The Composition of Ribonucleic Acid and Deoxyribonucleic Acid of Normal and Neoplastic Tissue

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The essential role of nucleic acids as prototypes, templates, or as carriers of coded information in biological processes has become progressively significant. In the concept of cancer the importance of the nucleic acids has become generally accepted. Differences in the composition of nucleic acids of normal and neoplastic tissues would be of fundamental importance, for they would provide a biochemical basis for the biological differences manifested in the two tissues.

Comparison of results of analyses of the nucleic acids of normal and neoplastic tissues reported since 1950 show differences in purine and pyrimidine composition. de Lamirande et al. (4) analyzed the RNA1 of rat liver tumor induced by 4-dimethylaminoazobenzene and found an exceedingly high molar ratio of guanine. Levy and Snellbaker (10) reported relatively high quantities of guanine in the RNA extracted from Ehrlich ascites tumor cells of the mouse. Beale et al. (1) also found high amounts of guanine in the RNA of the G.R.C.H. 15 fowl sarcoma tissue. Schneider and Ebner (14) examined the DNA of Ehrlich ascites tumor cells and of normal mouse liver and kidney, and they observed higher quantities of guanine and less thymine in the tumor tissue DNA. The reported value for the thymine content of the DNA of a mouse sarcoma induced by 1,2,5,6-dibenzanthracene (9) is also low. Differences in the DNA composition, ascribed to differences in the methods of extraction, were observed by Khovine and Vieyres (6) in rat epithelioina and Ehrlich ascites tumor cells. The initial saline extract of the Ehrlich ascites cells, however, gave the purine and pyrimidine values for DNA generally found in mammalian tissues.

The analyses above were made on extracted and purified nucleic acid preparations. Such preparations are known to vary in composition, depending on the methods of extraction employed (6, 11). Furthermore, the susceptibility of RNA to enzymatic degradation during isolation may be the cause of differences in the base ratios of RNA of neoplastic and normal tissues.

It was desirable, therefore, to analyze directly without prior isolation the nucleic acids of normal and neoplastic tissues under conditions which would eliminate their degradation. Analyses of the nucleic acids of the Ehrlich ascites tumor of the mouse, the Rous sarcoma of the chicken, and of normal tissues of these two animals were made. The results show no significant difference in base composition of the nucleic acids of these neoplastic tissues from that of normal tissues.

MATERIALS AND METHODS

Nucleic acid analysis.—The analytical procedures employed have been described previously (8). Essentially, the method of determination of RNA and DNA consists of the hydrolysis of small amounts of dried, defatted tissue, isolation of base constituents of the nucleic acids by two-dimensional paper chromatography, and measurement of the separate bases by their ultraviolet absorption.

Preparation of the tissues for analysis was modified slightly. Ethanol was substituted for acetone and petroleum ether in the extraction of lipide. The frozen tissues were fragmented in a high-speed blender with hot ethanol, transferred to a flask containing hot ethanol, extracted by refluxing for 1 hour, and then filtered. The residue was extracted twice more by refluxing with ethanol for 1 hour. Extraction with ethanol permitted faster preparation of the tissue and in most instances produced improvement in the chromatography of the hydrolysates because of further removal of interfering extraneous substances.

The RNA and DNA of the intestine, spleen, and Rous sarcoma of the chicken and of the spleen, kidney, and S-180 ascites tumor of the mouse were analyzed. In each case twenty animals were employed to procure a pool of each tissue. The "nor-

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1 The following abbreviations have been used in the paper: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; A, adenine; G, guanine; C, cytosine; U, uracil; T, thymine; 6 Am/6 Keto, ratio of 6-amino to 6-keto bases.

Received for publication June 4, 1959.
normal tissues were obtained from animals not bearing neoplasms. In the analysis two replicate samples of a tissue pool were weighed and hydrolyzed. Duplicate chromatograms were made of each hydrolysate.

Rous sarcoma tissue.—Fourteen-day-old chicks were given inoculations of the Rous sarcoma virus in the wing web, as described by Johnson and Baker (5). After 10 days the sarcoma tissue was excised, quickly freed of adhering skin, and frozen immediately in an acetone-dry ice bath.

Mouse ascites tumor cells.—Swiss mice were inoculated with 1–2 × 10⁶ mouse ascites tumor cells of the S-180 carcinoma and 7–8 days later were sacrificed by cervical dislocation. The tumor cells were removed with a syringe from the intraperitoneal cavity of the mice. The cells were delivered into a chilled tube containing a modified Krebs-Ringer buffer solution (13) to which had been added a small amount of heparin to prevent the cells from clotting. The cells were washed free of erythrocytes by centrifugation in the cold Krebs-Ringer buffer, were resuspended in the buffer, and were counted by measuring the light transmission on a Klett-Summerson photoelectric colorimeter, employing a No. 44 filter. The number of cells was determined from a standard curve prepared by calibration of the direct cell count, obtained with a Levy counting chamber, against the transmission of light.

The analysis of the mouse ascites tumor cells offered some problems. Erratic results were obtained when the cells were suspended in distilled water or organic solvents at any point in the procedure. Therefore, the cells were washed in the modified Krebs-Ringer solution, were counted, and were freeze-dried. In the calculations the weight of the buffer components was subtracted to obtain the weight of cells.

### TABLE 1

RNA COMPOSITION OF NORMAL AND NEOPLASTIC TISSUES

<table>
<thead>
<tr>
<th>Sample</th>
<th>A</th>
<th>U</th>
<th>A/U</th>
<th>G</th>
<th>C</th>
<th>A/U G/C</th>
<th>6 Am</th>
<th>6 Keto</th>
<th>RNA μg X 10⁶/ cell</th>
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</thead>
<tbody>
<tr>
<td>Chicken intestine</td>
<td>21.9</td>
<td>19.2</td>
<td>1.14</td>
<td>29.8</td>
<td>29.3</td>
<td>1.02</td>
<td>1.11</td>
<td>1.08</td>
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<td>Chicken spleen</td>
<td>24.4</td>
<td>18.4</td>
<td>1.32</td>
<td>31.0</td>
<td>28.4</td>
<td>1.09</td>
<td>1.02</td>
<td>1.02</td>
<td>3.15</td>
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<td>Rous chicken sarcoma</td>
<td>20.7</td>
<td>18.8</td>
<td>1.10</td>
<td>30.8</td>
<td>29.8</td>
<td>1.05</td>
<td>1.02</td>
<td>1.02</td>
<td>2.23</td>
</tr>
<tr>
<td>Mouse spleen</td>
<td>21.0</td>
<td>21.0</td>
<td>1.00</td>
<td>29.7</td>
<td>28.4</td>
<td>1.04</td>
<td>0.97</td>
<td>0.98</td>
<td>3.81</td>
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<td>Mouse kidney</td>
<td>22.6</td>
<td>19.4</td>
<td>1.16</td>
<td>28.4</td>
<td>29.6</td>
<td>0.96</td>
<td>1.21</td>
<td>1.69</td>
<td>1.87</td>
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<td>Mouse ascites tumor cells</td>
<td>19.7</td>
<td>22.1</td>
<td>0.89</td>
<td>28.7</td>
<td>29.5</td>
<td>0.97</td>
<td>0.92</td>
<td>1.03</td>
<td>6.30</td>
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</tbody>
</table>

### TABLE 2

DNA COMPOSITION OF NORMAL AND NEOPLASTIC TISSUES

<table>
<thead>
<tr>
<th>Sample</th>
<th>A</th>
<th>T</th>
<th>A/T</th>
<th>G</th>
<th>C</th>
<th>A/T G/C</th>
<th>6 Am</th>
<th>6 Keto</th>
<th>DNA μg X 10⁶/ cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken intestine</td>
<td>28.2</td>
<td>28.8</td>
<td>0.98</td>
<td>20.8</td>
<td>22.9</td>
<td>0.91</td>
<td>1.07</td>
<td>1.03</td>
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<td>Chicken spleen</td>
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<td>32.0</td>
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<td>Rous chicken sarcoma</td>
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<td>0.98</td>
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<td>1.07</td>
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<td>0.96</td>
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<td>29.0</td>
<td>0.99</td>
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<td>1.04</td>
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<td>1.17</td>
<td>0.87</td>
<td>0.93</td>
<td>2.65</td>
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<tr>
<td>Mouse ascites tumor cells</td>
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<td>30.0</td>
<td>0.93</td>
<td>21.4</td>
<td>20.9</td>
<td>1.05</td>
<td>0.89</td>
<td>0.95</td>
<td>4.90</td>
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</table>

RESULTS AND DISCUSSION

The results of the determinations are presented for RNA in Table 1 and for DNA in Table 2. The results are averages of two analyses on a single tissue pool. In our experience with the analytical method the coefficient of variation from replicate to replicate in the determination of each nucleic acid base constituent of the various tissues is 3–8 per cent (8). Agreement in the results of the two replicate determinations in the present study was equally as good or better.

The range of values for the nucleic acids in the various tissues is essentially that found previously for tissues of the calf, chick embryo, and the rat (8). The amounts of RNA and DNA per S-180
mouse ascites tumor cell are slightly higher than the range of values for the RNA and DNA in various mouse ascites tumor cells found by Klein (7) employing phosphorus determination.

If the molecular weight of a nucleic acid molecule were assumed to be $1 \times 10^6$, then a difference of only 1 per cent in the molar amount of a nucleotide in the above results would be equivalent to about 30 nucleotide residues per molecule. Therefore, the data do not permit one to state absolutely that the respective nucleic acids of the normal and tumor tissues do not differ in composition. It also should be noted that the analysis of whole tissue nucleic acids represents only an average of the composition of all the individual nucleic acid molecules present. Hence, a small amount of a nucleic acid with an unusual molar ratio would not significantly change these average values. However, within the limits of the method (8) no significant differences exist between the nucleic acids of the normal and the neoplastic tissues. The molar ratios of A/T of DNA and G/C of RNA approach 30/30 and A/U of RNA and G/C of DNA approach the expected 20/20. If the respective nucleic acids of the normal and neoplastic tissues do contain a population of molecules varying quantitatively in nucleotide composition, they would appear to approximate equal proportions in each tissue. Certainly, no unusually high molar ratio of guanine of the magnitude reported by de Lamirande (4) in the RNA of a rat liver tumor is observed.

The variation in composition of nucleic acids of normal and neoplastic tissues previously reported (1, 4, 9, 10, 14) may possibly be explained by changes produced by enzymic and other degradations during the isolation and preparation of the material for analysis. Magasanik and Chargaff (12) have demonstrated that RNA acted upon by ribonuclease yielded nondialyzable residues with very high proportions of guanine. Present analyses of tissue under conditions which eliminate the probability of alteration of the molecules result in nucleic acids to explain such biological differences as are exhibited by normal and neoplastic tissues must await methods for separation, without accompanying degradation, of the population of nucleic acids present in such tissues plus methods for determination of nucleotide sequences.

SUMMARY

The ribonucleic acid and deoxyribonucleic acid composition and content of normal tissues of the mouse and chicken and of the S-180 mouse ascites tumor and chicken Rous sarcoma were determined. No significant differences in base composition between the respective nucleic acids of the normal and neoplastic tissues were found.

ACKNOWLEDGMENTS

I wish to express my appreciation to Mr. L. A. Baker, Mrs. A. Folger, Miss P. Libbey, and Mr. H. E. Martlage for their valuable technical assistance.

REFERENCES


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