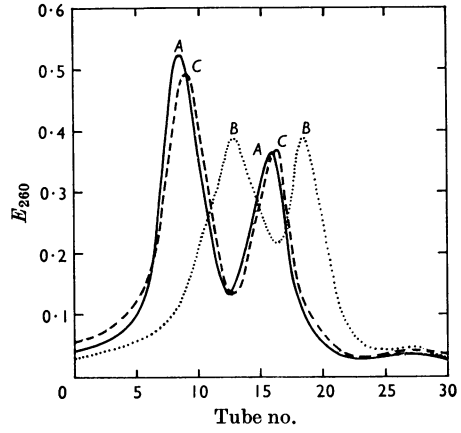

Fig. 5. Centrifugation patterns in parallel sucrose density gradients [5–20% (w/v) sucrose; 24500 rev./min. for 14hr. at 4° in the Spinco model L centrifuge (rotor SW25)] of *Drosophila* ribosomal RNA (0·8mg. of sample/gradient); no ribonuclease inhibitors were used. Curve *A* (——), sample dissolved initially at 0° in 0·15M-NaCl–15mM-trisodium citrate buffer, pH7: gradient made up in the same buffer; curve *B* (····), sample dissolved initially (10mg./ml.) at 0° in 0·2M-NaCl, pH7, then held at 25° for 7hr. in the presence of M-NaCl, then diluted with water at 4°: gradient made up in 10mM-sodium acetate, pH7; curve *C* (----), sample treated as for curve *B*, but gradient buffer as for curve *A*.

Fig. 5). After slow dissolution at 0° in 0·2M-NaCl–10mM-sodium acetate, pH7, the RNA (at 10mg./ml.) was kept for 7hr. at 25° in the presence of M-NaCl. It was then diluted and run on 10mM-sodium acetate and also on 0·15M-NaCl–15mM-trisodium citrate buffer, pH7. Curves of the type *B* shown in Fig. 5, showing distinct twin peaks but partial retardation of sedimentation, were also obtained when dissolution took place directly at 0° in 10mM-sodium acetate buffer, pH5·2, before running in the same buffer. When, however, the co-precipitate of RNA with much glycogen was recovered and dried at the stage immediately before the usual 3M-sodium acetate extraction, then redissolved at 0° and run in 10mM-sodium acetate buffer, pH5·2, the pattern of ribosomal peaks remained undistorted and resembled curves *A* in Figs. 4 and 5.

DISCUSSION

Previous attempts to isolate *Drosophila* nucleic acids in good yield in an undegraded state have met with little success. Estimated RNA/DNA ratios have ranged from 10:1 in salivary glands (Steele, Sfortunato & Ottolenghi, 1949) to 15:1 in whole *Drosophila* larvae (Leslie, 1955), whereas the deoxyribose/ribose ratio found in the metabolic pool of *Drosophila* eggs (cold-perchloric acid extract) was 20:1 (Travaglini, Levenbook & Schultz, 1958). RNA and DNA phosphorus estimated in larvae corresponded (at 7% of phosphorus in dried nucleic acid) to 40–45mg. of DNA and 600–750mg. of RNA/100g. of tissue (Leslie, 1955). RNA of *Drosophila* eggs was extracted with hot 10% sodium chloride (Levenbook, Travaglini & Schultz, 1958), giving base analyses with relatively high adenine+uracil and low guanine+cytosine contents, but the method used and variability of results suggested degradation. Direct application of the 4-aminosalicylate method for DNA to *Drosophila* larvae and adults (Kirby, 1959) gave a degraded impure non-fibrous product. A fibrous product, but of variable base composition, was obtained from *Drosophila* eggs by using a combination of sodium diethyldithiocarbamate and phenolphthalein diphosphate for deproteinization (Kirby, 1962*a*) and it was concluded that nuclease activity was high in the initial homogenates; the method was applied to *Drosophila* adults (Argyrakis & Bessman, 1963), and the 'DNA' obtained after repeated subjection to alkali and acid was similar in yield and base composition to the products now described.

Mead (1964) isolated from *Drosophila* flies and larvae DNA of similar base composition to that which we have reported but the $S_{20,w}$ value was

11·7s before ribonuclease treatment and 9·0s afterwards. He also isolated an RNA associated with the DNA that he assumed, from the similarity of its base composition to DNA, to be a messenger RNA.

The phenol methods for extraction of RNA and DNA (Kirby, 1956, 1957) were applied to serial extraction of RNA followed by DNA from the same tissue (Georgiev, 1959). The present method depends on the inability of a hydrophilic salt such as naphthalene-1,5-disulphonate to disintegrate the chromosomal structure but, in the presence of phenol, RNA is released from the ribosomes and DNA with denatured protein separates at the interfacial layer after centrifugation. Some protein is still present in the aqueous phase and this can be removed by addition of sodium chloride (to maintain ionic strength and depress the solubility of the phenol in the aqueous phase) and toluene-*p*-sulphonate and extracting with a phenol–cresol mixture. Toluene-*p*-sulphonate was considered a suitable detergent as it is easily soluble in aqueous ethanol and should not interfere in the separation of transfer RNA. Phenol–cresol mixture (Kirby, 1965) is a better deproteinizing agent than phenol alone, but if used in the first extraction a considerable amount of DNA was liberated with the RNA. Separation of glycogen from RNA with 2-methoxyethanol–phosphate (Kirby, 1956) was unsuccessful as the resultant RNA had only one component (about 18s) on centrifugation in a sucrose gradient. However, on extraction with 3M-sodium acetate, glycogen, transfer RNA and small amounts of DNA were all removed, leaving the ribosomal RNA, which was stable and had good sedimentation characteristics (Fig. 1).

Though transfer RNA was separated from ribosomal RNA by the use of concentrated salt, it was difficult to purify because of the presence of DNA of low molecular weight, and the most suitable method was the removal of ribosomal RNA by precipitation with *m*-cresol (Kirby, 1965), which also removed most of the glycogen and DNA. Since ribosomal RNA from *Drosophila* has shown considerable lability the *m*-cresol precipitation technique has not, however, generally been used during isolation of this RNA, although we now believe that under suitable conditions this technique should be practicable.

Phenol–cresol mixtures with 4-aminosalicylate and sodium chloride were used for the extraction of DNA from the interfacial material. The method for the purification, which obviated reprecipitation steps and minimized exposure to organic solvents or low ionic concentrations, aimed at preservation of the maximum level of native structural integrity, borne out by the sharp melting profile and large increase in extinction after thermal denaturation (40%). Purification of the DNA with 2-methoxyethanol–phosphate (Kirby, 1957) after extraction without cresol resulted in DNA with a higher protein contamination (1·1%) and a lower increase in extinction on thermal denaturation (30–35%) than found for DNA prepared by the method described.

The method now used for judging the degree of contamination of DNA by RNA was chosen for the following reasons. Established colorimetric methods for RNA such as that of Dische & Schwartz (1937) are unreliable in the presence of a large excess of DNA. The mean melting temperature (38°) of *Drosophila* ribosomal RNA under the ionic conditions now used is considerably below that for *Drosophila* DNA (70°). Moreover, at 50° the *Drosophila* RNA exhibited about 80% of its maximum thermal hyperchromicity and, on recooling (to 20°) of samples that had previously been completely denatured (by heating to 70°), the reversibility of the hyperchromic effect was almost complete (probably owing in large degree to random renaturation, in view of the marked changes in sedimentation profile after thermal denaturation; see Fig. 4). The specific absorptions and maximum thermal hyperchromicities of both purified *Drosophila* RNA and DNA are of the same order. Hence it was concluded that, if the DNA samples showed zero hyperchromic effect between 20° and 50°, then the DNA was likely to be free from RNA (see Fig. 3).

Analyses of the base compositions of the nucleic acids from *Drosophila* showed that though transfer RNA (separated by three different methods) and DNA had compositions similar to those already found for transfer RNA and DNA from mammalian tissues, the ribosomal RNA was completely different. A comparison of the base compositions of ribosomal RNA from rat liver, *Escherichia coli* and yeast with those of *Drosophila* is shown in Table 2.

A similar base composition with a high adenine + uracil content has been found for RNA from *Chironomus* (Edström & Beerman, 1962). There seemed little doubt that our material was indeed ribosomal RNA as it was present in large quantities (up to 25 times the amount of DNA), and contained two components that behaved similarly to mammalian ribosomal RNA on centrifugation in a sucrose gradient. Additional confirmation came from the separation of each component and determination of its individual sedimentation coefficient. Finally, material with the same base composition and the same sedimentation properties has been obtained after isolating and extracting ribosomes from *Drosophila* (Fig. 6). In this case, however, the yield of RNA was much less, owing probably to relatively low efficiency in separation of the ribosome–polysome–microsome fraction from the

Table 2. *Base composition of ribosomal RNA from different sources*

Results for rat-liver RNA are from Crosbie, Smellie & Davidson (1953) and those for *E. coli* and yeast RNA are from Midgley (1962). Results for *Drosophila* RNA were obtained as described in the text.

	Base composition (moles/100 moles of total bases)			
Source of RNA	Guanine	Adenine	Cytosine	Uracil
Rat liver	32·6	19·3	28·3	19·8
Drosophila	23·0	29·9	19·7	27·3
E. coli	32·6	25·1	21·9	20·4
Yeast	28·1	27·2	19·2	25·4

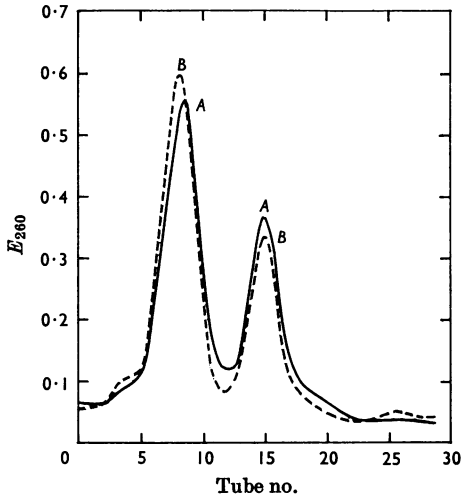

Fig. 6. Centrifugation patterns in parallel sucrose density gradients [5–20% (w/v) sucrose in 0·1 M-sodium acetate, pH 7; 24000 rev./min. for 14 hr. at 4° in the Spinco model L centrifuge (rotor SW 25)] of *Drosophila* ribosomal RNA. Samples (0·8 mg./gradient) were dissolved initially at 2° in 0·1 M-sodium acetate, pH 7; no ribonuclease inhibitors were used. Curve *A* (——), ribosomal RNA prepared by direct extraction of whole flies, then stored in the dry state for 6 months before testing; curve *B* (– – – –), ribosomal RNA prepared from ribosomes previously separated from homogenates of whole flies by differential centrifugation. Base compositions (moles/100 moles) determined in parallel were as follows:

	Guanine	Adenine	Cytosine	Uracil
A	23·2	30·1	20·2	26·5
B	23·3	30·5	19·9	26·3

remainder of the material during differential centrifugation of homogenates of whole tissue. It is also probable that the RNA with DNA-like base composition that Mead (1964) isolated contained ribosomal RNA, possibly derived from ribosomes that were associated with chromosomes.

If at any stage during the extraction and purification process of the *Drosophila* ribosomal RNA insufficient attention was paid to ionic concentration, temperature or concentration of organic solvent, the sucrose-gradient profiles of the purified RNA in 10 mM-salt tended towards a single peak.

The ease of conversion of the normal faster-sedimenting component of *Drosophila* ribosomal RNA into material sedimenting in the region of the slower component may be compared with the reported conversion of 23 s and 16 s ribosomal RNA in *E. coli* strain B ribonucleoprotein (Midgley, 1965); however, in the bacterial RNA the breakdown was considered to be due not so much to changes in its secondary structure dependent on intramolecular hydrogen bonds but to the breaking of certain atypical molecular internucleotide bonds, forming links between sub-units during development of the ribosome.

Rat-liver ribosomal RNA can be heated to 70° in 10 mM-sodium acetate and, on cooling and centrifuging in a sucrose gradient with the same salt concentration, much of the material that originally appeared in the 28 s peak had migrated to the 18 s region. If, however, the solution of heated ribosomal RNA was made 200 mM with respect to sodium acetate and centrifuged in a sucrose gradient made up in this salt concentration then the original patterns of centrifugation largely reappeared (Kirby, 1965). We attribute the difference in behaviour of the ribosomal RNA from *Drosophila* to the lower guanine + cytosine content compared with that of the RNA from rat liver. Rat-liver ribosomal RNA also shows sensitivity to organic solvents. Thus there is an increase in the amount of the 18 s compared with the 28 s component when the tetraethylammonium salt remains at room temperature in aq. 50% (v/v) ethanol and the pattern on centrifugation in a sucrose density gradient is never as good after recovery from 2-methoxyethanol–phosphate as by the method described (Kirby, 1965).

We regard the changes found in *Drosophila* ribosomal RNA as analogous to those found with RNA from rat liver, the main differences being the milder conditions under which they occur with

RNA from *Drosophila* and their irreversibility under the conditions studied.

An understanding of the secondary structure of ribosomal RNA is essential to investigations on the nature of ribosomes and protein biosynthesis, and a comparative study of RNA from different sources may be of value in determining the essential structural features.

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