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Effects of Tumor Necrosis Factor α on Skeletal Muscle and Walker 256 Carcinosarcoma Protein Metabolism Studied *in Vivo*[1]

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ABSTRACT

Human tumor necrosis factor α (TNF) inhibits tumor growth, but its effects on tumor and skeletal muscle protein metabolism *in vivo* have not been adequately studied. Walker 256 carcinosarcoma growth rate was followed over an 11-day period in Sprague-Dawley rats. Tumor-bearing rats received either saline or 50 μg of TNF (Genentech, Inc.) s.c. on day 8 of tumor growth. This single dose of TNF reduced tumor protein growth during a 2-day posttreatment period from 27.6 ± 4.4 to 10.5 ± 3.7%/day (mean ± SE; $P < 0.01$). The rate of *in vivo* incorporation of L-[1-^{14}C]leucine into skeletal muscle protein was significantly increased ($P < 0.05$) from 5.1 ± 0.2%/day in the saline-treated tumor-bearing rats to 7.7 ± 1.3%/day in the TNF-treated tumor-bearing rats. The latter value was not statistically different from the 9.2 ± 0.9%/day observed in the tumor-free control animals. TNF administration significantly increased both the total and individual acid-soluble skeletal muscle amino acid concentrations in tumor-bearing rats by an average of 86 ± 7%, compared to values in saline-treated tumor-bearing rats. Similarly, acid-soluble skeletal muscle 3-methyl-histidine concentrations increased from 66 ± 14 to 113 ± 19 pmol/g protein ($P < 0.05$). Tumor protein synthesis in the TNF-treated group was 50% greater than in the saline-treated group, whether expressed as %/day (72.7 ± 9.1 *versus* 47.9 ± 4.8; $P < 0.05$) or was μg/g tumor/min (58.7 ± 7.7 *versus* 40.7 ± 4.5; $P < 0.05$). In contrast, estimated tumor protein degradation rates were increased by over 200% in the TNF-treated rats, compared to the values in the saline-treated rats [62.1 ± 10.7 *versus* 20.3 ± 6.0%/day ($P < 0.01$) and 50.0 ± 8.9 *versus* 17.5 ± 5.4 μg/g tumor/min ($P < 0.01$)]. Thus, TNF appears to stimulate tumor protein degradation more than protein synthesis, explaining the overall decrease in tumor growth.

INTRODUCTION

TNF[3] has been associated with *in vitro* and *in vivo* killing of tumor cells. This killing activity was first discovered in the sera of Calmette-Guerin bacillus-infected animals (1). Murine TNF causes a dose-related regression of certain murine and human tumors transplanted into mice (2, 3). Chronic administration of TNF produces muscle wasting similar to that observed in chronic disease (4, 5). In this study the effects of a single dose of recombinant human TNF (6) on muscle and tumor protein metabolism have been examined during the early phase of transplanted tumor growth in the rat.

MATERIALS AND METHODS

Fourteen 67–70-g male Sprague-Dawley rats were inoculated s.c. in the right flank on day 0 with approximately 1 × 10^6 Walker 256 carcinosarcoma cells (Arthur D. Little, Inc., Cambridge, MA) in 0.1 ml and eight control rats received saline. Tumor growth was evident by day 5 and estimates of tumor volume were made daily thereafter as: $V = L \times W \times D \times \pi/6$, where V = volume, L = length, W = width; and D = depth in cm (7) (Fig. 1). This increase in tumor volume has been used as an index of growth in weight and in protein content prior to killing in this study. The use of volume in this manner is based on the defined relationships of volume to weight in Walker 256 tumors, as shown by the equation: $y = 0.74 + 1.08x$ ($r = 0.95$), where y is volume in cm^3 and x is mass in g (8). Furthermore, nitrogen concentration has been reported to be constant over a wide variety of tumor weights (5 to 90 g) (8, 9). In the present study the TNF-treated tumor protein content, at killing, of 11.8 ± 1.6% was not different from the saline-treated tumor protein content of 12.1 ± 0.9%, and both of these are similar to the previously reported values for the Walker 256 carcinosarcoma (8, 9).

On day 8 at 2:00 p.m., the 14 tumor-bearing rats were given 0.1 ml of either 50 μg (2.51 × 10^6 units) of recombinant human TNF or saline s.c. Tumor protein content prior to killing was estimated from the derived weight and the measured percentage of protein content at killing for each individual animal. The equation describing tumor protein growth for each animal between days 9 to 11 was determined using standard linear regression analysis and the equation: $\ln y = a + bx$, where $\ln y$ equals the natural logarithm of the tumor protein content in mg of protein and x equals time in days. The mean y intercept, a, and the mean slope, b, were calculated for each group of data. When log-transformed, the equation becomes: $y = e^a \cdot e^{bx}$. The slope b represents K_g, in mg protein per day. Doubling time in days was calculated from K_g as follows: doubling time = $\ln 2/\ln(1 + K_g)$.

Tissue protein synthesis was measured using the flooding dose technique (10) 60 h after TNF or saline injection. This interval was chosen based on the observation of previous workers that the effect of TNF on tumor growth becomes evident by 48 h (3) and lasts a minimum of 96 h (2). After an overnight fast, on the 11th day after inoculation the rats were given injections into the tail vein of 150 μmol of unlabeled and 30 μCi of labeled L-[1-^{14}C]leucine (50 mCi/mmol; ICN Radiochemicals, Irvine, CA) per 100 g of body weight. Such doses of leucine do not significantly stimulate skeletal muscle or tumor protein synthesis (11, 12). An i.v. bolus injection of label permits rapid near-equilibration of the leucine specific activity within both the acid-soluble muscle and tumor intracellular pools (SA_i) and the plasma (SA_p) pool. Equilibration was confirmed by the finding that the SA_i/SA_p ratios were similar and approached unity in the TNF- and saline-treated tumor tissue (0.91 ± 0.05 *versus* 0.98 ± 0.14, respectively) and skeletal muscle samples were 0.91 ± 0.03 *versus* 0.80 ± 0.04, respectively). At 10 min after injection, the animals were killed by decapitation. Mixed arterial-venous blood was obtained and 1–2 g samples of abdominal rectus muscle and of tumor tissue were collected, weighed, and frozen in liquid nitrogen within 2 to 4 min, and stored at −20°C until later analysis. Plasma and tissue preparation and scintillation counting were performed as described earlier (7). Amino acid determination was performed using a Beckman amino acid analyzer (Model 119CL) and protein determination was according to the method of Bradford (13).

The fractional K_s, expressed as mg protein per day, was experimentally determined on day 11 from the isotopic appearance of tracer leucine into tissue protein, as described by the equation: $K_s = SA_b/(SA_i \times T) \times 100$, where SA_b is the leucine specific radioactivity in the protein-bound acid-insoluble fraction after time T (time from injection to freezing of tissue as a fraction of a day) and SA_i is the leucine specific radioactivity in the acid-soluble fraction (10, 14). SA_i was calculated from the radioactivity and leucine concentration in the acid-soluble tissue fraction. SA_b was calculated from the radioactivity, the measured protein in the acid-insoluble fraction, and the average percentage of leucine content in rat skeletal muscle (15) and tumor tissue (8). Protein synthesis rate per g of tissue was calculated by dividing the synthesis rate by the wet weight of tumor tissue. In order to estimate K_d in the tumor tissue, we have made two assumptions: (*a*) that the protein synthesis in tumor tissue is constant over the 13-min period from injection of isotope to freezing of the tissue and (*b*) that protein

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[3] The abbreviations used are: TNF, tumor necrosis factor α; SA_i, intracellular specific activity; SA_p, plasma specific activity; SA_b, bound specific activity; K_s, rate of protein synthesis; NEAA, nonessential amino acids; EAA, essential amino acids; 3MH, 3-methyl-histidine; K_g, growth rate; K_d, rate of protein degradation.

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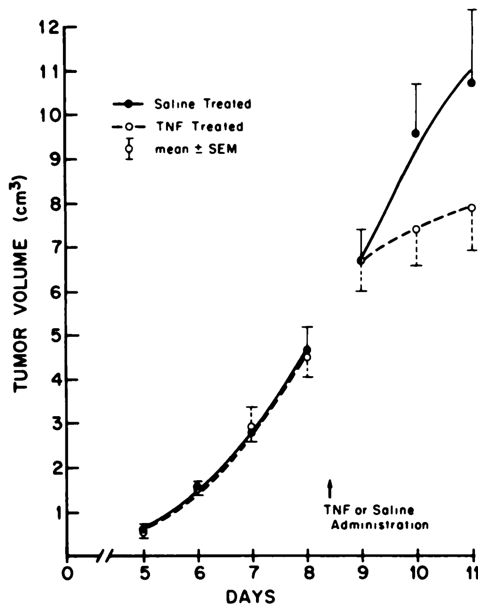

Fig. 1. Tumor growth was detectable in all animals by day 5. The volume of the tumor was calculated as follows: $V = L \times W \times D \times \pi/6$, where L = length, W = width, and D = depth in cm (see "Materials and Methods"). The growth of the tumor volume prior to any treatment (day 5 to day 8) is expressed by the equation: $V = e^{a} \times e^{bx}$, where V is the tumor volume, a is the y intercept, e is an exponential function, b is the tumor growth rate based upon volume measurements, and x is time in days. Tumor volume growth for both groups prior to saline or TNF injection was identical. The regression analysis equations for tumor volume growth in the pretreatment period were not statistically different: tumor (saline), $V = e^{-3.63} \cdot e^{0.653x}$ ($r^2 = 0.97$); tumor (TNF), $V = e^{-3.78} \cdot e^{0.670x}$ ($r^2 = 0.96$). The post-treatment regression analysis equations were statistically different: tumor (saline), $V = e^{-0.01} \cdot e^{0.213x}$ ($r^2 = 0.78$); and tumor (TNF), $V = e^{1.19} \cdot e^{0.076x}$ ($r^2 = 0.78$; $P < 0.01$). There is a significant reduction in the tumor volume growth after TNF administration.

synthesis, degradation, and tumor growth rates follow first-order kinetics. This is supported by the fact that previous work has demonstrated that this tumor grows in an exponential fashion until day 30 (16).

Comparison between groups was done by the Student t test and analysis of variance. Regression analysis was performed by the method of least squares. All data are represented as mean ± SE. Significance was defined as $P < 0.05$ or less, when modified to adjust for multiple comparisons.

RESULTS

Similar food intake and weight gain were observed in the three groups (Table 1). The final 34-h food intake was similar for the TNF-treated (21 g), saline-treated (23 g), and non-tumor-bearing control (24 g) rats. Although the final TNF-treated weight of 8.1 ± 0.7 g was not significantly less than the weight of the saline-treated tumors at sacrifice (10.5 ± 1.3; $P = 0.07$), the change in tumor volume during the final 2 days before killing was significantly less in the TNF group (0.6 ± 0.1 *versus* 1.8 ± 0.6 cm^3; $P < 0.025$). The regression analysis equations for tumor protein growth ($y = e^{a} \cdot e^{bx}$) in the pretreatment period were not statistically different: tumor (saline), $y = e^{-17.02} \cdot e^{2.08x}$; tumor (TNF), $y = e^{-18.03} \cdot e^{2.19x}$. The post-treatment regression analysis equations, however, were statistically different: tumor (saline), $y = e^{-2.97} \cdot e^{0.30x}$; tumor (TNF), $y = e^{-1.22} \cdot e^{0.10x}$ ($P < 0.01$). At killing, the tumor volumes of both groups (Y) correlated well with the weights (X) of the dissected tumors ($r = 0.95$; $Y = 1.86 \pm 0.80X$; $n = 14$; $P < 0.001$). Doubling time was prolonged from 2.8 to 6.9 days in the TNF-treated group.

Muscle protein synthesis rates were significantly reduced in the tumor-bearing saline-treated group, compared to values in the control group (5.1 ± 0.2 *versus* 9.2 ± 0.9%/day; $P < 0.01$) (Fig. 2). The TNF-treated group demonstrated a significant increase in muscle protein synthesis (7.7 ± 1.3%/day; $P < 0.05$) compared to the rate in saline-treated rats; this rate was not different from that in tumor-free control rats.

In addition, TNF treatment was associated with a 50% increase in the tumor K_s, compared to the value with saline treatment (72.7 ± 9.1 *versus* 47.9 ± 4.8%/day; $P < 0.05$) (Table 2). This change in the rate of protein synthesis led to a modest increase in the amount of protein formed per g of tumor in the TNF group, compared to the amount in the saline-treated group (58.7 ± 7.7 *versus* 40.7 ± 4.5 μg/g/min; $P < 0.05$). Tumor K_d values in the TNF-treated rats were 206% greater than in the saline-treated rats (62.1 ± 10.7 *versus* 20.3 ± 6.0%/day; $P < 0.01$) (Table 2). These saline-treated tumor degradation rates fall within the range of 57.0% for smaller tumors to 14.3% for larger tumors previously reported after 13 days of Walker 256 tumor growth (8). The amount of tumor protein degradation per g of tumor more than doubled in the TNF-treated tumors, compared to saline-treated tumors (50.0 ± 8.9 *versus* 17.5 ± 5.4 μg/g/min; $P < 0.01$).

The administration of TNF significantly increased the concentration of total acid-soluble plasma amino acids (Table 3), total acid-soluble plasma NEAA, total acid-soluble skeletal muscle EAA, and total acid-soluble skeletal muscle NEAA, compared to values in saline-treated tumor-bearing animals (Fig. 3). Total acid-soluble skeletal muscle NEAA were also significantly increased compared to levels in tumor-free controls. In contrast, total acid-soluble plasma EAA and NEAA levels were significantly decreased in the saline-treated tumor-bearing rats, compared to the tumor-free control rats. Individual acid-soluble amino acid levels are listed in Table 3. Various individual amino acids did differ among the experimental groups; those differences and the correlations between individ-

Table 1 *Body growth and mean intake data during the 11-day study*

Group	No. of rats	Initial body weight (g)	Final body weight[a] (g)	Food intake (g/day)	Weight gain (g/day)
Control	8	68 ± 1[b]	121 ± 2	12.3 ± 0.2	5.3 ± 1.0
Tumor, saline	7	67 ± 1	121 ± 2	12.2 ± 0.3	5.4 ± 1.3
Tumor TNF	7	70 ± 1	121 ± 1	12.1 ± 0.1	5.1 ± 1.6

[a] Final body weight includes tumor weight.
[b] Mean ± SE.

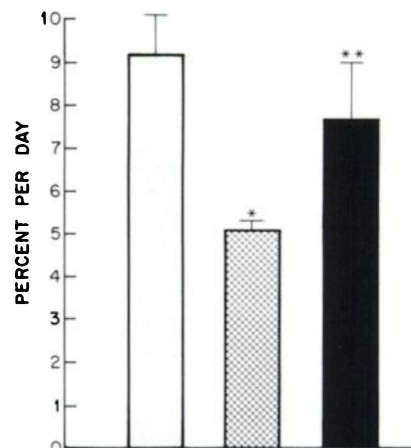

Fig. 2. Muscle protein synthesis rates are represented for the tumor-free controls and the two tumor-bearing groups. Abdominal rectus muscle was used for these determinations. The saline-treated tumor-bearing rats demonstrated a significantly ($P < 0.05$) reduced muscle protein synthesis rate (5.1 ± 0.2%/day), compared to both the TNF-treated and the tumor-free control groups. The administration of 50 μg of human recombinant TNF was associated with a near normalization of the muscle protein synthesis rate in the tumor-bearing rats (7.7 ± 1.3%/day), compared to the tumor-free controls (9.2 ± 0.9, $P > 0.05$). □, tumor-free controls; ■, tumor-bearing (saline); ■, tumor-bearing (TNF); *, $P < 0.01$ *versus* control; **, $P < 0.05$ *versus* saline.

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Table 2 *K_g, K_s and K_d in the Walker 256 carcinosarcoma studied in vivo*

Tumor growth rate is the mean growth rate between days 9 and 11 calculated from the change in tumor protein content over 2 days. K_s was determined on day 11. Tumor protein degradation is determined as the difference between tumor protein growth in %/day and tumor protein synthesis in %/day ($K_d = K_s - K_g$).

	No. of rats	K_g (%/day)	K_s (%/day)	K_d (%/day)
Tumor, saline	7	27.6 ± 4.1[a]	47.9 ± 4.8	20.3 ± 6.0
Tumor TNF	7	10.5 ± 3.5[b]	72.7 ± 9.1[c]	62.1 ± 10.7[b]

[a] Mean ± SE.
[b] $P < 0.01$.
[c] $P < 0.05$.

ual amino acid levels and tumor weight, growth, protein synthesis, and degradation are also shown in Table 3. The values for 3MH are of note, since muscle and plasma acid-soluble concentrations of this amino acid can increase only as a result of degradation of muscle protein. Although plasma 3MH values are significantly increased in both tumor groups, muscle concentrations are elevated only in the TNF-treated tumor group.

DISCUSSION

TNF and interleukin 1 are proposed mediators of many metabolic responses to endotoxemia (17, 18); these include the catabolic responses. In cases of bacteremia or endotoxin administration, muscle protein synthesis is variably reported to be either increased (15, 19, 20) or decreased (21, 22). The administration of interleukin 1 is associated with a decrease (23) or no change (24, 25) in muscle protein synthesis. TNF applied *in vitro* fails to affect skeletal muscle protein synthesis after a 2-h incubation (25, 26). In this study, the reduced muscle protein synthesis rate of the tumor-bearing rat was corrected towards normal by TNF administration (Fig. 2). This could be due to a direct stimulatory effect, or a secondary effect resulting from reduced tumor growth. Previous research has demonstrated that muscle protein synthesis is negatively correlated with tumor size (7, 8), so that some factor associated with the reduced tumor growth induced by TNF might secondarily modify muscle protein synthesis. The absence of a TNF-treated non-tumor-bearing control group prevents the drawing of any conclusions about a direct effect of TNF on skeletal muscle protein synthesis in this study.

All measured acid-soluble skeletal muscle amino acids, except for arginine, were significantly increased after TNF administration in the tumor-bearing rats of this study (Table 3). Total acid-soluble muscle NEAA increased 2-fold, from 52.8 ± 10.9 to 99.3 ± 9.0 nmol/g protein ($P < 0.01$), and total acid-soluble muscle EAA increased from 21.5 ± 3.0 to 46.1 ± 3.8 ($P < 0.01$) with TNF treatment (Fig. 3). Increased acid-soluble amino acid concentrations in skeletal muscle could be due to increased transport in, decreased transport out, or an increased muscle protein degradation. Significant increases in the forearm release of alanine, glutamine, and total amino acids are noted 6 h following *in vivo* administration of TNF in humans (27). Others have recently demonstrated that a 20-μg dose of i.v. TNF increases muscle breakdown by approximately 40% in non-tumor-bearing rats (28). Since the 3MH concentration significantly increased in the TNF-treated skeletal muscle (+70%; $P < 0.01$) (Table 3) and since muscle acid-soluble 3MH concentration has been shown to correlate ($r = 0.96$) in hindlimb preparations with 3MH release (29), the data imply increased muscle protein degradation. The increased amino acid concentrations observed in plasma may also be related to the elevations of amino acids in the muscle of TNF-treated rats. As shown in Table 3, there are a number of significantly increased muscle amino acids in the TNF group which are also significantly increased in the plasma of these animals. A TNF-related increase in muscle acid-soluble amino acid concentrations could lead to increased delivery, via plasma, of these compounds to

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Table 3 *Plasma and tissue acid-soluble amino acid concentrations and correlations with tumor weight, tumor growth rate, protein synthesis rate, and protein degradation rate*

	Amino acid concentration								Correlations (r) between acid-soluble tumor amino acid concentrations and:			
	Plasma (μmol/liter)			Muscle (nmol/g protein)			Tumor (nmol/g protein)					
	Control	Tumor (saline)	Tumor (TNF)	Control	Tumor (saline)	Tumor (TNF)	Tumor (saline)	Tumor (TNF)	Tumor weight	K_g	K_s	K_d
EAA												
THR	279 ± 9[a]	220 ± 23[b]	284 ± 21[c]	5.6 ± 0.7	4.1 ± 0.9	8.0 ± 0.8[b, d]	1.7 ± 0.2	22.8 ± 1.6[d]	−0.41	−0.63	0.62	0.69
VAL	99 ± 6	113 ± 14	109 ± 4	0.5 ± 0.1	1.1 ± 0.3[b]	2.1 ± 0.2[d, e]	5.5 ± 0.8	3.4 ± 0.2[c]	−0.33	−0.18	−0.37	−0.17
ISO	41 ± 3	51 ± 3[b]	53 ± 3[e]	0.6 ± 0.1	0.7 ± 0.1	1.2 ± 0.1[c, e]	1.8 ± 0.3	2.1 ± 0.2	−0.70	−0.75[f]	0.05	0.27
LEU	580 ± 33	577 ± 28	613 ± 29	5.8 ± 0.1	4.9 ± 0.9	8.9 ± 1.0[b, c]	10.7 ± 2.3	12.8 ± 1.2	−0.59	−0.51	0.26	0.38
PHE	51 ± 3	67 ± 4[e]	66 ± 3[e]	0.5 ± 0.1	0.6 ± 0.1	1.1 ± 0.1[c, e]	2.3 ± 0.4	2.3 ± 0.2	−0.61	−0.64	−0.01	0.26
LYS	561 ± 24	359 ± 32[e]	418 ± 14[e]	18.7 ± 3.2	8.3 ± 1.7[b]	21.5 ± 1.7[d]	7.2 ± 0.8	9.8 ± 0.7[c]	−0.72	−0.66	0.36	0.51
HIS	44 ± 1	46 ± 3	46 ± 2	1.9 ± 0.3	1.8 ± 0.4	3.3 ± 0.3[d, e]	2.1 ± 0.3	2.4 ± 0.3	−0.70	−0.64	0.22	0.44
NEAA												
ASP	15 ± 1	16 ± 1	18 ± 1	2.4 ± 0.3	1.8 ± 0.2	3.7 ± 0.3[d, e]	6.8 ± 0.8	8.2 ± 1.0	−0.47	−0.66	0.29	0.63
SER	211 ± 5	127 ± 9[e]	178 ± 11[b, d]	6.7 ± 1.1	3.6 ± 0.9[b]	7.8 ± 0.8[d]	3.9 ± 0.7	5.5 ± 0.9	−0.67	−0.62	−0.03	0.24
GLU	117 ± 6	108 ± 6	104 ± 6	9.4 ± 2.3	6.1 ± 1.4	12.2 ± 1.3[c]	29.2 ± 4.1	32.3 ± 2.3	−0.62	−0.60	0.22	0.41
GLY	201 ± 6	134 ± 1[e]	193 ± 11[d]	25.7 ± 3.8	19.9 ± 3.8	38.0 ± 4.0[b, d]	20.1 ± 1.9	27.9 ± 1.4[d]	−0.62	−0.72	0.50	0.64
ALA	234 ± 18	288 ± 23	343 ± 16[e]	17.6 ± 2.9	15.2 ± 3.0	28.3 ± 2.4[b, d]	43.9 ± 3.7	52.5 ± 5.0	−0.46	−0.53	0.39	0.50
TYR	56 ± 5	69 ± 4[b]	67 ± 3	0.9 ± 0.1	1.0 ± 0.2	1.6 ± 0.1[c, e]	2.6 ± 0.4	2.9 ± 0.2	−0.77[f]	−0.75[f]	0.13	0.39
ORT	40 ± 4	62 ± 5[e]	61 ± 5[e]	0.7 ± 0.1	0.7 ± 0.2	1.2 ± 0.1[c, e]	1.7 ± 0.2	2.0 ± 0.1	−0.79[f]	−0.46	−0.18	0.02
ARG	96 ± 4	63 ± 4[e]	72 ± 5[e]	4.5 ± 0.7	4.4 ± 1.3	6.4 ± 0.6	1.6 ± 0.5	2.4 ± 0.5	−0.53	−0.61	−0.05	0.24
3MH	3.8 ± 0.2	4.9 ± 0.3[b]	4.9 ± 0.4[b]	0.049 ± 0.009	0.066 ± 0.014	0.113 ± 0.019[c, e]	0.155 ± 0.015	0.164 ± 0.030	−0.01	−0.41	−0.05	0.21
Total AA amino acids	2629 ± 72	2305 ± 139[b]	2630 ± 83[c]	101 ± 16	74 ± 13	145 ± 13[b,c]	141 ± 16	190 ± 12[c]				

[a] Mean ± SE. Statistical analysis was performed by multivariant analysis of variance.
[b] $P < 0.05$ *versus* control.
[c] $P < 0.05$ *versus* tumor (saline).
[d] $P < 0.01$ *versus* tumor (saline).
[e] $P < 0.01$ *versus* control.
[f] $P < 0.0033$ was defined as the level of significance to take into account the multiple comparisons.

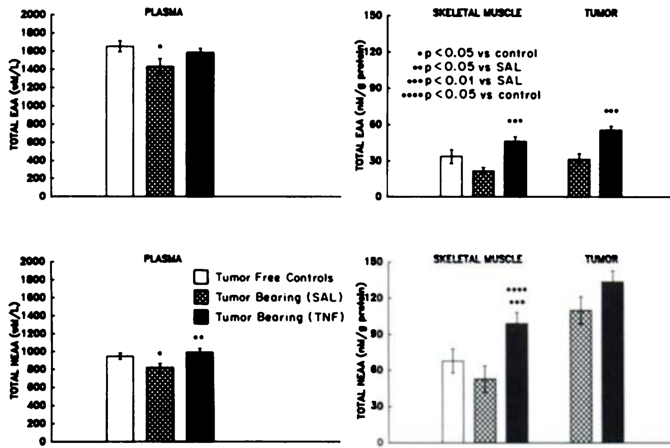

Fig. 3. Total acid-soluble EAA and total acid-soluble NEAA concentrations in plasma, skeletal muscle, and tumor tissue were compared by multivariant analysis of variance. Significant reductions in concentrations of plasma total EAA and total NEAA were observed in the saline-treated tumor-bearing group, compared to tumor-free controls. Total plasma NEAA were significantly increased in the TNF-treated *versus* the saline-treated tumor-bearing group. Skeletal muscle total EAA and total NEAA were both significantly elevated in the TNF- *versus* saline-treated tumor-bearing group. Furthermore, the total skeletal muscle NEAA were significantly greater in the TNF-treated rats compared to the tumor-free controls. Total tumor EAA were also increased in the TNF-treated group of rats. *SAL*, saline.

the tumor. Tumor tissue amino acid concentrations were negatively correlated with tumor weight and tumor growth (Table 3). This relationship could be due to increased tumor degradation or to an inhibitory effect of excess amino acids on tumor growth. In fact, *in vitro* studies with the Walker 256 carcinosarcoma cell line have demonstrated that elevated concentrations of either lysine or glycine can inhibit tumor growth (30). Clearly, further work is needed to investigate the possibility of amino acid inhibition of tumor growth.

TNF administration in mice reduces tumor growth after 24 to 48 h and the effects last for as long as 7 days (2, 3). In the present study, the Walker 256 carcinosarcoma demonstrated a significant reduction in growth after a single administration of TNF. Although TNF increased both tumor protein synthesis and degradation rates, the net effect was a slowing of tumor growth. The process(es) of decreasing tumor growth may be enhanced synthesis of proteases and/or other peptides which enhance protein catabolism in the tumor tissue. In fact, intratumor injections of proteases have been recently demonstrated to reduce tumor growth (31). Other authors have suggested that TNF may initiate the production of procoagulants (17) or fibrin-like deposition in tumor capillaries (32), with subsequent injury to the microcirculation leading to microthrombosis and hemorrhage. Hemorrhage into the tumor tissue was not grossly evident in this study but, if present, would not be expected to be associated with an increase in protein synthesis. Thus TNF-induced increases in the synthesis of specific degradative enzyme proteins, followed by increased rates of tumor protein degradation, are the possible explanation of the net effect of TNF. Any such protein products have yet to be determined.

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