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INTRACELLULAR RIBONUCLEIC ACID COMPOSITION IN REGENERATING LIVER CELLS*

GASTON DE LAMIRANDE**, CLAUDE ALLARD, AND ANTONIO CANTERO
Research Laboratories, Montreal Cancer Institute, Notre Dame Hospital, Montreal (Canada)

Evidence exists in the literature that the nucleotide composition of ribonucleic acid (RNA) is characteristic of each liver cellular fraction[1,2,3,4]. On the other hand, the nucleotide composition of the RNA isolated from rat-liver tumor induced by 4-dimethylaminoazobenzene was shown to be more similar in all cell fractions[5]. The study of RNA composition was undertaken in fast growing liver in order to establish whether the changes observed in the intracellular RNA composition of liver tumor were characteristic of neoplastic growth or mainly of the high rate of mitosis of this tissue. Very few studies have been carried out on the RNA composition of regenerating rat liver. CROSBIE *et al.*[6] have reported that the nucleotide composition of the RNA isolated from cytoplasmic fractions of rat liver was not affected 26 hours post-hepatectomy. A very recent report by Cox[7] indicated that no changes could be detected 72 hours post-hepatectomy.

In this report results obtained on the nucleotide composition of RNA isolated from cell fractions of regenerating liver 18 and 72 hours post-hepatectomy are presented. 18 hours post-hepatectomy correspond to a period prior to the first wave of mitosis and 72 hours to the period of maximal cell concentration per unit of tissue[8]. Fasted and sham-operated animals were used as controls. The present study indicates a tendency to homogeneity in the nucleotide composition of particulate RNA in normal fast growing liver tissue.

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** Research Associate of the National Cancer Institute of Canada.

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METHODS

Wistar rats of an average weight of 200 gm were used. The hepatectomy (66%) was performed according to the method of HIGGINS AND ANDERSON[9]. Sham-operated animals were used as controls. Since the operated rats do not eat for a period of 18 hours after operation, another group of rats was fasted for a similar period and used as another control. After periods of 18 and 72 hours following partial hepatectomy, four to seven of the operated rats and of the control groups were stunned and decapitated, the livers rapidly excised and pooled. A 10% homogenate was immediately prepared, from the pooled livers of each group, and four cellular fractions namely nuclear, mitochondrial, and microsomal fractions and supernatant fluid, were isolated according to the method of SCHNEIDER AND HOGEBOOM[10].

The RNA was then extracted from each cellular fraction and its nucleotide composition determined by column chromatography using previously described procedures[4,5].

RESULTS AND DISCUSSION

Purity of isolated cellular fractions

The quantitative examination of each cellular fraction by phase-microscopy in a Petroff-Hauser bacteria counter was performed as previously described[11]. The nuclear fraction was contaminated by mitochondria but this contamination was apparently insignificant as previously found with normal liver, and liver tumor[4,5] due to the very low content of RNA (14% of whole homogenate RNA content) in isolated mitochondrial fractions. The mitochondrial and microsomal fractions were freed from nuclei but were contaminated by an intermediate particulate first recognized by DE DUVE and named lysosomes[12]. This intermediate particulate characterized also by other workers[13,14] is mostly concentrated in the mitochondrial fraction obtained by the centrifugation procedure presently and previously used. The supernatant fluid was freed from any visible particulate.

RNA composition of cellular fractions of fasted, sham-operated and regenerating rat liver (18 hours)

Table I shows the nucleotide composition of RNA in moles per hundred moles of nucleotides for the nuclear, mitochondrial, and microsomal fractions and the supernatant fluid isolated from regenerating and control rat livers. The liver RNA of each cellular fraction from the three groups of animals contained predominantly cytidylic (23.2 to 34.0 moles) and guanylic (29.4 to 41.2 moles) acids. The uridylic (17.2 to 21.5 moles) and adenylic (16.1 to 24.0 moles) acids were found in smaller proportions.

Fasting (18 hours)

Table I shows that the concentration of the individual nucleotide is different in the cellular fractions isolated from liver of fasted rats indicating that the RNA composition is different in the various cellular fractions. The microsomal RNA has the highest content of guanylic acid (38.4 moles) and the supernatant fluid RNA the lowest (29.4 moles). The nuclear and mitochondrial RNA have intermediate concentrations, 33.9 and 32.1 moles respectively. The nuclear and microsomal RNA have lower content of both uridylic and adenylic acids than the mitochondrial and supernatant RNA. The nuclear RNA has the highest content of cytidylic acid.

The difference in the RNA composition of each cellular fraction is also apparent when the ratio of the different nucleotides to guanylic acid are calculated. For instance, the adenylic acid ratios are 0.58, 0.69, 0.47 and 0.77 respectively for nuclear, mito-

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TABLE I

NUCLEOTIDE COMPOSITION OF THE RIBONUCLEIC ACID ISOLATED FROM CELL FRACTIONS OF FASTED, SHAM-OPERATED AND REGENERATING (18 HOURS) RAT LIVER

	Moles per hundred moles of nucleotides			
	Cytidylic acid	*Uridylic acid*	*Adenylic acid*	*Guanylic acid*
Fasting (18 hours)				
Nuclei	27.3	19.0	19.8	33.9
Mitochondria	24.5	21.4	22.0	32.1
Microsomes	26.3	17.2	18.1	38.4
Supernatant	26.6	21.5	22.5	29.4
Sham-operated				
Nuclei	28.5	18.6	17.1	35.8
Mitochondria	26.7	18.3	21.3	33.7
Microsomes	23.2	19.3	16.9	40.6
Supernatant	26.6	21.8	22.3	29.3
Regenerating (18 hours)				
Nuclei	30.2	19.7	15.0	35.1
Mitochondria	29.1	18.4	17.9	34.6
Microsomes	26.8	15.9	16.1	41.2
Supernatant	34.0	13.1	24.0	28.9

chondrial, microsomal, and supernatant fluid RNA. A variation of the same magnitude is observed for the uridylic–guanylic acid ratio. Thus on the basis of an identical guanylic acid content, the supernatant fluid, mitochondrial and nuclear RNA contain 64, 47 and 23 % more adenylic and uridylic acid than the microsomal RNA. The ratio of cytidylic to guanylic acid varies to a much lesser degree. These facts suggest that each cellular fraction isolated from liver of rats fasted 18 hours contains a different RNA.

Sham-operated and regenerating (18 hours)

The nucleotide composition of the RNA of each cellular fraction isolated from the liver of sham-operated and hepatectomized rats (Table I) shows the same general picture as obtained for the fasted rats, and thus suggest heterogeneity in the RNA composition in these conditions.

Comparison of the RNA composition of individual fraction in fasted, hepatectomized, and sham-operated rats.

Comparison between the RNA composition of the same cellular fraction from regenerating liver and liver of fasted and sham-operated animals shows minor differences. Similarly if the ratios of each nucleotide to guanylic acid are calculated small variations are observed. The uridylic–guanylic acid ratios of the nuclear fraction RNA in fasted, sham-control and hepatectomized rats are respectively 0.56, 0.52 and 0.56; for the mitochondrial fraction RNA, 0.67, 0.54 and 0.53; for the microsomal RNA, 0.45, 0.47 and 0.47; for the supernatant fluid RNA, 0.73, 0.74 and 0.71. Similar values are obtained for the adenylic–guanylic acid ratios. Thus, on the basis of identical guanylic acid content, the proportions of adenylic and uridylic acids are practically the same in the RNA of each individual cell fraction whether isolated from 18 hours fasted, hepatectomized or sham-operated rats. It is concluded that in liver of rats 18 hours

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following hepatectomy the nucleotide composition of the RNA extracted from the various cellular fractions is not affected as compared to adequate controls. The composition of intracellular RNA is therefore not affected by the processes occurring before liver cell division. This is in accordance with the results obtained 26 hours post-hepatectomy by CROSBIE *et al.*[6].

Comparison of fed and fasted rats (18 hours)

The RNA composition of the cellular fractions isolated from liver of rats fasted for 18 hours is different from that of the corresponding fractions of normal liver[4]. There are increased proportions of cytidylic and guanylic acids especially in the nuclear and supernatant fluid RNA. The cytidylic acid content of the nuclear and supernatant RNA from liver of fasted rats is respectively 27.3 and 26.6 moles % whereas the corresponding values for normal rat liver were 21.3 and 20.4 moles %. Similarly the guanylic acid content is respectively 33.9 and 29.4 moles % for fasted rats whereas they were 30.0 and 22.7 moles % for normal animals[4]. Thus it would seem that an 18 hour period of fasting is sufficient to influence the RNA composition of isolated cellular fractions of rat liver.

RNA composition of cellular fractions of sham-operated and regenerating rat liver (72 hours)

Table II shows the nucleotide composition of RNA in moles per hundred moles of nucleotides for the cellular fractions isolated from liver of rat under the conditions mentioned above. A statistical analysis of the results is also included. The mean concentration of each nucleotide in a cellular fraction was compared to the corresponding concentration in the other fractions and the level of significance of the differences is indicated. For example, the letters M and g at the right of the concentration of cytidylic acid in the nuclear fraction means that the probability of difference between this concentration and that of the mitochondrial fraction is below 50%.

Table II shows that guanylic and cytidylic acids predominate when the composition of RNA is expressed in moles per hundred moles of nucleotides. This Table shows also that the concentration of the individual nucleotide is different in the cellular fractions isolated from liver of sham-control rats indicating that the RNA composition is different in the various cellular fractions. This conclusion supported by statistical analysis was also reached from the results of Table I.

The conclusion that the RNA composition is different in the cytoplasmic fractions of rat liver differs from that of ELSON *et al.* [15]. These authors reported that the RNA composition of the small particles and of the supernatant fluid of rat liver was the same. The present results show that the concentrations of uridylic, adenylic and guanylic acids in these cytoplasmic fractions are different. ELSON AND CHARGAFF emphasized also regularity in the RNA composition because the ratio of 6-amino (adenylic and cytidylic acids) to 6-keto (guanylic and cytidylic acids) derivatives in RNA was practically the unity[16]. The present results gave a value of 0.86 with a coefficient of variation of 9.8 for the ratio 6-amino/6-keto derivatives. This ratio is the same as the one obtained for 50 analysis of RNA carried out in this laboratory. ELSON AND CHARGAFF mentioned a mean value of 0.93 for 151 samples reported in the literature. It would thus seem that more work is needed to ascertain a possible regularity in RNA composition.

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TABLE II

NUCLEOTIDE COMPOSITION OF THE RIBONUCLEIC ACID ISOLATED FROM CELL FRACTIONS OF SHAM-OPERATED AND REGENERATING (72 HOURS) RAT LIVER

Fractions	*Moles per hundred moles of nucleotides*							
	Cytidylic acid		*Uridylic acid*		*Adenylic acid*		*Guanylic acid*	
	Mean ± *S.D.**	*Level of significance***	*Mean* ± *S.D.*	*Level of significance*	*Mean* ± *S.D.*	*Level of significance*	*Mean* ± *S.D.*	*Level of significance*
Sham-operated								
Nuclei	26.7 ± 1.2	M*** g Mc g S f	21.5 ± 1.7	M f Mc f S f	22.5 ± 0.7	M g Mc C S f	29.3 ± 1.7	M f Mc D S f
Mitochondria	27.5 ± 0.7	N g Mc f S f	24.1 ± 1.1	N f Mc D S g	21.9 ± 1.0	N g Mc D S D	26.5 ± 1.4	N f Mc D S g
Microsomes	25.7 ± 1.1	N g M f S f	19.5 ± 0.4	N f M D S B	18.0 ± 0.5	N C M D S B	36.8 ± 1.7	N D M D S C
Supernatant fluid	25.1 ± 1.1	N f M f Mc g	24.9 ± 0.8	N f M g Mc B	23.3 ± 0.5	N f M D Mc B	26.7 ± 1.2	N f M g Mc C
Regenerating								
Nuclei	24.7 ± 1.6	M g Mc f S g	20.4 ± 1.3	M g Mc f S g	21.5 ± 0.5	M f Mc D S g	33.4 ± 0.1	M e Mc D S g
Mitochondria	25.6 ± 1.5	N g Mc g S g	20.8 ± 0.7	N g Mc f S f	17.8 ± 1.6	N f Mc g S f	35.8 ± 0.7	N e Mc f S e
Microsomes	27.8 ± 1.7	N f M g S g	18.5 ± 1.7	N f M f S g	16.9 ± 1.0	N D M g S D	36.8 ± 0.8	N D M f S D
Supernatant fluid	26.2 ± 1.4	N g M g Mc g	19.1 ± 1.1	N g M f Mc g	21.2 ± 0.9	N g M f Mc D	33.5 ± 0.4	N g M e Mc D

* S.D. means standard deviation.

** Level of significance = Probability (*t* test) that the means are different. A, > 0.999; B, 0.999–0.990; C, 0.99–0.98; D, 0.98–0.95; e, 0.95–0.90; f, 0.90–0.50; g, < 0.50. Capital letters are used when the probability of difference exceeds 95%.

*** The abbreviations N, M, Mc and S stand for nuclear, mitochondrial and microsomal fractions and for supernatant fluid.

The RNA composition of regenerating liver appear different from that of the controls. The range of guanylic acid content in the intracellular RNA of regenerating liver (33.4 to 36.8 moles) is narrower than in the controls (26.5 to 36.8 moles). This would indicate that the RNA of the various cellular fractions of regenerating liver is more similar than in the liver of the sham-control rats. The statistical analysis of the results supports this indication since most of the significant differences observed in the RNA composition of the sham-controls disappear and those remaining show a lesser degree of probability. The tendency towards homogeneity of the RNA composition in the various cellular fractions of regenerating rat liver is also apparent when the ratio

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of each nucleotide to guanylic acid is calculated. The A/G ratios of the RNA of each cellular fraction of regenerating liver are 0.61, 0.58, 0.50 and 0.50 for the nuclear, mitochondrial, microsomal and supernatant fraction respectively, whereas they are 0.73, 0.91, 0.53 and 0.93 in the controls. Then, on the basis of a constant amount of guanylic acid, the nuclear, mitochondrial and supernatant RNA of regenerating liver would contain only 22, 16 and 0 % more adenylic acid than the microsomal RNA; whereas they would contain 38, 72 and 76 % more adenylic acid in the control. A similar picture is obtained for uridylic acid.

It is apparent from these facts that in regenerating livers (72 hours) the ribonucleic acid composition of the various cellular fractions is affected. These changes indicate that RNA composition tends to be similar in the liver cell fractions during rapid growth, and it is concluded from this that a high rate of cellular division in liver can influence the composition of RNA. These results differ from those reported by Cox[7].

Comparison with normal liver and liver tumor

The RNA composition of the cellular fractions of regenerating liver (72 hours) is intermediate to that observed in normal liver and liver tumor[4,5]. The proportions of guanylic acid in liver RNA of 72 hours post-hepatectomized rats are higher than in normal liver but lower than in liver tumor. For example, the guanylic acid value for mitochondrial RNA in regenerating liver is 35.8 moles as compared to 25.8 moles in normal liver, and 40.7 moles in liver tumor induced by 4-dimethylaminoazobenzene. Furthermore, the concentration of cytidylic acid is much higher than in the two other conditions. However, as in liver tumor, a marked tendency to homogeneity is observed. These results suggest that some of the alterations in RNA composition observed in primary liver tumor might be due to the high rate of mitosis in that tissue.

The changes in the pattern of intracellular ribonucleic acids observed in primary liver tumor were accompanied by variations in the protein composition of the intracellular fractions of this tissue[5]. This was thought to bring support to the hypothesis that specific ribonucleic acids play a role in the synthesis of the various intracellular proteins[17,18,19]. In the present study, the RNA composition of the cellular fractions of regenerating liver (72 hours) was found to be intermediate between that of normal liver and liver tumor. Previously it was found that the composition of intracellular proteins of regenerating liver was also intermediate between that of liver and liver tumor[20]. Therefore, these facts are in line with the above-mentioned hypothesis.

SUMMARY

The nucleotide composition of the ribonucleic acid of isolated cellular fractions from liver of fasted, sham-operated and hepatectomized (18 and 72 hours) rats has been examined. It was found on one hand that the RNA composition of each cellular fraction differs from the others in the case of 18 hours-fasted, sham-operated and hepatectomized rats. On the other hand, in regenerating liver (72 hours post-hepatectomy) the nucleotide composition of the RNA of the various cellular fractions was observed to be more similar than in sham-operated controls.

Fasting (18 hours) induced changes in the RNA composition of liver as compared to liver of fed animals. These variations consisted in increases of cytidylic and guanylic acids proportions. In regenerating liver, 72 hours post-hepatectomy, increases of cytidylic and guanylic acids were also apparent but these variations were less marked than those previously observed in liver tumor. The alterations in RNA nucleotide composition of primary liver tumor might be explained in part by the high mitotic rate of this tissue.

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REVERSED PHASE PARTITION CHROMATOGRAPHY OF SOME C_{27}-STEROIDS*

BILE ACIDS AND STEROIDS 38

HENRY DANIELSSON

Department of Physiological Chemistry, University of Lund (Sweden)

For metabolic studies and synthetic work on a micro-scale we needed a method for the separation of different, neutral C_{27}-hydroxy- and keto-steroids. The chromatographic systems worked out by MOSBACH *et al.*[1] for the separation of the air-oxidation products of cholesterol were not entirely satisfactory for our purposes, since cholesterol and less polar compounds move with the solvent front in these systems. The reversed phase partition chromatography technique of HOWARD AND MARTIN[2] has been found very useful for the separation of such compounds as higher fatty acids (HOWARD AND MARTIN[2]) and bile acids (BERGSTRÖM, SJÖVALL AND NORMAN[3-5]), and it was to be expected that this method could be used for the separation of neutral C_{27}-steroids.

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