Protein Metabolism in the Small Intestine during Cancer Cachexia and Chemotherapy in Mice

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ABSTRACT

The impact of cancer cachexia and chemotherapy on small intestinal protein metabolism and its subsequent recovery was investigated. Cancer cachexia was induced in mice with colon 26 adenocarcinoma, which is a small and slow-growing tumor characteristic of the human condition, and can be cured with 100% efficacy using an experimental nitrosourea, cystemustine (C₆H₄ClN₃O₄S). Both healthy mice and tumor-bearing mice were given a single i.p. injection of cystemustine (20 mg/kg) 3 days after the onset of cachexia. Cancer cachexia led to a reduced in vivo rate of protein synthesis in the small intestine relative to healthy mice (−13 to −34%; P < 0.05), resulting in a 25% loss of protein mass (P < 0.05), and decreased villus width and crypt depth (P < 0.05). In treated mice, acute cytotoxicity of chemotherapy did not promote further wasting of small intestinal protein mass, nor did it result in further damage to intestinal morphology. In contrast, mucosal damage and a 17% reduction in small intestinal protein mass (P < 0.05) were evident in healthy mice treated with cystemustine, suggesting that the effects of chemotherapy on the small intestine in a state of cancer cachexia are not additive, which was an unexpected finding. Complete and rapid recovery of small intestinal protein mass in cured mice resulted from an increase in the rate of protein synthesis compared with healthy mice (23–34%; P < 0.05). Northern hybridizations of mRNA encoding components of the major proteolytic systems suggested that proteolysis may not have mediated intestinal wasting or recovery. A major clinical goal should be to design methods to improve small intestinal protein metabolism before the initiation of chemotherapy.

INTRODUCTION

Central to the process of cancer cachexia is the gradual loss of host protein mass. Reductions of skeletal muscle mass can be as high as 75% (1). Surprisingly, very few studies have addressed the effects of cancer cachexia on major organs such as the small intestine (2, 3). The small intestine is responsible for the digestion and absorption of nutrients, serves as a physical barrier to endotoxins, bacteria, and viruses, as well as being involved in apolipoprotein metabolism, mucous secretion, and immune function (4). Conditions that compromise the integrity of the small intestine may interfere with these processes and, therefore, may impact the rest of the body. Although wasting of small intestinal mass has been reported during cancer cachexia (2, 3), the mechanisms responsible for this atrophy remain unknown.

The ultimate goal of antineoplastic therapy is to cure cancer, allowing recovery to ensue. Unfortunately, nonspecific cytotoxicity resulting from antineoplastic therapy remains to be a serious complication during the treatment of cancer. The treatment of patients suffering from cancer cachexia becomes even more problematic. Body wasting prior to chemotherapy treatment reduces expected patient survival (5), and there is evidence suggesting that chemotherapy may directly contribute to the cachexia observed during cancer, further complicating the treatment of this disease and subsequent recovery by the patient (3). Small intestinal damage after chemotherapy is also well established. Treating healthy animals with a variety of chemotherapeutic agents results in reductions in villus height, crypt depth, and mitotic counts (6, 7) and can lead to impaired nutrient absorption and opportunistic infections (7, 8). However, no study has defined the mechanisms mediating recovery of the small intestine from cancer cachexia after chemotherapy.

Understanding the mechanisms responsible for both the progressive deterioration of tissue mass attributable to cancer cachexia and ensuing recovery after chemotherapy are essential in developing therapies to reduce wasting and enhance recovery from cancer cachexia. The aim of this study was to investigate: (a) the mechanisms responsible for causing small intestinal atrophy during cancer cachexia; (b) the effect of chemotherapy on small intestine during a state of cancer cachexia; and (c) the mechanisms regulating recovery of small intestine after chemotherapeutic treatment of cancer. Small intestinal protein mass and histology, the in vivo rate of protein synthesis, and indirect measurements of proteolysis assessed through the expression of proteolytic genes were measured to meet these objectives.

MATERIALS AND METHODS

Animals, Housing, and Diet. All animal studies were completed in accordance with the guidelines of the Canadian Council of Animal Care. Male BALB/c By mice (20 g, 4–5 weeks of age; The Jackson Laboratory, Bar Harbor, ME) were housed in individual cages and maintained at 22–23°C on a 12-h light-dark cycle starting at 8:00 a.m. Mice were given at least 4 days to adjust to their new environment and diet before experimental treatments were imposed. Mice were given free access to water and food (standard rodent chow; UBC Animal Care Centre) with the exception of pair-fed mice. Pair-fed mice received one-third of their allotted food between 10:00 and 11:00 a.m. The remaining two-thirds was given between 5:00 and 6:00 p.m.

Experimental Model. Colon 26 adenocarcinoma (C26) serves as an appropriate model for investigating cancer cachexia. This murine tumor induces weakness, abnormal carbohydrate metabolism, hypercorticism (involved in initiating protein catabolism and growth inhibition), impaired hepatic function, leukocytosis, and elevated interleukin 6 levels (9, 10). All are characteristic of human cancer cachexia and were observed with a tumor burden of 3–6% of total body weight (9, 11, 12). Furthermore, this tumor can be cured with 100% efficacy using cystemustine, a member of the nitrosourea family of alkylating antineoplastic agents (13, 14). The cytotoxic mechanism of cystemustine is common to other nitrosoureas used clinically and is mechanistically similar to many commonly used alkylating agents (15, 16). Because cystemustine offers a complete cure for C26, recovery from cancer cachexia can be studied.

Tumor Inoculation, Transplantation, and Chemotherapeutic Regimen. Stock cells of C26 were used to generate the first tumor passage. Subsequent tumor passages were generated by serial transplantation from solid tumors. A volume of 0.1 ml of filtered tumor homogenates (0.5 g/ml saline) was injected s.c. into the upper dorsal region of recipient mice. Mice from passages three and four were used for the experiments described below. All tumor-bearing mice used experimentally displayed evidence of cancer cachexia. Cystemustine [N-(2-chloroethyl)-N′-[2-(methylsulfonyl)ethyl]-N-nitrosourea (C₂H₆ClN₃O₄S)] (1 mg/ml saline) was injected i.p. as a single dose at 20 mg/kg body weight between 10:15 and 10:45 a.m. The timing of injection was important, because cystemustine is highly

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chronotoxic (17). Cystemustine has been reported previously to cure C26 in mice (13). All control mice were injected with an equivalent volume of sterile, nonpyrogenic saline on the day of tumor transplantation and on the day of chemotherapy.

**Experimental Design.** Mice were randomly designated to one of five treatment groups: (a) healthy mice; (b) tumor-bearing mice; (c) tumor-bearing mice treated with cystemustine; (d) healthy mice treated with cystemustine; and (e) healthy pair-fed mice. Tumors were transplanted on day −18. Chemotherapy was administered on day 0 (about 3 days after the onset of cachexia). On day 0, tumor-bearing mice were randomly assigned to either the tumor-bearing group or the tumor-bearing group treated with cystemustine. Body weight and palpable tumor size were not different between these two groups. Pair-fed mice were initially fed the food intake of tumor-bearing mice. Once the chemotherapeutic agent was administered, healthy pair-fed mice received the food intake of tumor-bearing mice treated with cystemustine.

Healthy mice, tumor-bearing mice, and tumor-bearing mice treated with cystemustine were killed on days 2, 4, and 11. Days 2 and 4 were used to determine the acute effects of chemotherapy and the mechanisms initiating recovery. By day 11, the tumor had fully regressed and therefore served as a good indicator of long-term recovery. A group of tumor-bearing mice were also killed 2 days prior to chemotherapy (day −2) to determine the acute mechanisms responsible for initiating wasting in the small intestine. A second group of tumor-bearing mice was killed 1 day after chemotherapy (day 1) to further define the acute effects of the chemotherapeutic treatment. Both of these groups of mice were compared with healthy controls.

Healthy mice treated with cystemustine and healthy pair-fed mice were killed on days 2 and 4 only. Preliminary experiments showed that chemotherapy caused acute damage to the small intestine of healthy mice, but that recovery was complete by day 4. Pilot studies also showed that food intake in tumor-bearing mice had returned to normal by day 4. Therefore, measurements were not made on day 11 in these two groups.

The parameters measured in these experimental groups included: body weight, food intake, small intestinal protein mass, in vivo rate of protein synthesis, mRNA encoding components of proteolytic systems, and small intestinal histology. The experiment was completed in duplicate, and all measurements were combined.

**Protocol Used on Experimental Days.** Protein synthesis was measured in vivo using the flooding dose method as described previously (18, 19). This is considered to be the method of choice for determining protein synthesis in the intestine. Between 11:00 a.m. and 2:00 p.m., mice were injected i.p. with 150 μmol/100 g body weight of phenylalanine with a specific radioactivity of 0.6 μCi t-[2,6-3H]phenylalanine per μmol (Amersham Canada Limited, Oakville, Ontario, Canada). After precisely 15 min from time of injection, the mouse was killed, and its small intestine was removed and flushed with ice-cold saline. Consistent samples were taken starting 10 cm distal to the pyloric sphincter and immediately placed in liquid nitrogen. These samples were used to determine the rate of protein synthesis and for RNA isolation. Another sample was removed and placed in buffered formalin for histology. The remaining small intestine was placed in liquid nitrogen and used to determine the protein content of the small intestine. Intestinal samples were stored at −70°C until analyzed, except for the histology samples, which were stored at room temperature. Tumors were excised and weighed.

**Protein Mass and in Vivo Rate of Protein Synthesis.** Protein mass was determined using a LECO nitrogen analyzer (model FP428; Leco Instruments, Ltd., Mississauga, Ontario, Canada). The in vivo rate of protein synthesis was determined in a manner similar to that reported by McAllister et al. (19) and Garlick et al. (18). The specific radioactivity of free (Sa) and bound (SB) phenylalanine was measured in each sample. The fractional rate of protein synthesis (ks) (%/day) was calculated as: $k_s = (SB \times 100)/(SA \times t)$ (Ref. 18), where SB is the specific radioactivity of protein-bound phenylalanine, SA is the specific radioactivity of free phenylalanine in the tissue homogenate, and t is the time between injection and slaughter (in days). The absolute rate of protein synthesis (mg/day) was calculated by multiplying the fractional rate of protein synthesis by the total protein mass of the small intestine.

**Northern Hybridization.** Total RNA was isolated from small intestinal samples by phenol-chloroform extraction (20). Northern hybridizations were performed as described previously (21). Fifteen μg of RNA were electrophoresed on a 1% agarose gel containing formaldehyde and was electrophoretically transferred to a nylon membrane (GeneScreen; NEN Research Products, Boston, MA). RNA was covalently bound to the membrane using an UV cross-linker. The RNA on the membrane was then hybridized overnight at 65°C with [32P]cDNA probes labeled by random priming (21). The probes used encoded rat cathepsin B (22), human m-calpain (23), chicken polyubiquitin (24), rat 14-kDa ubiquitin conjugating enzyme E2 (25), and C8 (26) and C9 (27) rat 20S proteasome subunits. These probes were selected because they encode proteases and/or cofactors of the major proteolytic systems (lysosomal, calcium dependent, and ubiquitin-proteasome dependent). Probed membranes were washed four times in 0.1% SDS at 65°C for 15 min, and the stringency of washes was varied between 2× SSC and 0.2× SSC, depending on the probe. Washed membranes were placed in a phosphorimaging cassette and analyzed using a Phosphorimager (Molecular Dynamics, Sunnyvale, CA). After stripping the different probes, membranes were reprobed with a rRNA probe. The 18S ribosomal band was also quantified to test for loading differences.

**Histology.** Intestinal specimens were stained with H&E. Villus height, villus width, crypt depth, and muscularis width were measured in micrometers at 200-fold magnification, using a light microscope. A minimum of 10 consecutive villi and crypts were measured per animal. Treatment designation of slides was blinded at the time of measurement. Villus contour length was calculated using the following equation: villus contour length = (2 × villus height) + (0.3 × villus width) (Ref. 28).

**Statistics.** The effect of treatment was tested by ANOVA (29). Differences among means were assessed using Student’s t tests. Variability was expressed as SE. Differences were considered significant at P < 0.05.

**RESULTS**

**Experimental Model.** Tumors were first palpable in mice 1 week after transplantation; symptoms of cachexia began 4–5 days later. Weight loss began once tumors grew to ~0.5 g (day −2; Fig. 1) and was characterized by a rapid loss of body weight (Fig. 2). By day 2, body weights of tumor-bearing mice were nearly 20% lower than healthy controls (P < 0.05). On day 11, tumor-bearing mice weighed 30% less than healthy controls (P < 0.05).

Food intake was not different between tumor-bearing and healthy mice on a cumulative basis between days −8 and 10 (111.7 ± 6.3 g versus 111.1 ± 2.5 g, respectively; P > 0.05), which is consistent with the findings of Tanaka et al. (9). However, as mice became cachectic, food intake in tumor-bearing mice was reduced by ~20% but returned to healthy levels by day 1 and was slightly higher than healthy animals by the end of the study (P < 0.05; data not shown).

One day after chemotherapy, body weights of treated tumor-bearing mice was 7% lower (P < 0.05) and food intake was 20% lower (P < 0.05; data not shown) than that of untreated tumor-bearing mice. Recovery from cancer cachexia began by day 2. Body weight of
treated mice started to increase, and food intake returned to healthy levels. Tumor mass in treated mice was 25% smaller compared with untreated tumor-bearing mice (P > 0.05; Fig. 1). Mice were well into a state of recovery by day 4. Treated tumor-bearing mice were 15% heavier than untreated tumor-bearing mice (P < 0.05), and the food intake of treated mice was ~25% higher than that of healthy mice (P < 0.05). By day 11, tumor regression was complete with only trace amounts of tumoral tissue visible in a few mice. Body weights of cured mice were 5% lower than those of healthy mice (P < 0.05), and food intake had returned to normal.

The body weight of healthy mice given cystemustine was lower than that of healthy controls on day 2 (P < 0.05) but not on days 3 and 4. Food intake of healthy mice treated with cystemustine was lower than that of healthy mice 2 days after treatment (P < 0.05) and returned to normal 1–2 days later (data not shown).

**Small Intestinal Protein Mass and in Vivo Rate of Synthesis.**

Small intestinal protein mass of tumor-bearing mice at the onset of cachexia (day –2) was not different from healthy controls (P > 0.05); however, the fractional and absolute rates of protein synthesis were lower (~20%) in tumor-bearing mice (P < 0.05; Fig. 3A). The fractional (~15%) and absolute (~34%) rates of protein synthesis were still lower on day 2 (P < 0.05), contributing to a 25% loss in small intestinal protein mass (P < 0.05). This wasting of protein mass was not attributable to the small reduction in food intake. Pair-fed controls showed no evidence of wasting compared with healthy controls on day 2 (178.1 ± 8.9 versus 171.7 ± 6.1 mg protein, respectively; P > 0.05). On days 4 and 11, there were no further reductions in small intestinal protein mass; however, the rates of protein synthesis in tumor-bearing mice were still lower than in healthy control mice. There was no evidence indicating that chemotherapy had any additional negative effects on small intestinal protein mass of tumor-bearing mice because small intestinal protein mass of these mice just 1 day (133.6 ± 2.8 mg) and 2 days (140.0 ± 7.1 mg) after treatment was not different from untreated tumor-bearing mice on day 2 (128.7 ± 9.5 mg; P > 0.05). Furthermore, the effect on protein synthesis was minor because it was only 15% lower on day 1 in treated compared with untreated tumor-bearing mice (data not shown). This effect on protein synthesis was transient because the fractional rate of protein synthesis returned to normal by day 2.

The small intestine showed clear signs of recovery by day 4 in terms of protein mass and synthesis. Small intestinal protein mass of treated tumor-bearing mice had fully recovered and was actually 11% higher than that of healthy mice (P < 0.05). This corresponded to a 23 and 34% increase in the fractional and absolute rates of protein synthesis, respectively, on day 4 (P < 0.05). On day 11, protein mass was still 10% higher than in healthy control mice. Expressed relative
to body weight, small intestinal protein mass of treated tumor-bearing mice was higher than healthy mice on days 4 and 11 (P < 0.05; data not shown).

In contrast to tumor-bearing mice treated with cystemustine, evidence of acute toxicity from chemotherapy was detected on day 2 in healthy mice treated with cystemustine. Small intestinal protein mass was 17% lower compared with healthy controls (P < 0.05; Fig. 3B), whereas cystemustine in tumor-bearing mice did not appear to further reduce protein mass (Fig. 3A). These data suggest that the effect of chemotherapy in addition to the effect of cancer cachexia is not additive. Furthermore, small intestinal protein mass expressed relative to body weight in healthy mice treated with cystemustine was significantly lower (~13%) on day 2 compared with all other groups (P < 0.05; data not shown). The fractional rate of protein synthesis on day 2 was not lower in healthy mice treated with cystemustine compared with controls (Fig. 3B). It was likely not lower because recovery had begun by day 2, as evidenced by the increase in crypt depth (see “Histology” below), so that the fractional rate of protein synthesis had returned to normal by day 2. The decrease in intestinal protein mass in healthy mice treated with cystemustine led to a lower absolute rate of protein synthesis on day 2 (P < 0.05). Protein mass and synthesis were fully restored by day 4.

Northern Hybridization. Northern hybridizations of cathepsin B, ubiquitin, and C8 proteasome subunit are shown in Fig. 4. In general, during the protein wasting phase (days ~2 and 2), mRNA levels in the small intestine from tumor-bearing mice were lower than in healthy controls (P < 0.05). After day 4, when protein mass stabilized, there were no differences in mRNA levels. Chemotherapy in tumor-bearing mice did not appear to have any acute (day 2) effect on mRNA levels. During the recovery phase (days 4 and 11), mRNA levels either returned to or slightly exceeded (P < 0.05) healthy levels. Similar trends were also observed with mRNAs encoding the 14-kDa E2 ubiquitin conjugating enzyme and C9 proteasome subunit (data not shown). No differences were detected among levels of mRNAs from healthy mice treated with cystemustine, healthy pair-fed mice, and healthy controls for all mRNA species probed on all experimental days (P > 0.05; data not shown). There was no effect of any treatment on 18S rRNA signals (Fig. 4).

Histology. Cancer cachexia induced alterations to some of the morphological parameters of the small intestine. Villus height and contour length were only slightly lower in tumor-bearing mice than in healthy mice (P > 0.05), whereas villus width and crypt depth showed substantial evidence of atrophy (P < 0.05; Table 1 and Fig. 5). Wasting of smooth muscle in the muscularis layer was indicated by a reduction in muscularis width on days 2 and 11 (P < 0.05).

No evidence of acute morphological damage was detected in tumor-bearing mice treated with cystemustine; villus height, contour length, and width were not different from untreated tumor-bearing mice on day 2 (P > 0.05). Crypt depth in treated tumor-bearing mice returned to normal on day 2, indicating that recovery had started. On day 4, when protein mass was restored, these parameters were the same as in healthy mice.

In healthy mice treated with cystemustine, by contrast, villus height and contour length were drastically reduced (~30%; P < 0.05) compared with healthy control mice on day 2 (Figs. 5 and 6). These data corresponded to the wasting of small intestinal protein mass seen on day 2 in healthy mice given cystemustine (Fig. 3). In addition, villus height and contour length in healthy mice treated with cystemustine were 25 and 17%, respectively, lower than in treated tumor-bearing mice (data not shown). Evidence of crypt hypertrophy, indicating previous cytotoxic damage to crypt depth, in healthy mice

Table 1

<table>
<thead>
<tr>
<th>Day and parameter</th>
<th>Healthy</th>
<th>Tumor</th>
<th>Tumor + Cystemustine</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Village height</td>
<td>342 ± 19</td>
<td>312 ± 19</td>
</tr>
<tr>
<td></td>
<td>Village width</td>
<td>81 ± 3.5</td>
<td>72 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Crypt depth</td>
<td>121 ± 7.5</td>
<td>99 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>Village contour length</td>
<td>708 ± 39.0</td>
<td>646 ± 39.0</td>
</tr>
<tr>
<td></td>
<td>Muscularis width</td>
<td>46 ± 3.3</td>
<td>36 ± 2.9</td>
</tr>
<tr>
<td>4</td>
<td>Village height</td>
<td>367 ± 16.3</td>
<td>336 ± 18.1</td>
</tr>
<tr>
<td></td>
<td>Village width</td>
<td>80 ± 2.0</td>
<td>68 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Crypt depth</td>
<td>124 ± 2.6</td>
<td>98 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>Village contour length</td>
<td>757 ± 33.6</td>
<td>692 ± 36.6</td>
</tr>
<tr>
<td></td>
<td>Muscularis width</td>
<td>43 ± 2.9</td>
<td>42 ± 2.0</td>
</tr>
<tr>
<td>11</td>
<td>Village height</td>
<td>354 ± 18.1</td>
<td>345 ± 34.4</td>
</tr>
<tr>
<td></td>
<td>Village width</td>
<td>80 ± 2.2</td>
<td>63 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>Crypt depth</td>
<td>117 ± 3.6</td>
<td>88 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>Village contour length</td>
<td>732 ± 36.2</td>
<td>708 ± 69.0</td>
</tr>
<tr>
<td></td>
<td>Muscularis width</td>
<td>47 ± 4.0</td>
<td>34 ± 3.5</td>
</tr>
</tbody>
</table>

*Values within a given parameter on the same experimental day with different superscripts are significantly different (P < 0.05). See Fig. 1 legend.

Fig. 4. mRNA levels encoding cathepsin B, ubiquitin, and C8 proteasome subunit during cancer cachexia and chemotherapy. Values are means of phosphorimager signals expressed as a percentage of healthy controls with n = 5–6 mice/mean; bars, SE. Representative Northern blots are shown below the corresponding bars. No differences in loading were detected, determined by 18S rRNA signal analysis (P > 0.05). Northern blots for 18S rRNA are shown below blots for C8 proteasome subunit. A values are significantly different from healthy controls on the same experimental day (P < 0.05). B values are significantly different from tumor-bearing mice on the same experimental day (P < 0.05). H, healthy mice; T, tumor-bearing mice; TC, tumor-bearing mice treated with cystemustine. See Fig. 1 legend.
bearing mice. Atrophy of small intestinal protein mass occurred in cachexia caused wasting of small intestinal protein mass in C26-tumor-bearing mice treated with cystemustine (TC) are shown. Slides at ×200 from day 2 illustrate the acute effects of chemotherapy; day 11 represents the chronic effect of cancer cachexia (2 weeks after onset of cachexia). Jejunal segments were embedded in paraffin wax. Longitudinal sections were cut, fixed, and stained with H&E. Table 1 and Fig. 6 summarize mean values for morphological parameters.

DISCUSSION

This study showed for the first time that wasting of small intestinal protein mass occurs in tumor-bearing mice as a result of a decrease in the rate of protein synthesis, independent of reductions in food intake. Acute toxicity of chemotherapy did not appear to induce further wasting of small intestinal protein mass or alter histological parameters in tumor-bearing mice. Chemotherapy did promote wasting of small intestinal protein mass in healthy mice and caused characteristic morphological damage to small intestinal crypts and villi. The rapid recovery of small intestinal protein mass, in mice cured of cancer, was the result of an increase in protein synthesis.

Wasting of Small Intestine from Cancer Cachexia. Cancer cachexia caused wasting of small intestinal protein mass in C26-bearing mice. Atrophy of small intestinal protein mass occurred shortly after the onset of cachexia, with a relatively small tumor burden (2–3% of body weight), and independently of food intake. Chronic wasting of small intestinal protein mass did not occur. This is in contrast to skeletal muscle mass and body weight in which chronic wasting is well established in clinical studies (30–32) and animal models of cancer cachexia (21, 33–35), including C26 (9, 10). This also indicates that other body tissues wasted to a greater extent than small intestine. The lack of chronic wasting of small intestinal protein mass suggests an adaptive conservation by the small intestine during cancer cachexia. Conservation could be very important to the host but also to the tumor, because the small intestine is a portal of entry for nutrients needed to meet the elevated metabolic demands for both the host and tumor. To our knowledge, no other study has assessed the morphology of the small intestine during cancer cachexia. Histological analysis of the small intestine indicated that cancer cachexia induced a shrinkage of the barrier but did not cause specific morphological damage.

Protein wasting in the small intestine is mediated by changes in protein synthesis and/or proteolysis but also on the rate of cell sloughing and export protein secretion (systemic and luminal; Ref. 4). This study was the first to measure the rate of protein synthesis in the small intestine during cancer cachexia. Reductions in protein synthesis were detected in the small intestine from tumor-bearing mice at the very onset of cachexia. A decrease in protein synthesis was present before any atrophy of small intestinal protein mass was detected. Therefore, acute wasting of small intestinal protein mass was likely the result of a decrease in protein synthesis.

Protein degradation may also be involved in wasting of small intestinal protein mass. There are no direct methods available to measure the rate of protein degradation in the small intestine. It is possible, however, to assess changes in the expression of mRNA encoding components of the major proteolytic systems in the small intestine through Northern hybridization (36). Treatment differences among relative levels of mRNA may suggest directional changes in protein degradation as well as indicate transcriptional regulation or modulation of mRNA catabolism. Changes in mRNA levels have been shown to be associated with similar changes in the rate of proteolysis of skeletal muscle under a variety of conditions (37).

Overall, Northern hybridization indicated there was a down-regulation of mRNA encoding proteases and/or subunits of the lysosomal and ubiquitin-proteasome-dependent proteolytic pathways in the small intestine from cachectic mice during the protein wasting phase. If protein degradation had contributed to wasting of small intestinal protein mass in tumor-bearing mice, mRNA levels would likely have been up-regulated. Northern hybridization analysis suggests that pro-

![Fig. 5. Morphological features of the small intestine during cancer cachexia and chemotherapy. Representative histological slides of the small intestine from healthy mice (H), healthy mice treated with cystemustine (HC), tumor-bearing mice (T), and tumor-bearing mice treated with cystemustine (TC) are shown. Slides at ×200 from day 2 illustrate the acute effects of chemotherapy; day 11 represents the chronic effect of cancer cachexia (2 weeks after onset of cachexia). Jejunal segments were embedded in paraffin wax. Longitudinal sections were cut, fixed, and stained with H&E. Table 1 and Fig. 6 summarize mean values for morphological parameters.](image)

![Fig. 6. Histological parameters in healthy mice treated with cystemustine. Values of histological parameters are means (mm): bars, SE. A minimum of 10 consecutive villi and crypts were measured per animal, and the mean was used as the animal’s value. Sample sizes ranged from 5 to 6 mice/treatment group. A and B values within a given parameter with different letter annotations are significantly different (P < 0.05). Cyst, cystemustine. See Fig. 1 legend.](image)
teolysis was unlikely to have contributed to wasting. However, the first analyses were made on day −2, 16 days after tumors were implanted. Thus, we cannot exclude that changes in proteolysis may have contributed to intestinal wasting in tumor-bearing mice prior to day −2.

**Recovery of Small Intestine from Cancer Cachexia after Chemotherapy.** The small intestine is of particular interest when studying recovery from cancer cachexia after chemotherapy. Clinical studies report several toxic side effects related to the small intestine (38, 39), and it is well established that antineoplastic agents inadvertently target the epithelial lining of the small intestine in healthy animals. As expected, healthy mice injected with cystemustine in our study showed significant reductions in small intestinal protein mass and severe compromises to villus height and crypt hyperplasia, indicating previous cytotoxic damage to crypt depth.

Surprisingly, in treated tumor-bearing mice, chemotherapy did not appear to induce further wasting of small intestinal protein mass and it did not alter morphological features of the small intestine. This is in contrast to effect of cystemustine on healthy mice in this study, as well as numerous studies reporting intestinal atrophy in healthy mice after various other chemotherapies (6, 7, 40). Additional intestinal protein loss may not have been observed with chemotherapy because the mice were already cachectic. We speculate that the reduced rate of protein synthesis in cachectic mice rendered the small intestine less susceptible to the cytotoxic effects of chemotherapy, which normally targets the rapidly proliferating intestinal tissue. This study has shown for the first time that the small intestine in a state of atrophy induced by cancer cachexia appears to respond differently to chemotherapy than the normal, healthy small intestine.

Chemotherapy acutely decreased body mass without effect on intestinal protein mass in tumor mice. This is in agreement with Le Bricon et al. (3), who injected Morris hepatoma 7777-bearing rats with a high doses of several different cytostatic agents, and reported that small intestinal weight was either the same or higher in treated tumor-bearing rats compared with untreated tumor-bearing rats; in contrast, the body weights of the treated tumor-bearing rats were consistently lower than those of untreated tumor-bearing rats. This suggests that the small intestine may respond differently to chemotherapy during cancer cachexia than other tissues.

Complete recovery of small intestine requires repletion of protein mass. Rapid recovery of small intestinal protein mass occurred in C26-bearing mice cured with cystemustine and in healthy mice treated with cystemustine. Small intestinal protein mass recovered well before body and skeletal muscle mass (data not shown), suggesting that priority was given to recovery of small intestine, which would facilitate recovery of other tissues. Our study illustrated the resilient nature of the small intestine and showed that complete recovery occurs quickly. Also, cytotoxicity attributable to chemotherapy did not impede full recovery of small intestine in cured mice.

Small intestinal recovery was evident by the second day after chemotherapy. Recovery of small intestinal protein mass was driven primarily by an increased rate of protein synthesis, resulting in hypertrophy. The possible involvement of proteolysis in mediating recovery was assessed indirectly using Northern hybridization. Levels of mRNA encoding proteases and/or components of the major proteolytic pathways in the small intestine of treated cachectic mice were essentially normal. Thus, proteolysis was presumably not involved in the small intestinal recovery process. By the end of the experiment, normalization of the rate of protein synthesis and mRNA levels suggested that homeostasis of protein metabolism in small intestine of cured mice was not permanently affected by chemotherapy. Overall, this study also illustrates that chemotherapy was rapidly capable of reversing the deleterious effects of cancer cachexia on small intestinal protein metabolism.

**Future Directions and Clinical Implications.** Elucidating factors and mechanisms responsible for wasting in the small intestine during cancer cachexia may be useful for designing strategies aimed at minimizing wasting, because conservation of host tissue during cancer greatly increases expected survival time and tolerance to antineoplastic therapy (5, 41). The present study has clearly shown that reduced protein synthesis contributed to small intestinal wasting seen in untreated tumor-bearing animals. It has also demonstrated that the small intestinal recovery process, in terms of protein mass, protein synthesis, and histological parameters, is relatively rapid and complete in tumor-bearing animals cured by chemotherapy.

These data suggest that a major clinical goal should be to design methods to improve small intestinal protein metabolism before the initiation of chemotherapy. Numerous hormonal factors appear to be important to small intestinal growth in vivo. They include growth factors such as epidermal growth factor/urogastrone, peptide hormones produced in neuroendocrine cells (42, 43), and GLP-2 (44). Testing whether these factors partially prevent cancer cachexia is of obvious clinical importance. Our experimental model would be particularly suitable to study the effects of GLP-2 in cancer cachexia. GLP-2 was reported recently to reverse weight loss and improve histological and biochemical disease parameters in mice with experimental colitis (45). However, different tumors vary in regard to the severity of anorexia and cachexia, the associated cytokine environment, and release of cachexia-inducing factors from the tumor itself (41, 46), and there is variability in the cytotoxic impact from various antineoplastic agents. Research on intestinal protein metabolism using other tumor models and chemotherapeutic agents is also warranted to design effective clinical interventions.

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**REFERENCES**


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13. Bourut, C., Chenu, E., Godeneche, D., Madelmont, J. C., Maral, R., Mathe, G., and


22. Segundo, B. S., Chan, S. J., and Steiner, D. F. Identification of cDNA clones encoding

21. Temparis, S., Asensi, M., Taillandier, D., Aurousseau, E., Larbaud, D., Obled, A.,


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