($\lambda = 1.790 \text{ \AA}$) on a camera of diameter 114.6 mm. The principal diffraction lines measured, together with their intensities, are summarized in Tables 1 and 2.

Oscillation and Weissenberg photographs of a single crystal of the monohydrate showed the crystal to be orthorhombic, and together with powder data the unit cell is calculated to be $a = 19.62 \pm 0.20$, $b = 15.54 \pm 0.16$, $c = 7.56 \pm 0.07 \text{ \AA}$. The assignment of four molecules to the unit cell gives a calculated density of 1.505 g./cm.^3 compared with a measured density of 1.51 g./cm.^3 (pycnometer method).

X-Ray powder diffraction lines of the dihydrate were indexed on the basis of an orthorhombic cell

with dimensions $a = 19.80 \pm 0.20$, $b = 15.65 \pm 0.16$, $c = 7.51 \pm 0.07 \text{ \AA}$. The observed density (by flotation method) of 1.55 g./cm.^3 (cf. Pionchon, 1897) agrees with the calculated value of 1.54 g./cm.^3 .

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The Preparation and Properties of Ribonucleic Acids from Rat Liver

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Early methods of preparing ribonucleic acid (RNA) from mammalian tissues and yeast involved the use of alkali (Levene & Bass, 1931), but it is now recognized that this process necessarily involves a considerable amount of degradation (cf. Allen, 1954). On the other hand, if very mild conditions are used there is a danger of degradation by ribonuclease during the preparation.

Attempts have been made to inactivate the ribonuclease without affecting the RNA, but none of the substances used seems to be entirely satisfactory. Thus Grinnan & Mosher (1951) used 4-m-guanidine hydrochloride as a denaturing agent, followed by heat to remove the protein. This yielded a product stated to have a sedimentation coefficient $S = 10\text{s}$ corresponding to a high molecular weight (about 260 000). Gulland, Barker & Jordan (1945) have shown, however, that guanidine hydrochloride denatures deoxyribonucleic acid, and the probability of such action upon RNA, followed by aggregation, cannot be overlooked. Kay & Dounce (1953) have used sodium dodecyl sulphate as a protein precipitant and they obtained material in 50% yield having sedimentation coefficients of about 3s, corresponding to a molecular weight value of $2-3 \times 10^4$. It has since been shown by Allen (1954) that dodecyl sulphate does not completely inactivate ribonuclease under the conditions obtaining in this method of preparation. Strong salt will split a certain proportion of the cytoplasmic nucleoprotein, but only on prolonged standing, and

RNA thus obtained by Kerr & Seraidarian (1949) showed evidence of considerable enzymic depolymerization.

In the course of investigating these methods, it was found that after mild acid treatment a large part of the nucleic acid was easily separated from the associated protein and lipid by a short period of heating in salt solution. The protein flocculated and precipitated, leaving most of the nucleic acid in solution together with a small amount of protein which could readily be removed by digestion with chymotrypsin. It was also found that this process is effective with isolated cell fractions. The ribonucleic acids obtained have sedimentation coefficients in the range 2-3s, corresponding to molecular weights of $2-3 \times 10^4$. It is possible that the ribonucleic acids of high molecular weight obtained by some other workers are artifacts produced by aggregation of particles as a result of the reagents employed. It must be admitted, however, that some depolymerization may occur during the heating process.

EXPERIMENTAL AND RESULTS

Preparation of ribonucleic acids from whole cytoplasm. Freshly excised and chilled rat livers were minced and homogenized for 30 sec. in 9 vol. of 0.14M-NaCl in a Waring Blender, and nuclei, cell debris and unbroken cells were removed by centrifuging at 1200 g for 10 min. All operations up to the heating process were carried out below 3° and as quickly as possible to minimize enzymic degradation. The crude nucleoprotein was obtained as a white precipitate by

acidifying to pH 4.5 with M-HCl and was collected by centrifuging at 1200 *g* for 15 min. This step was found to be essential for efficient removal of protein at later stages. Attempts were made at this stage to remove protein by the addition of pepsin, trypsin and chymotrypsin; they were unsuccessful, perhaps owing to blocking of the enzymes by lipid material.

A fairly complete separation of the protein and nucleic acid was obtained by heating the acid precipitate at neutrality in salt solution. After dispersing in 2M-NaCl (1 l./150 g. of rat liver) and adjusting to pH 7 with 0.1M-NaOH, the suspension was heated for 10 min. at 80° with continuous mechanical stirring. These optimum conditions were found from trial runs, shown in Table 1, yields being calculated as a percentage of the RNA present in the starting material as given by Thomson, Heagy, Hutchison & Davidson (1953). While higher temperatures gave products with high sedimentation coefficients, these were not the result of increased extraction of highly polymerized material, as the yields were usually lower. Northrop & Sinsheimer (1954) have found aggregation of RNA to occur on heating at high temperature for several minutes.

After heating and cooling rapidly again to room temperature, the flocculated precipitate was removed by centrifuging, leaving a lemon-yellow supernatant. This contained the extracted RNA, which could be precipitated by the addition of 2 vol. of 98% ethanol to give products with N/P ratios in the region of 2. Purified products were obtained by digesting the supernatant with chymotrypsin at pH 7.5 for 16 hr. before precipitating with ethanol. The RNA was then reprecipitated from isotonic NaCl solution and finally washed with 70, 75, 80 and 90% (v/v) ethanol-water mixtures. It could be dried with ethanol and acetone, or by freeze-drying. This gave samples of RNA having N/P ratios of 1.7, which on hydrolysis and paper chromatography were shown to contain ribose, adenine, guanine, uracil and cytosine, with only a trace of thymine corresponding to less than 2% of DNA. The biuret reaction was negative and chromatography of the acid hydrolysate revealed less than 1% of protein.

Preparation of ribonucleic acids from cytoplasmic fractions. Three cell fractions were prepared by the method of Schneider & Hogeboom (1951). The cytoplasmic homogenate was prepared in 9 vol. of 0.25M-sucrose with a Potter-Elvehjem homogenizer fitted with a Teflon (polytetrafluoroethylene) pestle. Nuclei, unbroken cells and cell debris were removed by centrifuging at 1200 *g* for 15 min.

Fraction I was brought down at 10 000 *g* for 20 min. and washed once by resuspending in 0.25M-sucrose and centrifuging again. Fraction II was obtained at 36 000 *g* for 1.5 hr., leaving the supernatant (fraction III).

Fractions I and II were suspended in 0.14M-NaCl and all three were acidified and heated, as in the whole cytoplasm preparation. Fraction I, predominantly a 'mitochondrial' fraction, was contaminated with considerable amounts of 'microsomal' RNA (see Table 2), owing to the inadequate fractionation obtained by the method of Schneider & Hogeboom. The degree of contamination depends on whether the 'fluffy' layer (Schneider & Hogeboom, 1951) is added to fraction I (preparation A) or fraction II (preparation B). Sufficient resolution is obtained between fractions II and III to justify a comparison of their properties.

RNA was also prepared from fraction II by the sodium dodecyl sulphate method of Kay & Dounce (1953).

Base analysis of ribonucleic acids

Base analysis of the various preparations was made by the method of Wyatt (1951). Hydrolysis was carried out with 72% HClO₄ for 1 hr. Results

Table 1. *Influence of experimental conditions on amount and nature of the product*

Conditions of heating in 2M-NaCl	Product		Yield (pptd. by ethanol) (%)
	N/P	S _{20,w} (s)	
50°/30 min.	No flocculation	—	—
60°/10 min.	1.93	2.18	20
80°/10 min.	1.70	2.12	50
80°/30 min.	1.71	3.38	20
100°/5 min.	2.01	5.2	20
100°/10 min.	1.87	2.76	15
100°/30 min.	—	—	Negligible

Table 2. *Yields of RNA prepared from cell fractions*

Preparation	Fraction no. (mg./250 g. of liver)		
	I	II	III
A	340	80	225
B	100	160	215

Table 3. *Base composition of RNA prepared from rat liver*

SDS, Sodium dodecyl sulphate as protein precipitant. (Molar ratios with respect to adenine = 1.)

Preparation	Guanine	Cytosine	Uracil	6-Amino:6-keto	Purine:pyrimidine	(Adenine + uracil):(guanine + cytosine)
RNA sample 48 Whole cytoplasm	1.74	1.53	0.82	0.99	1.17	0.56
RNA sample 55 Whole cytoplasm	1.76	1.51	0.83	0.97	1.18	0.60
RNA sample 101/1 Fraction II	2.09	1.75	0.91	0.92	1.16	0.50
RNA sample 101/3 Fraction III	1.80	1.56	0.82	0.98	1.18	0.54
RNA Whole cytoplasm (SDS)	2.75	2.23	0.73	0.93	1.27	0.35
RNA sample 101/4 Fraction II (SDS)	2.47	2.01	0.90	0.89	1.19	0.42
RNA sample 100/2 Fraction II (SDS)	2.58	2.13	0.83	0.92	1.21	0.39
RNA Whole cytoplasm*	1.75	1.36	0.81	0.93	1.27	0.58

* Crosbie, Smellie & Davidson (1953): HClO₄ hydrolysis.

are given in Table 3 as moles of base:moles of adenine.

It is seen that ribonucleic acids obtained from cell fractions by the heat preparation do not differ appreciably in composition from the whole cytoplasm RNA as regards base composition. These results agree closely with those of Crosbie, Smellie & Davidson, which are included in Table 3. The ratio of 6-amino base to 6-oxo bases is constant and near to unity for all the preparations, as found by Elson & Chargaff (1955). [That the values are uniformly less than one may be due to the method of hydrolysis (Crosbie *et al.* 1953)].

RNA prepared from fraction II by the detergent method differs markedly in base composition from RNA prepared by the heating method, as shown by the ratio (adenine + uracil):(guanine + cytosine). However, the purine:pyrimidine ratio is unaffected, as is the ratio of 6-amino groups:6-oxo groups.

Sedimentation analysis

The sedimentation behaviour of the preparations was studied with a Spinco analytical ultracentrifuge. As a rule, only one broad peak was observed, as is shown for a 1% solution of RNA sample 82 in 10.4M-NaCl in Fig. 1 (c) ($S_{20,w} = 2.35$ s). However, in a few cases, a double peak was observed; e.g. with RNA sample 48, obtained by heating the whole cytoplasm homogenate at 100° for 10 min., without subsequent treatment with chymotrypsin, a 1% solution in water gave two peaks, as shown in Fig. 1 (a), with $S_{20,w} = 4.06$ s (A) and 2.32 s (B). It was thought that the material A might be an aggregated form of B and 2M-urea was added to the solution in an attempt to break up such aggregates. Under these conditions, however, two peaks were again observed [Fig. 1 (b)] with sedimentation coefficients, corrected for changes of density and viscosity ($S_{20,w} = 2.9$ s and 2.1 s). After dialysing

out the urea the sedimentation coefficients returned approximately to the original values ($S_{20,w} = 4.23$ s and 1.91 s). The results show that A undergoes some changes of shape or aggregation in urea, but it does not become identical with B. This means that they are probably two distinctly different substances. Since the N/P ratio for this preparation was 2.05, it contains about 20% of protein and the component A is thus probably a nucleic acid-protein complex. It may be noted that in preparations treated with chymotrypsin to remove protein no such double peaks have been observed.

An estimate of the molecular weight, as calculated from the mean sedimentation coefficients (determined in 1% solution in 0.14M-NaCl) and the diffusion coefficients, determined in the Perkin-Elmer electrophoresis apparatus (1% solutions of RNA, which had been dialysed against phosphate buffers, pH 6.8, at ionic strength 0.2) is given in Table 4. The value of the partial specific volume of RNA used in the calculations was 0.523 (Delcambe & Desreux, 1950).

Such values, calculated from sedimentation and diffusion coefficients measured at finite concentration, can be only approximate. However, since Grinnan & Mosher (1951) found that S_{20} for their RNA of rat liver varied little with concentration up to 1%, and since Cohen & Stanley (1942) found that D_{20} for the RNA of tobacco mosaic virus changed by only 20% between zero concentration and 0.6%, it is likely that these values for the molecular weights are of the right order of magnitude.

Table 4. *Sedimentation and diffusion data for RNA from cell fractions (see text)*

Fraction	$S_{20,w}$ (s)	$10^7 D_{20,w}$	Mol. wt.
II (microsomal)	3.70	7.0	26 000
III (supernatant)	2.4	10.6	11 000

Fig. 1. Sedimentation pictures of various preparations of RNA: (a) RNA sample 48, showing double peak in water (49 min.); (b) RNA sample 48, showing double peak in 2M-urea (55 min.); (c) RNA sample 82 (fraction III), showing single peak (25 min.). Pictures were taken after various times at 59 000 rev./min.

Fig. 2. Titration curves of RNA sample 55 (from whole cytoplasm): I, starting from neutrality to pH 2.6 and then to pH 11; II, starting at pH 7 to pH 11 and then to pH 2.6.

Titration curves

The preparation RNA sample 55 was titrated from neutrality both in the acid and alkaline direction and the pH values were obtained with a Doran Alkacid glass electrode (L.S.B. Components Ltd., Stroud), and in each the forward titration was succeeded by a back titration through the whole accessible range of pH (Fig. 2). No significant amount of hysteresis is observed between the forward and back titrations.

DISCUSSION

The method of preparation of RNA by heat denaturation of the protein has the great advantage of speed, since the time during which the nucleoprotein preparation is above 3° is much less than with some other processes. It would appear that as the RNA is quite stable after the heating process, the ribonuclease present is removed with the flocculated protein. The molecular weight of the product is similar to that of the preparation of Kay & Dounce (1953) and Volkin & Carter (1951). The higher molecular weights found by Grinnan & Mosher (1951) are not obtained. It is not certain, however, that no degradation occurs during the preparation, especially since heating for a longer period produces a more degraded product. The absence of hysteresis in the titration curves means that in the preparation examined no evidence of a hydrogen-bonded structure was obtained.

SUMMARY

1. A method of preparation of ribonucleic acid from rat liver is described involving heat denaturation and separation of the protein and lipid components. It has also been applied to cytoplasmic fractions. The yield is, in general, not greater than 50 %.

2. The ribonucleic acids obtained have molecular weights between 10 000 and 30 000. The composition of the ribonucleic acid obtained from the microsome fraction differs appreciably from that obtained by the sodium dodecyl sulphate method.

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