Altered Amino Acid Kinetics in Rats with Progressive Tumor Growth

Isao Kawamura, Lyle L. Moldawer, Ronald A. Keenan, Gerald Batist, Albert Bothe, Jr., Bruce R. Bistrian, and George L. Blackburn

Nutrition/Metabolism Laboratory, Cancer Research Institute, New England Deaconess Hospital/Harvard Medical School, Boston, Massachusetts 02215

ABSTRACT

The present study was designed to determine whether alterations in host metabolism associated with progressive tumor growth were a result of the anorexia frequently observed with cancer or could be attributed to other direct tumor effects. Rates of tyrosine flux, oxidation, and incorporation into protein, as well as fractional protein-synthetic rates in nonsecretory liver, muscle, and tumor, were determined in overnight-fasted rats, 5 to 6 (Stage I), 10 to 11 (Stage II), and 15 to 16 (Stage III) days following s.c. implantation of RNC-254 fibrosarcoma. Tumor-bearing rats were allowed to consume a purified diet containing 20% protein ad libitum, and results were compared to non-tumor-bearing rats pair fed quantities of food equivalent to tumor-bearing animals or allowed to consume the diet ad libitum.

Results demonstrate that during later stages of tumor growth (Stage III) calorie intake and nontumor body weight gain were reduced in tumor-bearing rats (p < 0.05). Fifteen and 16 days following implantation, there were significant changes in amino acid kinetics that were not observed after earlier periods of tumor growth and that could not be explained by any reduction in dietary intake. Rates of tyrosine appearance in the plasma and subsequent incorporation into whole-body protein were increased 33 and 34%, respectively (p < 0.05), when compared to non-tumor-bearing rats fed equivalent quantities of food. Whole-body tyrosine oxidation rates were unchanged. Skeletal protein synthesis, as reflected by gastrocnemius or rectus abdominus muscle, was reduced from 10.5 and 10.1%/day to 7.4 and 6.0%/day, respectively (p < 0.05), in tumor-bearing compared to pair-fed animals. The findings suggest that significant alterations in protein metabolism occur in advanced stages of experimental neoplastic disease which cannot be explained by reductions in dietary intake and are aimed at providing adequate quantities of endogenous amino acids for net tumor growth.

INTRODUCTION

Loss of body fat and lean tissue occurs with the progression of most neoplastic disease. However, there is no consensus as to whether cachexia is attributable only to the anorexia associated with cancer or whether an as yet unidentified tumor factor also increases energy and/or protein expenditure. Although anorexia resulting from disorders of swallowing, digestion, and absorption explain much of the weight loss associated with certain forms of cancer, particularly gastrointestinal tumors, in some tumor types these mechanisms cannot be invoked and anorexia appears to be a systemic tumor effect. Indeed, studies with forced enteral and parenteral feeding suggest the possibility that a metabolic abnormality in the host may be responsible for some of the weight loss of cancer (4, 7, 8, 13, 14, 22, 25).

It has been demonstrated that the presence of a tumor can significantly alter nontumor protein metabolism (27). There is insufficient evidence to establish whether these changes are a direct tumor effect or represent an adaptive mechanism by the host. In 1958, Wiseman and Ghadially (29) reviewed G. B. Mider's "nitrogen trap" theory to account for the ability of the tumor to preferentially concentrate amino acids for protein synthesis and growth while normal host tissues suffered as a consequence of insufficient amino acids to maintain protein synthesis. Although the concept has been revised following demonstration that tumor protein does recycle through the host (3), the recent ability to directly measure rates of protein synthesis and breakdown (28) in vivo affords the opportunity to retest this hypothesis. Furthermore, previous data are limited since they represent metabolic measurements made at single points in time without regard for either the stage of tumor development or the hormonal-nutritional status of the host.

The present study was designed to make sequential assessments of amino acid kinetics in tumor-bearing rats controlling for dietary intake. Rates of whole-body tyrosine flux, oxidation, and incorporation into protein, as well as fractional synthetic rates in individual tissues, were determined at 3 intervals of tumor growth. Controls were non-tumor-bearing animals either pair fed equivalent quantities of diet or allowed to consume the food ad libitum. The results suggest that development of cachexia cannot be attributed solely to any self-imposed reduction in dietary intake and subsequent decrease in whole-body protein synthesis. Rather, the development of cachexia during the later stages of tumor growth is associated with increased whole-body protein breakdown and synthesis while skeletal muscle protein is mobilized for increased net tumor synthesis.

MATERIALS AND METHODS

Experimental. Female NEDH/c rats with a final body weight of 100 to 150 g were used. The animals were obtained from the Cancer Research Institute Animal Farm, New England Deaconess Hospital, and were housed singly in stainless steel suspension cages in a light- and temperature-controlled room. Prior to investigation, the rats consumed a standard laboratory chow (Charles River D-3000; Agway Agricultural Products, Minneapolis, Minn.) ad libitum for 1 week in order to monitor weight gain to assure the health of the animals. On Day 0 of the study, under light diethyl ether anesthesia, a sterile trocar was used to implant approximately 1 cu mm of viable tumor s.c. in the infrascapular region. Non-tumor-bearing control rats had an identical sham procedure without tumor implantation. The tumor was RNC-254 fibrosarcoma originally induced in NEDH/c rats by irradiation. Previous...
work has demonstrated that this tumor is uniformly lethal within 21 to 25 days following implantation and does not either metastasize or become uniformly necrotic (15).

Tumor-bearing rats were allowed to consume ad libitum a semi-purified diet containing 20% casein (a detailed description of the diet is contained in Table 1). The powdered diet was mixed with an equal quantity of distilled water in which granulated agar had been dissolved into a final solution of 2% (w/v). The diets were made fresh every 48 to 72 hr and were stored at 4°C. Dietary intake in these animals was recorded daily by measuring the change in mass of the food dish after collecting scattered food and accounting for evaporative losses.

Non-tumor-bearing rats were divided into 2 groups, one pair fed the quantity of food consumed by tumor-bearing animals the previous day and the other allowed to consume the diet ad libitum.

Five or 6 days following tumor implantation (Stage I), one-third of the tumor-bearing (n = 9) and non-tumor-bearing pair-fed rats (n = 7) was investigated. Their food was withdrawn at 6:00 p.m., and the animals were fasted overnight. The following morning between 9:00 and 10:00 a.m., the rats were gently wrapped in cloth to restrain them, and a 26-g needle connected to medical-grade Silastic tubing (Dow-Corning Laboratories, Corning, N. Y.) was inserted into the lateral tail vein. A tracer quantity of L-[U-14C]tyrosine (New England Nuclear, Boston, Mass.) was added to a 0.9% sodium chloride solution and administered continuously (1.5 μCi/hr) for the next 6 hr at a rate between 2.2 and 2.4 ml/hr.

During this period, the rats were housed in plexiglass chambers which permitted the collection and analysis of expired breath. At the end of the 6-hr infusion, the rats were immediately sacrificed by decapitation. Blood was collected from the neck into chilled heparinized tubes. The liver and tumor were excised rapidly and weighed, and sections were homogenized immediately in vials containing ice-cold 20% trichloroacetic acid. Sections of gastrocnemius and rectus abdominus muscle were also homogenized in ice-cold 20% trichloroacetic acid. An additional section of tumor and liver were frozen in a 0.9% sodium chloride solution for subsequent analysis of protein content.

Ten and 11 days following implantation (Stage II), another third of the tumor-bearing (n = 9) and non-tumor-bearing pair-fed rats (n = 6) as well as healthy ad libitum-fed controls (n = 8) was studied in the same fashion as described above. After 15 to 16 days following implantation (Stage III), the remaining third of tumor-bearing (n = 8) and non-tumor-bearing pair-fed rats (n = 7) was investigated.

**Analytical Methods.** During the constant i.v. infusion of L-[U-14C]tyrosine, total carbon dioxide production and the appearance of 14CO2 were determined at hourly intervals using a modification of the method of Wolfe and Burke (30). Briefly, rats were housed in chambers open at the top to room air. Expired breath and room air were drawn from the bottom of the chamber with a circulating air pump at a rate of 1.6 liters/min (Masterflex; Cole-Palmer Instrument Co., Chicago, Ill.). The expired breath and room air mixture was first passed through drying tubes containing calcium chloride and then through weighing tubes containing barium hydroxide (Ascariate; Arthur H. Thomas Co., Philadelphia, Pa.) in 15-min collections for measurement of total carbon dioxide production. Immediately following, the dried room air:expired breath mixture was bubbled into a scintillation vial containing absolute ethanol, hyamine hydroxide (Hydroxide of Hyamine 10X; Packard Instrument Co., Inc., Downers Grove, Ill.), and 0.1% phenolphthalein to assure complete saturation of the solution. A commercial scintillant was added immediately to the scintillation vial (Betafluor; National Diagnostics, Inc., Somerville, N. J.), and the vial was set aside for analysis of total radioactivity. The efficiency of the collection system was determined by infusing a known quantity of carbon dioxide into the chamber and was 99 ± 2%. Room air served as a blank.

Plasma was treated with an equal quantity of 10% trichloroacetic acid, and the protein-free supernatant was separated by centrifugation. Free tyrosine specific radioactivity was determined using the methodology of Garlick and Marshall (11). L-Tyrosine was converted enzymatically to L-tyramine, extracted from other radioactive metabolites of tyrosine oxidation, and quantitated for both 14C radioactivity and concentration.

Protein precipitates of tumor, liver, rectus, and gastrocnemius were washed 3 times with 5% trichloroacetic acid, the original supernatant and first wash were saved for analysis of acid-soluble (intracellular) free tyrosine specific radioactivity in a similar manner as plasma. Prior to enzymatic conversion of L-tyrosine to L-tyramine, excess trichloroacetic acid was extracted from the protein-free supernatants of individual tissues with 3 diethyl ether extractions.

The protein precipitates were hydrolyzed with 6 n hydrochloric acid (1:150) at 120°C for 4 to 6 hr. The hydrolysis was neutralized with sodium hydroxide and assayed for free L-tyrosine specific radioactivity in a manner similar to plasma and acid-soluble free L-tyrosine fractions.

Total 14C radioactivity was determined using standard liquid scintillation techniques and was measured with a Beckman LS-8000 spectrometer (Beckman Instruments, Inc., Sunnyvale, Calif.). Aqueous samples of infusate, plasma, intracellular fluid, and acid-hydrolyzed protein were measured with a commercial scintillator (Instagel; Packard Instrument Co.). Efficiency of counting (between 65 and 70%) was determined using automated external standards, and all samples were counted to a 2-s error of less than 2.5%.

Total protein content (nitrogen × 6.25) of the liver and tumor were determined following a micro-Kjeldahl digestion, as described previously (21).

**Calculations.** The appearance and disappearance of tyrosine in the plasma compartment (flux) was estimated from the plasma specific radioactivity of free L-tyrosine obtained at the end of the 6-hr infusion (12). Plateau labeling of the plasma compartment was assumed once isotopic steady state in the expired breath was achieved. Rates of tyrosine appearance and disappearance in the plasma compartment were obtained from the equation:

$$Q = I/Sp_{max}$$

where Q was the rate of tyrosine flux through the plasma compartment, in μmol/100 g body weight/hr; I was the isotope infusion rate, in dpm/100 g body weight-hr, and Sp max was the specific radioactivity of plasma free L-tyrosine at plateau, in dpm/μmol.

Disappearance of tyrosine from the plasma compartment was used as an estimate for the quantity leaving the plasma pool for either synthesis into protein or oxidation. The rate at which tyrosine was oxidized to carbon dioxide was estimated directly from the appearance of 14C radioactivity in the expired breath (16):

$$O = E/Sp_{max}$$

where O was the oxidation rate, in μmol/100 g body weight-hr, and E was the appearance of 14C radioactivity in the breath, in dpm/100 g body weight-hr. The percentage of 14C-carbon dioxide that did not appear in the expired breath was estimated to be 8% (30). Rates of whole-body tyrosine incorporation into protein were derived from the difference between tyrosine flux and oxidation.

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### Table 1

<table>
<thead>
<tr>
<th>Composition of experimental dieta</th>
<th>g/kg diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
</tr>
<tr>
<td>dL-Methionine</td>
<td>3</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>150</td>
</tr>
<tr>
<td>Sucrose</td>
<td>500</td>
</tr>
<tr>
<td>Fiber</td>
<td>50</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
</tr>
<tr>
<td>AIN-76 mineral mix</td>
<td>35</td>
</tr>
<tr>
<td>AIN-76 vitamin mix</td>
<td>10</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2</td>
</tr>
</tbody>
</table>

a A detailed description of the diet is contained in a report prepared by the Ad Hoc Committee on Standards for Nutritional Studies, American Institute of Nutrition, and is presented in Ref. 1.
The fractional synthetic rate in individual tissues was obtained using the equation of Garlick et al. (12):

\[ \frac{S_b}{S} = \frac{\lambda}{(\lambda - k_s)} \left( 1 - e^{-k_s}\right) - \frac{k_s}{(\lambda - k_s)} \]

Where \( S_b \) and \( S \) were the specific radioactivities of tyrosine in bound protein and the free intracellular pool, respectively, in dpm/\mu g; \( \lambda \) was the rate constant for the rise in specific radioactivity of the true precursor, in days\(^{-1}\); and \( k_s \) was the fraction of protein mass renewed each day, in \%/day. For rapidly turning over tissues, such as liver and tumor, \( \lambda \) was replaced with \( \lambda_{\text{tr}} \), the rate constant for the rise in specific radioactivity of free tyrosine in the plasma compartment (12). For more slowly turning over tissues such as rectus and gastrocnemius muscle, \( \lambda \) was replaced with the ratio of tyrosine in bound protein and in the intracellular pool \((R)\) multiplied by the fractional synthetic rate.

Total protein-synthetic rates in liver and tumor were subsequently obtained by multiplying the fractional synthetic rate by the total protein content. Whole-body tyrosine kinetics was converted from \( \mu \text{mols}/100 \text{ g body weight-hr to g of protein/day} \) by assuming that, under the conditions of this study, tyrosine kinetics adequately reflected amino acids in general and that the rat is composed of 28 mg tyrosine/g protein (6).

Statistical Analysis. Data obtained from tumor-bearing animals were compared to pair-fed controls at the same stage using Student’s t test (2 tailed). Temporal changes in protein kinetics between Stages I, II, and III in tumor-bearing or non-tumor-bearing pair-fed animals was determined by one-way analysis of variance. Within-group comparisons were made using least significant differences. Significance, in all cases, was designated at the 95% confidence level.

RESULTS

There were no significant differences in initial body weight or total body weight gain over the 16-day period of the groups of animals (Table 2). However, as the study progressed, tumor weight increased at a greater rate than body weight. By Stage III (15 to 16 days following implantation), tumor weight averaged 15.2 ± 1.4 g (S.E.) and was 10.6 ± 1.2% of total weight. If the gain in body weight attributed to tumor growth was deducted from total weight gain, then significant \( p < 0.05 \) reductions in non-tumor body weight gain were observed in tumor-bearing rats at Stage III when compared to non-tumor-bearing rats pair fed equivalent quantities of food.

Dietary intake in tumor-bearing rats did not differ until the 11th to 15th days of the study when the intake was significantly less than either tumor-bearing animals during the first 2 stages of tumor growth or to \textit{ad libitum}-fed non-tumor-bearing animals \((p < 0.05)\). By the 11th to 15th days following implantation, dietary intake had fallen 18% when compared to the first 5 days of the study.

In contrast to nontumor body weight gain, liver weight was significantly greater in tumor-bearing rats at Stage III than in non-tumor-bearing pair-fed rats or rats fed the diet \textit{ad libitum} \((p < 0.05)\). In addition, liver weights at Stage III were significantly greater than at Stage I or II \((p < 0.05)\).

Amino Acid Kinetics. Rates of whole-body tyrosine flux, oxidation, and incorporation into protein, as well as fractional synthetic rates of liver and gastrocnemius and rectus muscle from non-tumor-bearing pair-fed rats, were similar over the 15-day period despite a significant reduction in dietary intake (Table 3). In addition, amino acid kinetics from non-tumor-bearing rats fed the diet \textit{ad libitum} for 10 to 11 days did not differ from non-tumor-bearing rats restricted in their caloric intake to quantities equivalent to amounts consumed by tumor-bearing animals. At Stages I and II, protein kinetics from tumor-bearing animals also did not differ either from pair-fed controls or non-tumor-bearing rats fed \textit{ad libitum}.

The only significant changes in protein dynamics were observed at Stage III, 15 to 16 days following tumor implantation. When compared to non-tumor-bearing pair-fed rats or to tumor-bearing animals at earlier stages of tumor growth, rates of whole-body tyrosine flux and incorporation into protein were significantly increased \((p < 0.05)\). However, these changes occurred without increases in tyrosine oxidation rates.

In individual tissues, there were significant reductions in muscle protein synthesis (Table 4). By Stage III, skeletal protein synthesis, as reflected by both gastrocnemius and rectus muscle, was 37% less than from non-tumor-bearing pair-fed animals \((p < 0.05)\). The contribution of individual tissue synthetic rates to whole-body protein synthesis is summarized in Chart 1. Despite a significantly larger liver, the contribution of hepatic structural protein synthesis to whole-body protein synthesis remained rather constant, even with a significant reduction in

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Table 2

<table>
<thead>
<tr>
<th>Rats</th>
<th>Final body wt (g)</th>
<th>Wt gain (g/day)</th>
<th>Tumor wt (g)</th>
<th>Non-tumor body wt gain (g)</th>
<th>Food intake (dry wt g/day)</th>
<th>Liver wt (g)</th>
<th>Liver wt (% of body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy \textit{ad libitum} fed</td>
<td>147.2 ± 11.3(^a)</td>
<td>1.45 ± 0.22</td>
<td>1.45 ± 0.22</td>
<td>14.25 ± 0.76</td>
<td>4.98 ± 0.30</td>
<td>3.42 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Tumor bearing</td>
<td>131.5 ± 5.5</td>
<td>1.12 ± 0.40</td>
<td>0.02 ± 0.004 (0.01%)(^b)</td>
<td>1.11 ± 0.40</td>
<td>14.44 ± 0.61</td>
<td>4.63 ± 0.15</td>
<td>3.53 ± 0.04</td>
</tr>
<tr>
<td>Non-tumor bearing pair fed</td>
<td>130.4 ± 5.4</td>
<td>1.06 ± 0.43</td>
<td>1.06 ± 0.43</td>
<td>14.73 ± 0.67</td>
<td>4.55 ± 0.25</td>
<td>3.49 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor bearing</td>
<td>124.7 ± 7.4</td>
<td>1.68 ± 0.36</td>
<td>1.00 ± 0.42 (0.5%)</td>
<td>1.30 ± 0.30</td>
<td>13.02 ± 0.97</td>
<td>4.37 ± 0.23</td>
<td>3.52 ± 0.10</td>
</tr>
<tr>
<td>Non-tumor bearing pair fed</td>
<td>130.5 ± 9.2</td>
<td>1.59 ± 0.31</td>
<td>1.59 ± 0.31</td>
<td>12.64 ± 1.00</td>
<td>4.20 ± 0.22</td>
<td>3.25 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor bearing</td>
<td>145.8 ± 10.7</td>
<td>2.02 ± 0.28</td>
<td>15.2 ± 1.4 (10.6%)</td>
<td>1.02 ± 0.24</td>
<td>12.39 ± 0.79(^c)</td>
<td>5.61 ± 0.50(^c)</td>
<td>3.81 ± 0.10</td>
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<tr>
<td>Non-tumor bearing pair fed</td>
<td>137.0 ± 8.9</td>
<td>1.73 ± 0.25</td>
<td></td>
<td>1.73 ± 0.25</td>
<td>12.19 ± 0.85(^c)</td>
<td>4.28 ± 0.29</td>
<td>3.17 ± 0.08</td>
</tr>
</tbody>
</table>

\(^a\) Mean ± S.E.
\(^b\) Numbers in parentheses, percentage of body weight.
\(^c\) p < 0.05, by analysis of variance and least significant difference (comparing similar rats, tumor bearing or non-tumor bearing pair fed, at different stages).
\(^d\) p < 0.05, by Student’s t test.
Whole-body tyrosine kinetics

<table>
<thead>
<tr>
<th>Rats</th>
<th>Tyrosine flux (μmol/100 g body wt-hr)</th>
<th>Tyrosine oxidation (μmol/100 g body wt-hr)</th>
<th>Tyrosine incorporation into protein (μmol/100 g body wt-hr)</th>
<th>% of tyrosine flux oxidized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy ad libitum fed</td>
<td>36.71 ± 3.90</td>
<td>6.40 ± 1.26</td>
<td>30.32 ± 3.85</td>
<td>18.95 ± 3.12</td>
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<tr>
<td>Stage I Tumor bearing</td>
<td>35.22 ± 3.47</td>
<td>6.42 ± 0.92</td>
<td>28.80 ± 2.88</td>
<td>19.75 ± 1.67</td>
</tr>
<tr>
<td>Non-tumor bearing pair fed</td>
<td>31.87 ± 4.75</td>
<td>6.19 ± 1.08</td>
<td>25.68 ± 3.88</td>
<td>19.24 ± 1.46</td>
</tr>
<tr>
<td>Stage II Tumor bearing</td>
<td>36.97 ± 2.39</td>
<td>6.04 ± 0.97</td>
<td>30.94 ± 1.70</td>
<td>15.57 ± 1.50</td>
</tr>
<tr>
<td>Non-tumor bearing pair fed</td>
<td>42.30 ± 5.48</td>
<td>5.86 ± 0.53</td>
<td>36.64 ± 5.12</td>
<td>14.26 ± 1.38</td>
</tr>
<tr>
<td>Stage III Tumor bearing</td>
<td>49.89 ± 1.77</td>
<td>7.89 ± 0.54</td>
<td>41.99 ± 1.80</td>
<td>15.95 ± 1.29</td>
</tr>
<tr>
<td>Non-tumor bearing pair fed</td>
<td>37.54 ± 2.07</td>
<td>6.26 ± 0.72</td>
<td>31.28 ± 0.72</td>
<td>17.48 ± 1.79</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
* c p < 0.05, by analysis of variance and least significant difference (comparing similar rats, tumor bearing or non-tumor bearing pair fed, at different stages).
* c p < 0.05, by Student's t test.

Table 4

<table>
<thead>
<tr>
<th>Rats</th>
<th>Liver fractional synthetic rate (%/day)</th>
<th>Rectus</th>
<th>Gastrocnemius</th>
<th>Rectus and gastrocnemius</th>
<th>Tumor fractional growth rate (%/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy ad libitum fed</td>
<td>126.0 ± 24.1</td>
<td>9.6 ± 3.8</td>
<td>8.9 ± 1.0</td>
<td>9.3 ± 1.8</td>
<td>ND</td>
</tr>
<tr>
<td>Stage I Tumor bearing</td>
<td>131.3 ± 10.5</td>
<td>9.7 ± 2.5</td>
<td>10.9 ± 1.8</td>
<td>10.4 ± 1.5</td>
<td>ND</td>
</tr>
<tr>
<td>Non-tumor bearing pair fed</td>
<td>126.3 ± 21.1</td>
<td>13.7 ± 2.6</td>
<td>9.5 ± 1.4</td>
<td>11.7 ± 1.7</td>
<td>ND</td>
</tr>
<tr>
<td>Stage II Tumor bearing</td>
<td>139.9 ± 15.6</td>
<td>13.6 ± 3.1</td>
<td>8.6 ± 1.8</td>
<td>11.0 ± 1.9</td>
<td>ND</td>
</tr>
<tr>
<td>Non-tumor bearing pair fed</td>
<td>135.7 ± 17.0</td>
<td>10.1 ± 1.6</td>
<td>10.5 ± 1.8</td>
<td>10.3 ± 1.2</td>
<td>ND</td>
</tr>
<tr>
<td>Stage III Tumor bearing</td>
<td>142.3 ± 15.7</td>
<td>6.0 ± 1.4</td>
<td>7.4 ± 1.5</td>
<td>6.5 ± 1.0</td>
<td>0.97 days⁻¹</td>
</tr>
<tr>
<td>Non-tumor bearing pair fed</td>
<td>120.3 ± 12.9</td>
<td>10.1 ± 2.8</td>
<td>10.5 ± 3.6</td>
<td>10.3 ± 2.4</td>
<td>(71.0%/day)</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
* ND, not determined due to insufficient tumor size or inaccuracy in weighing (see Table 1).
* c p < 0.05, by analysis of variance and least significant difference.
* d p < 0.05, by Student's t test.

Discussion

Significant differences exist between experimental neoplastic disease in rats and spontaneous human cancer. In human cancer, cachexia, as reflected by weight loss, is frequently observed. However, in the young rat, there was no decrease in total body weight despite an actively growing tumor which has resulted previously in death within 25 days following implantation. Indeed, other investigators have also reported a consistent weight gain in rats until a few days prior to death (19, 20). Nevertheless, the presence of advanced tumor growth did significantly alter the weight in nontumor body weight in these animals. By the 10th to 15th day following implantation of the fibrosarcoma, significant reductions in nontumor body weight gain were observed (Table 2) when comparing tumor-bearing to non-tumor-bearing animals. Though decreased intake or abnormalities of digestion and absorption often can explain this weight loss in humans (9), the tumor model used was chosen so as not to have a direct effect on gastrointestinal function. The present study demonstrated a tumor effect on body weight that could not be explained by a reduced dietary intake and suggests that the ultimate development of cachexia in this model could not be attributed to any tumor-induced anorexia.

With the pair-feeding technique, as recommended by the American Institute of Nutrition's Committee on Animal Nutrition Standards (1), we have been able to maintain without artificial feeding techniques such as i.v. hyperalimentation or continuous enteral administration dietary intake within 5% between tumor-bearing and their pair-fed non-tumor-bearing animals. Overall weight gain in both groups was comparable, suggesting that food utilization was similar but that the tumor was gaining mass at a proportionally greater rate than host tissue.

In an attempt to determine the changes in protein metabolism due to progressive tumor growth, a continuous i.v. infusion of L-[U-¹⁴C]tyrosine was administered to rats after an overnight fast. The use of isotopically labeled tyrosine for measuring rates of whole-body and individual tissue protein kinetics has been reported extensively (11, 12, 16, 28) and relies upon a...
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Chart 1. Contribution of individual tissues to whole-body protein kinetics in tumor-bearing and non-tumor-bearing rats. Whole-body and individual tissue protein synthesis was calculated as described in “Materials and Methods.” The contribution of muscle protein to whole-body protein synthesis was obtained by assuming that skeletal muscle represents 45% of nontumor body weight and was composed of 20% protein (31). Stage III tumor-bearing rats were compared to Stage III non-tumor-bearing pair-fed rats and non-tumor-bearing animals fed ad libitum for 10 to 11 days.

Whole-body and skeletal protein synthesis between tumor-bearing and non-tumor-bearing rats were significant (p < 0.05).

Stochastic model for whole-body protein metabolism and isotopic enrichment of protein-bound amino acid for fractional synthesis rates. Primarily, the measurement of tyrosine flux, oxidation, and incorporation into whole-body protein reflects the appearance and subsequent fate of tyrosine in the plasma compartment. As reported previously, the specific radioactivity in the intracellular (acid-soluble) pools at steady states during the constant i.v. infusion of isotopic tyrosine is always less than observed in plasma, presumably due to intracellular recycling of nonlabeled amino acids released from protein breakdown (12). Because the specific radioactivity of tyrosine at the sites of amino acid oxidation and protein synthesis is less than that in plasma (10), absolute rates of protein synthesis and amino acid oxidation must be subsequently underestimated. Therefore, results obtained from plasma enrichments and presented as whole-body tyrosine kinetics are in actuality measurements of whole-body tyrosine kinetics that occur solely in the plasma compartment. Nevertheless, it would be unlikely that changes in compartmentalization of free amino acids could explain the changes in whole-body tyrosine kinetics seen in Stage III tumor-bearing rats.

Although tyrosine is not an essential amino acid and its appearance in the plasma compartment from these postabsorptive animals can result from both protein breakdown and hydroxylation of phenylalanine, it is probable that the increase in tyrosine appearance seen in Stage III of tumor growth can be attributed to increased rates of whole-body protein breakdown. If the mechanism was primarily an increase in phenylalanine hydroxylation representing the first step in the ultimate oxidation of this amino acid, we would have expected to see a proportional increase in the percentage of tyrosine flux oxidized as well as absolute rates of oxidation. However, because neither was significantly greater, whereas tyrosine incorporation into protein was, it is unlikely that the increase in tyrosine appearance could largely represent an increase in the degradation of phenylalanine. It is more likely that, during advanced stages of tumor growth, whole-body tyrosine appearance in the plasma from protein breakdown was higher than in either pair-fed control animals or animals with earlier stages of tumor growth (Table 3).

If we assume that plasma tyrosine kinetics is representative of amino acids in general and the tyrosine composition of the rat is 28 mg/g protein (6), rates of tyrosine appearance and disappearance can be converted into g of protein synthesized and degraded. Although these values are merely estimates (26), they can reflect relative changes in whole-body protein dynamics. More importantly, they provide an opportunity to compare the relative contribution of individual tissues to whole-body kinetics, as presented in Chart 1.

Three basic conclusions can be obtained from this study. Initially, the presence of tumor in these animals per se did not result in abnormalities in protein metabolism. Alterations in protein metabolism due to the fibrosarcoma were only observed after the tumor had reached a considerable size (15.2 g or approximately 10.6% of total body weight). Furthermore, alterations in amino acid kinetics, like nontumor body weight gain, could not be attributed to any difference in dietary intake as amino acid kinetics in tumor-bearing rats were significantly different from that in healthy animals pair fed equivalent quantities of food.

Secondly, in the advanced stage of tumor growth, there was a significant increase in whole-body tyrosine appearance from protein breakdown and reincorporation into whole-body protein (protein synthesis) without any change in amino acid oxidation (Table 3). Unlike injury (5) and infection (17) where increased rates of protein turnover are associated with elevated amino acid oxidation rates, there was no significant increase in whole-body net protein catabolism in tumor-bearing rats.

In many forms of spontaneous and experimentally induced neoplasms, increased energy expenditure has been reported (12, 23, 24). Although oxygen consumption was not measured directly in this study, Young (32) has calculated the energy cost of protein synthesis and breakdown to be approximately 2.0 kcal/g. Given the calculated basal energy expenditure for a 150-g rat of 26 kcal/day (2) and a protein turnover rate estimated from this study at 7.2 g/day (Chart 1), approximately 55% of the basal energy expenditure can be explained by protein turnover. The increase in amino acid flux observed in advanced tumor growth (Table 3) would represent an increase in energy expenditure of 8 kcal/day or 27% of basal energy expenditure. The findings suggest that much of the increase in energy expenditure reported by other investigators from animals with active tumor growth may be explained by an increased amino acid flux.

Furthermore, it can be concluded that the increased whole-body amino acid release from protein breakdown can be attributed to enhanced protein degradation in nontumor tissues. Data contained in Table 4 demonstrate that the fractional growth rate of the tumor between Days 10 to 11 and 15 to 16 was similar in magnitude to the fractional synthetic rates at Stage II and Stage III. Although it is well established that fractional synthetic rates obtained with the constant i.v. infusion of labeled amino acid give only relative rates of protein synthesis (28), the similarity in fractional growth and synthesis imply...
that the tumor must have a substantially lower fractional rate of breakdown when compared to either synthesis or growth. As presented in Chart 1, the contribution of tumor to whole-body protein synthesis was only 13% in the advanced stages of tumor growth. If the fractional breakdown was substantially less, then the overall increases in whole-body amino acid release from protein breakdown must be attributed primarily to nontumor tissues.

Although whole-body resynthesis of protein was increased, the tumor represents a principal site of protein synthesis (13%) where an ever-increasing proportion of amino acids was incorporated into protein.

Finally, this increased uptake of amino acids by the tumor is in direct contrast to skeletal muscle where fractional synthetic rates were substantially reduced in the latter stage of tumor growth. In 2 different skeletal muscles, rectus and gastrocnemius, protein synthesis was 25 to 40% less in tumor-bearing rats than in either pair-fed or ad libitum-fed controls. Total hepatic nonsynthetic protein synthesis although slightly increased was not statistically different. These findings may explain the dramatic wasting of somatic tissue observed in some human cancers and confirms in vitro work by Lundholm (18) who also showed that synthetic rates in skeletal muscle were reduced in tumor-bearing patients.

Although it is difficult to extrapolate results from implantable tumors in rats to spontaneously occurring human cancer, it can be concluded that the changes in protein metabolism associated with advanced tumor growth in this model provide the tumor with adequate supplies of amino acids for growth even in the presence of a reduced dietary intake. By stimulation of whole-body protein breakdown and inhibition of skeletal protein synthesis, the tumor is guaranteed a supply of amino acids necessary for net anabolism. The resulting loss of host tissue represents a pathological redistribution of protein to the tumor which does in fact function like a nitrogen trap (29) leading to cachexia and, ultimately, death. The mechanism of this tumor effect on the host is unknown.

REFERENCES

Altered Amino Acid Kinetics in Rats with Progressive Tumor Growth


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