Evaluation of Anorexia as the Cause of Altered Protein Synthesis in Skeletal Muscles from Nongrowing Mice with Sarcoma

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ABSTRACT

The importance of decreased food intake as the mechanism behind altered protein metabolism in skeletal muscle in cancer was evaluated.

A methylcholanthrene-induced sarcoma (MCG 101) transplanted in weight-stable and nongrowing mice (C57BL/6J) was used as the tumor-animal model. Three study groups with appropriate control groups were used: sarcoma-bearing mice; pair-fed mice; and starved mice.

The synthesis of myofibrillar and sarcoplasmic proteins was decreased in sarcoma-bearing mice. This was correlated to decreased content of RNA in the muscles and caused a net loss of muscle tissue as measured by dry weight of skeletal muscles. The incorporation rate of amino acids into myofibrillar and sarcoplasmic proteins was decreased to the same extent in the pair-fed mice as that in the sarcoma-bearing mice. This probably reflected decreased protein synthesis, since the radioactivity (dpm/mg) did not differ significantly in the crude RNA fraction between the groups. Separation of soluble proteins from muscle tissue by means of ion-exchange chromatography showed that the pattern of decreased protein synthesis was not tumor specific when compared to muscle affected by starvation. The decrease in protein synthesis was more or less selective, since the synthesis of basic proteins was considerably decreased and was influenced more than were neutral and acidic proteins in both cancer and starvation.

Anorexia of a tumor-bearing host is a sufficient trigger to induce decreased protein synthesis in skeletal muscles, but other factors may also be of quantitative importance.

INTRODUCTION

A growing malignant tumor has a pronounced effect on protein metabolism in skeletal muscle (4, 5, 11, 22, 48). The alteration of protein metabolism in cancer has many features in common with those reported for malnutrition, trauma, and starvation (53). A key factor in the energy metabolism of the cancer patient is a loss of appetite (anorexia), which transforms the patient into a semistarved subject as pointed out by Munro (37). Insufficient control of food intake in cancer cachexia may be of greater importance (11, 14).

The aim of this study was to determine if the decrease in food intake per se is a sufficient trigger to induce alterations in the synthesis of skeletal muscle proteins in the nongrowing tumor host as found in conditions of pure caloric restriction.

MATERIALS AND METHODS

L-[guanido-14C]arginine (20 to 40 Ci/mmol), L-[U-14C]leucine (270 mCi/mmol), and L-[4,5-3H]leucine (5 mCi/mmol) were from New England Nuclear Chemicals (West Germany). DEAE-Sepharose CL-6B was from Pharmacia, Uppsala, Sweden. Yeast RNA and all other reagents were from Sigma Chemical Co., St. Louis, Mo.

Animal Model

Male and female C57BL/6J mice were used (24). All experiments were performed in 3-month-old, nongrowing, weight-stable mice (20 to 25 g). They were randomly allocated to the study groups and the corresponding control groups. A methylcholanthrene-induced sarcoma (MCG 101), which has been transplanted continuously in vivo for 4 years in our laboratory was used. The tumor was implanted s.c. in the flanks of the animals under aseptic conditions, and the controls were sham implanted. The tumor grows continuously and does not metastasize. The animals died with cachexia 14 ± 1 (S.D.) days after the tumor implantation. The tumor dry weight was 10 to 14% of the carcass dry weight (minus the tumor) when the animals died and about 7 to 10% of the body weight of the controls at the time when the metabolic experiments were performed 10 to 11 days after the tumor implantation (26). This tumor leads to metabolic host reactions similar to those found in human cancer (24, 26).

Housing of Animals and Feeding Experiments

Some experiments were performed with the animals in metabolism cages (Jacoby, Stockholm, Sweden) slightly modified by us for mouse experiments (26, 28) with one animal in each cage. Other experiments were performed with the animals in ordinary mouse cages with 5 animals in each cage. The experimental period in metabolism cages started and the tumor was implanted when the animals had spent 3 to 5 days in these cages, thus reaching constant body weight. The tumor was implanted when the animals had spent 3 to 5 days in these cages, thus reaching constant body weight. Body weight was recorded every morning. Food was supplied and food intake was registered at 8 a.m., 3 p.m., and 10 p.m. each day, with the animals in metabolism cages. Food was supplied in the metabolism cages (maintenance diet for rats and mice; AB Astra, Södertälje, Sweden) in a side box at floor level. It was not possible for the animals to climb in these metabolism cages. Thus, tumor-bearing and control animals kept in metabolism cages.
cages may have been less active compared with animals in ordinary cages. Metabolism cages may therefore represent a different experimental situation than do ordinary cages. This was evident, since the metabolism in skeletal muscle differed between control animals housed in metabolism cages compared with controls in ordinary cages.3 Because of this, studies with appropriate control animals were always run in parallel with those of a specific study group in all types of experiments. Survival time of the tumor-bearing animals in the metabolism cages did not differ from that of the tumor-bearing animals kept in ordinary cages. Experiments with pair-fed mice in metabolism cages were performed to elucidate the importance of the progressively decreasing food intake for the protein metabolism in the skeletal muscle of the tumor host. Control animals adapted to the metabolism cages were put on feeding schedules aimed at mimicking the spontaneously decreasing food intake in the tumor group (Chart 1A). This experimental group of animals will be referred to as "pair-fed" mice. Food was supplied as described above. The animals had free access to tap water in all experiments. The control animals had free access to food and tap water and will be referred to as controls. All experiments were performed 11 days after the tumor implantation.

**Experimental Protocol**

**Group 1.** The animals (10 sarcoma-bearing mice and 10 controls) were kept in ordinary cages, 5 animals in each cage, with free access to food and tap water. [3H]Leucine (1 µCi/g) was given i.p. 4 hr before the animals were killed. Myosin and soluble proteins were isolated as described by Weeds and Hartley (52). 3H radioactivity per protein was determined.

**Group 2.** These animals (13 sarcoma-bearing mice and 12 controls) were kept in metabolism cages with free access to food and tap water. The animals were given i.p. injections of [14C]leucine (0.1 µCi/g) 4 hr before they were killed for experiments. Radioactivity was measured in the myosin and in the soluble protein fraction and referred to protein weight.

**Group 3.** These animals (20 pair-fed mice and 20 controls) were kept in metabolism cages with free access to food and tap water. The animals were given i.p. injections of [14C]leucine (0.1 µCi/g) 4 hr before they were killed for experiments. Radioactivity was measured in the myosin and in the soluble protein fraction and referred to protein weight.

**Group 4.** These animals (5 sarcoma-bearing mice, 5 pair-fed mice, and 4 controls) were kept in metabolism cages. The tumor-bearing animals had free access to food, while pair-fed animals were supplied with food equivalent to the amount spontaneously eaten by the tumor-bearing animals in this experiment. Controls had free access to food. All animals had free access to water. The animals were given i.p. injections of [14C]leucine (0.6 µCi/g) 4 hr before they were killed. The specific radioactivity (dpm/mg RNA) was measured in the crude tRNA fraction and in acid-precipitable protein from skeletal muscle tissue.

**Group 5.** These animals (15 control mice) were kept in ordinary cages, had free access to food and water, and received both [14C]leucine (0.4 µCi/g) and [3H]leucine (4 µCi/g) i.p. as a single injection. The animals were killed 4 hr later. Soluble muscle proteins were prepared individually from each animal. These protein samples were then pooled to one sample, which was then applied to ion-exchange chromatography (DEAE-Sepharose CL-6B), and the proteins were separated into 250 fractions as shown in Chart 3. The 14C/3H radioactivity ratio was determined in each sample throughout the chromatogram. From these, the 99% confidence interval was calculated for determination of 14C/3H radioactivity ratio in soluble muscle proteins as illustrated in Chart 3.

**Group 6.** These animals (15 sarcoma-bearing mice and 15 controls) were kept in ordinary cages with free access to food and tap water. The tumor-bearing animals received [14C]leucine (0.4 µCi/g) and the controls received [3H]leucine (4 µCi/g) 4 hr before they were killed. Soluble muscle proteins were isolated from each of the 30 animals. These fractions were then pooled into one sample. This sample contained 14C-labeled proteins from the tumor animals and 3H-labeled proteins from the control mice. The proteins were then separated into 250 fractions as shown in Chart 3, and the 14C/3H radioactivity ratio was measured in each fraction and related to the 99% confidence interval as determined in the control experiment (Group 5).

**Group 7.** These animals (25 starved mice and 15 control mice) were kept individually in ordinary cages. The "starved group" was starved for 72 hr with free access to tap water, and the control mice had free access to food and water. Some of the starved animals died spontaneously between the second and third days. Fifteen of the least affected animals were selected from the starved group after 72 hr of starvation. These animals were given i.p. injections of [14C]leucine (0.4 µCi/g), and 15 control animals received [3H]leucine (4 µCi/g) 4 hr before they were killed. Soluble proteins were prepared individually from each animal as described for Groups 5 and 6, and the samples were then pooled to one protein sample. This sample contained 14C-labeled proteins from the starved animals and 3H-labeled proteins from the controls. The proteins were separated, and the 14C/3H radioactivity ratio was determined in each fraction as described for Group 6.

**Preparation of Muscle Tissue and Isolation of Proteins**

Skeletal muscle tissue was excised and trimmed of fat and connective tissue at 4°. The tissue was cut into small pieces and rinsed of blood and debris. Skeletal muscle tissue was always taken by excision of the muscles between the hip and the knee joint of the hind limb (24). Skeletal muscle tissue was homogenized in water (10% homogenate, v/v) with an Ultra Turrax homogenizer (Janke & Kunkel, West Germany). Triton X-100 was added to a final concentration of 0.2% (v/v) before preparation of soluble proteins. The homogenate was incubated at 4° for 60 min to solubilize the proteins (28). Acid-precipitable proteins were prepared by precipitation with trichloroacetic acid (final concentration, 10% w/v) after homogenization of the tissue. The procedures for the extraction of lipids, nucleic acids, and solutes have been described in detail elsewhere (22-24, 26-28). Proteins were solubilized in So-
Anorexia as Cause of Altered Protein Synthesis

luene 100 (Packard Instrument Co., Downers Grove, Ill.) before radioactivity was counted. The protein content was estimated in aliquots of the protein fractions according to the method of Lowry et al. (21) with bovine serum albumin as a standard. Soluble proteins intended for ion-exchange chromatography (Chart 3, Groups 5, 6, and 7) were extracted in 10 mM Tris-HCl, pH 7.4, instead of in deionized water. As judged from analytical isoelectrofocusing electrophoresis, these procedures extracted the same proteins. RNA content was estimated according to the method of Munro and Fleck (38), and protein contamination in RNA fractions was estimated in each sample and corrected for. Soluble proteins were separated by means of ion-exchange chromatography as follows. Muscle tissue was homogenized and incubated in the presence of Triton X-100 as described above. The homogenates were centrifuged at 15,000 × g for 20 min. The pellet was discarded, and the supernatant was centrifuged twice at 105,000 × g for 1 hr. The supernatants from different animal samples containing soluble proteins were pooled to one fraction (27 ml). This fraction was passed through a Sephadex G-25 column (1.6 x 30 cm) equilibrated in 10 mM Tris-HCl, pH 7.4, to remove solutes, Triton X-100, and amino acids. The protein fraction was concentrated to 13.5 ml by vacuum filtration (Catalog No. PTGC 11 KON; Millipore Corp., Bedford, Mass.). The concentrated protein fraction was then loaded onto a cationic exchange column (DEAE-Sepharose CL-6B, 1.6 x 30 cm) equilibrated in 10 mM Tris-HCl, pH 7.4. The flow rate through the column was 0.15 ml/min. Proteins were monitored in a flow cell as absorbance at 280 nm.

Proteins with isoelectric points less than pH 7.4 separated partially when eluted with the equilibration buffer. Proteins with isoelectric points higher than pH 7.4 were eluted with a linear gradient of sodium chloride. The gradient was generated according to standard procedures with 250 ml 10 mM Tris-HCl, pH 7.4, and 250 ml of the same buffer containing 500 mM NaCl. The gradient was run until the recorder reached the baseline. The ionic strength of the eluate was not determined, since this procedure gave highly reproducible results. The most acidic proteins were then eluted stepwise with 1 M NaCl in the buffer. Fractions (2 ml, n = 250) were collected throughout the chromatogram. The 14C/3H radioactivity ratio was determined by counting 1 ml from each fraction in 9 ml Dimilume 30 (Packard). Fractions that contained more than 200 dpm of either 14C or 3H radioactivity were used for the calculation of the 14C/3H radioactivity ratio. Counting efficiency did not vary among the fractions. Chromatography was performed at room temperature, and the columns were cooled by tap water (8–10°C). The resin bed was used for one separation procedure only.

The eluted fractions containing proteins were analyzed by means of analytical isoelectrofocusing electrophoresis in polyacrylamide gels at pH 3.5 to 10.0 (LKB Beckman, Sweden). The high ionic strength in some of the eluted fractions was not normalized before this electrophoresis.

Preparation of tRNA

The skeletal muscle tissue was immediately excised and frozen in nitrogen. The tissue was then homogenized as described above at 0–2°C in 0.1 M sodium acetate containing 0.9 M sodium chloride, pH 4.6 (20% homogenate, w/v). The homogenate was centrifuged at 15,000 to 20,000 × g for 20 min. The supernatant was extracted with redistilled phenol. Soluble RNA was precipitated with 2 volumes of 95% ethanol at 4°C overnight. The precipitate was washed twice with 95% ethanol at 4°C and then dissolved in water and filtered through a column of G-25 (PD column Pharmacia). The void volume in this chromatography was then analyzed on a column of G-75 superfine (1.6 x 90 cm) and was found to contain material absorbing at 260 nm with molecular weights between 18,000 and 30,000. This fraction was defined as consisting of crude tRNA according to cochromatography with commercially available pure tRNA from calf liver (Boehringer-Mannheim, Mannheim, Germany). Each muscle specimen was prepared separately for estimation of specific radioactivity tRNA. The specific radioactivity (dpm/mg protein) of acid-precipitable protein was measured in a piece of muscle from the same specimen as that for tRNA.

Counting of Radioactivity

Radioactive samples were counted in Packard Tri-Carb Model 3220 instrument. Counting efficiency was determined with the external standard method. The spillover of 3H radioactivity into the 14C channel was less than 1%, and the spillover of 14C radioactivity into the 3H channel was 15 to 20% when the 2 isotopes were counted in the same samples.

Statistics

The nonparametric Mann-Whitney U test (46) was used to evaluate the statistical significance between independent samples. The 99% confidence interval was calculated as x ± t001 • S.D.

RESULTS

The time courses of food intake and body weight of sarcoma-bearing pair-fed and control mice are shown in Chart 1. Body weight declined significantly in the pair-fed group at the end of the feeding experiments. This was mainly due to loss of body water (26). Starvation for 72 hr decreased body weight from 24.34 ± 1.10 (S.E.) to 18.10 ± 0.65 g (Group 7). Simultaneous i.p. injection of [3H]leucine and [guanido-14C]arginine in sarcoma-bearing mice and controls showed that the radioactivity in muscle proteins approached the maximal level within 4 hr of injection (Chart 2). The incorporation of [3H]leucine and [guanido-14C]arginine into muscle proteins was consistently lower between 4 and 36 hr in the tumor-bearing animals than in controls (Chart 2). This indicates that our results did not depend on the point in time chosen for measurements. The incorporation of [3H]leucine and [14C]leucine into acid-precipitable muscle proteins was decreased by about 40 to 50% in the tumor-bearing animals housed in ordinary cages and in metabolism cages, respectively, compared with controls (Chart 2; Table 1) as reported previously (24). This value agrees with that reported by Stein et al. (48) from studies with [15N]glycine infused for 18 hr at a constant rate into tumor-bearing animals. Amino acids were incorporated into myosin and into soluble proteins to approximately the same extent both in tumor-bearing animals and in controls within 4 hr (Table 2). The incorporation of labeled leucine into myosin and soluble proteins was
Chart 1. Time course of food intake (A) and body weight (B) in tumor-bearing mice, pair-fed mice, and controls. The experiments were performed with the animals in metabolism cages as described in "Materials and Methods." X, tumor-bearing mice (n = 14); A, pair-fed mice (n = 20); O, controls (n = 20); bars, S.E.

Chart 2. Time course of radioactivity in acid-precipitable proteins from skeletal muscle tissue in sarcoma-bearing mice and controls. A double isotope solution of [3H]leucine (1 μCi/g) and [guanido-14C]arginine (0.1 μCi/g) was injected i.p. at zero time. Determination of radioactivity as described in "Materials and Methods;" mean of 2 animals in each point.

Table 1
Specific radioactivity of crude tRNA fraction in relationship to that of acid-precipitable proteins in skeletal muscle tissue from sarcoma-bearing mice, pair-fed mice, and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Acid-precipitable proteins (dpm/mg)</th>
<th>tRNA (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Sarcoma-bearing mouse</td>
<td>340 ± 64</td>
<td>0.76 ± 0.02</td>
</tr>
<tr>
<td>II. Pair-fed mouse</td>
<td>262 ± 45</td>
<td>0.85 ± 0.02</td>
</tr>
<tr>
<td>III. Controls</td>
<td>208 ± 65</td>
<td>0.85 ± 0.02</td>
</tr>
</tbody>
</table>

a Statistical values (Mann-Whitney U test) were not significant for I versus II, I versus III, and II versus III.

b p < 0.01 (Mann-Whitney U test).

c p < 0.001 (Mann-Whitney U test).

d p < 0.025 (Mann-Whitney U test).

e Numbers in parentheses, number of animals.

Table 2
RNA content and incorporation in vivo of [3H]leucine into skeletal muscle proteins from sarcoma-bearing mice and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Myosin (dpm/mg)</th>
<th>Soluble proteins (dpm/mg)</th>
<th>RNA (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcoma-bearing mice</td>
<td>140 ± 12</td>
<td>150 ± 10</td>
<td>0.76 ± 0.02</td>
</tr>
<tr>
<td>Controls</td>
<td>169 ± 6</td>
<td>183 ± 7</td>
<td>0.85 ± 0.02</td>
</tr>
</tbody>
</table>

a Mean ± S.E.

Significantly decreased in tumor-bearing animals compared with controls. Pair-fed control mice, kept in metabolism cages, also had decreased incorporation rates of [14C]leucine into myosin and soluble proteins as compared to strictly matched control animals (Table 3). Incorporation of amino acid into myosin was also decreased in tumor animals kept in metabolism cages compared to the control animals. The corresponding value for the soluble proteins was lower in the tumor group but not significantly different. The decreased incorporation of radioactive amino acids into muscle proteins from tumor-bearing and pair-fed mice was associated with a decreased muscle tissue RNA content.

Double-isotope chase experiments were performed in order to determine if the protein synthesis in tumor-influenced muscle was altered in the same fashion as that during pure caloric restriction (Groups 5, 6, and 7) (20, 34, 39, 40, 47). Tumor-bearing mice (Group 6) and starved mice (Group 7) were given i.p. injections of [14C]leucine, and the corresponding control animals received [3H]leucine. Soluble proteins were isolated, and the protein fractions from the study and the control group were pooled before the proteins were separated by means of ion-exchange chromatography, as shown in Chart 3. The 14C/3H radioactivity ratio in the eluted protein fraction was deter-
mined and compared to the 99% confidence interval established from the specific control experiment (Group 5) described in "Materials and Methods." Basic proteins (Fractions 10 to 80) judged from iso-electrofocusing electrophoresis showed significantly decreased 14C/3H radioactivity ratios in all of 49 collected fractions containing proteins originating from the tumor-bearing animals and the starved mice. In the region of neutral proteins (Fractions 125 to 150), 16 of 27 fractions from tumor experiments deviated from the 99% confidence interval. In the starved group, 11 of 27 fractions deviated. In the acidic protein region (Fractions 155 to 250), these figures were 24 of 52 for tumor-bearing animals and 37 of 52 for starved animals.

The protein and water content in skeletal muscle tissue did not differ between sarcoma-bearing mice, pair-fed mice, and controls. Fig. 1 shows the soluble protein composition of muscle in sarcoma-bearing, starved, and control mice as determined by iso-electrofocusing electrophoresis. The dry weight of the tumor-influenced muscles decreased by 48 ± 11% (S.E.) (P < 0.025) compared with the controls, while the dry weight of muscle tissue from pair-fed mice decreased only 4 ± 7% (not significant).

**DISCUSSION**

The results in this study agree with previous reports showing that tumor-bearing animals and cancer patients have a decreased synthesis of skeletal muscle proteins illustrated by a decreased incorporation rate of radioactive amino acids into proteins both in vivo and in vitro (4, 11, 12, 22, 25). The results of this study show that this phenomenon is not tumor specific. This conclusion is based on pair-feeding experiments. Arginine gave lower values of incorporation into muscle proteins than did leucine when injected into the same animal (Chart 2). Therefore, results from isotopic studies of this kind must be interpreted with caution (30, 43). However, the decreased dry weight and the decreased RNA content of the muscle, together with the fact that muscle incorporation values were also decreased in sarcoma-bearing mice when the specific radioactivity of the crude tRNA fraction in muscle tissue was taken into account, shows that our results cannot be ascribed to isotopic kinetics only (34). Moreover, our results are consistent with those reported by Stein et al. (48) from studies with [15N]glycine infused for 18 hr at constant rate into tumor-bearing rats. With all possible limitations in mind, our results show that the synthesis of myofibrillar and soluble proteins was decreased to the same extent in sarcoma-bearing mice. These results agree with our previous report, which also suggested that cancer patients had an increased fractional degradation rate of muscle protein in vitro (22). In humans, the fractional degradation rate correlated to increased cathepsin D activity in muscle tissue. Our sarcoma-bearing mice also had increased cathepsin D activity, as reported previously (24, 28). Surgical trauma has been reported to elicit decreased muscle protein synthesis but an unchanged fractional degradation rate of myofibrillar proteins (18). These changes in protein turnover were not due to a decrease in food intake.

Experiments with pair-fed animals were performed in order to determine the importance of the malignant tumor per se versus the anorexia for the negative nitrogen balance in skeletal muscle in cancer. Our sarcoma-bearing mice decreased their food intake at the time when the tumor became palpable, as shown in Chart 1 and as previously reported (26). Pair-fed animals did not change their body lipid content or their lean body mass significantly, whereas the tumor-bearing animals did (26). This suggests that the pair-fed mice were able to alter their maintenance energy requirement as previously reported for rats (3, 44). This metabolic alteration is consistent with a decreased turnover of muscle protein as described from starvation studies (16, 33).

In agreement with such studies, our pair-fed animals showed a decreased synthesis of myosin and soluble proteins as well as decreased RNA content in muscle tissue to about the same level as that of the tumor-bearing mice at comparable experimental conditions. However, the decreased protein synthesis in pair-fed mice may have been concomitant with decreased or unchanged muscle degradation, since pair-fed and starved mice had unaltered cathepsin D activity in muscle tissue (28) and a significantly lower hepatic protein synthesis as compared to the tumor animals (27). Thus, it seems reasonable to assume that cancer may be associated with a decreased synthesis and increased degradation rate of muscle protein, rapidly leading to wasting of the muscle mass. On the other hand, pair-fed mice may instead have decreased protein synthesis concomitant with an unaltered or decreased degradation rate, but a negative nitrogen balance may still occur (33). This should lead to a slower loss of skeletal muscle mass than that in cancer, which is consistent with our finding that the dry weight of muscle tissue from pair-fed animals was approxi-
The decreased content of RNA in tumor-influenced muscle suggests that transcriptional mechanisms may be involved. The experiments illustrated in Chart 3 were performed in order to determine to what extent, compared to pure caloric restriction, the synthesis of different soluble proteins is altered in cancer and if a tumor-specific pattern exists among proteins. Starved animals were thought of as a comparable group for such qualitative analyses (41). The results show that the synthesis of all separated basic proteins was decreased in sarcoma-bearing mice as they were in starved mice, while the synthesis of neutral and acidic proteins was significantly less influenced ($p < 0.05$). Approximately 50% of the separated neutral and acidic proteins were significantly altered. The meaning of this differential response both in cancer and in starvation is unclear (2, 8, 9, 32, 35, 45). Acidic proteins in general have a shorter turnover time than do basic and neutral proteins, probably associated with regulatory functions (9, 15). This more or less selective decrease in the synthesis of proteins with respect to their turnover time may be one way of minimizing the deleterious consequences for cell function in an acute stress situation. It has been reported previously from in vitro experiments that decreased protein synthesis in muscle from Walker carcinoma-bearing rats was possibly due to a translational defect associated with the function of the 40S ribosomal subunit (5). However, our in vivo results do not support this translational defect as the explanation for decreased synthesis of muscle protein in vivo. This explanation would suggest specific ribosomes for the synthesis of specific proteins.

In conclusion, our results show that cancer is associated with decreased synthesis of both myofibrillar and sarcoplasmic proteins in skeletal muscles. Anorexia of the tumor-bearing host is a sufficient trigger to induce decreased synthesis of both contractile and sarcoplasmic proteins in skeletal muscles, but other factors may also be of quantitative importance. The response to anorexia and perhaps to other factors of the synthesis of muscle proteins mainly affects basic proteins. The results and the conclusions in this experimental study are further supported by the findings that protein synthesis in vitro and muscle RNA content increased considerably after total parenteral nutrition in cachectic patients with and without cancer (25).

REFERENCES


Chart 3. Relationship between synthesis rate and net charge of soluble proteins in skeletal muscles from sarcoma-bearing mice and starved mice. Soluble proteins were extracted in 10 mM Tris-HCl, pH 7.4, as described in "Materials and Methods." The proteins were partially separated by ion-exchange chromatography on Sepharose CL-6B (bottom). The salt gradient for elution of the proteins was linear and was generated with NaCl. Numbered protein fractions (Fractions 1 to 9) were subjected to isoelectrofocusing electrophoresis (top). Fractions 1 to 3, basic proteins; Fractions 4 to 5, neutral proteins; Fractions 6 to 9, acidic proteins. Protein fractions containing less than 200 dpm of either $^{14}$C or $^{3}$H radioactivity per ml are not indicated (middle). Parallel lines indicate the 99% confidence interval for determination of $^{14}$C/$^{3}$H radioactivity ratio in muscle soluble proteins as measured in the separate control experiment (Group 4 in "Materials and Methods"). Fractions with $^{14}$C/$^{3}$H ratios below the confidence interval indicate significantly decreased protein synthesis. Several fractions from sarcoma-bearing and starved mice (Groups 6 and 7) contained $^{14}$C radioactivity which gave ratios less than 0.02. These fractions are not shown since they contained less than 200 dpm of $^{14}$C radioactivity per ml. This indicates that these proteins were synthesized at very low rates.

Anorexia as Cause of Altered Protein Synthesis

Fig. 1. Isoelectrofocusing pattern of soluble muscle proteins from sarcoma-bearing mice (T), starved mice (S), and control mice (C). The electrophoresis was run in polyacrylamide gels, pH gradient 3.5 to 10.0. The proteins were visualized by Coomassie Brilliant Blue.
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