Quantitative Biochemical Differences between Tumor and Host as a Basis for Cancer Chemotherapy

III. Thiamine and Coenzyme A*

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A possible basis for the chemotherapy of neoplastic disease rests in the quantitative differences in the nutrient and coenzyme concentrations between the tumor and the host's normal tissues. Various observers have noted that, in general, vitamins tend to be in lower concentration in the malignancy (4, 7, 12, 14—16, 18, 19). Systematic measurement of various vitamins and vitamin-containing coenzymes has been part of a program in which the data obtained are utilized in conjunction with enzyme studies for the development of combinations of antimetabolites and hormones in treating mouse adenocarcinoma 755 in C57 mice (2, 8, 12—16). Detailed information on the concentrations of vitamins and coenzymes in various tissues of the individual mouse and its tumor are necessary to ascertain the uniformity and consistency of host-tumor differences. Data have been presented thus far on vitamin B6 (15) and on riboflavin and its phosphorylated coenzyme forms (16). This report gives the findings with respect to thiamine and coenzyme A in the same mouse strain and tumor.

MATERIALS AND METHODS

General.—C57BL mice, 2—4 months old, weighing between 18 and 22 gm., were maintained in plastic cages on an ad libitum diet of Rockland pellets and water. Animals were sacrificed by exsanguination (following light etherization) 21—23 days after the tumor was transplanted into the inguinal region by trocar. The trachea was first exposed, clamped, and the large vessels were cut above this level to allow exsanguination without aspiration of blood into the lungs.

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Coenzyme A (Co A) analysis.—Mice used for Co A analysis were immediately cooled on dry ice for 8 minutes following exsanguination. Organs and tissues were rapidly removed, put into small vials which were sealed and kept on dry ice, and weighed while still frozen within 2 hours of sacrifice. Stomach and intestine were washed with cold isotonic saline and blotted before freezing. Homogenization was carried out rapidly in ice water in a Potter-Elvehjem glass apparatus, and the tubes were placed in boiling water for 4 minutes, centrifuged, and stored overnight at —15° C. until assayed.

It was demonstrated by experiment that the procedure involving the cooling of the tissues on dry ice for 2 hours before homogenization and boiling did not result in any significant change in Co A content as compared with tissues that were frozen for only a few minutes and then homogenized and boiled.

The assay procedure was essentially that of Kaplan and Lipmann (8). However, certain modifications were made with respect to reagents, volumes, and procedure. The pigeon liver acetone powder was resuspended and aged with the use of 0.02 m potassium bicarbonate. The 'reaction mixture' consisted of 10 ml. 0.006 m sulfanilamide, 10 ml. 1.0 m sodium citrate, 2.5 ml. 1.0 m sodium acetate, and 8 ml. of 0.8 per cent magnesium chloride. For an analysis, 0.3 ml. aliquots of the thawed tissue supernates, which had been previously diluted so as to contain approximately 0.9—1.0 units of Co A/0.30 ml, were placed in incubation tubes which were maintained in an ice bath until incubation. Aqueous dilutions of Co A (Pabst) were used as a standard. A "basal mixture" was prepared, and 0.60 ml. of this was added by syringe pipette to the tubes containing the Co A standard or tissue extracts; this 0.60 ml. contained 0.12 ml. enzyme, 0.16 ml. "reaction mixture," 0.82 ml. H2O solution, with 2.56 mg. of the potassium salt of ATP, 3.36 mg. potassium bicarbonate, and 1.28 mg. cysteine • HCl. The tubes were corked, mixed, and incubated for 2 hours at 37° C. The remainder of the procedure followed that of Kaplan and Lipmann (8), except that the Co A content of the tissues was read from a standard curve constructed from the colorimetric readings obtained with standard Co A. It was found necessary in the case of lung, muscle, intestine, stomach, testis, and heart to pool the organs of two to six animals in order to perform an analysis. The tumors and other organs were analyzed on an individual basis.

Thiamine analysis.—Stomach, heart, and intestine were cut open and rinsed with cold isotonic saline and blotted. After organs were weighed, they were homogenized in a Potter-
Elvehjem glass apparatus with water slightly acidified with HCl as the homogenizing medium. After the homogenate was transferred to a 50-ml volumetric flask, it was brought to a volume of approximately 35 ml with sufficient HCl to make it 0.1 N; it was then placed in a boiling water bath for 1 hour. After being cooled, 2.5 ml of a freshly prepared and filtered solution of 4 per cent diastase in 2.5 ml sodium acetate was added. The hydrolysates were brought to volume, centrifuged, and filtered, and the supernates were kept at 5° C until ready for adsorption. Adsorption and elution procedures were essentially those of Hennessey (5) and Hennessey and Cerecedo (6). Oxidation of the vitamin to thiochrome was carried out by the method of Benes et al. (1). Tissue aliquots of 3.0 ml each were oxidized and compared with an external standard of 0.06 μg. thiamine in 3.0 ml. Only in the case of lung was the concentration of Co A concentration on a dry weight basis not appreciably changed, except that the markedly higher moisture content of testis increased its dry weight content of the coenzyme to above that of brain, intestines, and stomach. A close approximation to the pantothenic acid concentration in these tissues may be made by multiplying the Co A value by a factor of 0.29, which is the proportion of pantothenic acid in pure Co A (10).

**TABLE 1**

COENZYME A CONCENTRATION IN MAMMARY ADENOCARCINOMA 755 AND IN TISSUES OF MALE C57BL MICE*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Wet weight basis (μg/gm, Mean ± S.E.)</th>
<th>Dry weight basis (μg/gm, Mean ± S.E.)</th>
<th>No. pools analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>92.9 ± 0.85</td>
<td>192.5 ± 3.58</td>
<td>31</td>
</tr>
<tr>
<td>Muscle</td>
<td>10.1 ± 0.87</td>
<td>92.0 ± 4.21</td>
<td>12</td>
</tr>
<tr>
<td>Lung</td>
<td>34.9 ± 1.28</td>
<td>167.0 ± 6.12</td>
<td>9</td>
</tr>
<tr>
<td>Testis</td>
<td>63.4 ± 1.06</td>
<td>413.6 ± 12.85</td>
<td>13</td>
</tr>
<tr>
<td>Brain</td>
<td>63.9 ± 1.69</td>
<td>502.7 ± 8.06</td>
<td>22</td>
</tr>
<tr>
<td>Small</td>
<td>69.9 ± 2.81</td>
<td>340.0 ± 13.67</td>
<td>7</td>
</tr>
<tr>
<td>Intestine</td>
<td>6.7 ± 0.84</td>
<td>533.5 ± 20.4</td>
<td>11</td>
</tr>
<tr>
<td>Stomach</td>
<td>72.1 ± 3.41</td>
<td>854.5 ± 18.2</td>
<td>8</td>
</tr>
<tr>
<td>Heart</td>
<td>167.4 ± 7.04</td>
<td>793.3 ± 30.49</td>
<td>11</td>
</tr>
<tr>
<td>Kidney</td>
<td>288.4 ± 6.03</td>
<td>978.9 ± 34.78</td>
<td>24</td>
</tr>
<tr>
<td>Liver</td>
<td>280.2 ± 7.19</td>
<td>1008.4 ± 25.85</td>
<td>17</td>
</tr>
</tbody>
</table>

* Mice sacrificed 40-45 days after transplanting of tumor.
† The activity of the standard Co A preparation obtained from the Pabst Laboratories is expressed in terms of Lipmann units. We have converted these unita to micrograms on the basis of the factor:

\[ \text{μg/unit} = \frac{\text{Lipmann units per gram of standard}}{2.46} \]

1 Pooled tissues of two to six animals, depending on tissue.

**RESULTS**

Coenzyme A.—Comparison of the Co A concentration in the 755 tumor and nine normal tissues or organs is made in Table 1 on both a wet and dry weight basis. These average data reveal that, on a wet basis, skeletal muscle is lowest in Co A, with the tumor and lungs each containing approximately twice as much. Testis, brain, small intestines, and stomach fall into a group having about double the amount of Co A found in tumor; heart, liver, and kidney had much greater amounts. Calculation of Co A concentration on a dry weight basis does not appreciably change the order, except that the markedly higher moisture content of testis increased its dry weight content of the coenzyme to above that of brain, intestines, and stomach. A close approximation to the pantothenic acid concentration in these tissues may be made by multiplying the Co A value by a factor of 0.29, which is the proportion of pantothenic acid in pure Co A (10).

**Thiamine.**—The sensitivity of the photofluorometer employed allowed measurement of this vitamin in the tumor and normal tissues of individual animals. The values obtained with male mice are presented in Table 2. Only skeletal muscle had a thiamine concentration lower than that of the tumor, and this difference was slight on a wet weight basis. The other tissues rose in ascending order, as given in the table. The comparative order of concentrations of the vitamin in the different tissues has been found to be very uniform from mouse to mouse.

Determinations of this vitamin in the tumors of ten female mice yielded a mean value of 2.08 ± 0.05 μg/gm wet weight, which is not significantly different from that of the males.

**DISCUSSION**

Comparative data on the pantothenic acid and thiamine concentrations of some normal mouse tissues and of various experimental tumors were first presented by the Texas workers (12, 18, 19). Their analyses indicated that these vitamins were present in the tumors in comparatively low concentra-
tions. It has since been demonstrated (9, 11) that the method used by these investigators was inadequate for completely liberating the bound pantothentic acid; consequently, their absolute and relative values are questionable. With the discovery of Co A and the development of a method for its measurement in tissues, it has been found that almost all the tissue pantothentic acid is in the coenzyme form (11). It appeared desirable, therefore, to measure directly the biologically active form of this vitamin.

Higgins et al. (7) have reported that Co A is present in appreciably lower amounts in induced rat liver tumor than in the normal liver; however, no detailed analysis for this factor in tumor and various host tissues has appeared heretofore.

The concentrations of Co A in normal mouse tissues are in rather good agreement with those published by Kaplan and Lipmann (8) for rat tissues, with the exception that the mouse heart appears to have almost double the Co A found in rat heart. Muscle Co A in the mouse is about the same as that in the rabbit (8).

The thiamine content of the 755 tumor is very similar to that reported by Pollack et al. (12) for three samples of mammary adenocarcinoma in C3H female mice; another three mammary adenocarcinomas derived from a spontaneous tumor arising in DBA mice (12) had approximately one-half of the thiamine found in our 755 tumor.

It is of interest to compare the data obtained for the four factors which have thus far been studied in detail in this laboratory. In Chart 1, the concentrations of each of these nutrients or coenzymes have been plotted for each tissue in terms of percentage of the factor present in liver. In each instance the tumor content fell at the low end of the scale. Muscle was lower, however, with respect to thiamine and Co A, equal to it in riboflavin, and much higher than tumor in vitamin B6. Lung had concentrations of Co A and vitamin B6 equal to tumor and was significantly higher in the two other nutrients. All other tissues had higher concentrations of all four factors.

<table>
<thead>
<tr>
<th></th>
<th>Thiamine</th>
<th>Co A</th>
<th>Riboflavin</th>
<th>Pyridoxine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>14.9</td>
<td>11.5</td>
<td>9.8</td>
<td>13.1</td>
</tr>
<tr>
<td>Muscle</td>
<td>12.7</td>
<td>6.8</td>
<td>10.2</td>
<td>51.1</td>
</tr>
<tr>
<td>Lung</td>
<td>30.6</td>
<td>12.4</td>
<td>16.2</td>
<td>14.8</td>
</tr>
<tr>
<td>Testis</td>
<td>47.0</td>
<td>22.6</td>
<td>14.3</td>
<td>20.1</td>
</tr>
<tr>
<td>Brain</td>
<td>30.6</td>
<td>22.8</td>
<td>13.1</td>
<td>53.8</td>
</tr>
<tr>
<td>Intestine</td>
<td>32.8</td>
<td>24.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>49.3</td>
<td>25.7</td>
<td>26.4</td>
<td>28.4</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td>102.2</td>
<td>59.6</td>
<td>92.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>85.8</td>
<td>84.9</td>
<td>119</td>
<td>78.8</td>
</tr>
<tr>
<td>Liver</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**Chart 1.**—Comparative concentrations of four different nutritional factors in the 755 tumor and in normal tissues of the C57BL male mouse. The original analytical data (expressed on a wet weight basis) have been expressed as percent of the concentration of the nutrient in a given tissue as compared with the liver level (taken as 100 per cent). The figure at each bar is the percentage figure.
These data afford a basis for the possibility that the consistently low concentrations of nutrients and their cofactors in tumors may make the neoplasms more vulnerable to antagonists of these and other essential factors than tissues with higher contents. The fact that certain tissues, such as muscle and lung, may have values as low or lower in only certain of the factors supports the possibility that the use of combinations of antimitabolites will tend to interfere with the tumor more than with the latter group of normal tissues. In support of this hypothesis there has been presented evidence (13–15) that combination chemotherapy has decreased the growth of the tumor without obvious deleterious effect on the host.

SUMMARY

Coenzyme A and thiamine concentrations have been determined in the 755 mammary adenocarcinoma and in nine normal tissues of the C57 mouse. Co A was present in smaller amounts in skeletal muscle; tumor and lung each contained approximately twice as much, and the other seven normal tissues had appreciably more. Skeletal muscle had the lowest amount of thiamine, slightly less than the level in the tumor; all other normal tissue had significantly greater amounts. In comparing the distribution of these two factors with those of vitamin B5 and riboflavin, it is apparent that tumor falls consistently at the lower end of the scale in each instance, unlike the normal tissues. This fact furnishes support for the hypothesis that combinations of antimitabolites may injure neoplasms without seriously affecting normal tissues.

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