Fractionation of Abdominal Muscle in Tumor-bearing, Starved, and Cortisone-treated Rats

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Summary

Abdominal muscle of rats bearing Walker carcinosarcoma 256 was fractionated into 11 protein subfractions. The results were quantitatively compared with normal, starved, and cortisone-treated animals. Carcass nitrogen was determined as well. Although approximately equal losses of carcass protein were observed in severely starved animals, in animals which responded most severely to cortisone administration, and in animals bearing the largest tumors, the patterns of loss of individual protein fractions from abdominal muscle were distinctly different with each treatment.

Introduction

Nitrogen metabolism in the tumor-bearing host has been the subject of considerable interest (1, 2, 5, 7, 10, 12, 13, 16, 19, 20). Loss of protein in the tumor-bearing animal could result from reduced intake of food, the role of the tumor as a "nitrogen trap" (21), pressure of the tumor on vital organs, interference with organ function, adrenal hypercorticoidism (22), infection, erosion into blood vessels, and release by the tumor of substances which directly or indirectly interfere with normal nitrogen metabolism. Specific mechanisms are as yet unknown.

Studies of individual protein fractions in various organs have led to the discovery of some principles of nitrogen metabolism in vivo (27, 30, 31). These include the following: (a) individual proteins in an organ do not increase as a result of changes in demand depending on the need for their services; and (c) equal losses of total body nitrogen caused by different modalities do not necessarily result in equal responses of individual proteins (i.e., all nitrogen-losing situations are not equivalent). The last conclusion was derived from a study of individual heart and kidney protein fractions in rats following starvation and cortisone administration.

In order to obtain greater insight into the mechanisms which operate in cachexia in tumor-bearing animals, abdominal muscles of rats bearing Walker carcinosarcoma 256 were compared with those of animals which had been starved or had received injections of cortisone. These particular muscles were selected since it was felt that they would be less affected than the muscles of locomotion as a result of reduction in physical activity as the tumor grew to a large size.

Materials and Methods

Male rats, weighing approximately 150 gm, were obtained from a consistent commercial source and were maintained on Rockland rat pellets.

Rats, weighing approximately 200 gm, received either fragments of Walker carcinosarcoma 256 or cortisone injections, or were starved. Tumor implants were inserted into the left side. Tumors were permitted to grow to the desired size as indicated below.

Cortisone acetate was injected at a dose of 30 mg/kg daily. Some animals received daily injections until they lost approximately 15% of weight, and some, 30%. This required approximately 2 weeks and 4 weeks, respectively. Other rats were starved in 2 groups, 1 for a period of 10 days, the other for a period of 15 days; water was offered ad libitum.

The animals were sacrificed with ether anesthesia. The right femur and the complete abdominal muscle mass were removed from the right side in a uniform manner. The femurs were cleaned, and length was measured with a vernier caliper to the nearest 0.01 cm. Abdominal muscle, 0.5 gm, was subjected to fractionation as previously described for the heart (32). The fractionation procedure yielded 3 major fractions: Fraction I was obtained by extraction with 0.28 M sucrose-Versene buffer at pH 5.0, and Fraction IA, at pH 7.4; Fraction II was obtained by extraction with 0.6 M phosphate-pyrophosphate-Versene, and it contains the contractile proteins. Electrophoretic separation with the Kern electrophoretic apparatus resulted in 5 subfractions of Fraction I designated I₁, I₂, I₃, I₄, and I₅; 3 of Fraction IA designated IA₁, IA₂, and IA₃; and 3 of Fraction II designated α, β, and γ. Specific designations could be assigned only to Subfraction I₁, which is usually called myoalbumin, and to the subfractions of Fraction II which correspond to myosin, actomyosin, and contractin, respectively (32). Fraction I was thought to include cytoplasmic proteins, and Fraction IA, microsomal proteins. When the fractionations were completed the values were calculated for the total muscle mass which had been excised.

The tumor and remaining carcass were taken for the determination of protein content. The latter was determined as previously described (28) following solution in concentrated sul-
TABLE 1
RESULTS OF REGRESSION ANALYSIS OF OBSERVABLES OF 18 NORMAL RATS*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Equation</th>
<th>$b$</th>
<th>F*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle protein</td>
<td>In $Y_1 = 4.20130 + 2.36471 (ln Fl)*</td>
<td>0.9143</td>
<td>81.4831</td>
</tr>
<tr>
<td>Carcass protein</td>
<td>In $Y_2 = 6.53750 + 3.87508 (ln Fl)</td>
<td>0.9596</td>
<td>186.1809</td>
</tr>
<tr>
<td>Fraction $I_1$</td>
<td>In $Y_3 = 1.62104 + 4.27662 (ln Fl)</td>
<td>0.8775</td>
<td>33.5705</td>
</tr>
<tr>
<td>Fraction $I_2$</td>
<td>In $Y_4 = 1.37515 + 4.60556 (ln Fl)</td>
<td>0.8750</td>
<td>52.2650</td>
</tr>
<tr>
<td>Fraction $I_3$</td>
<td>In $Y_5 = 0.44500 + 3.94802 (ln Fl)</td>
<td>0.8525</td>
<td>42.5394</td>
</tr>
<tr>
<td>Fraction $I_4$</td>
<td>In $Y_6 = 1.21801 + 4.28192 (ln Fl)</td>
<td>0.8294</td>
<td>33.6835</td>
</tr>
<tr>
<td>Fraction $I_5$</td>
<td>In $Y_7 = 1.24125 + 4.85082 (ln Fl)</td>
<td>0.8876</td>
<td>59.4212</td>
</tr>
<tr>
<td>Fraction $I_{A_1}$</td>
<td>In $Y_8 = 3.23044 + 5.08201 (ln Fl)</td>
<td>0.6283</td>
<td>10.2741</td>
</tr>
<tr>
<td>Fraction $I_{A_2}$</td>
<td>In $Y_9 = 4.35581 + 5.84004 (ln Fl)</td>
<td>0.6986</td>
<td>15.2516</td>
</tr>
<tr>
<td>Fraction $I_{A_3}$</td>
<td>In $Y_{10} = 5.10822 + 6.59913 (ln Fl)</td>
<td>0.7890</td>
<td>26.383</td>
</tr>
<tr>
<td>Fraction $II$</td>
<td>In $Y_{11} = 2.09149 + 2.77450 (ln Fl)</td>
<td>0.6181</td>
<td>9.8034</td>
</tr>
<tr>
<td>Fraction $II$</td>
<td>In $Y_{12} = 1.83025 + 2.74971 (ln Fl)</td>
<td>0.6491</td>
<td>11.6522</td>
</tr>
<tr>
<td>Fraction $II$</td>
<td>In $Y_{13} = 1.11073 + 3.50086 (ln Fl)</td>
<td>0.7367</td>
<td>18.9865</td>
</tr>
</tbody>
</table>

* Values are expressed as mg of protein. Analysis of variance of total group significant at 0.01 for all 13 variables.
$^b$ Correlation coefficient.
$^b$ F, Ratio test derived by analysis of variance.
$^b$ Yn, Dependent variables.
$^b$ Femur length (cm).

Results
Principles Used in Evaluating the Data

These principles are based on previous experiences with material of this kind (25, 29).

1. Femur length is a function of maximum growth. There is no reduction in femur length with large losses of carcass nitrogen.

2. The total amount of a protein in an organ of a normal animal is a linear function of femur length when the data are expressed in an ln-ln relationship.

3. The femur length may be used to determine the quantity of a protein which was present in an organ prior to an induced nitrogen loss provided the animal was normal at that time when nitrogen loss was induced.

4. The difference between the observed quantity of a protein and the quantity calculated in Principle 3 above represents change which results from a given treatment.

Normal Regression Equations

In accordance with Principle 2 above, ln-ln regressions were derived for the 18 normal animals correlating the quantity of each observable with the femur length as the independent variable (Table 1).

Experimental Group

The experimental animals were subgrouped as shown in Table 2.

Loss of Carcass and Total Muscle Protein

The ranked mean residuals of carcass protein for each experimental subgroup, determined as the difference between the In-observed value and the ln value calculated from femur length, using the proper regression equation as well as similarly derived values for muscle protein, are shown in Table 3. Groups C-S, T-5, and S-S exhibited approximately equal losses of carcass protein. Animals which received more moderate treatment or which had smaller tumors lost less protein. There was no distinguishing pattern between the experimental conditions. However, a distinctly different pattern was seen in the case of the total muscle protein. The cortisone-treated animals lost considerably less protein from this source as compared with the tumor-bearing and starved animals. Although Group C-S ranked 1st with regard to carcass protein loss it ranked 8th with regard to loss of total muscle protein. Groups T-5, S-S, and T-4, which ranked 2nd, 3rd, and 4th in the former series, ranked 1st, 3rd, and 2nd, respectively, in the latter. The muscle in the...
Discussion

When an experimental variable is imposed on a growing organism the rate of growth is usually affected. Even when the weight loss is induced growth may continue for a time. Therefore, in evaluating the effect of a treatment on the content of a specific substance either in the total organism or in an individual organ, it is necessary to compare the quantity of that substance with the quantity present at the point of maximum growth prior to the cessation of growth, rather than at the time when treatment was started, i.e., in animals of identical weight. The latter would be valid only if growth ceased abruptly when the experiment was initiated.

Femur length is a function of maximum growth since there is no shortening with advanced nitrogen loss. In the absence of circumstances which may induce abnormal increase in length, e.g., hyperthyroidism (26) it may be used to compare the animal with itself prior to an induced nitrogen loss or some other experimental manipulation. This procedure has been used in this laboratory for a number of studies (25, 29).

The experiment described above is another example in which the patterns obtained serve to compare with normal patterns and other experimental situations much in the manner that serum proteins are used for diagnostic purposes.

The tumor-bearing animal restricts its food intake at the very time when additional protein and calories are needed for the synthesis of tumor tissue (21). In general, tumors contain more proteins for diagnostic purposes.

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The tumor-bearing animal restricts its food intake at the very time when additional protein and calories are needed for the synthesis of tumor tissue (21). In general, tumors contain more proteins than is stored in the host during tumor growth. The difference is obtained from the host, chiefly from muscle (24).

TABLE 2

<table>
<thead>
<tr>
<th>Designation</th>
<th>Number</th>
<th>Mean weight loss (%)</th>
<th>Tumor protein (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starved animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe starved</td>
<td>S-S</td>
<td>8</td>
<td>34</td>
</tr>
<tr>
<td>Moderately starved</td>
<td>S-M</td>
<td>8</td>
<td>23</td>
</tr>
<tr>
<td>Cortisone-treated</td>
<td>C-S</td>
<td>7</td>
<td>31</td>
</tr>
<tr>
<td>Maximum response</td>
<td>C-M</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Tumor-bearing animals</td>
<td>T-1</td>
<td>5</td>
<td>&lt;3</td>
</tr>
<tr>
<td></td>
<td>T-2</td>
<td>5</td>
<td>3 to &lt;5</td>
</tr>
<tr>
<td></td>
<td>T-3</td>
<td>5</td>
<td>3 to &lt;6</td>
</tr>
<tr>
<td></td>
<td>T-4</td>
<td>6</td>
<td>6 to &lt;7.5</td>
</tr>
<tr>
<td></td>
<td>T-5</td>
<td>6</td>
<td>7.5 to 12</td>
</tr>
</tbody>
</table>

T-groups seemed to exhibit a response similar to that of the starved groups, but not entirely in sequence of tumor size.

Loss of Protein from Individual Fractions

Inspection of the rankings of the experimental groups reveals differences in behavior of individual protein subfractions under the various treatments (Table 4).

Groups S-M and S-S exhibited the greatest losses of protein in Subfractions I5 and I4; and Group S-S, in Subfractions Iα and Iβ. For Subfractions I5 and I4, Groups S-M and S-S formed separate subsets, whereas the T-groups ranked with Groups C-S and C-M as subsets. For Fraction Iα Groups T-5, T-4, T-2, and T-3 formed a subset with Groups S-S and S-M. For Fraction Iβ, Group S-S formed a subset with Groups T-5, T-4, T-2, and T-3. Group T-5 exhibited the greatest protein loss in Fractions I5, I4, I3, Iα, Iβ, and it ranked second in Fractions I2 and Ia2. For Subfractions I5 and I4, Groups S-M and S-S exhibited the greatest losses of protein for these two subfractions. Fractions I2 and Ia2 were too dispersed to reveal statistical groupings which group the T-series with either the C- or S-subgroups.

Another subset included the T-group with C-M and C-S. The findings show that for the abdominal muscle, T-4, and T-1 occurred in a separate subset for Fraction I3. Likewise, the C-S and S-M subgroups did not always reveal greater losses than the C-M and S-M groups respectively.

The data revealed that the losses for the T-group were, in some cases, statistically similar to the C-groups (Fractions I5, I4, IA1, IA3), to the S-groups (Fractions I5, Iα, and Iβ), and to neither (Fractions I4 and Iγ).

Discussion

When an experimental variable is imposed on a growing organism the rate of growth is usually affected. Even when the majority of growth is induced growth may continue for a time. Therefore, in evaluating the effect of a treatment on the content of a specific substance either in the total organism or in an individual organ, it is necessary to compare the quantity of that substance with the quantity present at the point of maximum growth prior to the cessation of growth, rather than at the time when treatment was started, i.e., in animals of identical weight. The latter would be valid only if growth ceased abruptly when the experiment was initiated.

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The experiment described above is another example in which equal losses of carcass nitrogen induced by various means do not necessarily result in similar loss of individual proteins of an organ (31). The findings show that for the abdominal muscle, at least, the over-all pattern of nitrogen loss in the tumor-bearing animal is quite distinct from that produced by starvation and by cortisone administration. Adrenal cortical hyperplasia occurs in tumor-bearing rats (18). However, the substance elaborated by the rat adrenal is predominantly corticosterone (4).
TABLE 4
RANK OF MEAN RESIDUALS OF PROTEIN SUBFRACTIONS IN EXPERIMENTAL ANIMALS

<table>
<thead>
<tr>
<th>RANK</th>
<th>Fraction Ii</th>
<th>Fraction Ii</th>
<th>Fraction Ii</th>
<th>Fraction Ii</th>
<th>Fraction Ii</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subgroup</td>
<td>−Δln</td>
<td>Subgroup</td>
<td>−Δln</td>
<td>Subgroup</td>
</tr>
<tr>
<td>1</td>
<td>T-5</td>
<td>−1.5582</td>
<td>T-5</td>
<td>−1.4096</td>
<td>T-5</td>
</tr>
<tr>
<td>2</td>
<td>C-S</td>
<td>−1.4039</td>
<td>T-1</td>
<td>−1.2548</td>
<td>T-4</td>
</tr>
<tr>
<td>3</td>
<td>T-1</td>
<td>−1.3145</td>
<td>T-4</td>
<td>−1.2099</td>
<td>T-1</td>
</tr>
<tr>
<td>4</td>
<td>T-4</td>
<td>−1.2366</td>
<td>C-S</td>
<td>−1.2093</td>
<td>S-M</td>
</tr>
<tr>
<td>5</td>
<td>T-2</td>
<td>−1.1226</td>
<td>S-S</td>
<td>−1.2025</td>
<td>T-2</td>
</tr>
<tr>
<td>6</td>
<td>T-3</td>
<td>−1.0867</td>
<td>C-M</td>
<td>−0.9754</td>
<td>S-S</td>
</tr>
<tr>
<td>7</td>
<td>C-M</td>
<td>−1.0236</td>
<td>T-2</td>
<td>−0.9618</td>
<td>T-3</td>
</tr>
<tr>
<td>8</td>
<td>S-M</td>
<td>−0.6102</td>
<td>S-M</td>
<td>−0.9545</td>
<td>C-M</td>
</tr>
<tr>
<td>9</td>
<td>S-S</td>
<td>−0.3932</td>
<td>T-3</td>
<td>−0.9164</td>
<td>C-S</td>
</tr>
<tr>
<td>10</td>
<td>Control</td>
<td>0.0000</td>
<td>Control</td>
<td>0.0000</td>
<td>Control</td>
</tr>
</tbody>
</table>

- Homogenous subsets are shown by lines. Any 2 subgroups included by same line are not significantly different; any 2 not included by same line are significantly different; any 2 not included by same line are significantly different at 0.05 level.

In at least 2 subfractions (Ii and IIγ) the loss of protein was markedly greater for the T-series than for the C- or S-series, although equal losses of carcass protein were observed for groups Ii, C-S, and S-S.

It was pointed out previously (27, 30) that one of the major factors which controls the quantity of a cellular protein is the "demand or lack of demand for its service" either during an anabolic or antianabolic circumstance. Thus, depending on the circumstance, each protein would respond differently in the face of a nitrogen-losing situation. The result could be a different pattern of distribution of individual proteins in the presence of different causes of equal loss of body nitrogen. Of course, other factors, in addition to that of "work demand," could influence the result. Thus, some may induce or repress the synthesis of individual proteins, but feedback mechanisms would usually be encompassed under the concept of "work demand."

An explanation of cachexia has been offered in the fact that most cancer patients have a significant degree of hypoalbuminemia (10) and that tumors can absorb plasma protein (2, 5, 6, 7, 11, 15, 16) and exhibit a higher uptake of label from 14C-labeled albumin (3, 17, 23). The present study may support this as a partial explanation for cachexia. Plasma albumin travels with myoalbumin in most electrophoretic separations, including the present. Two separate fractions have been demonstrated (14) although the relationship between the 2 has not yet been clarified. It will be noted that subgroups of the T-series along with Group C-S, ranked highest in loss of Fraction Ii. However, the highly individualized behavior of the other subfractions demands additional explanations. The results again emphasize the fact that many aspects of the nature of cachexia are still poorly understood.

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References

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Harry Sobel

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