Additional components in ribonucleic acid of rat-liver fractions

Recent studies have demonstrated the presence in RNA from a number of sources of several additional components: these include pseudo-uridine[1-4], thymine[5], methylated adenines[5,6], methylated guanines[6,7], 5-methylcytosine[8,9] and an additional sugar[10]. In most instances the total cell RNA had been isolated and probably represented a mixture of components having different functions in the cells. As separate functions in protein synthesis have been suggested for the microsomal and soluble RNA fractions of rat liver[11,12] it was interesting to compare the proportions of additional components in these two materials. The additional sugar and the components shown in Table I were detected in both types of RNA, but there was a much higher proportion of most of them in the soluble RNA.

TABLE I

RELATIVE PROPORTIONS OF ADDITIONAL COMPONENTS IN RNA FROM RAT LIVER MICROSOMES AND SOLUBLE FRACTION

Values are mole/100 moles uridine.

Component	*Microsomes*	*Soluble*
Pseudo-uridine	7.5	25
5-Methylcytosine	0.4	10
6-Methylaminopurine	0.5	8.1
6-Dimethylaminopurine	0.1	0.1
1-Methylguanine	0.1	3.3
2-Methylamino-6-hydroxypurine	0.1	2.3
2-Dimethylamino-6-hydroxypurine	0.1	3.0

RNA was prepared from microsomal particles as previously described[5] and samples of rat-liver soluble RNA[12] were obtained from Dr. M. B. HOAGLAND. 6-Methylaminopurine and 6-dimethylaminopurine were estimated according to LITTLEFIELD AND DUNN[5] and the methylated guanines according to SMITH AND DUNN[7]. 5-Methylcytosine had previously been detected in RNA from *Bacterium coli* K 12[8] and I had detected it in RNA from *Aerobacter aerogenes* and wheat germ[9,13]. In these studies the 5-methylcytosine was isolated as a riboside using paper chromatography after dephosphorylation of the nucleotide present in an alkaline hydrolysate of the RNA[13]. The 5-methylcytidine was identified by comparison with a sample of 5-methylcytosine ribofuranoside synthesised by FOX *et al.*[14] and estimated in 0.1 *N* HCl using their value of ε of $12.5 \cdot 10^3$ at 287 mμ.

The nucleotide of pseudo-uridine was normally detected in the guanylic acid band separated from an alkaline hydrolysate of the RNA by chromatography in isopropanol–water–NH_3[15]. It was isolated by electrophoresis at pH 2.5[10]. In some instances part of this component was isolated together with uridylic acid in the initial chromatographic and electrophoretic separations. After removal of phosphate by chromatography in isopropanol–water–NH_3, the nucleotide was dephosphorylated with prostatic phosphomonoesterase[5] and separated from uridine by successive chromatography in isopropanol–water–NH_3 and *n*-butanol–water–formic acid[16]. In both these solvents the R_F of pseudo-uridine was lower than that of uridine, being half

Abbreviations: RNA, ribonucleic acid; ATP, adenosine triphosphate.

that of uridine in the latter solvent[2]. The nucleoside was identified by its spectra at pH 1 and 13[3] and by chromatographic and electrophoretic comparison with a sample of pseudo-uridine kindly supplied by Dr. WALDO E. COHN. For estimation a value of ε of $7.5 \cdot 10^3$ at 263 mμ in 0.1 *N* HCl was used[3].

From Table I it will be noted that the soluble RNA contains a higher proportion of all the additional components except 2-dimethylaminopurine. Preliminary observations indicate that a higher proportion in the soluble RNA also applies to the additional sugar. These results, together with the observation that the soluble RNA has a much lower molecular weight than that from microsomes[12], make it probable that the fraction of yeast RNA obtained by DAVIS AND ALLEN[2] which was soluble in 1 *M* NaCl and contained a high proportion of pseudo-uridine consisted mainly of this type of RNA. Studies already in progress seem to support this possibility. The high proportion of these additional bases in soluble RNA make it possible that they may be essential for the function of this RNA in protein synthesis.

As a template for protein synthesis we might expect a similarity between RNA from microsomes and from plant viruses. As at least two of the latter do not contain any of these additional components[5,7,9] it seemed possible that the microsomes contained a proportion of soluble RNA in addition to RNA free of all these compounds. To explain the composition obtained for microsomal RNA in this way it would be necessary to conclude that the soluble RNA was a mixture of polynucleotides of very different composition and that the relative proportions of these included in the microsomal and soluble fractions were markedly different.

As expected from other studies[17] the nucleosides obtained from the alkaline hydrolysis of the soluble RNA were mainly adenosine and cytidine with a small proportion of uridine. The total proportion of nucleosides (8.6 nucleosides/100 uridine residues) indicated an average chain length for the polynucleotides of 80–90 nucleotides. This value is similar to that obtained by labelling experiments with ATP[17] and corresponds with a molecular weight of about 30,000 which is similar to that obtained by physical measurements on this type of RNA[12].

I wish to express my thanks to Dr. M. B. HOAGLAND for the samples of soluble RNA, to Dr. W. E. COHN for the sample of pseudo-uridine and to Dr. J. J. FOX and his colleagues for the specimen of 5-methylcytidine. I am grateful to Dr. J. D. SMITH for much valuable discussion on this work.

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Isolation and identification of lysolecithin from lipid extracts of normal human serum

The major phospholipids of human plasma eluted from silicic acid columns were recently reported by HIRSCH AND AHRENS[1] to be cephalins, lecithin, and sphingomyelin in that order. PHILLIPS[2,3] has reported the isolation of lysolecithin from human serum using the silicic acid chromatography technique of LEA, RHODES AND STOLL[4].

Silicic acid columns were prepared according to the specifications of HIRSCH AND AHRENS[1] and maintained at 25° for all runs. Serum was extracted with 20 vol. 2:1 (v/v) chloroform–methanol, washed with distilled water, evaporated to dryness under nitrogen, and applied to columns in petroleum ether. Omission of the water washing, storage of serum or the lipid extracts up to one week at 4°, or the use of siliconized glassware did not affect the results. The recovery of lipid phosphorus from the extracts was 88.2–97.2 %. Glycerides and free and esterified cholesterol were eluted with 150 ml diethyl ether.

Fig. 1 shows the three peaks obtained by elution of the phospholipids with 500 ml 1:4 (v/v) chloroform–methanol when 360-drop fractions were collected using an automatic fraction collector. The recovery of total lipid phosphorus was from 90–101%.

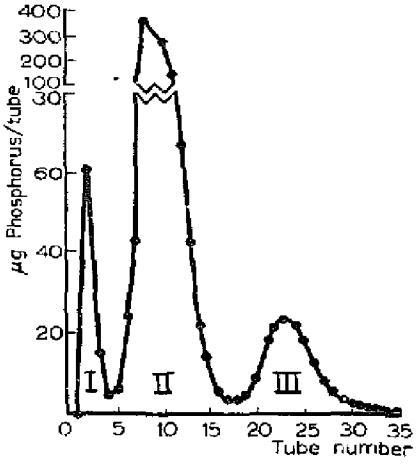

Fig. 1. Silicic acid chromatography of phospholipid from 15 ml normal human serum. Chloroform-methanol (1:4, v/v) as eluant with 360-drop fractions. Peak I, phosphatidylethanolamine + phosphatidylserine; Peak II, lecithin + sphingomyelin; Peak III, lysolecithin.

Peak I showed the presence of glycerol[5], a ninhydrin-positive reaction, and the absence of choline[6]. The carboxylic acid ester[7]/phosphorus[8] ratio was 2.01. Paper chromatography in three solvent systems[9–11] revealed the presence of phosphatidylethanolamine and phosphatidylserine. These could be separated by elution of the column with 200 ml 7:1 (v/v) chloroform–methanol followed by 200 ml 4:1 (v/v) chloroform-methanol. The Peak I material represented 6.4 % of the total lipid phosphorus and was present to the extent of 0.63 mg P/100 ml serum.

Peak II contained glycerol, but was negative to ninhydrin. The carboxylic acid ester/phosphorus ratio was 1.57 and the choline/phosphorus ratio was 0.73. Infrared spectra showed bands (1710, 1625, 1535, 1460 and 1170 cm^{-1}) which were compatible with lecithin (1710, 1460, 1170 cm^{-1}) and sphingomyelin (1625, 1535, 1460 cm^{-1}). Both