Tissue Nitrogen-sparing Effect of High Protein Diet in Mice with or without Ascites Tumor Treated with *Acinetobacter* Glutaminase-Asparaginase

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ABSTRACT

Forty-eight tumor-free mice and 32 mice bearing Ehrlich ascites tumors were randomized into 2 treatments, *Acinetobacter* glutaminase-asparaginase (AGA) (600 IU/kg/day for 7 days) and 0.9% NaCl controls, and into 2 or 3 isocaloric diets, normal protein (NP) (20 g protein/100 g diet), high protein (HP) (58 g protein/100 g diet), and zero protein (ZP) (tumor-free mice only). In tumor-free, NP-fed mice, AGA caused percentage reductions ($P < 0.01$) in the nitrogen content of liver (50%), intestine (42%), thymus (89%), spleen (75%), and carcass (20%), but HP prevented this effect on intestine and carcass and caused percentage increases in the nitrogen content of liver (53%), intestine (36%), thymus (122%), and carcass (25%). In Ehrlich ascites tumor mice (NP or HP fed) AGA caused markedly lower ($P < 0.01$) tumor burdens and increased nitrogen content of intestine (HP), kidney (NP and HP), and spleen (NP and HP). Ehrlich ascites tumor, AGA-treated, HP-fed mice ate 31% less food ($P < 0.01$) (compared to NP) but HP resulted in percentage increases in the nitrogen content of liver (18%; $P = 0.05$), intestine (25%; $P < 0.05$), and thymus (164%; $P < 0.01$). In the Ehrlich ascites tumor, AGA group the HP diet caused higher hemocrit and serum total protein (both, $P < 0.05$). Adverse nutritional effects of AGA seen in normal mice were markedly diminished in tumor-bearing animals. The observed nitrogen-sparing effects of the high protein: energy ratio may be relevant to humans and to other forms of neoplasia and chemotherapy.

INTRODUCTION

Malignant neoplasms can produce disease by interference with normal cell function via metabolic effects or by anatomic infiltration into normal tissues. Treatment of cancers by drugs or radiation interferes with the cellular biochemistry of cancer cells. However, almost inevitably such treatment results in at least some dysfunction of normal cells leading to host toxicity. These treatment-effectuated changes as well as the effects of cancer per se on host biochemistry lead to dysfunction of specific organs and to gross changes in body composition (1). One can consider the entire metabolic disturbance produced by cancer or its treatment as fundamentally a disruption of normal nutritional biochemistry (2). Hence it has seemed to us that one must approach the study of chemotherapy toxicity and the evaluation of how cancer produces disease by considering how nutrient requirements are altered by cancer and therapy. Because of our long-standing independent interests in the pharmacology of the anticancer drug, *Acinetobacter* glutaminase-asparaginase and in nitrogen metabolism, we studied the effects of AGA on nitrogen balance (humans) and nitrogen content of liver and carcass (mice) (3, 4). In mice with Ehrlich ascites tumors receiving relatively nontoxic (although effective) doses of AGA (300 IU/kg/day) and in children with acute leukemia, we could not discern a statistically significant effect of AGA on liver or carcass nitrogen content (3) or on net protein utilization (3, 4).

Net protein utilization refers to a classical nutritional method for evaluating the biological value of various dietary proteins. We have used this approach to determine how cancer or chemotherapy with an amino acid depleting enzyme (AGA) affects the biological value of a single protein source. We determined NPU by either evaluating the regression of nitrogen balance on nitrogen intake (humans) (4) or from the difference in carcass nitrogen content at two levels of nitrogen intake (mice) (3). While AGA has relatively broad anticancer activity, it has dose-limiting toxic effects including weight loss, hypoproteinemia, and neurotoxicity (5).

The present study was primarily designed to determine the effects of AGA on nitrogen utilization in various tissues of normal and tumor-bearing mice receiving a dose known to produce toxicity (600 IU/kg/day for 7 days). Because of such problems as anorexia, nausea, vomiting, diarrhea, and glucose intolerance, it is much more difficult practically to provide adequate energy to sick humans than to supplement the protein intake. With this clinical relevance in mind, our second goal was to investigate whether administering a diet with a higher P:E ratio would have nitrogen-sparing effects.

The results of the first experiment in normal mice demonstrated marked adverse effects of AGA on food intake, hematological and serum biochemical indices, and on the nitrogen content and utilization of mouse tissues, with thymus being most markedly affected. However, a high-protein diet had significant nitrogen-sparing effects and actually improved nitrogen utilization.

The second experiment was designed to test whether feeding a diet with a high P:E ratio is nutritionally beneficial in tumor-bearing animals treated with AGA and to determine whether such a diet alters the therapeutic effects of AGA. Our results show that the high-protein diet has the desired benefits without antagonizing the antitumor effects of AGA. Furthermore we have shown that the enzyme treatment has less effect on the nutritional status of tumor-bearing than on non-tumor-bearing mice.
NITROGEN-SPARING EFFECT OF DIET IN MICE TREATED WITH AGA

MATERIALS AND METHODS

Animal Care. Female CD1 mice weighing 15–20 g were studied at approximately 35 days of age (Charles River Mouse Farm, Cambridge, MA). The mice were housed in plastic filter-top cages with wire mesh on the floor to minimize coprophagy. There were 4 animals to a cage from Days 0 to 7 and two to a cage thereafter.

Experimental Design, Experiment 1. On Day 0, 56 mice were started on a normal protein diet (ICN Nutritional Biochemicals, Cleveland, OH). On the same day they were each given injections i.p. of 0.1 ml of a 0.1 dilution of pooled plasma from mice infected with lactate dehydrogenase-elevating virus in order to prolong the half-life of AGA (6). This treatment has no effect on liver or carcass nitrogen content (3); nevertheless a possible nutritional effect was controlled in the experiment because all animals were injected with the virus. On Day 7, eight mice were sacrificed to determine control values that were used to compute the rate of change in tissue nitrogen content (see below). On this day all the remaining 48 mice were randomized to 3 dietary protein groups: ZP, 0 g protein/100 g diet; NP, 20 g protein/100 g diet; and HP, 56 g protein/100 g diet. The diets were meant to be isocaloric with carbohydrate energy being substituted for protein energy as protein concentration diluted. Since the actual nitrogen concentration of HP was less than that thought to be present by the manufacturer the caloric density was slightly lower than that of the other two diets. On Day 8 animals in the ZP or HP groups were randomized (in equal groups) to receive either daily i.p. injections of AGA (600 U/kg) or 0.9% NaCl (saline), and these treatments were begun. On Day 9 the NP animals were randomized to either treatment. The animals and food intake were weighed daily. On Day 10, approximately 200 μl of blood were taken from 3 animals in each of the 6 groups for hematological analysis and for determination of plasma glucose and protein.

On Days 15 and 16, animals in the ZP-HP and NP groups, respectively, were sacrificed for nitrogen analysis. Under ether anesthesia blood was taken from the tail vein for serum glucose and total protein analysis and were sacrificed for nitrogen analysis. Under ether anesthesia blood was taken from the tail vein for serum glucose and total protein analysis and for determination of WBC, differential, and hematocrit. The animal was then killed by fracturing the cervical spine, and the following tissues were removed: blood; small intestine; kidney; thymus; and spleen. The entire small intestine was softened by autodigestion for 15 min and then homogenized with a Potter-Elvehjem tissue homogenizer, and the total nitrogen content was determined with a Beckman glucose analyzer. Blood counts were determined with a Coulter Counter.

Calculation and Statistics. Final tissue nitrogen contents were expressed per unit initial body weight (on Day 3) to avoid differential effects on body weight by diet and/or treatment. As described in "Results" (Table 1), AGA caused a significant decrease in body weight. By expressing the tissue nitrogen content per initial body weight (and not by final body weight) we controlled for spurious relative increases in tissue nitrogen content in AGA-treated mice that could be related only to a decrease in body weight associated with enzyme treatment (Table 2). However, the changes in weight during the study were not of sufficient magnitude to affect significantly the major conclusions of this study regardless of the reference weight used to express the data. This was true particularly with respect to the effects of the HP diet in AGA-treated mice since we observed no significant effects of this diet on body weight.

NPUs for each tissue and for the remaining carcass was calculated as follows (8)

\[ NPU = \left(\frac{\text{final tissue nitrogen content, mg nitrogen/g initial body wt, on NP or HP}}{\text{final average tissue nitrogen content, mg nitrogen/g initial body wt, of the zero protein saline group in the first experiment}}\right) + \text{nitrogen intake, mg nitrogen/8 days, on NP or HP} \]

Using specially developed computer programs and packaged programs, statistical analyses of the effects of treatment, diet, and tumor were accomplished using two-way analysis of variance and analysis of covariance (9–11). For each treatment group, AGA or saline, linear contrasts between dietary groups were analyzed using the SEs of the means from the two-way analysis of variance (11).

RESULTS

General Aspects

All animals survived the experiment except one mouse which died while blood was being taken midway through the study in the high protein, saline-treated group of the first experiment.

Effects of AGA on Food Intake and Body Weight Change (Table 1)

Experiment 1. Treatment with AGA resulted in a significant \( P < 0.01 \) decrease in food intake (and thus in energy intake) in each of the three diet groups (Tables 1 and 3). Within both the AGA-treated and saline-treated groups the mean total food intake was less in the HP group than in the NP group \( P < 0.01 \). Moreover, since the caloric density of HP was apparently lower than NP, the energy intake was even more decreased in the HP group.

AGA had similar effects on nitrogen intake, but it is of interest that nitrogen intake was 60% higher in the HP, AGA group \((1393 \pm 314 \text{ (SD) mg nitrogen/8 days})\) compared to the NP, saline-treated animals \((871 \pm 70)\). Saline treatment was associated with mean weight gain in the NP and HP groups but weight loss in the ZP group. Treatment with AGA resulted in significant \( P < 0.01 \) negative weight changes in each of the dietary groups.

Experiment 2. In the NP group, AGA-treated mice ate 29% more food than did the saline-treated mice \( P < 0.01 \). In contrast a 13% decrease \( P < 0.05 \) was seen in the HP group as a result of enzyme treatment. In the AGA group, food intake was 31% less \( P < 0.01 \) in the HP group than in the NP group. Enzyme-treated animals gained less weight than did their saline-treated counterparts, but this undoubtedly was caused by their much smaller tumor burdens (vide infra).

Effects of AGA on Hematological and Serum Biochemical Indices (Tables 1 and 3)

Experiment 1. Within each of the dietary protein groups AGA caused significant decreases in total WBC count \( P < 0.01 \),
### Table 1
Miscellaneous effects of treatment with Acinetobacter glutaminase-asparaginase or nitrogen intake

<table>
<thead>
<tr>
<th>Enzyme group</th>
<th>Diet group</th>
<th>Food intake (g/8 days)</th>
<th>Wt change (% wt/day)</th>
<th>WBC (10^9 cells/mm³)</th>
<th>Hematocrit (%)</th>
<th>Total protein (g/d)</th>
<th>Plasma glucose (mg/d)</th>
<th>Tumor cells/body wt (10⁶/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor free</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>ZP</td>
<td>25.3 ± 2.3</td>
<td>-2.2 ± 0.3</td>
<td>6.1 ± 2.4</td>
<td>39 ± 10</td>
<td>3.5 ± 0.6</td>
<td>169 ± 28</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>NP</td>
<td>30.5 ± 2.4</td>
<td>1.6 ± 0.6</td>
<td>9.3 ± 2.8</td>
<td>49 ± 3</td>
<td>5.4 ± 0.4</td>
<td>257 ± 36</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>HP</td>
<td>25.7 ± 3.0</td>
<td>0.5 ± 0.6</td>
<td>7.5 ± 2.4</td>
<td>50 ± 3</td>
<td>5.3 ± 0.7</td>
<td>281 ± 84</td>
<td></td>
</tr>
<tr>
<td>AGA</td>
<td>ZP</td>
<td>16.8 ± 2.0</td>
<td>-4.4 ± 0.4</td>
<td>1.1 ± 3.9</td>
<td>50 ± 2</td>
<td>2.8 ± 0.5</td>
<td>172 ± 62</td>
<td></td>
</tr>
<tr>
<td>AGA</td>
<td>NP</td>
<td>19.1 ± 1.9</td>
<td>-2.0 ± 0.5</td>
<td>1.6 ± 0.4</td>
<td>44 ± 3</td>
<td>4.3 ± 0.5</td>
<td>154 ± 69</td>
<td></td>
</tr>
<tr>
<td>AGA</td>
<td>HP</td>
<td>15.1 ± 3.4</td>
<td>-1.7 ± 1.4</td>
<td>2.5 ± 0.6</td>
<td>43 ± 2</td>
<td>5.0 ± 0.2</td>
<td>179 ± 42</td>
<td></td>
</tr>
</tbody>
</table>

- See text for details on diets.
- Mean ± SD.

### Table 2
Effects of treatment with Acinetobacter glutaminase-asparaginase or nitrogen intake on tissue nitrogen content

<table>
<thead>
<tr>
<th>Enzyme group</th>
<th>Diet group</th>
<th>Tissue nitrogen content (mg nitrogen/g initial body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor free</td>
<td></td>
<td>Liver 0.95 ± 0.07^b 0.42 ± 0.04 1.05 ± 0.18 0.25 ± 0.02 0.023 ± 0.012 0.068 ± 0.015 19 ± 2</td>
</tr>
<tr>
<td>Saline</td>
<td>ZP</td>
<td>1.92 ± 0.21 0.41 ± 0.06 1.32 ± 0.16 0.40 ± 0.02 0.083 ± 0.021 0.157 ± 0.063 20 ± 2</td>
</tr>
<tr>
<td>Saline</td>
<td>NP</td>
<td>2.00 ± 0.09 0.40 ± 0.04 1.25 ± 0.23 0.44 ± 0.06 0.056 ± 0.013 0.132 ± 0.040 20 ± 2</td>
</tr>
<tr>
<td>AGA</td>
<td>ZP</td>
<td>0.68 ± 0.07 0.39 ± 0.05 0.44 ± 0.06 0.29 ± 0.02 0.008 ± 0.004 0.015 ± 0.005 12 ± 3</td>
</tr>
<tr>
<td>AGA</td>
<td>NP</td>
<td>0.95 ± 0.15 0.38 ± 0.05 0.77 ± 0.24 0.39 ± 0.04 0.009 ± 0.005 0.039 ± 0.009 16 ± 4</td>
</tr>
<tr>
<td>AGA</td>
<td>HP</td>
<td>1.45 ± 0.37 0.42 ± 0.03 1.03 ± 0.27 0.50 ± 0.10 0.020 ± 0.014 0.051 ± 0.034 20 ± 4</td>
</tr>
</tbody>
</table>

- See text for details on diets.
- Mean ± SD.

### Table 3
Effect of ascites tumor, diet, and AGA treatment on nutritional status

| Percentage of values of tumor-free saline-treated mice on normal protein diet |
|----------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                                  | Food intake      | WBC              | Neutrophil count | Lymphocyte count | Hematocrit       | Plasma total protein | Plasma glucose   | Tissue nitrogen content |
| Liver                            | Tumor free 60±b  | 227±b 128±b 102b 109a | 1582±b 72a 92b 91a 83b | 102±b 84b 102±b 102±b | 82±b 81±b 93±b 83±b | 103±b 103±b 97±b 107±b | 103±b 103±b 97±b 107±b | 103±b 103±b 97±b 107±b |
| High protein                     | Tumor free 62±b  | 227±b 128±b 102b 109a | 1582±b 72a 92b 91a 83b | 102±b 84b 102±b 102±b | 82±b 81±b 93±b 83±b | 103±b 103±b 97±b 107±b | 103±b 103±b 97±b 107±b | 103±b 103±b 97±b 107±b |
| Normal protein                   | Tumor free 78±c  | 54±d e 50±b c 76±e | 129±e 120±c 98±f 148±b 100±c 175±e c 80±b 80±b | 116±e 116±e 110±f 120±c 98±f 140±b 125±b 145±b 24±c f 178±e c 32±c 32±c 32±c | 116±e 116±e 110±f 120±c 98±f 140±b 125±b 145±b 24±c f 178±e c 32±c 32±c 32±c |

* Significance of tumor (same diet, enzyme-treatment group), P < 0.01.
* Significance of high-protein diet (same tumor, enzyme-treatment group), P < 0.01.
* Significance of AGA (same tumor, diet group), P < 0.01.
* Significance of AGA (same tumor, diet group), P < 0.05.
* Significance of tumor (same diet, enzyme-treatment group), P < 0.05.
* Significance of high-protein diet (same tumor, enzyme-treatment group), P < 0.05.
absolute neutrophil count \((P < 0.05)\), and absolute lymphocyte count \((P < 0.01)\); for each of these hematological indices the HP diet was associated with higher values (56–67% increases) compared to normal but the changes were not statistically significant. In the NP group hematocrit was not affected by AGA treatment, but there was a significant decrease in the HP group. Plasma total protein concentration was significantly \((P < 0.01)\) decreased in the NP group after AGA treatment, but there was no effect in the HP group. Within the AGA group the HP diet was associated with a higher protein concentration compared to the NP \((P < 0.05)\) or ZP \((P < 0.01)\) groups. Serum glucose concentration was significantly decreased \((P < 0.01)\) after AGA treatment in the NP and HP groups. The blood counts, plasma protein, and glucose showed similar but smaller changes on the third day of treatment (data not shown).

**Experiment 2.** Treatment with AGA did not alter WBC except for one exception; the HP, AGA mice exhibited a 100% higher absolute lymphocyte count \((11,400 \pm 4,600)\) \((P < 0.01)\) than did their nontreated, saline controls \((5,700 \pm 2,700)\). Within each of the two treatment groups there were no dietary effects on cell counts. Hematocrit was lower in the NP, AGA group compared to the NP, saline controls but the HP diet appeared to prevent this effect of AGA since the hematocrit in this group was the same as that in the saline-treated groups. There was no effect of AGA on plasma total protein concentration, but in each treatment group the HP diet resulted in a slight increase \((P < 0.05)\).

**Effects of AGA and Diet on Tumor Burden (Experiment 2)**

(Table 1)

Tumor burden was estimated by tumor volume, packed tumor cell volume, tumor cell count, number of tumor cells per body weight, and tumor nitrogen content. By each criterion AGA resulted in significant \((P < 0.01)\) diminution of tumor size to values that approximated zero and the statistical conclusion was the same in either dietary group. Within the AGA group there were no statistical differences between the HP and NP groups and the means for tumor nitrogen content of the two groups were identical, 0.10 mg nitrogen/g initial body weight. In the HP, AGA group the mean absolute tumor cell number appeared considerably larger than in the NP, AGA group (Table 1), but the value was very small relative to the saline-treated group. In the saline group the only parameter that differed between the diet groups was a 20% increase \((P < 0.05)\) in the tumor nitrogen content in the HP group \((5.58 \pm 1.14 \text{ mg/g weight})\) compared to the NP group \((4.66 \pm 1.18)\).

**Effects of AGA or Diet on Tissue Nitrogen Content (Tables 2 and 3; Charts 1 and 2)**

**Experiment 1.** In the NP group AGA resulted in significant \((P < 0.01)\) decreases in the final nitrogen content of liver, intestine, thymus, spleen, and carcass (Tables 2 and 3). Brain and kidney were resistant to the nitrogen-depleting effect of AGA. The HP group treated with saline showed no significant differences from the NP group in the nitrogen content of all tissues except thymus (decreased). In contrast HP appeared to ameliorate the effects of AGA on nitrogen content. Moreover among the AGA-treated animals the HP diet resulted in increased nitrogen content (compared to NP or ZP) of the liver or kidney \((P < 0.01)\) and intestine, thymus, or carcass \((P < 0.05)\). The ZP group treated with saline showed decreased nitrogen content relative to the NP group in all tissues except brain and carcass. Within the AGA-treated group the ZP diet compared to the NP diet resulted in significantly lower nitrogen content liver, intestine, kidney, and carcass. The thymic nitrogen contents of the NP or ZP, AGA groups were both very low but equal.

Chart 1 shows the degree of depression of tissue nitrogen content induced by AGA. The HP diet resulted in the following...
relative percentage increments in tissue nitrogen content compared to NP, AGA: liver (53%); intestine (36%); thymus (122%); and carcass (25%). Even though the HP diet was associated with more than doubling of the thymic nitrogen content there was still a very marked reduction compared to normal. The capacity for synthesis of glutamine (as measured by glutamyl transferase activity) of normal mouse liver (343 nmol/min/mg protein) is approximately 1000 times that of intestine (0.3 nmol/min/mg protein) (6). In our study carcass comprises several important tissues including lung, heart, muscle, and skin. Of these muscle would contribute the major proportion of the free amino acid and protein-bound amino acid pool (12). The glutamyl transferase activity of muscle is 14 nmol/min/mg protein (6). Thus the relative increase in nitrogen content by HP in the AGA-treated group appears to be unrelated to glutamyl transferase activity (Chart 1).

We also computed and statistically analyzed the rate of change of tissue nitrogen content by making a comparison to tissue composition on Day 7 of the experiment in control animals sacrificed at that time (before AGA or saline treatment) (data not shown). In the saline-treated NP group tissue nitrogen content either increased (intestine, kidney, carcass) or remained unchanged (liver, brain, thymus, spleen) over the subsequent 9 days. After AGA treatment animals in the NP group lost nitrogen from liver, intestine, thymus, and spleen, and carcass. Relative to the NP diet the HP diet prevented the net loss of intestinal nitrogen (rate of change = 0) and significantly decreased the rate of nitrogen loss from liver and thymus.

Experiment 2. In tumor-bearing mice AGA did not cause statistically significant decreases in the nitrogen content of any individual tissue or the carcass. Moreover AGA caused a significant increase in nitrogen content for the following diet-tissue groups: HP, intestine; NP and HP, kidney; NP and HP, spleen (Tables 2 and 3). Within the AGA treatment group the HP diet resulted in moderate increases in the nitrogen content of the following tissues relative to the NP group: liver (18%, $P = 0.05$); intestine (26%, $P < 0.05$); kidney (17%, $P < 0.01$). The nitrogen content of thymus was increased 164% by the HP diet ($P < 0.01$) (Chart 2). In the untreated (saline) group the HP diet significantly improved the nitrogen content of liver and brain.

**Net Protein Utilization**

Experiment 1. In the saline-treated animals, feeding the HP diet resulted in a significantly reduced NPU (relative to NP) for the liver, intestine, kidney, and thymus. These results are to be anticipated since for a given source of dietary protein increased nitrogen intake is usually associated with decreased NPU (8).

In the NP group treatment with AGA resulted in significantly decreased NPU for the following tissues (percentage decrease from saline, NP: liver (100); brain (542); intestine (282); thymus (137); spleen (95); and carcass (1857). Treatment with HP appeared to block this negative effect of AGA on NPU in the liver, brain, intestine, and carcass. Moreover, in marked contrast to what was observed in normal animals, among the AGA-treated animals NPU was significantly higher in the HP group compared to the NP group for liver, kidney, and carcass (Table 3).

The mechanism for the improvement of NPU in the AGA-treated HP group was not related to enhanced energy intake since food intake was significantly ($P < 0.01$) reduced in this group compared to NP animals (Tables 1 and 3). However, the general effect of AGA on decreased NPU in the NP group could have been related to the decreased energy intake in AGA-treated animals (Tables 1 and 2) (13, 14). To explore this hypothesis further we assessed the covariate effect of food (energy) intake using a two-way analysis of variance model. After adjusting for this covariate effect AGA treatment had no statistically significant effect on NPU for liver, brain, intestine, spleen, and carcass. The NPU of thymus was still significantly ($P = 0.006$) decreased by enzyme treatment after adjustment for the covariate effect.

Experiment 2. In the NP-fed animals AGA resulted in decreased NPU for carcass ($P < 0.01$) but increased NPU for kidney ($P < 0.05$) and spleen ($P < 0.05$). In the HP group, AGA treatment was associated with increased NPU for intestine ($P < 0.01$), kidney ($P < 0.01$), and spleen ($P < 0.01$) compared to similarly fed saline controls. In both the AGA and saline groups feeding the HP diet resulted in significant decreases in NPU relative to the NP group for liver, kidney, and carcass. However, in AGA-treated animals (only) the NPU for thymus was increased ($P < 0.01$) by feeding the HP diet. The mean NPU for intestine was 76% greater in HP, AGA mice than in NP, AGA mice, but the result was not statistically significant.

**Correlations of Tissue Weight with Tissue Nitrogen Content (Experiment 1)**

One of the purposes of this experiment was to evaluate a more detailed approach to the study of nutritional effects of chemotherapy. Our major conclusions are related to tissue nitrogen content. We wondered whether conclusions similar to our own may have been found using only tissue weights instead of nitrogen analysis. There were highly significant correlations between tissue nitrogen content and tissue weight for all tissues with $R^2$ ranging from only 0.14 (brain) to 0.94 (thymus and spleen). The intestine, kidney, thymus, and spleen had $R^2$ values greater than 0.8. Thus it may be possible to screen for certain effects of AGA or other chemotherapy by merely weighing the tissues, but there will be considerable predictive error with this approach.

**Comparison of Dietary AGA Effects Between Tumor-free and Tumor-bearing Animals (Table 3)**

It is of interest to note how the presence of ascites tumor modified the effects of AGA or dietary protein on nutritional status. Table 3 shows selected data in tumor-bearing mice all expressed as a percentage of values in tumor-free, saline-treated mice fed NP. First of all in AGA-treated mice one notes that in marked contrast to the tumor-free mice, tumor-bearing mice exhibited normal nitrogen content in all tissues except thymus. Perhaps this finding is related in part to the fact that the effect of tumor per se was to increase food intake in the AGA-treated groups rather than to lower food intake as seen in both of the saline-treated groups.

Except for the lymphocyte count in the saline group (NP and HP) where there were no significant changes, blood counts were increased in the tumor-bearing animals. Plasma protein was not affected by the presence of tumor in the saline group but was increased in both AGA groups. Plasma glucose was lower in the
tumor-saline groups relative to non-tumor-bearing controls, but tumor had no effect in AGA-treated animals.

There were effects of tumor on tissue nitrogen in the saline- or AGA-treated animals (Table 3). Thus tumor resulted in greater intestinal nitrogen in both AGA groups but lower nitrogen content in the two saline groups. In the saline groups tumor caused a significant decrease in thymic nitrogen whereas in either of the AGA groups the mean thymic nitrogen content was much greater (though not to a statistically significant extent). Except for the saline NP group tumor-bearing animals exhibited greater liver nitrogen. Brain nitrogen was increased in the HP saline, and NP, AGA groups. Tumor resulted in a change in kidney nitrogen only for the AGA, NP group where it was greater. Splenic nitrogen was increased by tumor in both AGA groups but decreased (NP) or unchanged (HP) in the saline group. Carcass nitrogen was increased in association with tumor in the AGA, NP group but unchanged in the three other groups. Using two-way analysis of variance we also examined the effect of tumor on NPU (not shown in Table 3). There were two major patterns: (a) an increase in NPU in liver, spleen, and carcass for all four treatment x diet groups; and (b) an increase in NPU of intestine and thymus of AGA-treated animals (NP and HP) but a decrease in saline controls (NP and HP).

DISCUSSION

General Effects of AGA and Diet on Tissue Nitrogen Content. In the first experiment of the present study we observed that in otherwise healthy mice treatment with AGA caused decreased nitrogen content of liver, intestine, thymus, spleen, and carcass. Brain and kidney were resistant to the nitrogen-depleting effect of AGA. Previous studies of nitrogen metabolism also have shown that these two tissues are affected less than are other tissues by low protein diets (8). A diet with a high P:E ratio resulted in lower energy intake, but in AGA-treated mice a high nitrogen content of liver, kidney, intestine, thymus, and carcass.

In mice bearing Ehrlich ascites tumors AGA did not cause tissue nitrogen depletion relative to saline-treated mice. However, in those mice treated with AGA, increasing the P:E ratio of the diet still improved the nitrogen content of liver, intestine, and thymus and increased the hematocrit and the plasma protein concentration without antagonizing the therapeutic effect of AGA. The eventual relevance of these observations to the further use of AGA as an anticancer agent will depend largely on whether the drug can be applied perhaps in large doses to other test systems, especially human, where previous therapy with this agent has had to be limited or curtailed because of toxicity (5). This study offers no explanation for the neurotoxicity seen in humans treated with AGA (5). In the brain there is a relatively high activity of glutamyl transferase activity (6) and glutamine synthetase is localized in the glial cells (15). Therefore it is not surprising that AGA treatment would not have an observable effect on total brain nitrogen. However, specific significant nutritional effects on neurons still could play a role in neurotoxicity since these cells do lack the capacity to synthesize glutamine (15).

Net Protein Utilization in Tumor-free Mice. In tumor-free mice one of the obvious effects of AGA was decreased intake of food and total nitrogen in both the NP and HP groups. However the nitrogen-depleting effect of AGA was related not only to decreased nitrogen intake but also to decreased NPU. The NPU of a food protein is affected not only by the amino acid pattern but also by the level of protein and energy intake; NPU is higher on a low-protein intake (8) and lower on a low-energy intake (7). Indeed covariate analysis suggested that except for thymus one could explain the adverse effect of AGA on NPU of tissues by its causing lowered-energy intake. Previous studies of healthy mice on normal protein intakes have shown that reduction of energy intake to the level ingested by our tumor-free mice in the NP, AGA group resulted in a similar reduction of liver and intestinal nitrogen content as we observed in these mice (14). Future studies with pair feeding would be necessary to evaluate this point further. However, in mice receiving AGA (± tumor) the HP diet had a nitrogen-sparing effect even though it caused a reduction in energy intake. For several tissues in tumor-free mice and for thymus in tumor-bearing mice this nitrogen-sparing effect of HP was associated with improved NPU.

Nitrogen utilization by thymus was markedly affected by AGA via a mechanism that was apparently independent of the lower-energy intake found in enzyme-treated animals. This emphasizes the potential significance of normal blood levels of glutamine in maintaining normal thymic protein metabolism, composition, and function (16). Glutaminase treatment also could affect purine metabolism, but previous data suggest that omission of glutamine from a culture medium has a lesser effect on either purine synthesis de novo or guanine nucleotide synthesis by cultured T-lymphoblasts than by B-cell- or null cell-derived lymphoblasts (17). AGA also could exert a general or specific (e.g., thymus) "toxic" or "stress" effect directly on cell growth, division, or repair without necessarily affecting protein or amino acid metabolism directly. There are two lines of evidence against this hypothesis. Escherichia coli asparaginase which has glutaminase activity causes in tumor-free mice reduction in splenic size and apparently (from preliminary data) also reduction in the size of the thymus, but Vibrio succinogenes asparaginase which has no glutaminase activity lacks this effect (18). Also Bendich et al. (19) administered to normal mice a polyethylene glycol adduct of E. coli asparaginase which is nonantigenic and nonimmunogenic but which retains both catalytic and antitumor activities. This modified enzyme still caused depression of the splenocyte response to phytohemagglutinin and lipopolysaccharide. Thus this type of immunosuppression probably is not related to a toxic or stress response to a foreign protein.

The relative tissue-specific effects of a low energy intake on nitrogen utilization will depend on the ability of each tissue to supply energy and amino acids for its needs from endogenous synthesis, protein degradation, and from the circulation. With AGA treatment the circulation is removed as a source of glutamine and asparagine. A tissue may have to adapt to decreased plasma concentrations of asparagine and glutamine by decreasing its utilization of these amino acids for energy. This mechanism could be very important for the intestine of AGA-treated animals because normally the intestinal cells use glutamine preferentially and to a significant extent as a source of energy (20). In the AGA-treated animals the mechanism for the improvements in nitrogen content and NPU of various tissues by the HP diet is not clear. The effect may not be a trivial one related to providing more exogenous glutamine and asparagine since the liver and intestine with widely different glutamyl transferase activities exhibited approximately equal percentage increments in nitrogen content with the HP diet compared to NP (respec-
Nitrogen-sparing effects of diet in mice treated with AGA

Relevance to the Nutritional Support of Cancer Patients.

From our data one might derive several generalizations that could have future relevance to nutritional studies of cancer patients. Other antineoplastic agents (e.g., asparaginase or methotrexate) also may affect amino acid utilization directly or particularly indirectly by interfering with energy intake and absorption (2). The nitrogen-sparing effect of the increased protein:energy ratio on tissue nitrogen content is of interest since providing adequate energy to the sick patient may be difficult and even unsafe because of fluid, glucose, and lipid intolerance (5). Finally, this study suggests that the effect of AGA on certain tissues is greater than its effect on the carcass ("whole body"). It might be necessary to focus nutritional assessment on specific tissue (organ) mass, structure, or function.

Different Effects of AGA in Tumor-free and Tumor-bearing Mice and the Effect of Tumor per se.

AGA had different effects on nitrogen-sparing utilization in tumor-bearing and non-tumor-bearing mice. In non-tumor-bearing animals fed NP the toxic effects of AGA included (a) decreases in food intake, WBC, and plasma concentrations of total protein and glucose and (b) decreased nitrogen content and NPU of liver, intestine, thymus, spleen, and carcass. In contrast except for a decreased NPU for carcass none of these adverse effects of AGA was seen in mice inoculated previously with tumor.

The data on saline-treated NP-fed animals from the two studies also provide information on the effects of this tumor per se on nutritional status which included lower food intake and nitrogen content of intestine and spleen. The effects of tumor on food intake and nitrogen content of intestine and spleen were greatly modified or reversed in the AGA-treated mice (Table 3).

Thus although either condition alone, AGA treatment or tumor, caused decreased food intake and impaired nitrogen utilization, the existence of an "incipient tumor" during AGA therapy was associated with less adverse effect on nutritional status. Is this effect unique to this tumor and form of chemotherapy? The effects of an increased P:E ratio contrast with the usual ineffectiveness of simple hyperalimentation of cancer patients (21). Finally, the surprising interaction of the ascites tumors (albeit very small in mass) in nullifying some of the adverse nutritional effects of AGA requires more study as we attempt to understand the biology of neoplasia.

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Tissue Nitrogen-sparing Effect of High Protein Diet in Mice with or without Ascites Tumor Treated with *Acinetobacter* Glutaminase-Asparaginase

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