Effect of Ascorbic Acid and Glucoascorbic Acid on Nucleic Acids in Tumor Tissue

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In our previous paper (16), we described the retarding effect of the continuous administration of a nontoxic dietary level (1 and 2 per cent) of d-glucosascorbic acid on Crocker rat carcinoma and mouse adenocarcinoma E 0771. Apparently, the effects resulted from the low ascorbic acid concentration in blood plasma and tissue induced by the analog. Our present investigation concerns the effect of low ascorbic acid concentrations in tissue on the deoxypentosenucleic acid (DNA) and pentosenucleic acid (PNA) contents in fibrosarcoma of guinea pig and Crocker rat carcinoma. The total DNA and PNA and the nuclear DNA contents were estimated. Goldstein et al. (7) found that a scorbutogenic diet induced changes in the PNA and DNA contents of the spleen and adrenals of guinea pigs and increased the PNA/DNA ratio. On the basis of their experiments in vivo, with and without ascorbic acid added, they concluded that ascorbic acid is involved in the formation of DNA from PNA.

MATERIALS AND METHODS

A British brown and white strain of rats, bearers of Crocker carcinoma, originally obtained from the Crocker Laboratory of Columbia University, was used for this investigation. Both the control and treated animals, all males of the same age and weight, were kept on an isocaloric Sherman-LaMer scorbutogenic diet. The tumors used for the determination of nucleic acids were 0.75—1.0 cm. in diameter, fast-growing, and without any necrotic areas. A transplanted fibrosarcoma, AL-2, originally induced by injection of methylcholanthrene in guinea pigs of Family A, obtained from the Jackson Memorial Laboratory, was used for the same determination. The guinea pigs, both treated and control, were also placed on an isocaloric Sherman-LaMer diet, and the doses of sodium ascorbate, 3, 10, and 25 mg/day, were given orally in water solution.

The guinea pigs were placed on the Sherman-LaMer diet the day before the fibrosarcoma was transplanted. The rats were kept on the same diet 10 days prior to transplantation. d-Glucosascorbic acid was added in the amount of 2 per cent to this diet.

The Sherman-LaMer diet used in our experiments was composed of: 18 gm. vitamin-free casein; 65 gm. corn starch; 5 gm. hydrogenated vegetable oil (Crisco); 2 gm. cod liver oil; 6 gm. dried brewers' yeast; and 4 gm. salt mixture #1.

Ascorbic acid determination.—The technics of Farmer and Abt (5, 6) for the blood plasma and of Roe and Kuether (18) for determination in the tissues was employed. The procedure was described in our previous work (16).

Total DNA and PNA content estimation.—A slightly modified method of Barnum (1) and Payne et al. (10) was used for the extraction and separation of DNA and PNA. The technic of Schneider (14) was applied for the estimation of DNA and PNA in the tissues. The samples of tumor tissue were placed in the refrigerator for 50—40 minutes and homogenized for 5—3 minutes in 5 volumes of cold saline solution in the apparatus of Potter and Elvehjem. The homogenate was centrifuged for 5 minutes at about 1,500 X g. The purification of DNA was repeated 6 times, as suggested by Kelly and Jones (8). DNA was measured by the diphenylamine reaction and PNA by the orcinol reaction with a Coleman spectrophotometer as described by Dische (4) and elaborated by Schneider (14). The moisture content of fresh tumor tissue was determined on numerous control samples, and accordingly the total DNA and PNA content was estimated by the percentage of dry tissue.

Estimation of the nuclear DNA.—The procedure was essentially the same as recommended by Lessler (9). Tissues were fixed in 6 per cent neutral formalin, washed in running tap water, dehydrated, embedded in paraffin, sectioned, hydrated, and hydrolyzed for 18 minutes in 1 N HCl at 65° C. Following the hydrolysis, the preparations were rinsed in running water for 5 minutes, washed twice in distilled water, and exposed to the Feulgen reagent for 1 hour and 30 minutes, placed in 3 changes of water saturated with sulfur dioxide, and washed twice in distilled water. To remove the yellow dye impurity in the Feulgen reaction, sulfur dioxide was bubbled through a 0.5 per cent basic fuchsin solution for 2 hours, and 1 gm. of decolorizing charcoal was added to 900 cc. of solution; the

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solution was then agitated and filtered. The spectrophotometric apparatus and technic employed in this investigation were similar to those described by Pollster (11). The apparatus consisted of a photomultiplier tube mounted above a microscope. The DNA content per cell, in arbitrary units, was calculated according to the procedure of Lessler.

RESULTS

Twenty-five male guinea pigs, average weight 550 gm., were divided into five groups, each of five animals. Group I was fed the Sherman-LaMer scorbutogenic diet and received no ascorbic acid. Group II (control), fed the same diet, was given 3 mg sodium ascorbate/day, the equivalent of the daily required dose. Group III, on the same diet, received 10 mg. of ascorbic acid daily. Group IV was maintained on the same diet plus 25 mg. of the vitamin, and Group V was kept on the Sherman-LaMer diet plus 2 per cent of d-glucosaccharic acid.

On the same day that the animals were placed on their respective diets, fibrosarcoma AL-2 was transplanted.

Seven days later, another fragment of the same tumor was transplanted into the same animals. When each of the first series of tumors was 1 cm. in diameter, it was excised without sacrificing the animals. In about 7 days or more, each of the second series of tumors was removed when it had attained about the same size. At the time of the operation, samples of tumor tissue were taken for ascorbic acid estimation. All the tumors (in one instance there was no "take") were analyzed for DNA and PNA. Small samples of the tumor were preserved for the Feulgen reaction. The results are summarized in Table 1.

The analysis of the data presented in Table 1 indicates that neither low nor elevated concentrations of ascorbic acid in tumor tissue influenced significantly its total PNA content. This remained within the limits of variation observed in each of the series of the experiments. On the other hand, there was a trend for an increase in the DNA content on a diet rich in ascorbic acid and for a decline on a scorbutogenic diet. The average PNA/DNA ratio, which for the control guinea pigs on 3 mg/day of ascorbic acid was 0.84, was 1.1 for the animals on the scorbutogenic diet. The PNA/DNA ratio for those guinea pigs given large amounts of ascorbic acid was 0.59. The administration of d-glucosaccharic acid by itself had no effect on the PNA/DNA ratio in the fibrosarcoma of guinea pigs. This was to be expected, since the analog exerts no antagonistic activity against dietary vitamin C, as was demonstrated in our previous work (16).

<table>
<thead>
<tr>
<th>Group</th>
<th>Supplement</th>
<th>No.</th>
<th>PNA per cent of dry wt.</th>
<th>DNA per cent of dry wt.</th>
<th>PNA/DNA ratio</th>
<th>Ascorbic acid (mg/100 gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>No ascorbic acid</td>
<td>15</td>
<td>±0.52±0.09</td>
<td>±0.45±0.15</td>
<td>1.02</td>
<td>5.3±0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>±0.60±0.1</td>
<td>±0.2±0.01</td>
<td>1.1</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>II</td>
<td>3 mg. ascorbic acid daily, control</td>
<td>9†</td>
<td>±0.45±0.12</td>
<td>±0.06±0.02</td>
<td>0.84</td>
<td>18.4±1.55</td>
</tr>
<tr>
<td>III</td>
<td>10 mg. ascorbic acid per day</td>
<td>15</td>
<td>±0.88±0.01</td>
<td>±0.03±0.01</td>
<td>0.71</td>
<td>28.9±1.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>±0.37±0.08</td>
<td>±0.07±0.02</td>
<td>0.64</td>
<td>28.3±1.83</td>
</tr>
<tr>
<td>IV</td>
<td>25 mg. ascorbic acid per day</td>
<td>15</td>
<td>±0.88±0.01</td>
<td>±0.03±0.01</td>
<td>0.62</td>
<td>28.4±2.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>±0.40±0.09</td>
<td>±0.05±0.02</td>
<td>0.59</td>
<td>29.8±2.45</td>
</tr>
<tr>
<td>V</td>
<td>2 per cent glucosaccharic acid</td>
<td>15</td>
<td>±0.38±0.15</td>
<td>±0.05±0.02</td>
<td>0.90</td>
<td>6.04±0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>±0.45±0.08</td>
<td>±0.06±0.01</td>
<td>0.95</td>
<td>0.01±0.015</td>
</tr>
</tbody>
</table>

* One guinea pig died on 85th day of dieting.
† There was no take in one guinea pig.
‡ Two guinea pigs died, one on 16th day, second on 18th day of dieting.

In another series of experiments, thirty male rats of British brown and white strain with an average weight of 180 gm. were divided into three groups, ten animals each. Group I was fed the Sherman-LaMer diet; Group II the Sherman-LaMer diet plus 2 per cent of d-glucosaccharic acid; and Group III the Sherman-LaMer diet plus 2 per cent of sodium ascorbate. After 10 days on these diets, Crocker carcinoma was transplanted into all animals. The tumors were removed after about 25 days on the diets when they were approximately 0.75 cm. in diameter. Thirty hours before excision of the tumors, food was withdrawn from the animals. The total PNA and DNA of tumor tissues was estimated, as well as its ascorbic acid concentration. Table 2 summarizes the results of these trials.

The data of Table 2 suggest that there was an increase in the DNA content of Crocker rat carcinoma when the animals were kept on a diet rich...
in ascorbic acid and a decrease in the DNA content when low ascorbic acid concentrations were induced by the analog. No significant changes were observed in the PNA content under either condition of experimentation. The shift in the PNA/DNA ratio was smaller in Crocker rat carcinoma than in fibrosarcoma of guinea pigs. From 0.85 in the control group, it was increased to 1.16 in rats on D-glucosascorbic acid, while it declined to 0.77 in the group of animals receiving large doses of ascorbic acid.

The analysis of data presented in Table 3 indicates that a vitamin C-free diet resulted in a decrease in the nuclear DNA content of fibrosarcoma of guinea pigs. This decrease was more pronounced when the diet was fed for a period of 25 days, at which time the guinea pigs manifested the scurvy syndrome. From 3.48 (in arbitrary units) the nuclear DNA content was reduced to 2.61. Administration of large doses of ascorbic acid, above its daily requirement for guinea pigs, had no appreciable effect on the nuclear DNA content.

**TABLE 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Supplement</th>
<th>No. Tumors</th>
<th>PNA per cent of dry wt.</th>
<th>DNA per cent of dry wt.</th>
<th>PNA/DNA Ratio</th>
<th>Ascorbic Acid (mg/100 gm tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>None</td>
<td>9*</td>
<td>0.84±1.16</td>
<td>0.85±1.2</td>
<td>0.85</td>
<td>40.5±1.55</td>
</tr>
<tr>
<td>II</td>
<td>2 per cent glucoascorbic acid</td>
<td>10</td>
<td>0.87±0.92</td>
<td>0.81±0.22</td>
<td>1.16</td>
<td>4.5±0.67</td>
</tr>
<tr>
<td>III</td>
<td>2 per cent sodium ascorbate</td>
<td>10</td>
<td>0.85±0.17</td>
<td>0.89±0.19</td>
<td>0.77</td>
<td>63.3±1.75</td>
</tr>
</tbody>
</table>

* One rat died.

**TABLE 3**

<table>
<thead>
<tr>
<th>Group</th>
<th>Supplement</th>
<th>No. Specimens*</th>
<th>No. Cells</th>
<th>(E)(A)† (arbitrary units)</th>
<th>Feulgen reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>None</td>
<td>15</td>
<td>4</td>
<td>2.92±1.13</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>3 mg. ascorbic acid per day, control</td>
<td>25</td>
<td>3</td>
<td>3.43±1.15</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>10 mg. ascorbic acid per day 15</td>
<td>2</td>
<td>50</td>
<td>3.48±1.11</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>25 mg. ascorbic acid per day 15</td>
<td>2</td>
<td>60</td>
<td>3.82±1.11</td>
<td></td>
</tr>
</tbody>
</table>

* Each specimen taken from a different tumor.
† E = extinction coefficient.
A = nuclear area, in Df.

The low ascorbic acid concentrations in tissues induced by D-glucosascorbic acid, added to the Sherman-LaMer diet in the amount of 2 per cent, decreased the nuclear DNA content of Crocker rat carcinoma. Instead of 4.23 for the control animals, the average nuclear DNA content was 3.73 (in arbitrary units). This decrease, amounting to about 12 per cent, was smaller than the one observed in the guinea pig fibrosarcoma. Administration of large doses of ascorbic acid to rats did not alter the nuclear DNA content of Crocker carcinoma.

**DISCUSSION**

Davidson and Leslie (3) concluded, on the basis of their experiments with chick embryo, that “real growth could not be said to have occurred without a rise in DNA.” This opinion was concurred in by
Brachet (2) and others. In certain malignant tissues, the nuclear DNA content is increased, a phenomenon which seems to be associated in some instances with polyplody and polyteny. On the other hand, certain substances, exerting an antitumor activity, affect the synthesis of DNA and decrease the nuclear DNA content, according to Skipper et al. (15) and others.

Robertson et al. (12) found that a vitamin C-free diet resulted in a marked retarding effect on the growth of fibrosarcoma in guinea pigs. Sokoloff et al. (16) confirmed this observation on liposarcoma of guinea pigs. By keeping rats and mice on a scorbutogenic diet containing an analog of ascorbic acid and thus reducing the ascorbic acid concentrations in the tissue of animals, they observed a retardation of the growth of Crocker rat carcinoma and of mouse adenocarcinoma E 1771.

Goldstein et al. (7), on the basis of their experiments in vitro, reported that the addition of ascorbic acid or PNA to the media did not influence the DNA content of rat liver, while an addition of either ascorbic acid or PNA increased considerably the DNA content of rat sarcoma. These workers concluded that ascorbic acid is involved in the process of conversion of PNA to DNA.

In our present investigation, an attempt was made to evaluate possible alterations in the DNA and PNA contents of tumor tissue under various conditions where the ascorbic acid concentrations of tissue were decreased nearly to zero or were elevated above the normal levels.

The cytological evaluation with the Feulgen reaction revealed that low ascorbic acid concentrations in the tumor tissue resulted in a decrease in its nuclear DNA content. This decrease was more pronounced in fibrosarcoma of guinea pigs than in Crocker rat carcinoma. It is assumed that the synthesis of ascorbic acid was only partially suppressed in rats by its analog, and the presence of a sufficient amount of ascorbic acid in the organism prevented any more pronounced alteration in the nuclear DNA.

**SUMMARY**

A vitamin C-free diet resulted in a decrease in the total and nuclear DNA contents in the fibrosarcoma AL-2 of guinea pigs. Large amounts of ascorbic acid given to guinea pigs kept on a diet containing a daily requirement of vitamin C had no significant effect on the nuclear DNA content of the tumor.

Low ascorbic acid concentrations in the tissue of rats, induced by an ascorbic acid analog, decreased the total and nuclear DNA contents of Crocker rat carcinoma.

The alteration in the nuclear DNA content was more pronounced in the guinea pig fibrosarcoma than in the Crocker rat carcinoma.

**REFERENCES**

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