Ribonucleic Acid Composition of Rat Liver Tumor Induced by 4-Dimethylaminoazobenzene*

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Studies on normal tissues have suggested that the ribonucleic acid (RNA) of nuclei, mitochondria, microsomes, and supernatant fluid isolated from tissue homogenate has a characteristic nucleotide composition (7, 8, 16), while other data did not support such a view (6). Results obtained in this laboratory revealed, in fact, the occurrence of characteristic ribonucleic acids in rat liver fractions prepared from sucrose homogenate (13, 15). In the present investigation, the study was extended to cell fractions of primary liver tumors induced by 4-dimethylaminoazobenzene. The results indicated that, in liver tumors, the ribonucleic acids from the different cellular fractions are less heterogeneous than in normal liver.

METHODS

Adult, male, Wistar rats of an average weight of 150 gm. were fed, during 150 consecutive days, a semisynthetic diet containing p-dimethylaminoazobenzene at a concentration of 0.06 per cent (17). This was followed by a 2-month period on normal diet (Purina Fox Chow). At the end of this period, the animals were stunned and immediately decapitated. The whole liver with tumors was excised, placed on cracked ice, and chilled for 2 minutes. The liver was then dissected, and tumor masses were collected. Only small (less than 1 cm. in diameter) and firm masses of light buff color were taken. These tumors did not show any soft part when cut in small pieces.

In a first assay, tumors from eight animals and, in a second assay, tumors from five animals were pooled. These pooled tumors were immediately minced in a cold plexiglass squeezer, and the tissue pulp was homogenized in 0.25 M sucrose with a glass homogenizer. The final volume was adjusted to give a 10 per cent homogenate. Four cellular fractions, namely, nuclear, mitochondrial, microsomal, and supernatant fluid fractions, were isolated by differential centrifugation according to the method of Schneider and Hogeboom (20) with a Servall SS-1 and a Spinco (Model L) refrigerated centrifuge.

The nucleic acids were isolated from homogenate and cellular fractions by a slightly modified (5) Schmidt and Thannhauser procedure (19). A 10 per cent solution of trichloroacetic acid was used to extract the acid-soluble material from tissue pulp or cellular fraction. This was followed by three washings with water. The lipids were extracted as in the original procedure. The dried powder obtained was stored at 5° C. The nucleotides, obtained by the alkaline hydrolysis of the ribonucleic acid, were analyzed by ion exchange chromatography (4). The elution was followed by spectrophotometric measurements of the eluted fractions, and the concentrations of nucleotides in these fractions were determined as previously described (15).

RESULTS

Purity of the cellular fractions.—Each cellular fraction isolated by differential centrifugation was examined under the phase microscope in a Petroff-Hausser bacteria counter for cross-contamination of fractions (2). Mitochondrial counts on the nuclear fraction revealed that the latter contained 30 per cent of the total number of mitochondria present in the original homogenate. Such contamination of the nuclear fraction by mitochondria was nevertheless insignificant, since the amount of RNA in the mitochondrial fraction was very low. Table 1 shows that the differences between proportions of corrected and uncorrected nucleotides in the nuclear ribonucleic acid were smaller than those noted between the two groups of animals under observation. The nuclear fraction appeared practically free of microsomes.

The mitochondrial fraction was clear of nuclei and microsomes. However, microsomal contami-
nation is difficult to evaluate, since these particulars are near or below the limit of definition of the phase microscope.

The microsomal fraction appeared free of nuclei and mitochondria, and the supernatant fluid was uncontaminated by any visible particles under the phase microscope.

**RNA composition of homogenate and cell fractions of rat liver tumor.**—Table 1 shows the nucleotide composition of RNA in mg/100 mg of nucleotides. The homogenate RNA contained pre-

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<th>TABLE 1</th>
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<td><strong>NUCLEOTIDE COMPOSITION OF THE RNA ISOLATED FROM HOMOGENATE AND CELL FRACTIONS OF RAT LIVER TUMOR</strong></td>
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<tr>
<td>m/100 mg of nucleotides</td>
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<td></td>
</tr>
<tr>
<td>Cytidylic acid</td>
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<td>Uridylic acid</td>
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<td>Adenylic acid</td>
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<tr>
<td>Guanylic acid</td>
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<td><strong>Mitochondria:</strong></td>
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<td>1st assay</td>
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<td>Mean</td>
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<td><strong>Microsomes:</strong></td>
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<td>1st assay</td>
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<td><strong>Supernatant:</strong></td>
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<td>2nd assay</td>
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<td>Mean</td>
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*The nucleotide composition of the nuclear fraction RNA, when corrected for the number of mitochondria present in this fraction and according to the composition of the mitochondrial RNA, gives the respective corrected values for the first assay: 55.7 for cytidylic, 16.5 for uridylic, 18.8 for adenylic, 39.1 for guanylic acid.

dominantly guanylic (39.6 mg) and cytidylic (25.6 mg) acids. Uridylic and adenylic acids were found in smaller proportions (16.5 and 18.3 mg). This high level of guanylic acid was found in the RNA of all cellular fractions, especially in the nuclear and mitochondrial fractions (40.1 and 40.7 mg). In these two cellular fractions, the proportions of the three other nucleotides were similar. The RNA of the microsomal fraction and of the supernatant fluid showed a slightly lower level of guanylic acid (5.8 mg and 5.8 mg); the proportions of the other nucleotides varied to a greater extent than they did in the nuclear and mitochondrial fractions. Nevertheless, the composition of the ribonucleic acid was quite similar, the percentage of each nucleotide showing only slight differences from one fraction to another.

It can be noted that the concentration of cytidylic acid was lower in each fraction than in the homogenate. Values of uridylic and adenylic acids, on the other hand, were higher in the fractions than in the homogenate. The RNA composition of the homogenate calculated according to the composition of the RNA and the percentage of RNA in each fraction gives the following: cytidylic acid, 23.6 mg; uridylic acid, 19.1 mg; and guanylic acid, 39.3 mg. These calculated values compare well with those found by analysis of the original homogenate RNA even if the cytidylic acid value is slightly low and the uridylic acid value high. This could be owing to deamination of cytidylic acid to give uridylic acid during alkaline hydrolysis of RNA. However, deamination of cytidylic acid does not seem to proceed to an appreciable extent under these conditions, as shown previously (15).

Table 2 shows the absolute amounts of total RNA and RNA guanylic acid isolated from all cellular fractions. These were computed from the concentrations obtained by spectrophotometric determinations on eluted fractions. An absolute increase in guanylic acid was observed in tumor RNA as compared with that in normal RNA. Tumor nuclear RNA showed a 12.2 per cent increase of guanylic acid; mitochondrial RNA, 22.0 per cent; and supernatant fluid RNA, 51.7 per cent. However, no variation was observed for the microsomal RNA.

**DISCUSSION**

The most striking result appears to be the high content of guanylic acid in tumor ribonucleic acids of homogenate and cellular fractions, namely, 35.4-40.7 mg, as compared with the corresponding values in normal tissue, 22.7-34.6 mg (15). It is of interest to note that in tumors induced by azo dyes, both deoxyribonucleic acid (10) and ribo-
nucleic acid show an increased concentration of guanylic acid.

The high level of guanylic acid in the RNA of all cellular fractions of tumor was accompanied by low levels of uridylic and adenylic acids (20.9 and 20.3 M, respectively, as compared with 28.4 and 28.6 M for normal liver). This high level of guanylic acid actually represents an absolute increase, as shown in Table 2. This might be explained either by a preferential uptake of guanylic acid precursors in the RNA of each fraction (if the latter is to be considered as a chemical entity) or by a preferential formation of RNA with high levels of guanylic acid (if the RNA of each fraction is a mixture of several entities). In any case, an increased uptake of guanylic acid precursors by total RNA would take place.

The ribonucleic acid from tumor microsomal fraction was the only one which did not show an increase in guanylic acid and variations in the proportions of the various nucleotides, as compared with normal microsomal fraction (15). In relation to that fact, it seems of interest to point out that the changes in the ribonucleic acid of the other cellular fractions of tumor tend to approach the microsomal ribonucleic acid composition (predominance of guanylic acid and approximately equal distribution of the other nucleotides). This tendency toward homogeneity has also been reported with regard to the turnover of the phosphorus of mitochondrial and microsomal RNA (8).

The tendency toward homogeneity in the composition of the ribonucleic acid isolated from tumor cellular fractions, as compared with normal, is further emphasized by comparing the ratios of the different nucleotides to guanylic acid. For instance, the ratio uridylic/guanylic acid, which ranged between 0.64 and 1.25 in the nucleic acid of the normal cellular fractions (15), varied in tumor from 0.49 to 0.65. Variation of the same order was observed for the ratio of adenylic/guanylic acid. The greatest variations observed between the various ratios, as compared with those of the microsomal RNA, were those seen in the cytidylic/guanylic acid ratio, which was of about the same order as that of the smallest variations observed in normal ribonucleic acids. This emphasizes the uniformity of the RNA composition of all cellular fractions isolated from tumor homogenate.

The hypothesis that specific ribonucleic acids play a role in the synthesis of the various intracellular proteins (1, 12, 18) implies the existence of numerous types of RNA. Accordingly, a change in the pattern of ribonucleic acids would be accompanied by an alteration in the protein pattern. Such a relation seems to exist in primary liver tumor. Numerous changes in tumor proteins correspond to an altered RNA composition, as reported here. Variations in the protein composition of intracellular fractions have been reported (14, 21). Furthermore, a complete loss of specific proteins, glucose-6-phosphatase (22) and cystine desulphurase (9), as well as the presence of a new protein (11) has been reported in primary liver tumors. These facts bring further support to the above-mentioned hypothesis.

SUMMARY

The nucleotide composition of the ribonucleic acid of the homogenate and of isolated cellular fractions of primary liver tumor was determined. It was found that the RNA compositions of all cellular fractions resemble one another. Therefore, the ribonucleic acid composition is not specific for each cellular fraction, as was observed in normal liver. The relative homogeneity of RNA in the cellular fraction of tumor is characterized by a high guanylic acid content. These findings are discussed in relation to the possible role of ribonucleic acids in protein synthesis.

ACKNOWLEDGMENTS

The authors wish to thank Mr. Bernard Messier for valuable technical assistance in this work.

REFERENCES


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