

Brief Notes

Ribonucleic Acid Composition in Cytoplasmic Fractions Isolated from Rat Liver Cells.* By GASTON DE LAMIRANDE,† CLAUDE ALLARD,‡ AND ANTONIO CANTERO. (From the Research Laboratories, Montreal Cancer Institute, Notre Dame Hospital, and University of Montreal, Montreal, Canada.)§

INTRODUCTION

Differences in ribonucleic acid (RNA) composition were observed by various authors between the liver cell fractions isolated by differential centrifugation. While authors generally agree that the nuclear RNA differs from the cytoplasmic RNA, disagreement exists with respect to the composition of the RNA isolated from cytoplasmic fractions (1-8). Such analysis was usually carried out on three cytoplasmic fractions, namely the mitochondrial and microsomal fractions and the supernatant fluid. The present work represents an attempt to study the composition of the RNA isolated from nine different cytoplasmic fractions prepared by differential centrifugation.

Methods

Adult rats of the Wistar strain (average body weight 181 ± 4 gm.) were used. The animals were killed by decapitation and bled. The livers were removed, chilled on ice, blotted on filter paper, and weighed. The organs were minced and homogenized in 0.88 M sucrose to give a 10 per cent homogenate. Nine cytoplasmic fractions were obtained by differential centrifugation (9). Refrigerated centrifuges, International PR2 and Spinco model L, were employed.

The various cellular fractions were obtained from a total of 16.8 gm. of rat liver in four series of centrifugation to prepare enough material of each fraction for RNA analysis. In each series, the fractions isolated from 42 ml. of a 10 per cent homogenate were suspended in 40 ml. of ice cold 10 per cent trichloroacetic acid and centrifuged at 1500 R.P.M. during 10 minutes. The supernatant was discarded and the trichloroacetic acid treatment was repeated twice. This was followed by two washings with 25 ml. of distilled water. The residues were kept in ethanol in the cold until the four series of centrifugation were completed. The ethanol-suspended materials from corresponding fractions were then pooled

and treated according to a modified (10) Schmidt and Thannhauser procedure (11). The nucleotides obtained by the alkaline hydrolysis of the isolated RNA (3) were analysed by ion-exchange chromatography according to the method of Cohn (12).

RESULTS

The composition of the RNA isolated from the nine cytoplasmic particulate fractions is summarized in Fig. 1. These results are expressed in moles per hundred moles of nucleotides and each value, except for fraction 9, is the mean of two separate series of experiments. The average variation from the mean for the two series of analysis was less than 3 per cent for cytidylic acid, 5 per

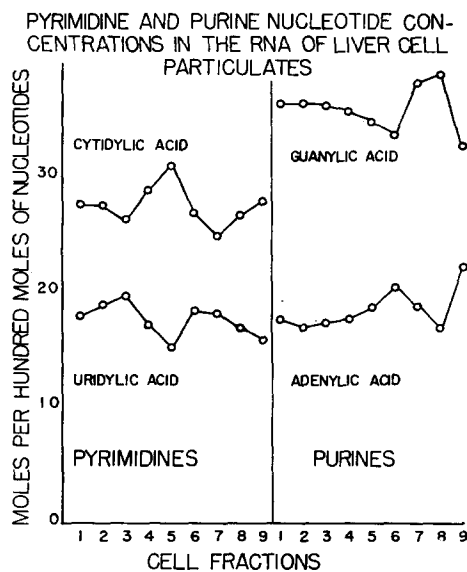


FIG. 1. Pyrimidine and purine nucleotide composition of ribonucleic acid in the cytoplasmic fractions isolated from rat liver cells. The following pairs of cytoplasmic fractions had statistically significant differences ($P < 0.05$) in their concentrations of cytidylic acid, 1-3, 1-7, 2-3, 2-7, 3-9; of uridylic acid, 3-5, 5-6, 5-7; of adenylic acid, 4-9, 5-9, 8-9; of guanylic acid, 4-8, 4-9, 5-7, 5-8, 6-7, 6-8, 6-9.

* Supported by grants from the National Cancer Institute of Canada.

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§ Received for publication, March 24, 1959.

cent for uridylic acid, 6 per cent for adenylic acid, and 2 per cent for guanylic acid.

The results (Fig. 1) indicate that the RNA of all the fractions studied contained predominantly cytidylic (25.0 to 31.0 moles) and guanylic (32.8 to 39.0 moles) acids. Uridylic (15.3 to 19.7 moles) and adenylic (16.8 to 23.4 moles) acids were found in smaller proportions. Fractions 1 to 5, as previously shown (9), contain the cytoplasmic elements found in the mitochondrial fraction obtained by the differential centrifugation method of Schneider and Hogeboom (13). The average nucleotide composition of the RNA extracted from fractions 1 to 5 is not, however, the same as the one previously reported for the whole mitochondrial fraction RNA (3), but no explanation can be found actually for this discrepancy. Fractions 6, 7, and 8 are, in fact, subfractions (9) of the classical microsomal fraction obtained by the method of Schneider and Hogeboom (13). The average nucleotide composition of the RNA of fractions 6, 7, and 8 confirms the one reported for the microsomal RNA (3).

The variations in the RNA composition of the different cytoplasmic fractions are continuous (Fig. 1). The cytidylic acid concentration decreases from fractions 1 to 3, then increases in fractions 4 and 5. The proportion of this nucleotide falls again in fractions 6 and 7 and rises in fractions 8 and 9. Reciprocal variations are observed in the relative amounts of uridylic acid.

The concentration of guanylic acid decreases from fractions 1 to 6, then increases in fractions 7 and 8. A fall is observed in fraction 9. Reciprocal variations are recorded for the proportions of adenylic acid. Statistical analysis, reported in the legend of Fig. 1, shows that many significant differences are observed from one fraction to another.

The ratios of cytidylic, uridylic, and adenylic acids to guanylic acid in the different cytoplasmic fractions were calculated. Their variations, even though larger, give essentially the same patterns as those shown in Fig. 1 for the concentration of the nucleotides. For this reason, they are not reported in this note. The ratio of 6-amino to 6-keto derivatives gives a value of 0.86 with a coefficient of variation of 11.5 for the seventeen analyses presently reported. This confirms values previously reported for a much larger number of analyses (4).

DISCUSSION

The results described above indicate that the concentrations of the pyrimidine as well as those

of the purine mononucleotides varied inversely one from the other. This variation in the proportions or ratios of the various mononucleotides from the largest to the smallest cytoplasmic structures was continuous. The continuity might be due to an overlapping of the cytoplasmic fractions obtained with the centrifugation procedure used (9). Nevertheless, it is of interest that in the present RNA analysis, fractions 3, 6, and 8 show inflection points in the curves representing the relative amounts of the various nucleotides. These fractions have been characterized in previous enzymatic studies as being mitochondria, lysosomes, and microsomes respectively (9).

SUMMARY

An attempt has been made to correlate the ribonucleic acid composition and the cytoplasmic structures prepared by differential centrifugation of rat liver homogenate. The ribonucleic acid was isolated from each fraction by a modified Schmidt and Thannhauser procedure and submitted to ion-exchange chromatography after alkaline hydrolysis. Continuous variations of the proportions of each mononucleotide present in ribonucleic acid from one fraction to the other were observed.

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